

Détection, distribution et impacts d'un parasite émergent (Tetracapsuloides bryosalmonae) chez les populations de truites fario (Salmo trutta)

Eloise Duval

► To cite this version:

Eloise Duval. Détection, distribution et impacts d'un parasite émergent (Tetracapsuloides bryosalmonae) chez les populations de truites fario (Salmo trutta). Parasitologie. Université Paul Sabatier - Toulouse III, 2022. Français. NNT: 2022TOU30179. tel-03975848

HAL Id: tel-03975848 https://theses.hal.science/tel-03975848

Submitted on 6 Feb 2023

HAL is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers. L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.





En vue de l'obtention du

DOCTORAT DE L'UNIVERSITÉ DE TOULOUSE

Délivré par : L'Université Toulouse 3 Paul Sabatier (UT3 Paul Sabatier)

Présentée et soutenue par :

Eloïse DUVAL

Le 07/10/2022

Detection, distribution, and impacts of the emerging parasite *Tetracapsuloides bryosalmonae* on wild populations of the brown trout *Salmo trutta*

> École doctorale et spécialité : ED SEVAB : Écologie, biodiversité et évolution

> > Unité de recherche :

Station d'Écologie Théorique et Expérimentale, CNRS – Université Paul Sabatier, UAR 2029, 2 route du CNRS, 09200 Moulis, France

> **Directeurs de Thèse :** Simon BLANCHET & Erwan QUÉMÉRÉ

Composition du Jury :

Karen McCOY	Directrice de recherches	Rapportrice
Anti VASEMÄGI	Professeur	Rapporteur
Delphine DESTOUMIEUX-GARZÓN	Directrice de recherches	Examinatrice
Sébastien BROSSE	Professeur	Examinateur
Nicolas POULET	Chargé de mission	Invité
Simon BLANCHET	Directeur de recherches	Directeur
Erwan QUÉMÉRÉ	Chargé de recherches	Co-directeur

Preface

This thesis at the SEVAB doctoral school from Toulouse III Paul Sabatier University was led at the Station d'Ecologie Théorique et Expérimentale of Moulis (CNRS), and at the DECOD UMR of Rennes (INRAE), financed by a PhD grant from the French Ministry of Higher Education, Research and Innovation (MESRI), and by the PKD project (French Office for Biodiversity grant), under the supervision of Simon Blanchet and Erwan Quéméré.

The thesis is written in English, and composed of a general introduction, followed by four chapters under scientific papers form, submitted for peer-review or in preparation for submission, a general discussion, and appendices complementing the writing. Chapter 1 is published in *Environmental DNA*, and Chapter 4 is published in *Biological Conservation*.

Acknowledgments

Tout d'abord, je tiens à remercier mes deux directeurs de thèse, Simon Blanchet et Erwan Quéméré. Merci Simon de m'avoir fait confiance pour mener à bien ce projet alors qu'on ne se connaissait pas encore. Merci Erwan de m'avoir accordé ce premier stage de M1 qui m'a ouvert la porte de la thèse et m'a permis de bénéficier d'une équipe encadrante d'exception. Merci d'avoir cru en moi et pour votre soutien sans failles dans les moments un peu plus difficiles. Je remercie également à Géraldine Loot et Lisa Jacquin qui ont toujours été présentes pour les réunions de mise au point et la participation aux productions scientifiques.

Ensuite, ce travail n'aurait pas pu aboutir dans de si bonnes conditions sans le travail et le soutien de Charlotte Veyssière, à qui je dois la majorité du travail de laboratoire avec Géraldine. Merci également à toutes les deux pour le temps que vous avez pris pour me former au labo. Merci Charlotte pour tous les bons moments partagés sur le terrain, ta transmission du sens de l'organisation et les nombreuses fois où tu m'as hébergée à Toulouse.

Je ne peux parler d'hébergement sans remercier Jérôme Prunier et Claire-Lise, qui m'ont également hébergée pendant le premier mois de la thèse. Merci à vous de m'avoir accueillie et initiée à la vie ariégeoise, à ses oiseaux et à ses produits locaux. Pour poursuivre sur le logement, je tiens à remercier Iris Lang pour les soirées passées à Toulouse entre deux journées de dispense de cours, je me souviendrai en particulier de cette fameuse veille de fermeture des Universités et écoles que nous avons vécue comme un début de fin du monde !

Je tiens à remercier les Fédérations de pêche de l'Ariège, de Haute-Garonne et des Hautes-Pyrénées pour leur collaboration tout au long de ce projet. Merci tout particulièrement à Laurent Garmendia, Allan Yotte, Marc Delacoste, Olivier Plasseraud et Gaël Durbe, qui ont toujours répondu présents à nos sollicitations. Je remercie également Armand Lautraite pour le partage de ses connaissances qui m'ont beaucoup apporté pour le lancement du projet.

Je remercie chaleureusement les membres de mon comité de thèse pour leurs précieux conseils lors des deux réunions concernant l'avancement du projet : Nicolas Poulet, Olivier Rey et Hanna Hartikainen.

Ensuite, je tiens à remercier tous les membres de la Station d'Ecologie Théorique et Expérimentale de Moulis, sans qui l'insertion dans le milieu ariégeois n'aurait pas été aussi simple. Tout d'abord un merci particulier à Marine Deluen, avec qui j'ai commencé et je finis cette aventure de thèse au même moment. Merci pour tous les bons moments, pour les très nombreux covoiturages entre Cazères et Moulis, et surtout pour le soutien mutuel pendant ces 4 années ! Ensuite, je tiens à remercier

toute l'équipe poisson avec laquelle j'ai eu la chance de partager un bureau en open space rempli de bonne humeur et de bons conseils. Merci à Camille Poésy pour les nombreux repas partagés le midi, les séances de nage et de rando, merci à Jérôme Prunier, Rik Verdonck, Keoni Saint Pé, Laura Fargeot, Maxim Lefort, Madoka Krick. Merci à toutes les personnes qui ont contribué de près ou de loin au bon déroulement du terrain, une bonne partie de l'équipe poisson mais aussi Murielle Richard, Michèle Huet, Thomas Deruelles, Olivier Calvez, Charlotte Veyssière, mes merveilleux stagiaires sans qui rien n'aurait été possible : Pierre Girard, Perrine Huet-Conan et Maxim Lefort. Un très grand merci également aux stagiaires qui ont travaillé sur les données récoltées pendant le terrain : Laurine Gouthier, Nedjma Morazé et Chiara Mercier. Ce fut un réel plaisir d'avoir contribué à votre encadrement pendant vos formations.

Merci à tous les Moulisiens présents durant mes deux années en Ariège (en plus de tous ceux déjà cités), pour les soirées, les marchés du samedi et les randos, notamment Mathieu, Léa, Diego, Laurane, Jérémie, Pierre, Laura, Hugo, Aisha, Elodie, Malo et Orlane ; merci à tous les deux pour le soutien et les moments partagés pendant cette année de coloc à St Gi.

Un immense merci également à tous les membres de l'ex-ESE de l'INRAE de Rennes. Merci à tous pour votre accueil en cours de route pour ces dernières années d'analyses et de rédaction de la thèse. Merci particulièrement à Gervaise Février pour ses réponses à toutes mes questions, à Anne-Laure Besnard pour l'aide aux manips au labo, à Gilles Maubert pour l'installation informatique. Je tiens également à remercier Ahmed, Cécile, Nolwenn, Margot, Clarisse, Gilles, Pierre-Yves, Marc, Sophie, les deux Eric, les deux Guillaume, les deux Olivier, François, Alan, Thibaut, Scott, Bastien, Quentin, Zoé, Bonnie, Jean-Marc, Alban, Laura, Dominique qui m'a sauvé la mise plusieurs fois, Flavie ma chère collègue de bureau pour tous les bons moments partagés, notamment lors des pauses repas. Merci à tous pour cette merveilleuse ambiance au labo qui fait qu'on s'y sent tout de suite à l'aise (et ce même en temps de covid).

Enfin, je tiens à remercier tout particulièrement ma famille qui m'a soutenue tout au long de cette aventure. Un merci tout particulier à mes parents, ma sœur et à toi Arthur pour tout ce que nous avons vécu pendant ces 4 années, avec des périodes de distance un peu plus rudes à traverser pour mieux se retrouver par la suite, mais aussi à Pyrène, adoptée juste avant le confinement qui m'a été d'une grande aide pour la rédaction.

Scientific actions statement

Scientific publications

- **Duval, E.**, Quéméré E., Loot, G., Jacquin, L., Veyssière, C. & Blanchet, S. (2022). A multifaceted index of population health to detect risk-prone populations and underlying stressors in wildlife. *Biological conservation*, 274, https://doi.org/10.1016/j.biocon.2022.109706 (Chapter 4)
- Duval, E., Blanchet, S., Quéméré, E., Jacquin, L., Veyssière, C., Lautraite, A., Garmendia, L., Yotte, A., Parthuisot, N., Côte, J., & Loot, G. (2021). Urine DNA (uDNA) as a non-lethal method for endoparasite biomonitoring: Development and validation. *Environmental DNA*, 3(5), 1035-1045. https://doi.org/10.1002/edn3.228 (Chapter 1)
- Duval, E., Skaala, Ø., Quintela, M., Dahle, G., Delaval, A., Wennevik, V., Glover, K. A., & Hansen, M. M. (2021). Long-term monitoring of a brown trout (Salmo trutta) population reveals kin-associated migration patterns and contributions by resident trout to the anadromous run. *BMC Ecology and Evolution*, 21(1), 143. https://doi.org/10.1186/s12862-021-01876-9 (Appendix 11)

Conferences and seminars participation

- 17/12/2019 ESEminaire : Détection, distribution et impacts du parasite Tetracapsuloides bryosalmonae sur les populations sauvages de truites fario, UMR ESE INRAE Rennes
- 14/01/2020 Scientifario : Distribution spatiale du parasite responsable de la PKD dans les Pyrénées centrales (09,31,65), association de pêche la truite luchonnaise, Bagnères-de-Luchon
- 13/10/2020 Epidec seminar: non-lethal method for biomonitoring an emerging infectious disease, UMR INRA-ENVT 1225 IHAP, Toulouse
- 09/03/2021 DNAQUA conference, Video poster: An eDNA-based method for monitoring a salmonid infectious disease: Development and application https://www.youtube.com/watch?v=DHTMOdgd1Hw&t=30s&ab_channel=DNAQUA2021
- 26/03/2021 Comité technique de l'Association Régionale Pêche Occitanie : Détection et distribution du parasite responsable de la PKD chez la truite fario dans les rivières du piémont pyrénéen
- 15/11/2021 Meeting réseau ADN-O : Détection non létale d'un pathogène émergent chez la truite grâce à l'ADN excrété *via* les urines, INRAE Villeurbanne

Teaching

- Interactions hôtes-parasites (Université Toulouse III Paul Sabatier, 12h)
- Biologie et Ecologie Expérimentales (Université Toulouse III Paul Sabatier, 40h)

Students co-supervision

Pierre Girard (BTS GPN LEGTA Mormont), **Perrine Huet-Conan** (ISARA-LYON), **Laurine Gouthier** (M1 puis M2 Ecologie et Evolution, Université Toulouse III Paul Sabatier), **Maxim Lefort** (Licence GADER, Institut Universitaire et Technologique de Perpignan), **Nedjma Morazé** (M1 IMABEE Rennes) et **Chiara Mercier** (M1 Ecologie et Evolution, Université Toulouse III Paul Sabatier).

Table of contents

Preface	i
Acknowledgments	iii
Scientific actions statement	v
Abstract	1
Résumé	2
General introduction	3
On the importance of species interactions	3
Parasitism and host-parasite interactions Coevolution of parasite virulence and host defences Genetic background of the host-parasite coevolution	6 7 8
Global change and emerging infectious diseases	11
Surveillance of infectious diseases in the wild Passive and active surveillance New tools for active surveillance	15 15 16
The proliferative kidney disease of salmonid fish: an emerging infectious disease linked to g warming	global 18
Study context and general aims	23
Literature cited	27
Chapter 1. Urine DNA (uDNA) as a non-lethal method for endoparasite biomonitoring: development and validation	39
Abstract	40
Introduction	41
Methods Brown trout sampling uDNA collection Gross lesions and kidney parasite prevalence and load (kDNA) uDNA detection Statistical analyses	44 44 45 46 46 48
Results	49
Inferring infection status and parasite prevalence from uDNA Inferring parasite load from uDNA	49 51
Discussion	54
Literature cited	59
Supporting information	63
Chapter 2. Investigating the environmental conditions shaping parasite distribution and	
infection prevalence in host populations: an integrative approach using eDNA	65
Abstract	66

Introduction	
Methods	
Studied host-parasite system eDNA sampling	
DNA extraction and amplification	
Infection prevalence	
Statistical analyses	
Results	74
T. bryosalmonae distribution	
Injection prevalence	
Discussion	
Literature cited	
Supporting information	
Chapter 3. Eco-evolutionary response of cold-water fish populations to the o	combined effect of an
emerging parasite and temperature	
Abstract	
Introduction	89
Methods	
Studied species	
Brown trout sampling Morphometric measures and fin-clip	
Infection prevalence	
Colouration measurement	
Neutral and adaptive genetic diversity Statistical analyses	
Results	97
Variation in population density	
Variation in genetic diversity	
Variation in body condition and colouration	
Discussion	
Impacts of temperature and infection on brown trout density	
Impacts of stressors on phenotypic variation: body condition	
Impacts of stressors on phenotypic variation: colouration	103
Conclusion and perspectives	
Literature cited	
Supporting information	
Chapter 4. A multifaceted index of population health to detect risk-prone po	opulations and
underlying stressors in wildlife	
Graphical abstract	122
Abstract	
Introduction	

Methods	
Brown trout sampling	
Pathological indicator (PI)	127
Ecological indicator (EI)	127
Genetic indicator (GI)	128
Multifaceted health index (MFI)	128
Environmental data	129
Statistical analyses	129
Results	
Covariance between the component indicators and with the multifaceted index	
Effect of environmental factors on the indicators	130
Discussion	133
Identifying risk prone populations	134
Identifying underlying environmental stressors	
Implementation and implications for wildlife conservation	
Conclusions	
Literature cited	
Supporting information	
General discussion	
Synthesis of the main results	
The use of non-lethal methods for wildlife disease surveillance	156
Monitoring population infection status	156
eDNA as a tool for studying the dynamics of host-parasite interactions	158
Reliability of eDNA quantification	161
The impacts of multiple stressors on host populations	
Monitoring wildlife populations intraspecific variation in time	
Monitoring wildlife populations health in space	166
Relevance of this work for brown trout management in the Pyrenees	
Literature cited	
Appendices	

Abstract

The epidemiological dynamics of infectious diseases result from a complex interplay between the parasite, the host and the environment. Changes in environmental conditions can affect key characteristics of either hosts or parasites, favouring the emergence of disease in wild populations. The proliferative kidney disease (PKD), caused by *Tetracapsuloides bryosalmonae*, is responsible for important declines in salmonid fish. *T. bryosalmonae*'s life cycle is complex and involves two hosts: a salmonid fish and a bryozoan. The different stages of its life cycle and PKD development in fish are temperature dependent, so that climate change may increase the severity of PKD outbreaks and extend further the disease range. Understanding the parasite distribution and its drivers, as well as its impacts on wild populations are thus key elements for the elaboration of management plans.

In my thesis, I investigated the distribution, the ecological and evolutionary impacts of T. bryosalmonae on wild brown trout (Salmo trutta) populations from Southern France, at the foothill of the Pyrenean mountains. The first objective was to map the parasite distribution and infection prevalence along environmental gradients to investigate the biotic and abiotic factors favouring the development of the disease. To this end, I developed a new non-lethal method for effective wide-scale detection of infected fish, building on the excretion of infective spores through fish urine, and an environmental DNA (eDNA) based approach. I demonstrated that the abundance of T. bryosalmonae in the water was tightly linked to the abundance of its hosts but poorly to abiotic factors, whereas the infection prevalence in fish was tightly linked to abiotic factors related to water quality and weakly to the abundance of parasites in the water. The second objective aimed to test the ecological and evolutionary consequences of selection conjointly mediated by the parasite and the water temperature on brown trout populations. I specifically compared juvenile density and intraspecific variation at both genetic and phenotypic levels, before and after PKD outbreak. I found a negative effect of the infection prevalence on the density of naive populations that was not observed on populations already heavily infected the year before. I also found that high infection reduced the diversity of immune-related genes, which was not observed at neutral loci. I further revealed a joint negative impact of temperature and infection prevalence on the body condition and ornamentation of juveniles, suggesting that these stressors might affect brown trout fitness. In a last part, I developed an integrative index combining the pathological, the ecological, and the genetic status of populations to inform on their global health. This multifaceted index, transposable to other ecosystems and species, highlighted the importance of the surrounding agricultural land and the oxygen concentration in the water as the main drivers of brown trout population health.

This thesis provides and uses new tools to investigate the distribution of an endoparasite, either within its vertebrate host or directly into the aquatic environment, and to assess the global health of host populations. The consequences of the disease outbreaks depended on the infection prevalence and the water temperature, with a pronounced effect on populations' phenotypes. These results fill some gaps in the understanding of this emerging disease settlements and impacts, and may serve as a guide for future management plans.

Résumé

La dynamique épidémiologique des maladies infectieuses résulte de l'interaction complexe entre le parasite, l'hôte et l'environnement. Des changements de conditions environnementales peuvent affecter des caractéristiques clés des hôtes ou des parasites, favorisant l'émergence de maladies dans les populations sauvages. La maladie rénale proliférative (PKD pour proliferative kidney disease en anglais), causée par *Tetracapsuloides bryosalmonae*, est responsable d'importants déclins chez les salmonidés. Le cycle de vie de *T. bryosalmonae* est complexe et implique deux hôtes : un poisson salmonidé et un bryozoaire. Les différentes étapes de son cycle de vie et le développement de la PKD chez les poissons étant température-dépendants, le changement climatique est susceptible d'augmenter la sévérité des épidémies de PKD et d'étendre davantage sa répartition géographique. Comprendre la répartition du parasite et ses déterminants, ainsi que ses impacts sur les populations sauvages sont donc des éléments clés pour l'élaboration de plans de gestion.

Dans ma thèse, j'ai étudié la distribution ainsi que les impacts écologiques et évolutifs de T. bryosalmonae sur les populations sauvages de truites fario (Salmo trutta) du sud de la France, au pied des Pyrénées. Le premier objectif était de cartographier la distribution du parasite et la prévalence de l'infection le long de gradients environnementaux afin d'étudier les facteurs biotiques et abiotiques favorisant le développement de la maladie. À cette fin, j'ai développé une nouvelle méthode non létale permettant une détection efficace à grande échelle des poissons infectés, en s'appuyant sur l'excrétion de spores infectieuses par l'urine des poissons et la détection d'ADN environnemental (ADNe). J'ai démontré que l'abondance de T. bryosalmonae dans l'eau était étroitement liée à l'abondance de ses hôtes et peu aux facteurs abiotiques, tandis que l'infection chez les poissons était étroitement liée aux facteurs abiotiques traduisant la qualité de l'eau et faiblement à l'abondance des parasites dans l'eau. Le second objectif visait à tester les conséquences écologiques et évolutives de la sélection exercée conjointement par le parasite et la température de l'eau sur les populations de truites fario. J'ai spécifiquement comparé la densité de juvéniles et la variation intraspécifique aux niveaux génétique et phénotypique avant et après l'épidémie de PKD. J'ai trouvé un effet négatif de la prévalence d'infection sur la densité des populations naïves, qui n'apparaissait pas chez les populations déjà fortement infectées l'année précédente. J'ai également montré qu'une forte infection réduisait la diversité des gènes liés au système immunitaire, ce qui n'était pas observé aux loci neutres. J'ai en outre révélé un impact négatif conjoint de la température et de la prévalence d'infection sur la condition corporelle et l'ornementation des juvéniles, suggérant que ces facteurs de stress affectent la fitness des truitelles. Dans une dernière partie, j'ai développé un indice intégratif combinant les statuts pathologique, écologique et génétique des populations afin d'informer leur santé globale. Cet indice à multiples facettes, transposable à d'autres écosystèmes et espèces, a mis en évidence l'importance des terres agricoles alentours et de la concentration en oxygène dans l'eau comme principaux déterminants de la santé des populations de truites fario.

Cette thèse fournit et utilise de nouveaux outils pour étudier la distribution d'un endoparasite au sein de son hôte vertébré ainsi que dans le milieu aquatique, et pour évaluer la santé globale des populations hôtes. Les conséquences des épidémies dépendaient de la prévalence de l'infection et de la température de l'eau, avec un effet prononcé sur les phénotypes des populations. Ces résultats comblent certaines lacunes dans la compréhension de l'établissement et des impacts de cette maladie émergente, et permettent d'orienter les futurs plans de gestion.

On the importance of species interactions

Ecology is the science studying the interactions among and between living organisms and their environment, at all the levels of biodiversity, from the cell to the biosphere (Molles & Sher, 2019). An ecological community is composed of coexisting species involved in a complex network of interactions (Montoya et al., 2006). Species interactions, just as the number of interacting species, are a core component of biodiversity. As such, it appears substantial for the study of an ecosystem to not only consider a list of present species, but also the network of interactions existing between the species, as they are keys to ecosystem functioning and services (Harvey et al., 2017). In an ecological community, all the species and their mutual interactions intertwined contribute to the maintenance of ecosystem stability (Mougi & Kondoh, 2012).

The outcomes of any biological interaction strongly depend on the environmental conditions experienced by the involved species (Chamberlain et al., 2014; Song et al., 2020). The complexity of the interaction network connecting the different species within an ecosystem makes the prediction of the effects of global change difficult. Indeed, changes in abiotic conditions may have both direct and indirect (i.e. due to the alteration of species interaction networks) impacts on species and ecosystems (Tylianakis et al., 2008; Ockendon et al., 2014). Famous examples of domino effects involving species interactions include the Yellowstone wolves (Canis lupus) restoring stream functionality (Ripple & Beschta, 2012). After 70 years of absence, this top predator was reintroduced into the Yellowstone Park from 1995. It widely contributed to the regulation of the elk (Cervus elaphus) population that had prospered in its absence, releasing the vegetation from an over-browsing pressure. This way, aspens (Populus tremuloides), willows (Salix spp.), and cottonwood (Populus spp.) could develop further, increasing the riparian vegetation along streams. This created favourable niche for beavers (Caster canadensis), that in turn increased in density. This latter engineer species, together with the riparian plant community, contributed to enhance the stability of the eroding streambanks. Dams built by the beavers impacted the morphology of the streams and created small pounds benefiting a whole community of waterfowls, amphibians, reptiles, fish and amphibious mammals (Ripple & Beschta, 2012; Beschta & Ripple, 2019). Another example involves the impact of an emerging infectious disease on a whole trophic chain in kelp forests along the central coast of California (Fig. 1a). In 2014, a series of climatic stressors coinciding with the development of the sea star (Pycnopodia helianthoides) wasting disease caused an unprecedented decline in kelp (Macrocystis pyrifera) density. This released urchins' (Strongylocentrotus purpuratus) populations from the sea star predation, and the exponential development of this grazer transformed the kelp forest into a patchy landscape of remnant kelp interspersed with sea urchin barrens (i.e., "naked" seabed covered with urchins suffering from starvation

because of the lack of kelp). The over-development of the urchins favoured an increase of this system's top-predator, the sea otter (*Enhydra lutris nereis*). In turn, by favouring healthy active urchins within its diet, the sea-otter contributes to the preservation of the remnant patchy kelp forest (Smith et al., 2021). Moreover, species' phenology, which is the timing of species life cycle, is guided by environmental clues (Körner & Basler, 2010). Species may shift their phenology in response to changing environmental conditions, but these shifts might be species-specific and lead to phenological mismatches in interspecies interactions (Chmura et al., 2019). For instance, great and blue tits' (*Parus major* and *Parus caeruleus*) reproduction is synchronised with the period favourable to the development of the winter moth (*Operophtera brumata*) caterpillars to ensure enough feeding to the offspring, while caterpillars' development is synchronised with the development of pedunculate oaks (*Quercus robur*) leaves. The increase in temperature leads to earlier oak budbursts, and earlier peak in the caterpillars' abundance. This creates a great selection pressure on tits for earlier laying dates, with failure to synchronise chick food needs with the caterpillar's availability (phenological mismatch, see Fig. 1b) leading to decreased recruitment (Van Noordwijk et al., 1995; Buse et al., 1999; Reed et al., 2013).

These examples illustrate how a change in the ecology of one species might affect the whole community of species within an ecosystem, so that the study of species interactions should be considered cautiously for the elaboration of appropriate wildlife conservation plans to anticipate unfortunate events (Harvey et al., 2017; Clark-Wolf et al., 2022). However, studies describing species interaction networks and their dynamic in such details are scarce, because of technical difficulties in acquiring information on the nature and strength of interactions in wild environments.



Figure 1. (a) Illustration of the trophic cascade in the kelp forest. The emergence of the sea star wasting disease caused a sharp decline in the sea star density (**D**). The decline of this mesopredator released predation pressure on the sea urchins, resulting in a massive development of this grazer (**C**), which together with the action of climatic stressors, caused a decline in kelp density (**B**). The abundance of urchins resulted in an increase of the system's top predator, the sea otter (**A**), which prevented further decline of the remnant patchy kelp forest by controlling the urchins' development. This figure is from Smith et al. (2021), ©Jessica Kendall-Barr. (**b**) Illustration of the synchronisation between tits chick food needs and the peak of caterpillar biomass (upper panel), and of the phenological mismatch that may occur because of early budburst. This figure is adapted from Reed et al. (2013).

Parasitism and host-parasite interactions

Among the different types of interspecies interactions, parasitism refers to the intimate interaction between a parasite and its host, resulting in potential harmful effects to the host (Crofton, 1971). Parasitism is ubiquitous in the living world and parasite species may represent a wide part of the biodiversity (Dobson et al., 2008). Parasites play a significant role in ecosystems by regulating host population dynamics, altering interspecific interactions, and shaping intra and interspecific biodiversity (Hudson et al., 2006). Parasite organisms depend, at least for one stage of their life cycle, on a host organism that provides them habitat, transport (especially when the host is an animal), and food (Combes, 2001). Before exploiting its host, the parasite must go through two main filters, namely the encounter filter and the compatibility filter (Fig. 2; Combes, 2001). The encounter filter describes the fact that the parasite must stray in the environment close to its hosts to maximise its chances of encountering one. The compatibility filter includes the ability of the parasite to pass through the defence mechanisms of its host once it is encountered. The strategies used by parasites to find, enter, and exploit their hosts are highly diversified and specialised. These strategies include high reproduction to counter the risk of mortality during dispersal as free-living stages between two hosts, synchronization in time and space with the hosts, and manipulation of the hosts' behaviour to ensure a better transmission (Rea & Irwin, 1994; Combes, 2005; Lefèvre et al., 2009). These elaborated strategies result from long-term coevolution between a parasite and its host species.



Figure 2. Schematic representation of the encounter and the compatibility filters that have to be overcome by a parasite to infect its host. The potential impacts of environmental factors are also shown as non-exhaustive examples. The dashed line separates between the hosts attributes on the left, and the parasite attributes on the right. However, this line is not closed because for instance, a parasite virulence might depend on host characteristics (see below). This figure is adapted from Budria & Candolin (2014).

Coevolution of parasite virulence and host defences

Parasitism describes, by essence, an antagonistic relationship between the parasite and its host. Pathogenic effects of parasitism on the hosts include decrease in growth, reproduction, and survival. Because of these deleterious impacts, parasites exert a selection pressure on their hosts, and the hosts showing better defences may have a higher survival or reproduction, leading to an increase of their genes' frequency in the host population (Combes, 2005). On the other hand, parasites with a higher chance of circumventing the hosts' defences may also be selected for, and their genes will also increase in frequency in the parasite population. This leads to a reciprocal selection favouring the coevolution of the host defence mechanisms against infection (resistance and tolerance, see below), and of the parasite virulence (Fig. 3; Råberg et al., 2014).

Parasite virulence is the reduction of host fitness caused by the infection. It is the product of the density of parasites within a host (parasite load) and the extent of damages caused by each parasite (pathogenicity) (Little et al., 2010). A high parasite load in the host confers a high transmission success to the next host benefiting the parasite, but an increase in density within the host leads to higher virulence, with the potential risk of killing the host before transmission (or too early in the case of obligate killers, Ebert & Weisser, 1997). This leads to a trade-off between transmission (positively linked to the parasite load) and pathogenicity, implying that the most successful parasite would show an intermediate level of virulence (Frank, 1996; Little et al., 2010). However, parasite virulence, which is the impact of parasite infection on the host, does not depend on the parasite only but also on the host ability to cope with the infection.

Hosts use two distinct mechanisms to reduce the impact of parasite infection: resistance to limit the parasite load, and tolerance to limit the damages caused by a given parasite load (Råberg et al., 2009; Medzhhitov et al., 2012). These two defence mechanisms are costly and may be negatively correlated. Their relative benefits may vary across different environmental conditions (Råberg et al., 2007; Read et al., 2008). A parasite with high transmission but low pathogenicity may favour increased parasite tolerance, while a parasite with low transmission but high pathogenicity would have a tendency to favour increased resistance mechanisms (Read et al., 2008). Resistance has a direct negative impact on the parasites by limiting their establishment in the host population, whereas tolerance is expected to have limited effect on the parasite to overcome the hosts defence (see Fig. 3), while tolerance is not expected to impose such selection (Råberg et al., 2009; see below).

Host-parasite coevolution is defined as the reciprocal, adaptive genetic changes between the two species, each increasing its fitness at the other's expense (Little et al., 2010; Van Oosterhout, 2021). Each step of evolution for an increase in parasite pathogenicity or in host resistance has indeed a negative

effect on host or parasite fitness respectively (Fig. 3). However, just as the increase in parasite pathogenicity may have a cost for its transmission potential (see above), an increase in resistance or in tolerance may be costly to the host. There is an energetic trade-off between traits involved in limiting parasite load and its effects and other costly functions for maintenance (basal metabolism), growth or reproduction. An over-reactive response of the immune system to infection may lead to collateral damages such as immunopathology (i.e., when the immune system turns against host tissues; Svensson et al., 1998; Stjernman et al., 2008). Furthermore, the investment in high resistance can become useless and decrease host fitness when the parasite is no longer in the environment, and resistance to a specific parasite might be negatively correlated to resistance to other parasites (Stjernman et al., 2008; Auld et al., 2013). Tolerance might be negatively correlated to resistance, so that increased tolerance represents a cost of resistance and *vice versa* (Råberg et al., 2007, 2009).



Figure 3. Schematic representation of host-pathogen coevolution, adapted from Woolhouse et al. (2002). Green arrows represent intraspecific selection and yellow arrows represent interspecific selection.

Genetic background of the host-parasite coevolution

To evolve, host resistance or tolerance to parasite infection needs to be at least partially genetically determined (Sorci et al., 1997). A genetic variant (allele) conferring benefits to the host by limiting the effects of parasite infection will be selected for and will increase in frequency in the population. Once this allele becomes common in the population, the parasite will adapt to it through coevolution and the allele will lose its benefits, so that another allele might be selected for (see Fig. 4). This process is referred to as the frequency-dependent selection (Tellier & Brown, 2007). Changes in genetic

frequencies in the host population as a result of parasite-induced selection may lead to changes in genetic frequencies in the parasite population, and so on (Woolhouse et al., 2002). This represents the Red Queen dynamics where to stay at the same place (i.e., to exist and maintain its fitness), a species has to continuously run (evolve) to keep track with its interacting species' evolution (Van Valen, 1973; Brockhurst et al., 2014). While Red Queen dynamics shaping host resistance and parasite virulence may lead to an increase in the diversity of host genes involved in resistance through negative-frequency dependence, tolerance may result in other evolutionary outcomes. Because tolerance to parasite pathogenicity has no negative effect on the parasite, increased tolerance may favour its transmission and increase the infection prevalence in a population, so that tolerant genes might be selected for until fixation (Best et al., 2014). Tolerance may turn host–parasite interactions into apparent commensalism, that may cause severe damages upon transmission to other non-tolerant host populations, or a shift in virulence due to a change in the ecological context or host population genetic background (Little et al., 2010; Best et al., 2014).



Frequency of allele

Figure 4. Graphical representation of the negative frequency dependence, from Lenz et al. (2018). A new allele enters the population at low frequency, originating either from mutation or from immigration (orange dot). Under the negative frequency dependence selection hypothesis, this new allele is expected to confer higher fitness than common alleles, because these latter have been the main target of selection by coevolving parasites, so that the new allele frequency increases ①. Once common, the allele's fitness is expected to decrease again, because it now becomes itself the main target of selection by parasites, resulting in two possible trajectories: Once the allele is rare again, it loses its fitness disadvantage, because parasites adapt to other, more common alleles, and it ultimately gains again in frequency, leading to a full cycle in the allele frequency trajectory ②. Instead, the allele can also be lost from the population, because of either continuous fitness disadvantage or neutral drift effects ③. The blue shading illustrates the time dependence of the process.

The outcome of the host-parasite interaction is partially determined by the standing genetic variation of the host that encompasses all the genetic variation across the genome available for disease resistance/tolerance. Indeed, a high level of standing genetic variation maximises the chances that some alleles conferring advantage to a potentially new parasite (or strain) are already present in the population (Barrett & Schluter, 2008). The standing genetic variation within a population is shaped by several neutral and selective evolutionary forces (including parasite-mediated selection) that operated in synergy during its recent demographic history. While genetic drift leads to the random loss of alleles at each generation (because of the random contribution of breeders to the next generation), especially in small populations, mutation and migration favour the appearance of new alleles. However, mutation is a slow process, especially in long-lived species, and migration depends on the connectivity between populations (Lacy, 1987).

In their natural systems, host species are confronted with a wide range of different parasites acting all at once in a different manner on the host genotype (Maizels & Nussey, 2013). Moreover, the selective pressures exerted by these multiple pathogens may strongly vary in space and time depending on the environmental conditions. As a result, host-parasite coevolution and the selection pressure imposed by multiple parasites on host species may favour the maintenance of high genetic diversity (balancing selection) in host genomic regions involved in pathogen recognition and immunity (Ebert & Fields, 2020). The host immunity to infection is composed of two mechanisms. The innate immune response, that is rapidly effective, represents a broad-spectrum defence because of its non-specificity, but in turn may result in collateral damages to the host through immunopathology and destruction of self-tissues and confers long-lasting immune memory so that immune response to previously encountered parasites is faster and stronger. The adaptive immune system is launched by the innate immune system, and therefore takes more time to be efficient (Fearon & Locksley, 1996). The individuals possessing the adequate alleles involved in the adaptive immune system will mount a more specific response more rapidly, therefore avoiding the costs of immunopathology (Råberg et al., 2009).

Three non-exclusive selection mechanisms have been proposed for the high genetic diversity of immune loci: heterozygote advantage, rare-allele advantage (resulting from a frequency-dependent selection, see above and Fig. 4) and fluctuating selection in time and space (Spurgin & Richardson, 2010). To disentangle these mechanisms, most work focused on the major histocompatibility complex (MHC), a complex of genes involved in the adaptive immunity that show an exceptional polymorphism in vertebrates (Bernatchez & Landry, 2003). Their structure and mechanisms of interaction with antigens are well described (Swain, 1983). Furthermore, their ability to trigger a rapid microevolutionary response to varying pathogen-mediated selective pressures has been demonstrated experimentally (Eizaguirre et al., 2012; Phillips et al., 2018). MHC genes code for molecules that bind to self and non-

self antigens and present them to T-cells which can initiate the immune response process. MHC class I genes are associated with the recognition of intracellular pathogens, and class II with the recognition of extracellular pathogens (Neefjes et al., 2011). Because different MHC molecules bind to a different set of antigens, heterozygous hosts may be better armed against different pathogens than homozygous hosts, illustrating the heterozygote advantage (Lewontin & Hubby, 1966). Alternatively, it is expected that the most common alleles within the host population will select pathogens capable of circumventing their action, so that hosts presenting rare alleles will get the advantage and these rare alleles will get more common, thus maintaining a wide variety of alleles in the host population through negative frequency-dependent selection (mechanism illustrated by Fig. 4). For instance, rare alleles at the human leukocyte antigen (HLA, the human MHC) represent an advantage to guppies experimentally infected by an ectoparasite (Phillips et al., 2018). Lastly, the fluctuating selection suggests that the variation in space and time of the parasite pressure maintains the polymorphism at MHC loci through context-dependent advantage of the different alleles (Levene, 1953; Hill, 1991).

Parasite-mediated selection acting on MHC genes is expected to modulate allelic frequencies in a different way than when only neutral evolutionary forces (drift, mutation and migration) are acting. Therefore, the comparison of patterns of genetic diversity at the MHC to that of non-functional loci, that are expected to be under the influence of neutral forces only, may inform on the parasite-mediated selective pressure (Spurgin & Richardson, 2010). For instance, populations that are not connected by gene flow (migration) may be different at neutral loci, but similar at MHC loci because of similar environmental pressure acting on the MHC (e.g. in Fraser & Neff, 2010).

Global change and emerging infectious diseases

As presented above, parasites are ubiquitous, and they regulate wild populations by decreasing their reproduction and causing disease-related mortality at a background level (Okamura & Feist, 2011; Adlard et al., 2015). The spread and severity of an infection are the outcome of the interaction between the parasite, the host, and the environment (Fig. 5). There are therefore many sources of variation that can affect infection parameters. These include heterogeneity among hosts and parasite strains, but also environmental abiotic and biotic factors (such as hosts and parasite density), leading to context-dependent disease outcomes. Abiotic factors directly modulate hosts and parasite physiology and life cycles, so that they are often used to predict parasite distribution and forecast disease outbreaks (Turner et al., 2021). Changes in environmental conditions may affect key characteristics of either hosts or parasites, such as their geographic range, parasite virulence and transmission rate, or host sensitivity to infection (Schrag & Wiener, 1995; Lafferty, 2009; Gallana et al., 2013; Altizer et al., 2013). Moreover, when infections are governed by seasonal patterns, changes in environmental conditions may lead to an

alteration of the timing window for infection and disease development, or to phenological mismatching (Koelle et al., 2005; Paull & Johnson, 2014). These changes, acting at both the spatial and the temporal scales, may cause an imbalance in the dynamics of the host-parasite interaction and favour "emerging infectious disease" (EID), i.e. an infectious disease that recently appeared within a population or whose incidence is rapidly increasing in an existing host population (Schrag & Wiener, 1995; Engering et al., 2013). EIDs may therefore result from the introduction of pathogen in new areas by natural range extension, or by human intervention or displacement (novel pathogen hypothesis), but also from an increase in host range, in parasite virulence or in the period favourable for parasite transmission (endemic pathogen hypothesis, Rachowicz et al., 2005). Generally benign infections may indeed express increased virulence under new environmental conditions (Brown et al., 2003). For instance, Vale et al. (2011) showed experimentally a strong influence of temperature and food availability on the virulence of a bacterial infection in daphnia hosts.



Figure 5. Visual representation of the interplay between the host, the pathogen and the environment leading to disease: "the epidemiological triangle", adapted from Arkoosh et al. (1998). The disease outcome (D) depends on the interactions between the three parts of the diagram and may extend for instance where the environment is altered, the pathogen is more virulent and/or the host is more sensitive to the infection. These changes in environment, host or pathogen attributes and their impacts on the disease extent are represented by the double-arrows.

Studying EIDs is critical to anticipate host population health issues and demographic declines. EIDs are widely studied in human and livestock populations (Morse 2004) but have received comparatively less attention in wild plant and animal populations (Spalding & Forrester, 1993; Cunningham et al., 2017). This is an important issue because wildlife can serve as a reservoir for zoonotic diseases, transmitted from wild to domestic animal populations and to humans, such as brucellosis, Lyme disease, the recently emerged virus COVID-19, or mosquitoes-transmitted diseases (Steere et al., 2004; Godfroid et al., 2005; Jones et al., 2008; Karesh et al., 2012; Rizzoli et al., 2019; Zhou et al., 2020). EIDs have a deep economic impact, either directly when affecting human health, or

indirectly when affecting food production and ecosystem services (Daszak, 2000; Jones et al., 2008; Sileshi & Gebeyehu, 2021; Brooks et al., 2022). Investigating EIDs dynamics in space and time in wild populations is also crucial for management and conservation purposes (Smith et al., 2006).

A renowned example of the impacts of a wildlife EID caused by a newly introduced pathogen is the use of an American myxoma virus as a biological control for rabbits (Sylvilagus brasiliensis and Sylvilagus bachmani) in Australia (Kerr, 2012). After an initial severe decline in some rabbit populations (up to 95% mortality), an attenuation of the virus virulence and the emergence of host resistance reducing the disease impacts occurred within a decade, illustrating real-time host-pathogen coevolution. Other notorious examples of EIDs in the wild include the chytridiomycosis, responsible for a large share of the global amphibian decline (Berger et al., 1998; Daszak et al., 1999; Scheele et al., 2019; Fisher & Garner, 2020). This disease is caused by fungi of the genus Batrachochytrium, and likely results from the introduction of the pathogen into new areas (thus corresponding to the novel pathogen hypothesis), as suggested by molecular analysis revealing that the global pandemic lineages of the fungus would originate from Asia (Fu & Waldman, 2019; Fisher & Garner, 2020). In its supposed origin area, the infection by B. dendrobatidis is highly prevalent and virulent, as revealed by the experimental infection of naive populations, but causes no apparent harm on its indigenous host populations, suggesting that they have coevolved with endemic B. dendrobatidis infection and express defence mechanisms (Fu & Waldman, 2019). However, environmental factors have also been shown to play an important role in disease outcomes and infection dynamics outside Asia, thus corresponding to patterns of the endemic pathogen hypothesis. Temperature, precipitations, altitude, seasonality, ultraviolet exposure or pesticides, together with the density of zoospore exposure have indeed been identified as determinants of the disease outcomes by diverse studies, either experimentally or in the field (Fisher & Garner, 2020). Similarly, the bat white-nose syndrome, caused by the fungus *Pseudogymnoascus destructans*, is responsible for big declines in bat populations of North America (Hoyt et al., 2021). This fungus originated from Europe where it remained unnoticeable until investigations after disease outbreaks in North America, also substantiating the novel pathogen hypothesis (Warnecke et al., 2012; Hoyt et al., 2021). Temperature and humidity have been identified as factors modulating the fungus development and disease outcomes in bats (Langwig et al., 2012; Hoyt et al., 2021). Another example of a wild EID involves Trichomonas gallinae, a common protozoan parasite of pigeons. Unprecedented levels of finch birds (Carduelis chloris and Fringilla coelebs) mortality were reported in Great Britain in 2006, attributable to an epidemic of trichomonosis, until then never reported in finches (Robinson et al., 2010). Here, the hypothesis of the endemic pathogen that has switched its host range seems the most likely. The spread of the infection in finches might be exacerbated by garden feeders where the birds preferentially feed in degraded habitats, favouring high densities of susceptible hosts. The epidemic has then been reported in other European countries, with bird migration as the most probable means of transmission (Lawson et al., 2011; Chavatte et al., 2019).

As emphasised by the above examples of wildlife EIDs, parasite infections may interact with multiple environmental stressors, modulating the disease outcomes in different host populations. The extinction of wildlife populations rarely has a single cause, and most of the time results from the interaction of multiple stressors. Global change may lead to an increase in severity of most environmental stressors, and lead to the emergence of novel stressors combinations (Niinemets et al., 2017). Faced with environmental change, species may show three different responses: they may shift their geographical range to a more suitable environment, they may adapt to the new environmental conditions, or they may become extinct (Hoffmann & Sgrò, 2011). The adaptation can occur by shifting phenotype through phenotypic plasticity, i.e., the ability of a genotype to express different phenotypes in different environmental conditions, and/or by microevolution and natural selection of adapted genotypes (Nunney, 2016). Therefore, to be able to stay within their current range, species must possess enough standing genetic variation to be able to respond rapidly to the environmental changes and produce the adequate phenotypes (Barrett & Schluter, 2008). Temperature appears as the main driver of species extinction, with future global temperatures estimated to threaten up to one in six species (Urban, 2015). Ectotherm species, including amphibians, fish, reptiles, crustaceans, and insects, might be particularly at risk because their body temperature is directly driven by ambient temperature, influencing physiological functions such as locomotion, growth and reproduction (Deutsch et al., 2008). Optimal performances of ectotherms are characterised by a thermal performance curve which rises gradually from a minimum critical temperature to an optimum temperature and then drops to a critical thermal temperature (Huey & Kingsolver, 1989). Phenotypic plasticity in ectotherm thermal tolerance might be limited and insufficient to prevent species extinction, so that behavioural and evolutionary response (through the increase in tolerant genotype frequencies due to natural selection) may play a substantial role for their conservation (Gunderson & Stillman, 2015). Nonetheless, other non-ectotherm taxa might suffer as much from the temperature increase, probably as a result of biotic interactions including trophic chains (decrease in prey abundance, phenological mismatch), species introduction to novel environments due to range shift, or the increase of parasite development (Cahill et al., 2013; Urban, 2015).

Habitat fragmentation, climate change, pollution, increased displacement of human populations, livestock and wild organisms, which are leading factors of biodiversity loss, may act synergistically as multiple stressors to modulate the outcomes of host-parasite interactions (Fig. 6; Williams et al., 2002). EIDs, in addition to the other exacerbated environmental stressors, may increase further the biodiversity loss (Keesing et al., 2010), as illustrated by Fig. 1a and the impact of the sea star wasting disease on the kelp forest through a trophic cascade. For instance, a wild population of rattlesnake (*Crotalus horridus*) was driven towards extinction because of the synergistic effects of inbreeding due to fragmentation, and a context-dependent skin infection exacerbated by high summer rainfall (Clark et al., 2011). Likewise, Rohr et al. (2008) showed that the exposure of amphibians to pesticides had both positive and negative

effects on trematode transmission, and that the net outcome of both stressors (infection and pesticide) would most likely lead to increased infection in the amphibian hosts (somewhere between additive and antagonistic effect, Fig. 6). On the other hand, global change could also have a negative effect on parasites and alter their regulation function in some ecosystems (reverse effect illustrated in Fig. 6; Lafferty & Kuris, 1999).



Figure 6. Net effect of multiple stressors acting together additively, synergistically, antagonistically or in a reverse direction compared to their single effects, adapted from Johnson & Penaluna (2019).

These examples illustrate that population characteristics may modulate the impact of environmental change in both abiotic and biotic conditions. For instance, the amount of standing genetic variation available within a population appears substantial to increase the chances of populations to adequately respond to the environmental change, either through phenotypic plasticity or through the selection of the most tolerant genotypes (Barrett & Schluter, 2008). Intraspecific genetic diversity thus informs on the adaptive potential of populations in changing environments (Lande & Shannon, 1996; Hoban et al., 2013). Similarly, environmental change might lead to stochastic demographic fluctuations that might have devastating consequences in small populations, buffered within more dense populations. It therefore appears important to monitor wildlife populations characteristics enabling to anticipate their potential response to environmental change.

Surveillance of infectious diseases in the wild

Passive and active surveillance

The complexity of the host-parasite relationships, together with the action of multiple stressors, make the surveillance of EIDs a crucial step in the elaboration of management plans for biodiversity conservation. However, surveillance of EIDs in wild populations is notoriously challenging because of the elusive features of the host species, for which we often lack knowledge on the ecological and

behavioural characteristics (Ryser-Degiorgis, 2013). Surveillance of wildlife disease consists in the process of monitoring population health status to set up management plans when a certain threshold in disease/infection prevalence is reached. Surveillance of wildlife disease can either be passive or active (Christensen, 2001; Artois et al., 2009; Guberti et al., 2014). Passive surveillance of wildlife disease refers to the recording of disease cases as they occur, typically through the finding of carcasses (Ryser-Degiorgis, 2013). Although informative to detect new infection events, passive surveillance generally fails to provide precise estimates of parasite prevalence and abundance in host populations, which are two essential parameters to model and predict the dynamics of disease in host populations (Perez & Dragicevic, 2009). Indeed, passive surveillance records individuals showing symptoms, which is only the tip of the iceberg. Asymptomatic parasite carriers are an invisible parasite reservoir that plays a key role in the spread of the disease (Kalajdzievska & Li, 2011). Therefore, an active surveillance involving the random testing of host individuals without prior knowledge is essential to monitor the spatio-temporal dynamics of EIDs and guide management decisions (Artois et al., 2009).

The active surveillance of EIDs in wild host populations is often a highly challenging task, especially for endoparasitic diseases. Indeed, screening the presence of endoparasites often requires sampling organs or tissues to detect the infectious agent, which generally implies killing the host (Fig. 7a; e.g. in Böhm et al., 2006; Sjöberg et al., 2009; McAllister et al., 2016; Cilia et al., 2020). Lethal approaches for monitoring key parameters such as parasite prevalence and abundance are problematic, because of ethical issues and low achievable sample size in host populations. Such approaches can obviously not be considered in the case of protected, endangered, or even exploited species for which EIDs monitoring is however particularly important (Smith et al., 2006; Breed et al., 2009). Moreover, lethal sampling precludes individual-based follow-up, which is useful to monitor the individual health state all along the infection. Faced with this challenge, an increasing number of studies have developed indirect methods to non-lethally detect and monitor endoparasitic infections in wild animal populations (Fig. 7b, 7c). Most of these studies involve faces collection (Meijer et al., 2011; Honma et al., 2011; Riepe et al., 2019; Kumar et al., 2019), and/or the use of environmental DNA (eDNA, see below) for detecting the infectious agents in the environment and evaluating disease risks (Huver et al., 2015; Bass et al., 2015; Sengupta et al., 2019).

New tools for active surveillance

Contrary to studies lead in a laboratory under controlled conditions, studies in the wild are subjected to great fluctuations in environmental conditions, so that large sampling over a wide spatial scale and along environmental gradients is needed to ensure enough statistical power for unravelling ecological mechanisms. Studies in the wild require appropriate and powerful tools to ensure an accurate monitoring of environmental conditions, as for instance remote sensing to map land use, vegetation type, canopy cover or even the ocean floor (Turner et al., 2003), loggers for air, water temperature or physico-

chemicals sensors (Rogers, 2006). Then, the monitoring of wildlife populations and their characteristics has been revolutionised notably by bio-loggers such as GPS or sensors devices directly borne by the studied animals (Wilmers et al., 2015). Another breakthrough in wildlife monitoring, allowed by the development of molecular technologies, is the use of environmental DNA (eDNA) (Bohmann et al., 2014).

eDNA is defined as the DNA of all organisms present in environmental samples, including intracellular or free DNA molecules released from organisms that can then be found in water, soil or faeces samples, witnessing the presence of a species (Bohmann et al., 2014; Pawlowski et al., 2020). eDNA detection has quickly become a standard in ecological studies due to the increasing availability of powerful genetic tools allowing the detection of species even from slight amount of DNA available in the environment (Jerde et al., 2011; Shokralla et al., 2012; Miotke et al., 2014). In the case of infectious diseases, eDNA can be used to detect parasites either from the open environment (i.e. air, water, soil) to get information about host exposition (Fig. 7c; Rusch et al., 2018), or from host fluids (i.e. blood, faeces or urine), which allows to get host individual infection status, as well as individual follow-up all along the infection stages (Fig. 7b; e.g. in Etienne et al., 2012). The approach using host fluids has until now rarely been used for aquatic species (but see Berger & Aubin-Horth, 2018).



Figure 7. Methods for investigating infectious agents and their invasiveness. Examples from this thesis are also given. (a) Brown trout (*Salmo trutta*) euthanised for gross lesion examination and kidney sampling for parasite DNA detection. This method was used in Chapter 1 to validate the non-lethal method shown in (b). (b) Filtration of brown trout excreted urine for detection of parasite DNA. The fish is at the bottom of the bottle and will be released after manipulation. This method was used in the four chapters of this thesis. (c) Filtration of river water for the detection of parasite environmental DNA. This method was used in Chapter 2. The figure is adapted from Chapman et al. (2021).

The proliferative kidney disease of salmonid fish: an emerging infectious disease linked to global warming

The proliferative kidney disease (PKD) is an EID of salmonid fish caused by the myxozoan parasite *Tetracapsuloides bryosalmonae*. During the last two decades, PKD outbreaks have been increasingly reported in salmonid populations of Europe and North America (see Fig. 8 for a map; Hedrick et al., 1993; Sudhagar et al., 2020). PKD has been reported in most of the salmonid species, and in the northern pike (*Esox lucius*) (Fig. 8; Seagrave et al., 1981; Bucke et al., 1991; Sudhagar et al., 2020). This disease is responsible for important losses in aquaculture and worrying declines in wild salmonid populations, with up to 95% mortality reported in fish farms (Okamura et al., 2011). For instance, several studies have shown that brown trout (*Salmo trutta*) declines in Swiss rivers were linked to PKD development (Burkhardt-Holm & Scheurer, 2007; Wahli et al., 2007). More recently, an impressive outbreak of the disease occurred in the Yellowstone River (Montana, USA) with over tens of thousands of fish deaths reported including > 99% of mountain whitefish (*Prosopium williamsoni*), brown trout, rainbow trout (*Oncorhynchus mykiss*) and cutthroat trout (*Oncorhynchus clarkii*) (Hutchins et al., 2021). Disease outbreaks are seasonal and occur during the summer, tightly linked with the increase in water temperature (Okamura et al., 2011; see below). Therefore, more PKD outbreaks are to be expected under the context of global warming (Koelle et al., 2005; Ros et al., 2022).

If the first occurrence of PKD was reported in Germany in 1924 (Plehn, 1924), *T. bryosalmonae*'s taxonomy and complex life cycle were resolved far more recently (Kent & Hedrick, 1985). Because transmission from fish to fish had never been shown, it suggested that there was a missing piece in the parasite life cycle, involving another host species. The observation of similar cellular structures between myxozoans infecting bryozoans and the parasite causing salmonid PKD provided the first clue, and comparison through molecular analyses further confirmed the identity of *T. bryosalmonae*. The parasite life cycle was finally resolved after the confirmed experimental parasite transmission from bryozoans to the rainbow trout (Anderson et al., 1999; Feist et al., 2001; Okamura & Wood, 2002).



Figure 8. Occurrence of the proliferative kidney disease reported in the world. Cases have been reported in Northern America and in Europe. Figures indicate the species involved when detection occurred in wild populations. When no figure is indicated, PKD has been detected in fish farms, most of the time in rainbow trout (Onchorhynchus mykiss). This figure illustrates the successive surveys by Clifton-Hadley et al. (1984), Hedrick et al. (1993) and Sudhagar et al. (2020). Photo credits: 1,6,7,9: Patrick Clayton; 2: Ross Roberton; 3: Kevin Cass; 4: Alaska stock; 5: Mossy Oak; 8: Johan Frederiksson; 10: Gilbert van Ryckevorsel; 11: Jobomir Hlasek: 12: Robert J. Erwin; 13: rktl.fi.

Myxozoans are microscopic endoparasites of vertebrates and invertebrates, mostly from fresh and marine waters (Goater et al., 2014). They are part of the Cnidaria phylum, that also includes anemones, corals, hydrozoans, and jellyfish, and they are characterized by multicellular spores (Atkinson et al., 2018). T. bryosalmonae is part of the Malacosporea, a class of myxozoans that alternates between freshwater bryozoan as invertebrate host and fish as vertebrate host (see Fig. 9 for an illustration of the life cycle). In its bryozoan host, T. bryosalmonae alternates between two distinct infection phases. During covert infection, T. bryosalmonae is present as single cells within the bryozoan body cavity and develops into multicellular sac structures containing up to 4000 spores during overt infection (Okamura et al., 2011). Covert infection is costless to the bryozoan host and may persist throughout the year (Fontes et al., 2017). Overt infection develops when the environmental conditions are favourable to the bryozoan growth and causes temporary castration of the bryozoan colony as well as reduced growth (Hartikainen & Okamura, 2015). Favourable environmental conditions include an increase in temperature and nutrient abundance in the water (Fontes et al., 2017), and the onset of spore release from the sacs occurs in spring when water temperature reaches 9°C (Gay et al., 2001). T. bryosalmonae spores released by bryozoans during overt infection are infective to the fish hosts. These spores are ovoid with soft outer valves, two sporoplasms (infective cells), and four polar capsules (Okamura & Canning, 2003; Gruhl, 2015).

When reaching their fish host, parasite spores enter the body through gills or skin, circulate through the blood and settle for multiplication (sporogony) when they reach their target organs, mainly the kidney and spleen (Okamura et al., 2011). The infection can remain benign, producing asymptomatic parasite carriers (Fig. 9). However, parasite proliferation in the kidney interstitial tissue may cause an excessive immune response, responsible for kidney swelling (Fig. 9; Chilmonczyk et al., 2002). This results in impaired kidney functions, including blood cell production, leading to anemia and decreased oxygen transportation that may eventually cause fish death from asphyxia (Hedrick et al., 1993; Palikova et al., 2017). As they are ectotherm organisms, fish physiology, including the immune response, is directly affected by the ambient temperature (Bowden et al., 2007). In agreement, the development of PKD is tightly linked to water temperature, and disease symptoms are mostly reported when the temperature reaches 15°C (Clifton-Hadley et al., 1986; Bailey et al., 2017). Fish surviving their first infection by T. bryosalmonae likely develop an adaptive immune response as they do not develop PKD upon reinfection (Foott & Hedrick, 1987; Feist & Longshaw, 2006). Therefore, young-of-the-year fish, by definition exposed for the first time, are the most concerned with PKD issues. When surviving the infection, fish most probably become long-term parasite carriers, as spore shedding has been reported up to 5 years post infection in the brown trout (Soliman et al., 2018). After multiplication in kidney interstitium, spores may migrate into the lumen of kidney tubules (Morris & Adams, 2008), and be excreted in the river water through fish urine excretion (Hedrick et al., 2004). These spores, infective to

the bryozoan hosts, are also ovoid with soft outer valves, but with only one sporoplasm and two polar capsules (Hedrick et al., 2004; Gruhl, 2015). Up to now, only brown trout and brook trout (*Salvelinus fontinalis*) have been shown to successfully transmit *T. bryosalmonae* to bryozoan colonies (Grabner & El-Matbouli, 2008), suggesting that the other fish hosts may represent dead-ends for the parasite.

Bryozoans, T. bryosalmonae's definitive hosts, are moss-like colonial animals leaving attached to the substrate, often within small cavities, or attached to the roots of riparian trees, that they use as a shelter from the mainstream flow, or even within submerged artificial pipes (Wood & Marsh, 1999; Hartikainen & Okamura, 2015). Tree roots are also attractive to salmonid fish for shelter and feeding, so that parasite transmission might be enhanced in this microhabitat (Hartikainen & Okamura, 2015; Mathieu-Bégné et al., 2021). Bryozoan colonies are formed of connected zooids that feed with their ciliated tentacles on organic particles in suspension (Massard & Geimer, 2008). Their growth is favoured in spring by increased water temperature and nutrient concentration in the water (Hartikainen et al., 2009; Okamura et al., 2011). Increased bryozoan growth leaves more available niche for parasite infection, and increased temperature also favours T. bryosalmonae's multiplication within the bryozoan body cavity, so that more spores may be released to infect fish hosts (Tops et al., 2006). Furthermore, T. bryosalmonae can exploit its bryozoan host clonal reproduction for transmission. Branches may detach from the mother colony under the river flow, disperse and attach to new surfaces to form new colonies via fragmentation (Fig. 9). Moreover, T. bryosalmonae can also be vertically transmitted within bryozoan statoblasts. These dormant resistant propagules are emitted by senescent colonies to survive the winter and germinate to form new bryozoan colonies when conditions become favourable again (Abd-Elfattah et al., 2013). Transmission through colony fragmentation and within bryozoan statoblasts allow the parasite to persist and disperse further within the stream system, balancing the fact that its free-living stages are fragile and remain infectious only for 12-24h after emission (De Kinkelin et al., 2002). Besides, T. bryosalmonae has been found in bryozoans inhabiting a lake deprived of is potential fish hosts (salmonids and pike), suggesting that it can persist even in the absence of its fish hosts (Okamura et al., 2001).



Figure 9. Illustration of *Tetracapsuloides* bryosalmonae's life cycle and some environmental factors' effects. The transfer between the fish and bryozoan hosts is seasonal. T. bryosalmonae is non-virulent to its bryozoan host during fall (covert infection) and develops into sacs containing thousands of spores (overt infection) during spring when conditions become favourable for bryozoan growth (increased water temperature and nutrient abundance). Spores infectious to fish are then excreted in the water. Once in the fish, the infection can be asymptomatic in tolerant hosts, or provoke proliferative kidney disease during summer. The right picture shows a cross section of a diseased fish with swollen kidney (dark red), in comparison with a healthy kidney (left picture). T. bryosalmonae is excreted through fish urine in the water during early fall and may then infect a bryozoan host to complete its cycle. T. bryosalmonae uses the bryozoan asexual reproduction by fragmentation and statoblasts for dispersion and winter survival. A picture of a typical Pyrenean river is also shown. This representation is adapted from Gallana et al. (2013) and Hartikainen & Okamura (2015). Juvenile brown illustration trout ©americanfishes.co

Study context and general aims

The salmonid species brown trout *Salmo trutta* originally occurred naturally in Europe, North Africa and Western Asia. This species has been subsequently successfully introduced into 24 countries outside its initial range, resulting in a worldwide distribution encompassing all continents (Elliott 1994). Its wide range of occupied habitats, together with a wide diversity of morphologies and a continuum between different life-history strategies has led to troubles regarding classification of the brown trout (Elliott, 1994; Cucherousset et al., 2005). Some taxonomists had indeed favoured the description of about fifty species for all the varieties of brown trout that could be described (Klemetsen et al., 2003). The high plasticity in life-history strategies of this species (e.g., brown trout from the same population may stay as resident in their natal river, or migrate to other streams, lakes or to the sea; Cucherousset et al., 2005) is presumably responsible for its successful worldwide establishment (Elliott 1994). The establishment of this ectotherm cold-water species is mainly determined by the water temperature, with an upper thermal limit for feeding of 19.5°C in brown trout adults, and a critical maximum temperature of 26°C when exposed more than 10min (Fig. 10; Elliott, 1994; Elliott & Elliott, 2010). This species also needs well-oxygenated streams (tightly linked to cool water temperatures) and non-polluted waters.



Figure 10. Schematic representation of the thermal requirements of the brown trout. Stressing and lethal temperature zones are indicated in red. This representation is adapted from Elliott (1994).

The anthropogenic global change represents a multi-level threat to the freshwater habitat and to brown trout populations because of the increase in water temperature, eutrophication due to pollution, fragmentation from dams' construction, fishing pressure and fish stocking (Dudgeon et al., 2006; Jonsson & Jonsson, 2009; Frank et al., 2011). These multiple stressors may lead to a loss of brown trout intraspecific diversity, in line with the increasing report of population declines in Europe, its native region (Burkhardt-Holm & Scheurer, 2007; Stelkens et al., 2012; Santiago, 2017; O'Briain et al., 2019). Moreover, the recent development of the proliferative kidney disease, a temperature-dependent disease

(see above), has added a new biotic threat to brown trout populations' survival (Hari et al., 2006; Borgwardt et al., 2020).

The proliferative kidney disease was first detected in wild brown trout populations of the Pyrenees (Southern France) in 2016. High mortality of juveniles was reported through the finding of dead carcasses (passive surveillance) presenting the typical signs of the disease along the riverbanks, i.e., opened gills revealing death from asphyxia and kidney swelling (Garmendia & Lautraite, 2017). The infection by *T. bryosalmonae* was further confirmed by parasite DNA amplification from kidney samples. These findings alerted the local angling federations and annual surveys through active surveillance were thereafter conducted in Ariège. Brown trout is an emblematic fish of the Pyrenean streams, with high socio-economic value, and declines of this fish populations (all ages included) are reported since 2007. The aim of the present project was to build a methodological framework for an integrative study of the different compartments of the disease tryptic (Fig. 5), including multiple environmental stressors, at a large geographical scale to cover environmental gradients and at the intra and inter host population levels to better grasp the mechanisms involved in populations declines and in the epidemiological dynamics of the disease. To this end, I investigated in my thesis the distribution, the ecological and evolutionary impacts of T. bryosalmonae on wild brown trout in nearly a hundred sites spread over 200 kilometers at the foothill of the Pyrenean mountains. I developed and applied a set of new environmental DNA tools to detect the parasite in the river but also within its fish host fluids. This allowed me to investigate the respective roles of abiotic and biotic factors (hosts and parasite propagules density) in the parasite distribution and the infection prevalence within fish populations. I also used a before/after study design to assess the ecological and evolutionary impacts of the combination of the infectious and thermal stressors. Finally, for management perspectives, I aimed at assessing the global health of wild brown trout populations by combining complementary indicators informing on their genetic, ecological and pathological status, and investigated the role of multiple abiotic environmental factors.

Chapter 1. Urine DNA (uDNA) as a non-lethal method for endoparasite biomonitoring: development and validation.

Endoparasite infections are usually assessed through the sampling of specific organs targeted by the parasite. Such sampling is obviously lethal and may prevent large and repeated samplings that appear necessary to understand disease dynamics because of ethical and conservation reasons (Okamura et al., 2011). This first chapter aimed at developing a new operational tool to monitor an endoparasite infection without killing the sampled host. I have sought to answer to this specific question:

• Can we accurately assess the infection status and the parasite load of a host by using its fluids excretion?

Building on *T. bryosalmonae*'s life cycle, I isolated and quantified parasite DNA after filtration of juvenile brown trout urine excretion (uDNA) and compared the results to the classical method that quantifies parasite DNA from kidney samples.

Chapter 2. Investigating the environmental conditions shaping parasite distribution and infection prevalence in host populations: an integrative approach using eDNA

Wildlife disease surveillance usually relies on host sampling, and the presence of a parasite outside its host in the open environment (parasite pressure) is rarely assessed, so that there is a missing piece for the understanding of the disease dynamics. Moreover, a parasite life cycle may include several host species so that it is necessary to study the ecology of all the compartments of the life cycle to better understand and predict the eco-evolutionary dynamics of the disease. In this second chapter, I aimed at mapping and explaining the parasite distribution in the water and within its fish host (infection prevalence) at a wide scale, answering these specific questions:

- What are the main drivers of the parasite propagules' distribution in flowing water? What is the relative role of abiotic and biotic environmental factors?
- What are the main causes of the parasite infection in the fish host?

Since a parasite relies on its hosts for surviving, spreading, and completing its life cycle, we expected its distribution to be tightly linked to that of its hosts. However, the abiotic environmental conditions may also act directly on its free-living stages (Pietrock & Marcogliese, 2003). As temperature and eutrophication may favour the parasite and its bryozoan host development, we expected water quality parameters to be linked to the parasite distribution. Fish infection may depend on the intensity of the propagule pressure (i.e., the abundance of infective parasite propagules in the milieu, e.g. for the infection of amphibians by a trematode in Johnson et al., 2007), but also on the abiotic condition acting on the fish physiology. As water temperature is the main factor influencing brown trout physiology, we expected that fish immune response might be altered in warm environments, leading to higher infection prevalence. The infection prevalence could thus be affected by the abiotic conditions either directly by their effect on fish host, or indirectly by their effect on the parasite and its hosts abundances (biotic factors) using water eDNA and digital PCR and measured relevant abiotic environmental factors at 83 sites located on 54 different streams, and I measured population infection prevalence with the uDNA method in 46 brown trout populations.

Chapter 3. Eco-evolutionary response of cold-water fish populations to the combined effect of an emerging parasite and temperature.

Wild species are confronted to multiple stressors acting synergistically on their integrity, but the impacts of multiple stressors on population intraspecific variation are rarely assessed conjointly in the wild. Moreover, the impact of infectious diseases in the wild is hardly ever assessed with before/after study
designs (due to the lack of opportunity and often unpredictable disease development), leading to potentially biased overview of the disease impacts as individuals with low resistance or tolerance may have died before the sampling. PKD develops seasonally during the summer, so that brown trout juveniles (the most sensitive stage to the disease) sampling before and after the summer may give insights on the infection impacts. Because water temperature and infection by *T. bryosalmonae* may act synergistically on brown trout populations through their impact on fish physiology and parasite multiplication, I raised these specific questions within chapter 3:

- How does the combination of infection and water temperature influence the survival of brown trout juveniles?
- What are the eco-evolutionary responses of the populations to the combined effect of water temperature and infection?

Literature suggests a strong combined impact of temperature and infection on naïve brown trout survival (Okamura et al., 2011; Waldner et al., 2021). We thus expected that these factors would act conjointly to decrease populations' density. Moreover, provided that host populations show genetic variation in parasite control (tolerance and/or resistance) (Debes et al., 2017), we expected to find a shift in genetic variation at a locus involved in the adaptive immune response. We expected that pathogen- and temperature- mediated selection would also translate at the phenotypic level, with either only the fittest individuals surviving the summer, or a decrease in the mean fitness traits (body condition, ornamentation) due to trade-offs with a costly immune response (Rauw, 2012). To answer these questions, we sampled juveniles from 15 brown trout populations exposed to gradients of temperature and parasite infection to compare their genetic and phenotypic variations before and after the summer.

Chapter 4. A multifaceted index of population health to detect risk-prone populations and underlying stressors in wildlife.

The adaptive potential of wild populations (i.e., their ability to respond to environmental change) depends on intrinsic factors related to their global health. However, most studies on wild populations focus on only one facet of population health, for instance their pathological status or genetic variation. The use of a single facet may miss a part of the story, leading to erroneous conclusions and misguided management plans. In this last chapter, I developed an integrative multifaceted index combining the pathological, ecological and genetic status of wild populations to inform on their global health, aiming at answering these questions:

• Can we develop an integrative index of population health that accurately represents multiple facets of population integrity?

• Using this index, can we identify the major environmental drivers of wild population health? I therefore developed and tested this index on 46 wild populations of brown trout and assessed the impact of a wide range of environmental factors in shaping this multifaceted index.

Literature cited

- Abd-Elfattah, A., Fontes, I., Kumar, G., Soliman, H., Hartikainen, H., Okamura, B., & El-Matbouli, M. (2013). Vertical transmission of *Tetracapsuloides bryosalmonae* (Myxozoa), the causative agent of salmonid proliferative kidney disease. *Parasitology*, 141(04), 482–490. https://doi.org/10.1017/S0031182013001650
- Adlard, R. D., Miller, T. L., & Smit, N. J. (2015). The butterfly effect: Parasite diversity, environment, and emerging disease in aquatic wildlife. *Trends in Parasitology*, *31*(4), 160–166. https://doi.org/10.1016/j.pt.2014.11.001
- Altizer, S., Ostfeld, R. S., Johnson, P. T. J., Kutz, S., & Harvell, C. D. (2013). Climate Change and Infectious Diseases: From Evidence to a Predictive Framework. *Science*, 341(6145), 514–519. https://doi.org/10.1126/science.1239401
- Anderson, C. L., Canning, E. U., & Okamura, B. (1999). Molecular data implicate bryozoans as hosts for PKX (Phylum Myxozoa) and identify a clade of bryozoan parasites within the Myxozoa. *Parasitology*, 119(6), 555–561. https://doi.org/10.1017/S003118209900520X
- Arkoosh, M. R., Casillas, E., Clemons, E., Kagley, A. N., Olson, R., Reno, P., & Stein, J. E. (1998). Effect of Pollution on Fish Diseases: Potential Impacts on Salmonid Populations. *Journal of Aquatic Animal Health*, 9.
- Artois, M., Bengis, R., Delahay, R. J., Duchêne, M.-J., Duff, J. P., Ferroglio, E., Gortazar, C., Hutchings, M. R., Kock, R. A., Leighton, F. A., Mörner, T., & Smith, G. C. (2009). Wildlife Disease Surveillance and Monitoring. In *Management of Disease in Wild Mammals* (Delahay R.J., Smith G.S., Hutchings M. R. (Eds.), pp. 187–213). Springer Tokyo Berlin Heidelberg New York.
- Atkinson, S. D., Bartholomew, J. L., & Lotan, T. (2018). Myxozoans: Ancient metazoan parasites find a home in phylum Cnidaria. *Zoology*, 129, 66–68. https://doi.org/10.1016/j.zool.2018.06.005
- Auld, S. K. J. R., Penczykowski, R. M., Housley Ochs, J., Grippi, D. C., Hall, S. R., & Duffy, M. A. (2013). Variation in costs of parasite resistance among natural host populations. *Journal of Evolutionary Biology*, 26(11), 2479–2486. https://doi.org/10.1111/jeb.12243
- Bailey, C., Segner, H., Casanova-Nakayama, A., & Wahli, T. (2017). Who needs the hotspot? The effect of temperature on the fish host immune response to *Tetracapsuloides bryosalmonae* the causative agent of proliferative kidney disease. *Fish & Shellfish Immunology*, 63, 424–437. https://doi.org/10.1016/j.fsi.2017.02.039
- Barrett, R., & Schluter, D. (2008). Adaptation from standing genetic variation. *Trends in Ecology & Evolution*, 23(1), 38–44. https://doi.org/10.1016/j.tree.2007.09.008
- Bass, D., Stentiford, G. D., Littlewood, D. T. J., & Hartikainen, H. (2015). Diverse Applications of Environmental DNA Methods in Parasitology. *Trends in Parasitology*, 31(10), 499–513. https://doi.org/10.1016/j.pt.2015.06.013
- Berger, C. S., & Aubin-Horth, N. (2018). An eDNA-qPCR assay to detect the presence of the parasite Schistocephalus solidus inside its threespine stickleback host. The Journal of Experimental Biology, 221(9). https://doi.org/10.1242/jeb.178137
- Berger, L., Speare, R., Daszak, P., Green, D. E., Cunningham, A. A., Goggin, C. L., Slocombe, R., Ragan, M. A., Hyatt, A. D., McDonald, K. R., Hines, H. B., Lips, K. R., Marantelli, G., & Parkes, H. (1998). Chytridiomycosis causes amphibian mortality associated with population declines in the rain forests of Australia and Central America. *Proceedings of the National Academy of Sciences*, 95(15), 9031–9036. https://doi.org/10.1073/pnas.95.15.9031
- Bernatchez, L., & Landry, C. (2003). MHC studies in nonmodel vertebrates: What have we learned about natural selection in 15 years?: MHC studies in nonmodel vertebrates. *Journal of Evolutionary Biology*, *16*(3), 363–377. https://doi.org/10.1046/j.1420-9101.2003.00531.x
- Beschta, R. L., & Ripple, W. J. (2019). Can large carnivores change streams via a trophic cascade? *Ecohydrology*, 12(1). https://doi.org/10.1002/eco.2048
- Best, A., White, A., & Boots, M. (2014). The coevolutionary implications of host tolerance: Coevolution of tolerance. *Evolution*, 68(5), 1426–1435. https://doi.org/10.1111/evo.12368
- Böhm, M., White, P. C. L., Daniels, M. J., Allcroft, D. J., Munro, R., & Hutchings, M. R. (2006). The health of wild red and sika deer in Scotland: An analysis of key endoparasites and

recommendations for monitoring disease. *The Veterinary Journal*, 171(2), 287–294. https://doi.org/10.1016/j.tvjl.2004.10.020

- Bohmann, K., Evans, A., Gilbert, M. T. P., Carvalho, G. R., Creer, S., Knapp, M., Yu, D. W., & de Bruyn, M. (2014). Environmental DNA for wildlife biology and biodiversity monitoring. *Trends in Ecology & Evolution*, 29(6), 358–367. https://doi.org/10.1016/j.tree.2014.04.003
- Borgwardt, F., Unfer, G., Auer, S., Waldner, K., El-Matbouli, M., & Bechter, T. (2020). Direct and Indirect Climate Change Impacts on Brown Trout in Central Europe: How Thermal Regimes Reinforce Physiological Stress and Support the Emergence of Diseases. *Frontiers in Environmental Science*, 8, 59. https://doi.org/10.3389/fenvs.2020.00059
- Bowden, T. J., Thompson, K. D., Morgan, A. L., Gratacap, R. M. L., & Nikoskelainen, S. (2007). Seasonal variation and the immune response: A fish perspective. *Fish & Shellfish Immunology*, 22(6), 695–706. https://doi.org/10.1016/j.fsi.2006.08.016
- Breed, A. C., Plowright, R. C., Hayman, D. T. S., Knobel, D. L., Molenaar, F. M., Garder-Roberts, D., Cleaveland, S., Haydon, D. T., Kock, R. A., Cunningham, A. A., Sainsbury, A. W., & Delahay, R. J. (2009). Disease Management in Endangered Mammals. In *Management of Disease in Wild Mammals* (Delahay R.J., Smith G.S., Hutchings M. R. (Eds.)). Springer Tokyo Berlin Heidelberg New York.
- Brockhurst, M. A., Chapman, T., King, K. C., Mank, J. E., Paterson, S., & Hurst, G. D. D. (2014). Running with the Red Queen: The role of biotic conflicts in evolution. *Proceedings of the Royal Society B: Biological Sciences*, 281(1797), 20141382. https://doi.org/10.1098/rspb.2014.1382
- Brooks, D. R., Hoberg, E. P., Boeger, W. A., & Trivellone, V. (2022). Emerging infectious disease: An underappreciated area of strategic concern for food security. *Transboundary and Emerging Diseases*, 69(2), 254–267. https://doi.org/10.1111/tbed.14009
- Brown, M. J. F., Schmid-Hempel, R., & Schmid-Hempel, P. (2003). Strong context-dependent virulence in a host–parasite system: Reconciling genetic evidence with theory. *Journal of Animal Ecology*, 72, 994–1002.
- Bucke, D., Feist, S. W., & Clifton-Hadley, R. S. (1991). The occurrence of proliferative kidney disease (PKD) in cultured and wild fish: Further investigations. *Journal of Fish Diseases*, 14(5), 583– 588. https://doi.org/10.1111/j.1365-2761.1991.tb00614.x
- Budria, A., & Candolin, U. (2014). How does human-induced environmental change influence hostparasite interactions? *Parasitology*, *141*(4), 462–474. https://doi.org/10.1017/S0031182013001881
- Burkhardt-Holm, P., & Scheurer, K. (2007). Application of the weight-of-evidence approach to assess the decline of brown trout (*Salmo trutta*) in Swiss rivers. *Aquatic Sciences*, 69(1), 51–70. https://doi.org/10.1007/s00027-006-0841-6
- Buse, A., Dury, S. J., Woodburn, R. J. W., Perrins, C. M., & Good, J. E. G. (1999). Effects of elevated temperature on multi-species interactions: The case of Pedunculate Oak, Winter Moth and Tits. *Functional Ecology*, 13(Suppl. 1), 74–82.
- Cahill, A. E., Aiello-Lammens, M. E., Fisher-Reid, M. C., Hua, X., Karanewsky, C. J., Yeong Ryu, H., Sbeglia, G. C., Spagnolo, F., Waldron, J. B., Warsi, O., & Wiens, J. J. (2013). How does climate change cause extinction? *Proceedings of the Royal Society B: Biological Sciences*, 280(1750), 20121890. https://doi.org/10.1098/rspb.2012.1890
- Chamberlain, S. A., Bronstein, J. L., & Rudgers, J. A. (2014). How context dependent are species interactions? *Ecology Letters*, 17(7), 881–890. https://doi.org/10.1111/ele.12279
- Chapman, J. M., Kelly, L. A., Teffer, A. K., Miller, K. M., & Cooke, S. J. (2021). Disease ecology of wild fish: Opportunities and challenges for linking infection metrics with behaviour, condition, and survival. *Canadian Journal of Fisheries and Aquatic Sciences*, 78(8), 995–1007. https://doi.org/10.1139/cjfas-2020-0315
- Chavatte, J.-M., Giraud, P., Esperet, D., Place, G., Cavalier, F., & Landau, I. (2019). An outbreak of trichomonosis in European greenfinches *Chloris chloris* and European goldfinches *Carduelis carduelis* wintering in Northern France. *Parasite*, 26, 21. https://doi.org/10.1051/parasite/2019022
- Chilmonczyk, S., Monge, D., & de Kinkelin, P. (2002). Proliferative kidney disease: Cellular aspects of the rainbow trout, *Oncorhynchus mykiss* (Walbaum), response to parasitic infection. *Journal of Fish Diseases*, 25(4), 217–226. https://doi.org/10.1046/j.1365-2761.2002.00362.x

- Chmura, H. E., Kharouba, H. M., Ashander, J., Ehlman, S. M., Rivest, E. B., & Yang, L. H. (2019). The mechanisms of phenology: The patterns and processes of phenological shifts. *Ecological Monographs*, 89(1), e01337. https://doi.org/10.1002/ecm.1337
- Christensen, J. (2001). Epidemiological Concepts Regarding Disease Monitoring and Surveillance. *Acta Veterinaria Scandinavica*, 42(Suppl 1), S11. https://doi.org/10.1186/1751-0147-42-S1-S11
- Cilia, G., Bertelloni, F., Mignone, W., Spina, S., Berio, E., Razzuoli, E., Vencia, W., Franco, V., Cecchi, F., Bogi, S., Turchi, B., Cerri, D., & Fratini, F. (2020). Molecular detection of *Leptospira* spp. In wild boar (*Sus scrofa*) hunted in Liguria region (Italy). *Comparative Immunology, Microbiology and Infectious Diseases*, 68, 101410. https://doi.org/10.1016/j.cimid.2019.101410
- Clark, R. W., Marchand, M. N., Clifford, B. J., Stechert, R., & Stephens, S. (2011). Decline of an isolated timber rattlesnake (*Crotalus horridus*) population: Interactions between climate change, disease, and loss of genetic diversity. *Biological Conservation*, 144(2), 886–891. https://doi.org/10.1016/j.biocon.2010.12.001
- Clark-Wolf, T. J., Hahn, P. G., Brelsford, E., Francois, J., Hayes, N., Larkin, B., Ramsey, P., & Pearson, D. E. (2022). Preventing a series of unfortunate events: Using qualitative models to improve conservation. *Journal of Applied Ecology, Advance online publication*. https://doi.org/10.1111/1365-2664.14231
- Clifton-Hadley, R. S., Bucke, D., & Richards, R. H. (1984). Proliferative kidney disease of salmonid fish: A review. *Journal of Fish Diseases*, 7, 363–377.
- Clifton-Hadley, R. S., Richards, R. H., & Bucke, D. (1986). Proliferative kidney disease (PKD) in rainbow trout *Salmo gairdneri*: Further observations on the effects of water temperature. *Aquaculture*, 55(3), 165–171. https://doi.org/10.1016/0044-8486(86)90112-2
- Combes, C. (2001). *Parasitism: The Ecology and Evolution of Intimate Interactions* (The University of Chicago Press).
- Combes, C. (2005). *The art of being a parasite* (The University of Chicago).
- Crofton, H. D. (1971). A quantitative approach to parasitism. *Parasitology*, 62(2), 179–193. https://doi.org/10.1017/S0031182000071420
- Cucherousset, J., Ombredane, D., Charles, K., Marchand, F., & Baglinière, J.-L. (2005). A continuum of life history tactics in a brown trout (*Salmo trutta*) population. *Canadian Journal of Fisheries and Aquatic Sciences*, 62(7), 1600–1610. https://doi.org/10.1139/f05-057
- Cunningham, A. A., Daszak, P., & Wood, J. L. N. (2017). One Health, emerging infectious diseases and wildlife: Two decades of progress? *Philosophical Transactions of the Royal Society B: Biological Sciences*, 372(1725), 20160167. https://doi.org/10.1098/rstb.2016.0167
- Daszak, P. (2000). Emerging Infectious Diseases of Wildlife—Threats to Biodiversity and Human Health. *Science*, 287(5452), 443–449. https://doi.org/10.1126/science.287.5452.443
- Daszak, P., Berger, L., Cunningham, A. A., Hyatt, A. D., Green, D. E., & Speare, R. (1999). Emerging Infectious Diseases and Amphibian Population Declines. *Emerging Infectious Diseases*, 5(6), 735–748. https://doi.org/10.3201/eid0506.990601
- De Kinkelin, P., Gay, M., & Forman, S. (2002). The persistence of infectivity of *Tetracapsula* bryosalmonae-infected water for rainbow trout, *Oncorhynchus mykiss* (Walbaum). Journal of Fish Diseases, 25, 477–482.
- Debes, P. V., Gross, R., & Vasemägi, A. (2017). Quantitative Genetic Variation in, and Environmental Effects on, Pathogen Resistance and Temperature-Dependent Disease Severity in a Wild Trout. *The American Naturalist*, *190*(2), 244–265. https://doi.org/10.1086/692536
- Deutsch, C. A., Tewksbury, J. J., Huey, R. B., Sheldon, K. S., Ghalambor, C. K., Haak, D. C., & Martin, P. R. (2008). Impacts of climate warming on terrestrial ectotherms across latitude. *Proceedings* of the National Academy of Sciences, 105(18), 6668–6672. https://doi.org/10.1073/pnas.0709472105
- Dobson, A., Lafferty, K. D., Kuris, A. M., Hechinger, R. F., & Jetz, W. (2008). Homage to Linnaeus: How many parasites? How many hosts? *Proceedings of the National Academy of Sciences*, 105(supplement_1), 11482–11489. https://doi.org/10.1073/pnas.0803232105
- Dudgeon, D., Arthington, A. H., Gessner, M. O., Kawabata, Z.-I., Knowler, D. J., Lévêque, C., Naiman, R. J., Prieur-Richard, A.-H., Soto, D., Stiassny, M. L. J., & Sullivan, C. A. (2006). Freshwater

biodiversity: Importance, threats, status and conservation challenges. *Biological Reviews*, 81(02), 163. https://doi.org/10.1017/S1464793105006950

- Ebert, D., & Fields, P. D. (2020). Host-parasite co-evolution and its genomic signature. *Nature Reviews Genetics*, 21(12), 754–768. https://doi.org/10.1038/s41576-020-0269-1
- Ebert, D., & Weisser, W. W. (1997). Optimal killing for obligate killers: The evolution of life histories and virulence of semelparous parasites. *Proceedings of the Royal Society of London. Series B: Biological Sciences*, 264(1384), 985–991. https://doi.org/10.1098/rspb.1997.0136
- Eizaguirre, C., Lenz, T. L., Kalbe, M., & Milinski, M. (2012). Rapid and adaptive evolution of MHC genes under parasite selection in experimental vertebrate populations. *Nature Communications*, 3(1), 621. https://doi.org/10.1038/ncomms1632
- Elliott, J. M. (1994). Quantitative Ecology and the Brown Trout (Oxford University Press).
- Elliott, J. M., & Elliott, J. A. (2010). Temperature requirements of Atlantic salmon *Salmo salar*, brown trout *Salmo trutta* and Arctic charr *Salvelinus alpinus*: Predicting the effects of climate change. *Journal of Fish Biology*, 25.
- Engering, A., Hogerwerf, L., & Slingenbergh, J. (2013). Pathogen-host-environment interplay and disease emergence. *Emerging Microbes & Infections*, 2(1), 1–7. https://doi.org/10.1038/emi.2013.5
- Etienne, L., Locatelli, S., Ayouba, A., Esteban, A., Butel, C., Liegeois, F., Aghokeng, A., Delaporte, E., Mpoudi Ngole, E., & Peeters, M. (2012). Noninvasive Follow-Up of Simian Immunodeficiency Virus Infection in Wild-Living Nonhabituated Western Lowland Gorillas in Cameroon. *Journal* of Virology, 86(18), 9760–9772. https://doi.org/10.1128/JVI.01186-12
- Fearon, D. T., & Locksley, R. M. (1996). The Instructive Role of Innate Immunity in the Acquired Immune Response. *Science*, 272(5258), 50–54. https://doi.org/10.1126/science.272.5258.50
- Feist, S., Longshaw, M., Canning, E., & Okamura, B. (2001). Induction of proliferative kidney disease (PKD) in rainbow trout Oncorhynchus mykiss via the bryozoan Fredericella sultana infected with Tetracapsula bryosalmonae. Diseases of Aquatic Organisms, 45, 61–68. https://doi.org/10.3354/dao045061
- Feist, S. W., & Longshaw, M. (2006). Phylum Myxozoa. In P. T. K. Woo, *Fish diseases and disorders* (CAB International, pp. 230–296).
- Fisher, M. C., & Garner, T. W. J. (2020). Chytrid fungi and global amphibian declines. *Nature Reviews Microbiology*, *18*(6), 332–343. https://doi.org/10.1038/s41579-020-0335-x
- Fontes, I., Hartikainen, H., Williams, C., & Okamura, B. (2017). Persistence, impacts and environmental drivers of covert infections in invertebrate hosts. *Parasites & Vectors*, 10(1). https://doi.org/10.1186/s13071-017-2495-8
- Foott, J. S., & Hedrick, R. P. (1987). Seasonal occurrence of the infectious stage of proliferative kidney disease (PKD) and resistance of rainbow trout, *Salmo gairdneri* Richardson, to reinfection. *Journal of Fish Biology*, 30(4), 477–483. https://doi.org/10.1111/j.1095-8649.1987.tb05771.x
- Frank, B. M., Piccolo, J. J., & Baret, P. V. (2011). A review of ecological models for brown trout: Towards a new demogenetic model: Towards a demogenetic model for brown trout. *Ecology of Freshwater Fish*, 20(2), 167–198. https://doi.org/10.1111/j.1600-0633.2011.00491.x
- Frank, S. A. (1996). Models of Parasite Virulence. *The Quarterly Review of Biology*, 71(1), 37–78. https://doi.org/10.1086/419267
- Fraser, B. A., & Neff, B. D. (2010). Parasite mediated homogenizing selection at the MHC in guppies. *Genetica*, 138(2), 273–278. https://doi.org/10.1007/s10709-009-9402-y
- Fu, M., & Waldman, B. (2019). Ancestral chytrid pathogen remains hypervirulent following its long coevolution with amphibian hosts. *Proceedings of the Royal Society B: Biological Sciences*, 286(1904), 20190833. https://doi.org/10.1098/rspb.2019.0833
- Gallana, M., Ryser-Degiorgis, M.-P., Wahli, T., & Segner, H. (2013). Climate change and infectious diseases of wildlife: Altered interactions between pathogens, vectors and hosts. *Current Zoology*, 59(3), 427–437.
- Garmendia, L., & Lautraite, A. (2017). Fédération de l'Ariège de pêche et de protection du milieu aquatique. Cas d'une Tétracapsuloïdose sur l'axe Ariège, Rapport d'étude de la tétracapsuloïdose (« PKD ») infectant les truites fario dans le réseau hydrographique de la région d'Ax-Les-Thermes (Ariège, Oriège, Lauze).

- Gay, M., Okamura, B., & de Kinkelin, P. (2001). Evidence that infectious stages of *Tetracapsula* bryosalmonae for rainbow trout *Oncorhynchus mykiss* are present throughout the year. *Diseases* of Aquatic Organisms, 46, 31–40. https://doi.org/10.3354/dao046031
- Goater, T. M., Goater, C. P., & Esch, G. W. (2014). Myxozoa: The spore-forming cnidarians. In *Parasitism. The Diversity and Ecology of Animal Parasites* (2nd edition, pp. 96–112). Cambridge University Press.
- Godfroid, J., Cloeckaert, A., Liautard, J.-P., Kohler, S., Fretin, D., Walravens, K., Garin-Bastuji, B., & Letesson, J.-J. (2005). From the discovery of the Malta fevers agent to the discovery of a marine mammal reservoir, brucellosis has continuously been a re-emerging zoonosis. *Veterinary Research*, 36(3), 313–326. https://doi.org/10.1051/vetres:2005003
- Grabner, D., & El-Matbouli, M. (2008). Transmission of *Tetracapsuloides bryosalmonae* (Myxozoa: Malacosporea) to *Fredericella sultana* (Bryozoa: Phylactolaemata) by various fish species. *Diseases of Aquatic Organisms*, 79, 133–139. https://doi.org/10.3354/dao01894
- Guberti, V., Stancampiano, L., & Ferrari, N. (2014). Surveillance, monitoring and survey of wildlife diseases: A public health and conservation approach. *Hystrix, the Italian Journal of Mammalogy*, 25(1). https://doi.org/10.4404/hystrix-25.1-10114
- Gunderson, A. R., & Stillman, J. H. (2015). Plasticity in thermal tolerance has limited potential to buffer ectotherms from global warming. *Proceedings of the Royal Society B: Biological Sciences*, 282(1808), 20150401. https://doi.org/10.1098/rspb.2015.0401
- Gruhl, A. (2015). Myxozoa. In A. Wanninger (Ed.), Evolutionary Developmental Biology of Invertebrates, Springer, Vol. 1, pp. 165–177.
- Hari, R. E., Livingstone, D. M., Siber, R., Burkhardt-Holm, P., & Güttinger, H. (2006). Consequences of climatic change for water temperature and brown trout populations in Alpine rivers and streams. *Global Change Biology*, 12(1), 10–26. https://doi.org/10.1111/j.1365-2486.2005.001051.x
- Hartikainen, H., Johnes, P., Moncrieff, C., & Okamura, B. (2009). Bryozoan populations reflect nutrient enrichment and productivity gradients in rivers. *Freshwater Biology*, 54(11), 2320–2334. https://doi.org/10.1111/j.1365-2427.2009.02262.x
- Hartikainen, H., & Okamura, B. (2015). Ecology and Evolution of Malacosporean-Bryozoan Interactions. In B. Okamura, A. Gruhl, & J. L. Bartholomew, *Myxozoan Evolution, Ecology and Development* (Springer International Publishing, pp. 201–216).
- Harvey, E., Gounand, I., Ward, C. L., & Altermatt, F. (2017). Bridging ecology and conservation: From ecological networks to ecosystem function. *Journal of Applied Ecology*, *54*(2), 371–379. https://doi.org/10.1111/1365-2664.12769
- Hedrick, R. P., Baxa, D. V., De Kinkelin, P., & Okamura, B. (2004). Malacosporean-like spores in urine of rainbow trout react with antibody and DNA probes to *Tetracapsuloides bryosalmonae*. *Parasitology Research*, 92(1), 81–88. https://doi.org/10.1007/s00436-003-0986-3
- Hedrick, R. P., MacConnell, E., & de Kinkelin, P. (1993). Proliferative kidney disease of salmonid fish. *Annual Review of Fish Diseases*, *3*, 277–290. https://doi.org/10.1016/0959-8030(93)90039-E
- Hill, A. V. S. (1991). HLA Associations with Malaria in Africa: Some Implications for MHC Evolution. In J. Klein & D. Klein (Eds.), *Molecular Evolution of the Major Histocompatibility Complex* (pp. 403–420). Springer Berlin Heidelberg. https://doi.org/10.1007/978-3-642-84622-9_33
- Hoban, S., Hauffe, H. C., Pérez-Espona, S., Arntzen, J. W., Bertorelle, G., Bryja, J., Frith, K., Gaggiotti, O. E., Galbusera, P., Godoy, J. A., Hoelzel, A. R., Nichols, R. A., Primmer, C. R., Russo, I.-R., Segelbacher, G., Siegismund, H. R., Sihvonen, M., Vernesi, C., Vilà, C., & Bruford, M. W. (2013). Bringing genetic diversity to the forefront of conservation policy and management. *Conservation Genetics Resources*, 5(2), 593–598. https://doi.org/10.1007/s12686-013-9859-y
- Hoffmann, A. A., & Sgrò, C. M. (2011). Climate change and evolutionary adaptation. *Nature*, 470(7335), 479–485. https://doi.org/10.1038/nature09670
- Honma, H., Suyama, Y., & Nakai, Y. (2011). Detection of parasitizing coccidia and determination of host crane species, sex and genotype by faecal DNA analysis. *Molecular Ecology Resources*, 11(6), 1033–1044. https://doi.org/10.1111/j.1755-0998.2011.03048.x
- Hoyt, J. R., Kilpatrick, A. M., & Langwig, K. E. (2021). Ecology and impacts of white-nose syndrome on bats. *Nature Reviews Microbiology*, 19(3), 196–210. https://doi.org/10.1038/s41579-020-00493-5

- Hudson, P. J., Dobson, A. P., & Lafferty, K. D. (2006). Is a healthy ecosystem one that is rich in parasites? *Trends in Ecology & Evolution*, 21(7), 381–385. https://doi.org/10.1016/j.tree.2006.04.007
- Huey, R. B., & Kingsolver, J. G. (1989). Evolution of Thermal Sensitivity of Ectotherm Performance. *Trends in Ecology & Evolution*, 4(5), 131–135.
- Hutchins, P. R., Sepulveda, A. J., Hartikainen, H., Staigmiller, K. D., Opitz, S. T., Yamamoto, R. M., Huttinger, A., Cordes, R. J., Weiss, T., Hopper, L. R., Purcell, M. K., & Okamura, B. (2021). Exploration of the 2016 Yellowstone River fish kill and proliferative kidney disease in wild fish populations. *Ecosphere*, 12(3). https://doi.org/10.1002/ecs2.3436
- Huver, J. R., Koprivnikar, J., Johnson, P. T. J., & Whyard, S. (2015). Development and application of an eDNA method to detect and quantify a pathogenic parasite in aquatic ecosystems. *Ecological Applications*, 25(4), 991–1002. https://doi.org/10.1890/14-1530.1
- Jerde, C. L., Mahon, A. R., Chadderton, W. L., & Lodge, D. M. (2011). "Sight-unseen" detection of rare aquatic species using environmental DNA. *Conservation Letters*, 4(2), 150–157. https://doi.org/10.1111/j.1755-263X.2010.00158.x
- Johnson, P. T. J., Chase, J. M., Dosch, K. L., Hartson, R. B., Gross, J. A., Larson, D. J., Sutherland, D. R., & Carpenter, S. R. (2007). Aquatic eutrophication promotes pathogenic infection in amphibians. *Proceedings of the National Academy of Sciences*, 104(40), 15781–15786. https://doi.org/10.1073/pnas.0707763104
- Johnson, S. L., & Penaluna, B. E. (2019). Climate Change and Interactions With Multiple Stressors in Rivers. In *Multiple Stressors in River Ecosystems* (pp. 23–44). Elsevier. https://doi.org/10.1016/B978-0-12-811713-2.00002-9
- Jones, K. E., Patel, N. G., Levy, M. A., Storeygard, A., Balk, D., Gittleman, J. L., & Daszak, P. (2008). Global trends in emerging infectious diseases. *Nature*, 451(7181), 990–993. https://doi.org/10.1038/nature06536
- Jonsson, B., & Jonsson, N. (2009). A review of the likely effects of climate change on anadromous Atlantic salmon *Salmo salar* and brown trout *Salmo trutta*, with particular reference to water temperature and flow. *Journal of Fish Biology*, 75(10), 2381–2447. https://doi.org/10.1111/j.1095-8649.2009.02380.x
- Kalajdzievska, D., & Li, M. Y. (2011). Modeling the effects of carriers on transmission dynamics of infectious diseases. *Mathematical Biosciences and Engineering*, 8(3), 711–722. https://doi.org/10.3934/mbe.2011.8.711
- Karesh, W. B., Dobson, A., Lloyd-Smith, J. O., Lubroth, J., Dixon, M. A., Bennett, M., Aldrich, S., Harrington, T., Formenty, P., Loh, E. H., Machalaba, C. C., Thomas, M. J., & Heymann, D. L. (2012). Ecology of zoonoses: Natural and unnatural histories. *The Lancet*, 380(9857), 1936– 1945. https://doi.org/10.1016/S0140-6736(12)61678-X
- Keesing, F., Belden, L. K., Daszak, P., Dobson, A., Harvell, C. D., Holt, R. D., Hudson, P., Jolles, A., Jones, K. E., Mitchell, C. E., Myers, S. S., Bogich, T., & Ostfeld, R. S. (2010). Impacts of biodiversity on the emergence and transmission of infectious diseases. *Nature*, 468(7324), 647– 652. https://doi.org/10.1038/nature09575
- Kent, M. L., & Hedrick, R. P. (1985). PKX, the Causative Agent of Proliferative Kidney Disease (PKD) in Pacific Salmonid Fishes and Its Affinities with the Myxozoa. *The Journal of Protozoology*, 32(2), 254–260. https://doi.org/10.1111/j.1550-7408.1985.tb03047.x
- Kerr, P. J. (2012). Myxomatosis in Australia and Europe: A model for emerging infectious diseases. *Antiviral Research*, 93(3), 387–415. https://doi.org/10.1016/j.antiviral.2012.01.009
- Klemetsen, A., Amundsen, P.-A., Dempson, J. B., Jonsson, B., Jonsson, N., O'Connell, M. F., & Mortensen, E. (2003). Atlantic salmon Salmo salar L., brown trout Salmo trutta L. and Arctic charr Salvelinus alpinus (L.): A review of aspects of their life histories. Ecology of Freshwater Fish, 12(1), 1–59. https://doi.org/10.1034/j.1600-0633.2003.00010.x
- Koelle, K., Pascual, M., & Yunus, M. (2005). Pathogen adaptation to seasonal forcing and climate change. *Proceedings of the Royal Society B: Biological Sciences*, 272(1566), 971–977. https://doi.org/10.1098/rspb.2004.3043
- Körner, C., & Basler, D. (2010). Phenology Under Global Warming. *Science*, *327*(5972), 1461–1462. https://doi.org/10.1126/science.1186473

- Kumar, S., Kumara, H. N., Santhosh, K., & Sundararaj, P. (2019). Prevalence of gastrointestinal parasites in lion-tailed macaque *Macaca silenus* in central Western Ghats, India. *Primates*, 60(6), 537–546. https://doi.org/10.1007/s10329-019-00751-y
- Lacy, R. C. (1987). Loss of Genetic Diversity from Managed Populations: Interacting Effects of Drift, Mutation, Immigration, Selection, and Population Subdivision. *Conservation Biology*, 1(2), 143–158. https://doi.org/10.1111/j.1523-1739.1987.tb00023.x
- Lafferty, K. D. (2009). The ecology of climate change and infectious diseases. *Ecology*, 90(4), 888–900. https://doi.org/10.1890/08-0079.1
- Lafferty, K. D., & Kuris, A. M. (1999). How environmental stress affects the impacts of parasites. *Limnology* and *Oceanography*, 44(3part2), 925–931. https://doi.org/10.4319/lo.1999.44.3_part_2.0925
- Lande, R., & Shannon, S. (1996). The Role of Genetic Variation in Adaptation and Population Persistence in a Changing Environment. *Evolution*, 50(1), 434–437.
- Langwig, K. E., Frick, W. F., Bried, J. T., Hicks, A. C., Kunz, T. H., & Kilpatrick, A. M. (2012). Sociality, density-dependence and microclimates determine the persistence of populations suffering from a novel fungal disease, white-nose syndrome. *Ecology Letters*, 15, 1050–1057.
- Lawson, B., Robinson, R. A., Neimanis, A., Handeland, K., Isomursu, M., Agren, E. O., Hamnes, I. S., Tyler, K. M., Chantrey, J., Hughes, L. A., Pennycott, T. W., Simpson, V. R., John, S. K., Peck, K. M., Toms, M. P., Bennett, M., Kirkwood, J. K., & Cunningham, A. A. (2011). Evidence of Spread of the Emerging Infectious Disease, Finch Trichomonosis, by Migrating birds. *EcoHealth*, 8(2), 143–153. https://doi.org/10.1007/s10393-011-0696-8
- Lefèvre, T., Adamo, S. A., Biron, D. G., Missé, D., Hughes, D., & Thomas, F. (2009). Invasion of the Body Snatchers: The Diversity and Evolution of Manipulative Strategies in Host-Parasite Interactions. In J. P. Webster, *Natural History of Host-Parasite Interactions* (Elsevier, pp. 45– 83).
- Lenz, T. L. (2018). Adaptive value of novel MHC immune gene variants. *Proceedings of the National Academy of Sciences*, *115*(7), 1414–1416. https://doi.org/10.1073/pnas.1722600115
- Levene, H. (1953). Genetic Equilibrium When More Than One Ecological Niche is Available. *The American Naturalist*, 87(836), 331–333. https://doi.org/10.1086/281792
- Lewontin, R. C., & Hubby, J. L. (1966). A molecular approach to the study of genic heterozygosity in natural populations. II. Amount of variation and degree of heterozygosity in natural populations of *Drosophila pseudoobscural. Genetics*, *54*, 595–609.
- Little, T. J., Shuker, D. M., Colegrave, N., Day, T., & Graham, A. L. (2010). The Coevolution of Virulence: Tolerance in Perspective. *PLoS Pathogens*, 6(9), e1001006. https://doi.org/10.1371/journal.ppat.1001006
- Maizels, R. M., & Nussey, D. H. (2013). Into the wild: Digging at immunology's evolutionary roots. *Nature Immunology*, 14(9), 879–883. https://doi.org/10.1038/ni.2643
- Massard, J. A., & Geimer, G. (2008). Global diversity of bryozoans (Bryozoa or Ectoprocta) in freshwater: An update. 11.
- Mathieu-Bégné, E., Blanchet, S., Rey, O., Scelsi, O., Poesy, C., Marselli, G., & Loot, G. (2021). A finescale analysis reveals microgeographic hotspots maximizing infection rate between a parasite and its fish host. *Functional Ecology*, *36*(2), 380–391. https://doi.org/10.1111/1365-2435.13967
- McAllister, C. T., Bursey, C. R., & Connior, M. B. (2016). New Host and Distributional Records for Helminth Parasites (Trematoda, Cestoda, Nematoda) from Amphibians (Caudata, Anura) and Reptiles (Testudines: Ophidia) of Oklahoma. *Proceedings of the Oklahoma Academy of Science*, 96, 76–82.
- Medzhhitov, R., Schneider, D. S., & Soares, M. P. (2012). Disease Tolerance as a Defense Strategy. *Science*, 335, 936–941.
- Meijer, T., Mattsson, R., Angerbjörn, A., Osterman-Lind, E., Fernández-Aguilar, X., & Gavier-Widén, D. (2011). Endoparasites in the endangered Fennoscandian population of arctic foxes (Vulpes lagopus). European Journal of Wildlife Research, 57(4), 923–927. https://doi.org/10.1007/s10344-011-0505-2
- Miotke, L., Lau, B. T., Rumma, R. T., & Ji, H. P. (2014). High Sensitivity Detection and Quantitation of DNA Copy Number and Single Nucleotide Variants with Single Color Droplet Digital PCR. *Analytical Chemistry*, 86(5), 2618–2624. https://doi.org/10.1021/ac403843j

- Molles, M. C., & Sher, A. (2019). *Ecology: Concepts and applications* (Eighth edition). McGraw-Hill Education.
- Montoya, J. M., Pimm, S. L., & Solé, R. V. (2006). Ecological networks and their fragility. *Nature*, 442(7100), 259–264. https://doi.org/10.1038/nature04927
- Morris, D. J., & Adams, A. (2008). Sporogony of *Tetracapsuloides bryosalmonae* in the brown trout *Salmo trutta* and the role of the tertiary cell during the vertebrate phase of myxozoan life cycles. *Parasitology*, *135*(09). https://doi.org/10.1017/S0031182008004605
- Morse, S. S. (2004). Factors and determinants of disease emergence. *Revue Scientifique et Technique de l'OIE*, 23(2), 443–451. https://doi.org/10.20506/rst.23.2.1494
- Mougi, A., & Kondoh, M. (2012). Diversity of Interaction Types and Ecological Community Stability. *Science*, 337(6092), 349–351. https://doi.org/10.1126/science.1220529
- Neefjes, J., Jongsma, M. L. M., Paul, P., & Bakke, O. (2011). Towards a systems understanding of MHC class I and MHC class II antigen presentation. *Nature Reviews Immunology*, 11(12), 823–836. https://doi.org/10.1038/nri3084
- Niinemets, Ü., Kahru, A., Mander, Ü., Nõges, P., Nõges, T., Tuvikene, A., & Vasemägi, A. (2017). Interacting environmental and chemical stresses under global change in temperate aquatic ecosystems: Stress responses, adaptation, and scaling. *Regional Environmental Change*, 17(7), 2061–2077. https://doi.org/10.1007/s10113-017-1196-3
- Nunney, L. (2016). Adapting to a Changing Environment: Modeling the Interaction of Directional Selection and Plasticity. *Journal of Heredity*, 107(1), 15–24. https://doi.org/10.1093/jhered/esv084
- O'Briain, R., Coghlan, B., Shephard, S., & Kelly, F. L. (2019). River modification reduces climate resilience of brown trout (*Salmo trutta*) populations in Ireland. *Fisheries Management and Ecology*, 26(6), 512–526. https://doi.org/10.1111/fme.12326
- Ockendon, N., Baker, D. J., Carr, J. A., White, E. C., Almond, R. E. A., Amano, T., Bertram, E., Bradbury, R. B., Bradley, C., Butchart, S. H. M., Doswald, N., Foden, W., Gill, D. J. C., Green, R. E., Sutherland, W. J., Tanner, E. V. J., & Pearce-Higgins, J. W. (2014). Mechanisms underpinning climatic impacts on natural populations: Altered species interactions are more important than direct effects. *Global Change Biology*, 20(7), 2221–2229. https://doi.org/10.1111/gcb.12559
- Okamura, B., Anderson, C. L., Longshaw, M., Feist, S. W., & Canning, E. U. (2001). Patterns of Occurrence and 18S rDNA Sequence Variation of PKX (*Tetracapsula bryosalmonae*), the Causative Agent of Salmonid Proliferative Kidney Disease. *Journal of Parasitology*, 8.
- Okamura, B., & Canning, E. U. (2003). Orphan worms and homeless parasites enhance bilaterian diversity. *Trends in Ecology & Evolution*, 18(12), 633–639. https://doi.org/10.1016/j.tree.2003.09.017
- Okamura, B., & Feist, S. W. (2011). Emerging diseases in freshwater systems: Emerging freshwater diseases. *Freshwater Biology*, 56(4), 627–637. https://doi.org/10.1111/j.1365-2427.2011.02578.x
- Okamura, B., Hartikainen, H., Schmidt-Posthaus, H., & Wahli, T. (2011). Life cycle complexity, environmental change and the emerging status of salmonid proliferative kidney disease. *Freshwater Biology*, *56*(4), 735–753. https://doi.org/10.1111/j.1365-2427.2010.02465.x
- Okamura, B., & Wood, T. S. (2002). Bryozoans as hosts for *Tetracapsula bryosalmonae*, the PKX organism. *Journal of Fish Diseases*, 25(8), 469–475. https://doi.org/10.1046/j.1365-2761.2002.00376.x
- Palikova, M., Papezikova, I., Markova, Z., Navratil, S., Mares, J., Mares, L., Vojtek, L., Hyrsl, P., Jelinkova, E., & Schmidt-Posthaus, H. (2017). Proliferative kidney disease in rainbow trout (*Oncorhynchus mykiss*) under intensive breeding conditions: Pathogenesis and haematological and immune parameters. *Veterinary Parasitology*, 238, 5–16. https://doi.org/10.1016/j.vetpar.2017.03.003
- Paull, S. H., & Johnson, P. T. J. (2014). Experimental warming drives a seasonal shift in the timing of host-parasite dynamics with consequences for disease risk. *Ecology Letters*, 17(4), 445–453. https://doi.org/10.1111/ele.12244

- Pawlowski, J., Apothéloz-Perret-Gentil, L., & Altermatt, F. (2020). Environmental DNA: What's behind the term? Clarifying the terminology and recommendations for its future use in biomonitoring. *Molecular Ecology*, 29(22), 4258–4264. https://doi.org/10.1111/mec.15643
- Perez, L., & Dragicevic, S. (2009). An agent-based approach for modeling dynamics of contagious disease spread. *International Journal of Health Geographics*, 8(1), 50. https://doi.org/10.1186/1476-072X-8-50
- Phillips, K. P., Cable, J., Mohammed, R. S., Herdegen-Radwan, M., Raubic, J., Przesmycka, K. J., van Oosterhout, C., & Radwan, J. (2018). Immunogenetic novelty confers a selective advantage in host–pathogen coevolution. *Proceedings of the National Academy of Sciences*, 115(7), 1552– 1557. https://doi.org/10.1073/pnas.1708597115
- Pietrock, M., & Marcogliese, D. J. (2003). Free-living endohelminth stages: At the mercy of environmental conditions. *Trends in Parasitology*, *19*(7), 293–299. https://doi.org/10.1016/S1471-4922(03)00117-X
- Plehn, M. (1924). Praktikum der Fischkrankheiten. Schweizerbart'sche Stuttgart, 423–424.
- Råberg, L., Alacid, E., Garces, E., & Figueroa, R. (2014). The potential for arms race and Red Queen coevolution in a protist host-parasite system. *Ecology and Evolution*, 4(24), 4775–4785. https://doi.org/10.1002/ece3.1314
- Råberg, L., Graham, A. L., & Read, A. F. (2009). Decomposing health: Tolerance and resistance to parasites in animals. *Philosophical Transactions of the Royal Society B: Biological Sciences*, 364(1513), 37–49. https://doi.org/10.1098/rstb.2008.0184
- Råberg, L., Sim, D., & Read, A. F. (2007). Disentangling Genetic Variation for Resistance and Tolerance to Infectious Diseases in Animals. *Science*, 318(5851), 812–814. https://doi.org/10.1126/science.1148526
- Rachowicz, L. J., Hero, J.-M., Alford, R. A., Taylor, J. W., Morgan, J. A. T., Vredenburg, V. T., Collins, J. P., & Briggs, C. J. (2005). The Novel and Endemic Pathogen Hypotheses: Competing Explanations for the Origin of Emerging Infectious Diseases of Wildlife. *Conservation Biology*, 19(5), 1441–1448. https://doi.org/10.1111/j.1523-1739.2005.00255.x
- Rauw, W. M. (2012). Immune response from a resource allocation perspective. *Frontiers in Genetics*, 3. https://doi.org/10.3389/fgene.2012.00267
- Rea, J. G., & Irwin, S. W. B. (1994). The ecology of host-finding behaviour and parasite transmission: Past and future perspectives. *Parasitology*, *109*(S1), S31–S39. https://doi.org/10.1017/S0031182000085061
- Read, A. F., Graham, A. L., & Råberg, L. (2008). Animal Defenses against Infectious Agents: Is Damage Control More Important Than Pathogen Control. *PLoS Biology*, 6(12), e1000004. https://doi.org/10.1371/journal.pbio.1000004
- Reed, T. E., Jenouvrier, S., & Visser, M. E. (2013). Phenological mismatch strongly affects individual fitness but not population demography in a woodland passerine. *Journal of Animal Ecology*, 82(1), 131–144. https://doi.org/10.1111/j.1365-2656.2012.02020.x
- Riepe, T., Calhoun, D., & Johnson, P. (2019). Comparison of direct and indirect techniques for evaluating endoparasite infections in wild-caught newts (*Taricha torosa* and *T. granulosa*). *Diseases of Aquatic Organisms*, 134(2), 137–146. https://doi.org/10.3354/dao03365
- Ripple, W. J., & Beschta, R. L. (2012). Trophic cascades in Yellowstone: The first 15years after wolf
reintroduction.*BiologicalConservation*,145(1),205–213.https://doi.org/10.1016/j.biocon.2011.11.005
- Rizzoli, A., Tagliapietra, V., Cagnacci, F., Marini, G., Arnoldi, D., Rosso, F., & Rosà, R. (2019). Parasites and wildlife in a changing world: The vector-host- pathogen interaction as a learning case. *International Journal for Parasitology: Parasites and Wildlife*, 9, 394–401. https://doi.org/10.1016/j.ijppaw.2019.05.011
- Robinson, R. A., Lawson, B., Toms, M. P., Peck, K. M., Kirkwood, J. K., Chantrey, J., Clatworthy, I.
 R., Evans, A. D., Hughes, L. A., Hutchinson, O. C., John, S. K., Pennycott, T. W., Perkins, M.
 W., Rowley, P. S., Simpson, V. R., Tyler, K. M., & Cunningham, A. A. (2010). Emerging Infectious Disease Leads to Rapid Population Declines of Common British Birds. *PLoS ONE*, 5(8), e12215. https://doi.org/10.1371/journal.pone.0012215
- Rogers, K. R. (2006). Recent advances in biosensor techniques for environmental monitoring. *Analytica Chimica Acta*, 568(1–2), 222–231. https://doi.org/10.1016/j.aca.2005.12.067

- Rohr, J. R., Raffel, T. R., Sessions, S. K., & Hudson, P. J. (2008). Understanding the net effects of pesticides on amphibian trematode infections. *Ecological Applications*, 18(7), 1743–1753. https://doi.org/10.1890/07-1429.1
- Ros, A., Schmidt-Posthaus, H., & Brinker, A. (2022). Mitigating human impacts including climate change on proliferative kidney disease in salmonids of running waters. *Journal of Fish Diseases*, 45(4), 497–521. https://doi.org/10.1111/jfd.13585
- Rusch, J. C., Hansen, H., Strand, D. A., Markussen, T., Hytterød, S., & Vrålstad, T. (2018). Catching the fish with the worm: A case study on eDNA detection of the monogenean parasite *Gyrodactylus salaris* and two of its hosts, Atlantic salmon (*Salmo salar*) and rainbow trout (*Oncorhynchus mykiss*). *Parasites & Vectors*, 11(1), 333. https://doi.org/10.1186/s13071-018-2916-3
- Ryser-Degiorgis, M.-P. (2013). Wildlife health investigations: Needs, challenges and recommendations. BMC Veterinary Research, 9(1), 223. https://doi.org/10.1186/1746-6148-9-223
- Santiago, J. M. (2017). Thermal ecology of Brown trout and the climate change challenge. In B. Richardson (Ed.), *Tilapia and Trout: Harvesting, Prevalence and Benefits* (pp. 79–121). Nova Science Publishers.
- Scheele, B. C., Pasmans, F., Skerratt, L. F., Berger, L., Martel, A., Beukema, W., Acevedo, A. A., Burrowes, P. A., Carvalho, T., Catenazzi, A., De la Riva, I., Fisher, M. C., Flechas, S. V., Foster, C. N., Frías-Álvarez, P., Garner, T. W. J., Gratwicke, B., Guayasamin, J. M., Hirschfeld, M., ... Canessa, S. (2019). Amphibian fungal panzootic causes catastrophic and ongoing loss of biodiversity. *Science*, 363(6434), 1459–1463. https://doi.org/10.1126/science.aav0379
- Schrag, S. J., & Wiener, P. (1995). Emerging infectious disease: What are the relative roles of ecology and evolution? *Trends in Ecology & Evolution*, *10*(8), 319–324. https://doi.org/10.1016/S0169-5347(00)89118-1
- Seagrave, C. P., Bucke, D., Hudson, E. B., & McGregor, D. (1981). A survey of the prevalence and distribution of proliferative kidney disease (PKD) in England and Wales. *Journal of Fish Diseases*, 4(5), 437–439. https://doi.org/10.1111/j.1365-2761.1981.tb01155.x
- Sengupta, M. E., Hellström, M., Kariuki, H. C., Olsen, A., Thomsen, P. F., Mejer, H., Willerslev, E., Mwanje, M. T., Madsen, H., Kristensen, T. K., Stensgaard, A.-S., & Vennervald, B. J. (2019). Environmental DNA for improved detection and environmental surveillance of schistosomiasis. *Proceedings of the National Academy of Sciences*, *116*(18), 8931–8940. https://doi.org/10.1073/pnas.1815046116
- Shokralla, S., Spall, J. L., Gibson, J. F., & Hajibabaei, M. (2012). Next-generation sequencing technologies for environmental DNA research. *Molecular Ecology*, 21(8), 1794–1805. https://doi.org/10.1111/j.1365-294X.2012.05538.x
- Sileshi, G. W., & Gebeyehu, S. (2021). Emerging infectious diseases threatening food security and economies in Africa. *Global Food Security*, 28, 100479. https://doi.org/10.1016/j.gfs.2020.100479
- Sjöberg, N. B., Petersson, E., Wickström, H., & Hansson, S. (2009). Effects of the swimbladder parasite *Anguillicola crassus* on the migration of European silver eels *Anguilla anguilla* in the Baltic Sea. *Journal of Fish Biology*, 74(9), 2158–2170. https://doi.org/10.1111/j.1095-8649.2009.02296.x
- Smith, J. G., Tomoleoni, J., Staedler, M., Lyon, S., Fujii, J., & Tinker, M. T. (2021). Behavioral responses across a mosaic of ecosystem states restructure a sea otter–urchin trophic cascade. *Proceedings of the National Academy of Sciences*, 118(11), e2012493118. https://doi.org/10.1073/pnas.2012493118
- Smith, K. F., Sax, D. F., & Lafferty, K. D. (2006). Evidence for the Role of Infectious Disease in Species Extinction and Endangerment. *Conservation Biology*, 20(5), 1349–1357. https://doi.org/10.1111/j.1523-1739.2006.00524.x
- Soliman, H., Kumar, G., & El-Matbouli, M. (2018). *Tetracapsuloides bryosalmonae* persists in brown trout *Salmo trutta* for five years post exposure. *Diseases of Aquatic Organisms*, 127(2), 151– 156. https://doi.org/10.3354/dao03200
- Song, C., Von Ahn, S., Rohr, R. P., & Saavedra, S. (2020). Towards a Probabilistic Understanding About the Context-Dependency of Species Interactions. *Trends in Ecology & Evolution*, 35(5), 384–396. https://doi.org/10.1016/j.tree.2019.12.011

- Sorci, G., Møller, A. P., & Boulinier, T. (1997). Genetics of host-parasite interactions. *Trends in Ecology* & *Evolution*, 12, 196.
- Spalding, M. G., & Forrester, D. J. (1993). Disease Monitoring of Free-Ranging and Released Wildlife. Journal of Zoo and Wildlife Medicine, 24(3), 271–280.
- Spurgin, L. G., & Richardson, D. S. (2010). How pathogens drive genetic diversity: MHC, mechanisms and misunderstandings. *Proceedings of the Royal Society B: Biological Sciences*, 277(1684), 979–988. https://doi.org/10.1098/rspb.2009.2084
- Steere, A. C., Coburn, J., & Glickstein, L. (2004). The emergence of Lyme disease. *The Journal of Clinical Investigation*, 113(8), 1093–1101.
- Stelkens, R. B., Jaffuel, G., Escher, M., & Wedekind, C. (2012). Genetic and phenotypic population divergence on a microgeographic scale in brown trout. *Molecular Ecology*, 21(12), 2896–2915. https://doi.org/10.1111/j.1365-294X.2012.05581.x
- Stjernman, M., Råberg, L., & Nilsson, J.-Å. (2008). Maximum Host Survival at Intermediate Parasite Infection Intensities. *PLoS ONE*, *3*(6), e2463. https://doi.org/10.1371/journal.pone.0002463
- Sudhagar, A., Kumar, G., & El-Matbouli, M. (2020). The Malacosporean Myxozoan Parasite *Tetracapsuloides bryosalmonae*: A Threat to Wild Salmonids. *Pathogens*, 9(1), 16. https://doi.org/10.3390/pathogens9010016
- Svensson, E., Råberg, L., Koch, C., & Hasselquist, D. (1998). Energetic stress, immunosuppression and the costs of an antibody response. *Functional Ecology*, *12*(6), 912–919. https://doi.org/10.1046/j.1365-2435.1998.00271.x
- Swain, S. L. (1983). T Cell Subsets and the Recognition of MHC Class. *Immunological Reviews*, 74(1), 129–142. https://doi.org/10.1111/j.1600-065X.1983.tb01087.x
- Tellier, A., & Brown, J. K. M. (2007). Stability of genetic polymorphism in host-parasite interactions. *Proceedings of the Royal Society B: Biological Sciences*, 274(1611), 809–817. https://doi.org/10.1098/rspb.2006.0281
- Tops, S., Lockwood, W., & Okamura, B. (2006). Temperature-driven proliferation of *Tetracapsuloides* bryosalmonae in bryozoan hosts portends salmonid declines. Diseases of Aquatic Organisms, 70, 227–236. https://doi.org/10.3354/dao070227
- Trachtenberg, E., Korber, B., Sollars, C., Kepler, T. B., Hraber, P. T., Hayes, E., Funkhouser, R., Fugate, M., Theiler, J., Hsu, Y. S., Kunstman, K., Wu, S., Phair, J., Erlich, H., & Wolinsky, S. (2003). Advantage of rare HLA supertype in HIV disease progression. *Nature Medicine*, 9(7), 928–935. https://doi.org/10.1038/nm893
- Turner, W. C., Kamath, P. L., van Heerden, H., Huang, Y.-H., Barandongo, Z. R., Bruce, S. A., & Kausrud, K. (2021). The roles of environmental variation and parasite survival in virulence– transmission relationships. *Royal Society Open Science*, 8(6), 210088. https://doi.org/10.1098/rsos.210088
- Turner, W., Spector, S., Gardiner, N., Fladeland, M., Sterling, E., & Steininger, M. (2003). Remote sensing for biodiversity science and conservation. *Trends in Ecology & Evolution*, 18(6), 306– 314. https://doi.org/10.1016/S0169-5347(03)00070-3
- Tylianakis, J. M., Didham, R. K., Bascompte, J., & Wardle, D. A. (2008). Global change and species interactions in terrestrial ecosystems. *Ecology Letters*, 11(12), 1351–1363. https://doi.org/10.1111/j.1461-0248.2008.01250.x
- Urban, M. C. (2015). Accelerating extinction risk from climate change. *Science*, *348*(6234), 571–573. https://doi.org/10.1126/science.aaa4984
- Vale, P. F., Wilson, A. J., Best, A., Boots, M., & Little, T. J. (2011). Epidemiological, Evolutionary, and Coevolutionary Implications of Context-Dependent Parasitism. *The American Naturalist*, 177(4), 510–521. https://doi.org/10.1086/659002
- Van Noordwijk, A. J. V., McCleery, R. H., & Perrins, C. M. (1995). Selection for the Timing of Great Tit Breeding in Relation to Caterpillar Growth and Temperature. *The Journal of Animal Ecology*, 64(4), 451. https://doi.org/10.2307/5648
- Van Oosterhout, C. (2021). Mitigating the threat of emerging infectious diseases; a coevolutionary perspective. *Virulence*, *12*(1), 1288–1295. https://doi.org/10.1080/21505594.2021.1920741
- Van Valen, L. (1973). A new evolutionary theory. *Evolutionary Theory*, 1, 1–30.

- Wahli, T., Bernet, D., Steiner, P. A., & Schmidt-Posthaus, H. (2007). Geographic distribution of Tetracapsuloides bryosalmonae infected fish in Swiss rivers: An update. Aquatic Sciences, 69(1), 3–10. https://doi.org/10.1007/s00027-006-0843-4
- Waldner, K., Borkovec, M., Borgwardt, F., Unfer, G., & El-Matbouli, M. (2021). Effect of water temperature on the morbidity of *Tetracapsuloides bryosalmonae* (Myxozoa) to brown trout (*Salmo trutta*) under laboratory conditions. *Journal of Fish Diseases*, 44(7), 1005–1013. https://doi.org/10.1111/jfd.13361
- Warnecke, L., Turner, J. M., Bollinger, T. K., Lorch, J. M., Misra, V., Cryan, P. M., Wibbelt, G., Blehert, D. S., & Willis, C. K. R. (2012). Inoculation of bats with European Geomyces destructans supports the novel pathogen hypothesis for the origin of white-nose syndrome. Proceedings of the National Academy of Sciences, 109(18), 6999–7003. https://doi.org/10.1073/pnas.1200374109
- Williams, E. S., Yuill, T., Artois, M., Fischer, J., & Haigh, S. A. (2002). Emerging infectious diseases in wildlife. *Revue Scientifique et Technique de l'OIE*, 21(1), 139–157. https://doi.org/10.20506/rst.21.1.1327
- Wilmers, C. C., Nickel, B., Bryce, C. M., Smith, J. A., Wheat, R. E., & Yovovich, V. (2015). The golden age of bio-logging: How animal-borne sensors are advancing the frontiers of ecology. *Ecology*, 96(7), 1741–1753.
- Wood, T. S., & Marsh, T. G. (1999). Biofouling of wastewater treatment plants by the freshwater bryozoan, *Plumatella vaihiriae* (Hastings, 1929). *Water Research*, 33(3), 609–614. https://doi.org/10.1016/S0043-1354(98)00274-7
- Woolhouse, M. E. J., Webster, J. P., Domingo, E., Charlesworth, B., & Levin, B. R. (2002). Biological and biomedical implications of the co-evolution of pathogens and their hosts. *Nature Genetics*, 32(4), 569–577. https://doi.org/10.1038/ng1202-569
- Zhou, P., Yang, X.-L., Wang, X.-G., Hu, B., Zhang, L., Zhang, W., Si, H.-R., Zhu, Y., Li, B., Huang, C.-L., Chen, H.-D., Chen, J., Luo, Y., Guo, H., Jiang, R.-D., Liu, M.-Q., Chen, Y., Shen, X.-R., Wang, X., ... Shi, Z.-L. (2020). A pneumonia outbreak associated with a new coronavirus of probable bat origin. *Nature*, 579(7798), 270–273. https://doi.org/10.1038/s41586-020-2012-7

Chapter 1. Urine DNA (uDNA) as a non-lethal method for endoparasite biomonitoring: development and validation

This chapter is published as a scientific article in *Environmental DNA*.

Eloïse Duval^{1,3}, Simon Blanchet^{1,2}, Erwan Quéméré³, Lisa Jacquin², Charlotte Veyssière², Armand Lautraite⁴, Laurent Garmendia⁵, Allan Yotte⁵, Nathalie Parthuisot², Jessica Côte² & Géraldine Loot^{2,6}.

¹ CNRS, Université Toulouse III Paul Sabatier, Station d'Écologie Théorique et Expérimentale du CNRS à Moulis, UMR-5321, 2 route du CNRS, F-09200 Moulis, France.

² Université Toulouse III Paul Sabatier, CNRS, IRD, UMR-5174 EDB (Laboratoire Evolution & Diversité Biologique), 118 route de Narbonne, F-31062, Toulouse, France.

³ INRA, Agrocampus Ouest, UMR-985 ESE (Ecologie et Santé des Ecosystèmes), 65 rue de Saint Brieuc F-35042 Rennes, France

⁴ Independant Fish veterinarian, 34 Rue des Déportés, F-82170 Grisolles, France

⁵ Fédération Départementale des Associations Agréées de Pêche et de Protection du Milieu Aquatique de l'Ariège, 336 rue Antoine de Saint-Exupéry F-09340 Verniolle, France

⁶ Institut Universitaire de France, Paris, France.

Abstract

Changes in environmental conditions alter host-parasite interactions, raising the need for effective epidemiological surveillance. Developing operational, accurate, and cost-effective methods to assess individual infection status and potential for pathogen spread is a prerequisite to anticipate future disease outbreaks in wild populations. For endoparasites, effective detection of infections usually relies on hostlethal approaches, which are barely compatible with wildlife conservation objectives. Here, we used the brown trout (Salmo trutta) - Tetracapsuloides bryosalmonae host-parasite system to develop a nonlethal method for endoparasite infection detection, hereafter called "uDNA" for urine DNA. The uDNA diagnostic test is based on the amplification of endoparasite DNA from host urine. We sampled wild fish (N = 111) from eight sites, let them excrete in individual buckets filled with mineral water and performed parasite DNA amplification from water filtration. We compared the results of the uDNA diagnostic test for host infection status and parasite load to those from kidney samples (the current standard method). uDNA was sensitive in determining host infection status (even for infected hosts showing no sign of the disease), since up to 90% of fish individuals were correctly assigned to their infection status. The quantity of uDNA detected from the hosts depended on the sampling sites, suggesting a spatial variation in the parasite spread. uDNA was positively, but weakly correlated with parasite load in the kidney. This correlation depended on the severity of macroscopic lesions caused by the disease, and was negative in fish with severely damaged kidney, likely due to impaired urine excretion. The uDNA approach provides novel avenues to non-lethally infer infection parameters from wildlife populations at large spatial scales. By targeting parasite transmission stage, uDNA is also valuable to get insights on the parasite fitness and the ecological and evolutionary dynamics of this hostparasite interaction.

Keywords: parasitology, proliferative kidney disease, non-lethal sampling, wildlife, fresh water, Salmonids

Introduction

Changes in environmental conditions can affect both host and pathogen geographic ranges as well as the outcomes of host-parasite interactions, such as parasite virulence and transmission rate, host susceptibility to infection and ultimately disease development (Schrag & Wiener, 1995; Lafferty, 2009; Gallana et al., 2013). Investigating the dynamics of parasites in space and time in wild host populations is crucial for management and conservation plans (Smith et al., 2006). However, parasite surveillance in wild populations is notoriously challenging because of sampling difficulties (Ryser-Degiorgis, 2013), especially when it involves endoparasites. For instance, screening for the presence of endoparasites often requires sampling specific organs or tissues, which generally implies killing a substantial number of hosts to reliably monitor key parameters such as parasite prevalence and abundance (Sjöberg et al., 2009; McAllister et al., 2016; e.g., Cilia et al., 2020). These lethal approaches raise ethical issues and often fail to provide sufficient sample sizes in host populations. These approaches can additionally hardly be considered for protected, endangered or even exploited species for which parasite infection monitoring is particularly important (Smith et al., 2006; Breed et al., 2009). Moreover, lethal sampling impedes repeated individual-based survey, which would be useful to monitor individual infection over time. To overcome this challenge, an increasing number of studies have developed indirect methods to non-lethally detect and monitor endoparasites in wild animal populations. Most of these studies involve parasite morphological identification in faeces collection (Riepe et al., 2019; Kumar et al., 2019), and/or the use of environmental DNA (eDNA) to detect the infectious agents and evaluate disease risks (Honma et al., 2011; Huver et al., 2015; Bass et al., 2015; Sengupta et al., 2019). eDNA is defined as free DNA molecules released from living bodies in surrounding environment without having to isolate target organisms, thereby indicating the presence of a species (Bohmann et al., 2014; Pawlowski et al., 2020). eDNA has quickly become a prominent tool due to the increasing availability of powerful genetic devices allowing the detection of species even from slight amounts of DNA (Jerde et al., 2011; Shokralla et al., 2012; Miotke et al., 2014). eDNA can be used to detect parasites from the open environment (i.e., air, water, soil) (Rusch et al., 2018) or from host fluids (i.e., blood, faeces or urine) to measure host individual infection status along the infection course (e.g., Etienne et al., 2012). Using host fluids may be particularly relevant for detecting endoparasites colonising internal organs, but is still rarely used, especially for aquatic species (but see Berger & Aubin-Horth, 2018; Jousseaume et al., 2021). Moreover, detection of parasite DNA into host fluids mediating parasite transmission could further provide valuable information on the parasite fitness and on its ability to produce infectious stages.

The myxozan endoparasite *Tetracapsuloides bryosalmonae* represents an ideal case study for testing the effectiveness of a non-lethal method to infer infection from its fish host fluids. This parasite has a complex life cycle involving two hosts: a salmonid fish species (intermediate host) and a bryozoan species (primary host). Parasite spores excreted from infected bryozoans enter salmonid fish through

gills and skin, circulate through the blood until they reach the kidney and spleen where they settle and develop (Hedrick et al., 1993; Okamura et al., 2011). Parasite multiplication and strong host inflammatory response can lead to renal lesions and impair renal functions, especially blood cell production (Hedrick et al., 1993; Bailey et al., 2020). As a result, fish can suffer from anaemia, which is considered to be the most severe health consequence of Proliferative Kidney Disease (PKD), often resulting in losses in aquaculture and declines of several wild salmonid populations (Hedrick et al., 1993; Okamura et al., 2011).

As its development strongly depends upon water temperature and quality, global environmental changes could lead to more recurrent and severe PKD outbreaks (Okamura et al., 2011), emphasizing the need for precise and operational tools for monitoring T. bryosalmonae infection in both wild and hatchery-reared salmonid populations. T. bryosalmonae spores are excreted from infected fish into the water through urine (Hedrick et al., 2004; Morris & Adams, 2006). Up to date, detection of T. bryosalmonae in open waters is based on eDNA approaches targeting free-circulating spores and/or DNA molecules (Fontes et al., 2017; Hutchins et al., 2018). Nonetheless, the amount of T. bryosalmonae DNA detected with these approaches does inform neither on its developmental stage (i.e. relative number of spores released by the salmonid fish or by the bryozoan) nor on individual host infection status. The latter information is however important, because fish individuals are not equally infected even when exposed to the same concentration of parasite spores due to individual variation in resistance (Råberg et al., 2007; Debes et al., 2017). Current monitoring of fish parasite load and infection status relies on histological observation or T. bryosalmonae DNA amplification out of kidney samples (hereafter kDNA for "kidney DNA") (Hedrick et al., 1993; Bruneaux et al., 2017), involving fish euthanasia, and thus relatively small sample sizes when inferring infection prevalence (Okamura et al., 2011; Fontes et al., 2017). T. bryosalmonae DNA detection from brown trout excretion has been explored recently in an experimental setup to approximate the start of spore release by infected fish host (Strepparava et al., 2018), but it has never been used for individual infection status assessment.

Here, we exploited the fact that *T. bryosalmonae* spores are released in fish urine to develop a non-lethal diagnostic test to monitor *T. bryosalmonae* infection and parasite spore release at the individual fish level. This novel approach based on the detection and quantification of *T. bryosalmonae* DNA in the fish urine (hereafter uDNA for "urine DNA"), was developed on wild brown trout (*Salmo trutta*), a species known to be an intermediate host of *T. bryosalmonae* and for releasing infectious spores (only towards bryozoan host) through urine (Okamura et al., 2011). Specifically, we tested (i) whether the probability to detect *T. bryosalmonae* DNA in the excreted urine (uDNA) is positively correlated with the probability of detection directly in the kidney (kDNA) and to a lesser extent with the detection of PKD symptoms, i.e. typical gross renal lesions, and (ii) whether the quantity of *T. bryosalmonae* uDNA is a good proxy of individual fish parasite load. Infected brown trout should release

T. bryosalmonae spores or at least DNA traces in their surrounding water through urine, even though we have no information on the regularity of the release. However, by waiting for several excretion cycles, the uDNA could inform on the infection status (infected or uninfected) and parasite load (relative quantity of parasites in the kidney) of fish individuals, and be used to infer parasite prevalence and mean parasite load in fish populations. Moreover, as uDNA measures parasite transmission through fish excretion, it could be used as a proxy for the fitness of the parasite (the higher the quantity of DNA excreted in urine, the higher the fitness of the parasite), which has to our knowledge never been estimated. To assess the reliability of the uDNA method, we compared results of the uDNA test to the detection of T. bryosalmonae DNA in fish kidney (kDNA) that we used as a "reference" as it is the classical method currently used to detect fish infection status and parasite load (Bettge et al., 2009; Bruneaux et al., 2017). We expected the infection status inferred directly from kidney (kDNA) to be only partially correlated with the detection of gross PKD lesions, as some individuals can be asymptomatic parasite carriers (Abd Elfattah et al., 2014; Soliman et al., 2018). In addition, previous PKD studies did not find a clear relationship between the level of PKD lesions and the parasite load in the kidney (Gorgoglione et al., 2013; Bruneaux et al., 2017). More importantly, we expected to find a positive correlation between the infection status inferred from DNA detected in the urine and that inferred from DNA detected in the kidney, and that the uDNA diagnostic test would be more efficient than macroscopic examination of gross renal lesions to detect infected fish hosts, as asymptomatic infected fish should also release parasite DNA (Soliman et al., 2018). Moreover, we tested whether the amount of uDNA varied among environmental contexts (sampled sites), as ecological parameters such as water temperature may impact parasite development and disease severity (Okamura et al., 2011), which could influence uDNA detection success through variability in parasite release. We predicted a positive correlation between uDNA concentration and parasite load inferred from kidney, except for fish showing important gross renal lesions and/or fish living in the warmest sites, because of impaired excretion rate due to the disease development.

Methods

Brown trout sampling

The study area was located in southern France, at the foothills of the Pyrenean mountains and sampling took place in the Bouigane, Lez, Oriège, Arize and Ariège Rivers (Fig. 1). PKD was suspected in the area by the Fédération Départementale de Pêche de l'Ariège since late 2000s, and confirmed in 2016 after a high mortality of juvenile brown trout was reported through passive surveillance (Garmendia & Lautraite, 2017). Brown trout were sampled at eight sites; six of them were sampled during the first week of September 2018, while the last two sites were sampled during the first week of October 2018 (Table 1, Fig. 1). At this time of the year, all the infected fish should be shedding parasite spores (Strepparava et al., 2018). The sampled sites showed contrasted environmental conditions, especially in terms of thermal regime (see Table 1). Fish were sampled through electrofishing by the Fédération Départementale de l'Ariège de Pêche et de Protection des Milieux Aquatiques, in charge of the local angling management and the conservation of aquatic environment. We primarily targeted small juvenile trout (mean = 120 mm, range = 68 - 169 mm) corresponding mainly to young-of-year (0+) because brown trout are more prone to develop PKD when exposed to infectious spores of T. bryosalmonae for the first time (which generally occurs in Spring, some months after trout emergence from the gravel, Okamura et al. 2011). A total of 111 fish were sampled (4 to 16 individuals per site according to local abundances, Table 1).



Figure 1: Sampled sites location (blue dots with codes) at the foothills of Pyreneans Mountains, southern France. Main rivers of the sampled area (Ariège department) are also shown.

Table 1: Characteristics of sampled sites, date of sampling and number of brown trout individuals
sampled (N). Mean water temperature for summer 2018 was computed with SIEAG (Système
d'Information sur l'Eau du Bassin Adour Garonne) and the Fédération de pêche de l'Ariège data when
available.

Code	Site	Stream	Altitude (m)	Source distance (km)	Mean summer temperature (°C)	Sampling date	N
ARIPam	Pamiers	Ariège	277	102	-	07/09/2018	16
ARIVar	Varilhes	Ariège	324	87	15.6	07/09/2018	16
ARZDur	Durban	Arize	351	27	16	05/09/2018	16
LEZAub	Aubert	Lez	418	32	17.2	06/09/2018	12
BOUArg	Argein	Bouigane	531	21	15.8	06/09/2018	16
ARILuz	Luzenac	Ariège	590	40	15	10/10/2018	4
ARISav	Savignac	Ariège	677	32	14.6	10/10/2018	15
ORIAx	Ax-les-Thermes	Oriège	777	21	12.6	04/09/2018	16

uDNA collection

After capture, fish recovered for about 30 minutes in a bucket filled with stream water and aerated by a small air pump. Each individual was "rinsed" with commercial (i.e. uncontaminated) mineral water to avoid false-positive detection of parasite DNA potentially present in the stream water, and then placed in a plastic bag filled with 2L of commercial mineral water (Villéger et al., 2012; as in Raffard et al., 2019). To minimise physiological stress, we chose mineral water (Cristaline®) with physicochemical characteristics similar to those encountered in streams and stored at stream temperature. Each bag was maintained in a bucket to allow proper movements of the fish, and buckets were covered and shaded to reduce fish stress. Given that urine excretion occurs by bursts every ~20 minutes (Curtis & Wood, 1991), fish were kept in bags for at least one hour to ensure they had enough time for at least one excretion cycle. At each site, the experimental design included 1 negative control bucket with mineral water only per 4 sampled fish (i.e. 1-4 controls per site, Table 1).

After excretion, 1L from each bag was filtered onto a $1.2\mu m$ cellulose nitrate Sartorius® filter (Ø 50mm), using a Solinst® peristaltic pump (model 410) and Sartorius® filter holders. Filters were then individually stored in Eppendorf Tubes 5mL in a cooler on the field, and then at -80°C until DNA extraction. The material (filter holders, buckets, pipes...) was thoroughly disinfected in a 10% bleach bath overnight and rinsed with clear water after each field session to avoid cross-contamination.

Gross lesions and kidney parasite prevalence and load (kDNA)

After excretion, fish were euthanised with an overdose of benzocaine, measured to the nearest mm and weighed to the nearest 0.1g. PKD gross lesions were assessed after dissection by a trained fish veterinarian (AL) through a visual inspection of gills, spleen and kidney, showing typical PKD gross lesions ranging from 0 (no lesion) to 3 (very high PKD suspicion). A gross lesions score of 3 represents a pale kidney exhibiting severe swelling (due to hyperplasia). Medium kidney samples were collected from each individual, stored in 70% ethanol and sent to the laboratoire des Pyrénées et des Landes (LPL, Mont-de-Marsan), a certified laboratory for analyses, in order to assess fish kidney parasite load (kDNA) through quantitative Polymerase Chain Reaction (qPCR). 18S *T. bryosalmonae* rDNA was co-amplified together with 12S brown trout mitochondrial DNA to correct for the amount of kidney tissue used for extraction. kDNA is thus expressed as the ratio between the number of 18S *T. bryosalmonae* rDNA copies detected and the number of 12S brown trout mitochondrial DNA detected in the sample. As the LPL is commercially exploiting the kDNA approach described above, they held details about the laboratory protocol confidential. kDNA provides the actual infectious status and parasite load of a fish, i.e. the "reference" value (Bruneaux et al., 2017).

uDNA detection

DNA extraction was performed on excretion filters using the QIAGEN DNeasy PowerWater kit following manufacturer recommendations and under a strict laboratory environment for eDNA extractions. The 518F_Q and 680R_Q primers designed by Fontes et al. (2017) were used to amplify a 182bp fragment of T. bryosalmonae 18S SSU rDNA sequence. To accurately measure the quantity of parasite DNA released by fish individuals, DNA amplifications were run through quantitative PCRs (qPCRs). The PCR reactions were performed in a total volume of 20µL including 10µL SYBR® Green master mix, 2 μ l of sample DNA, 1 μ l of each primer (10 μ M) and 6 μ l of DNase/RNase-free water. The PCR program was run with a QuantStudio[™] 6 Flex System (Applied Biosystems), under the following thermal conditions: 95 °C for 15 min followed by 45 cycles of 95°C for 15s and 60 °C for 1 min. We performed a qPCR standard curve with one positive sample and a 1:10 serial dilution of this sample (n = 7 concentrations). The linear standard curve revealed an amplification efficiency above 100% suggesting too high concentration of samples. So, we diluted the most concentrated sample in the standard curve and all samples in our study to 1/10th. The 7 standards used encompassed the full range of sample tested in this study. The standard curve was applied to all runs to allow comparison of parasite DNA quantification across separately run qPCR plates. Each 96-well qPCR plate included 3 PCR negative controls (water only), 2 field negative controls (buckets with no fish), 2 dilution series and samples in triplicates. A sample would be considered positive for T. bryosalmonae DNA when at least two out of three replicates yielded positive results. Following qPCRs, mean Ct values, representing the

number of amplification cycles needed to get to a fluorescence threshold, ranged from 26.8 to 38.8 for positive samples. Ct values exceeding 40 were considered as artefacts and the corresponding assays as negative. After correcting Ct values for among-plates variation in PCR efficiency (see above), the initial relative concentration values (N0) ranged between 0 and 1, 1 being the individual with the highest *T*. *bryosalmonae* DNA concentration found in urine.

For a comparison purpose, we used a second method of DNA amplification and quantification that is expected to be more sensitive than classical qPCRs and eases comparisons among samples: the droplet digital PCR (ddPCR) (Doi et al., 2015). ddPCR is based on water-oil emulsion droplet technology and transforms the PCR mix into approximately 20000 droplets in which independent PCR reactions occur. If target DNA is present in the droplet, amplification occurs and the droplet fluoresces. This method provides concentration values (hereafter dC for droplet concentration) that are directly interpretable and that corresponds to the ratio of positive fluorescing droplets to total number of droplets, with no need for corrections, nor sample replicates, while limiting trouble with PCR inhibitors (Doi et al., 2015; Wood et al., 2019). ddPCRs were run with a BioRad QX200 Droplet Digital PCR systemTM (Bio-Rad, Temse, Belgium), with the following thermal conditions: 95°C for 5 min followed by 40 cycles of 95°C for 30s and 60°C for 1 min; and 4°C for 5 min and 90°C for 5 min. The PCR reactions were performed in a total volume of 22 μ l including 11 μ L EvaGreen digital PCR Supermix, 2.2 μ l of sample DNA, 1.1 µl of primer mix (same primers as for the qPCRs, 2µM) and 7.7 µl of DNase/RNasefree water. Each 96-well run included 4 PCR negative controls (water only), and a total of 18 field negative controls (buckets with no fish) were distributed among the different runs. The baseline threshold for separating positive and negative droplets was manually chosen per run, according to the distribution of the negative droplets from the negative control wells.

Ultimately, because urine excretion depends both on time spent in the plastic bag and on fish body mass (Hunn, 1982), we corrected the resulting uDNA values (N0 and dC for qPCR and ddPCR concentration values respectively) by the time of excretion (in minutes) and fish body mass (in grams).

Statistical analyses

Statistical analyses were conducted using the R environment (R 3.6.1, R Core Team 2019).

Inferring infection status and parasite prevalence from uDNA

We first used a Cochran's Q test (*RVAideMemoire* R package, Hervé 2020) to test whether the global prevalence (percentage of infected fish across all populations) varied among the methods (renal lesions examination, kDNA, uDNA amplified using either qPCR or ddPCR). When significant, pairwise Wilcoxon post-hoc tests were conducted. Then, we tested whether *T. bryosalmonae* prevalence estimated at the site level using the uDNA diagnostic test correlated (Spearman rank correlation) with the parasite prevalence estimated using kDNA.

Finally, the sensitivity and specificity of the uDNA test (using either qPCR or ddPCR) were quantified to provide a quantitative reliability of the method. Sensitivity is defined as the proportion of infected trout (i.e. positive with kDNA) that are also detected as infected with the uDNA test, while specificity corresponds to the proportion of non-infected trout (i.e. negative with kDNA) that are also identified as non-infected with the uDNA test (Akobeng, 2007). We thus classified each individual as "true positive" or "true negative" when the status (infected or non-infected) identified through uDNA (using either qPCR or ddPCR) was the same than the status identified through kDNA. On the contrary, we classified each individual as "false positive" or "false negative" when the status identified through kDNA. Sensitivity is then calculated as the ratio between the number of true positives and the sum of true positives and false positives.

Inferring parasite load from uDNA

Our second objective was to test whether the uDNA approach was a reliable method to estimate the parasite load of fish hosts.

We first used a Spearman rank correlation test to assess the relationship between individual *T*. *bryosalmonae* DNA concentration in urine samples obtained either from qPCR or ddPCR, so as to test whether these two methods yield similar information about *T. bryosalmonae* DNA excretion. A similar approach was used at the site level to assess and test the relationship between *T. bryosalmonae* DNA concentration estimated from kidney and the DNA concentration estimated from urine samples (amplified using either qPCR or ddPCR), in order to provide information about the reliability of uDNA for estimating the mean parasite intensity.

Finally, we tested whether the quantity of *T. bryosalmonae* DNA excretion in fish urine could vary depending on the environmental and epidemiological contexts. We assumed that different sites

corresponded to different environmental contexts, and that the extent of PKD gross lesions corresponded to different epidemiological contexts, i.e. different stages of disease development estimated by a trained veterinarian. We used a linear model with *T. bryosalmonae* DNA concentration estimated from urine at the individual level as the dependent variable, and *T. bryosalmonae* DNA concentration estimated from kidney as the explicative variable and we included in the full model the site identity, the extent of gross renal lesions and their two-terms interactions as additional fixed effects. Note that three out of the eight sites (LEZAub, ARZDur and BOUArg) were removed from this analysis because they had too few infected individuals. Based on a full model (all simple terms + the interactions between site identity and kDNA concentration and between renal lesions and kDNA concentration), we used an information-theoretic approach (based on the small-sample size corrected Akaike Information Criterion, AICc) to identify the most likely model(s) (Burnham & Anderson 2002) using the *MuMIn* package (Barton, 2019).

Results

Inferring infection status and parasite prevalence from uDNA

The overall *T. bryosalmonae* infection prevalence differed significantly among the four methods of detection (Cochran's Q test, Q=73, df=3, P < 0.001, Fig. 2a). We identified more infected individuals with the detection of parasite DNA from kidney (kDNA, 62%) or from urine (uDNA, qPCR = 53%, ddPCR = 56%) than with macroscopic examination of PKD gross lesions (28%, Fig. 2a, post-hoc tests, P < 0.001), meaning that 55% of the infected individuals were asymptomatic. *T. bryosalmonae* prevalence estimated from uDNA was slightly lower than from kDNA (Fig. 2a, Fig 3). Nonetheless, this difference was significant when uDNA was amplified using qPCR (post-hoc test, P = 0.02), but not with ddPCR (post-hoc test, P = 0.08). Using kDNA individual infection status as reference, uDNA methods provided a correct infection status (true positives + true negatives) for 87% (qPCR) and 90% (ddPCR) of the samples (Fig. 3).





Figure 2: (a) *T. bryosalmonae* infection prevalence recorded among the 111 brown trout sampled according to the detection method. kDNA indicates parasite DNA detected in kidney and serves as a reference. uDNA indicates *T. bryosalmonae* DNA detected in urine (either using ddPCRs or qPCRs). Gross lesions indicate suspicion of *T. bryosalmonae* infection based on macroscopic examination of the fish. Different letters above the bars indicate a significant difference after Cochran's Q test and post-hoc pairwise Wilcoxon tests ($\alpha = 0.05$). (b) *T. bryosalmonae* kDNA infection prevalence recorded at each site.

Infection prevalence (from kDNA) varied markedly among sites with three out of the eight sites (LEZAub, ARZDur, BOUArg) not or barely infected (0 to 6% infection prevalence), whereas five sites (ARILuz, ARIPam, ARISav, ARIVar, ORIAx) had a prevalence of 100% (Fig. 2b). In these highly infected sites, PKD gross lesions score varied between 0 and 3, except for ORIAx in which none of the individuals displayed any PKD gross lesions (Fig. S1). kDNA and uDNA prevalence measured at the site level were significantly positively correlated (rho = 0.85, P < 0.01 for uDNA prevalence estimated from ddPCR; rho = 0.85, P < 0.01 for uDNA prevalence estimated from qPCR). However, uDNA underestimated the prevalence for ARIVar and ARISav (Fig. 4a).





Figure 3: Qualitative comparison between *T. bryosalmonae* detection in the kidney (kDNA) and in the urine (uDNA) with qPCR and ddPCR amplifications respectively. True negative/positive: samples that were found positive and negative both with kDNA and uDNA methods. False negative/positive: samples that were found either positive or negative with uDNA while they were found at the opposite with kDNA (reference method).

The specificity of the uDNA diagnostic test was very high (95%) for both amplification methods (qPCR and ddPCR). The sensitivity was slightly lower for uDNA amplified using qPCR than using ddPCR (83% *vs.* 87%) because qPCR yielded more false negatives (11% vs 8% using ddPCR), even though the difference in distribution of the results categories between both methods was not significant (Stuart-Maxwell marginal homogeneity test, $\chi^2 = 1.8$, df = 3, P = 0.615) (Fig. 3).

Inferring parasite load from uDNA

T. bryosalmonae DNA concentrations estimated with uDNA from qPCR and ddPCR were strongly and positively correlated (rho = 0.96, P < 0.001, Fig. S2), indicating that both approaches yielded very similar estimates of DNA parasite concentrations in the urine. Only the results with *T. bryosalmonae* DNA concentrations obtained from ddPCR will be shown hereafter.

-Chapter 1-



Figure 4: (a) Relationship between *T. bryosalmonae* prevalence per site inferred from the parasite DNA contained in the urine (uDNA diagnostic test) and prevalence inferred from the parasite DNA contained in the kidney (kDNA, reference method). (b) Relationship between the mean *T. bryosalmonae* DNA concentration detected in urine (uDNA diagnostic test) per site and the mean parasite DNA concentration detected in kidney (kDNA). Values are scaled to the mean. Each dot represents the values for a single site, letters above dots are the code of each site (see Figure 1 for site location).

kDNA concentration, representing our standard measure of parasite load, ranged from 1.27 10⁻⁶ to 9.29 10⁻² *T. bryosalmonae* DNA copies per *S. trutta* DNA copy for infected trout. At the site level, mean *T. bryosalmonae* DNA concentrations measured in kidney were positively correlated with mean concentrations measured with uDNA, but this relationship was only marginally significant (rho = 0.71, P = 0.06). *T. bryosalmonae* kDNA/uDNA concentrations strongly departed from the 1:1 expectation for two sites (ARIPam and ARILuz, Fig. 4b) indicating both over- and underestimations of the *T. bryosalmonae* parasite load when measured with uDNA.

The most likely model to explain *T. bryosalmonae* DNA concentration measured in urine at the individual level included the identity of the sampling site, the score of fish PKD gross lesions, *T. bryosalmonae* DNA concentration measured in kidneys and the two-term interaction involving the two later variables as fixed effects (Table 2, Table S1, AICc = -8.4, W = 0.72). This model revealed that *T. bryosalmonae* DNA concentration measured in urine significantly varied among sites with a highest uDNA quantity excreted by fish from ARIPam (see Fig. 4b and Fig. 5). More importantly, the relationship between *T. bryosalmonae* DNA concentration measured in urine and *T. bryosalmonae* DNA concentration measured in urine and *T. bryosalmonae* DNA concentration measured in urine and in kidney was positive (as expected) for all gross lesions scores, except for score "3", corresponding to the most affected fish (in which case the relationship was negative, Fig. 5). In other words, fish with highly damaged kidneys excreted less *T. bryosalmonae* DNA in their urine than expected despite high concentrations of *T. bryosalmonae* DNA in their kidneys. It is noteworthy that a significant and positive relationship was observed between *T. bryosalmonae* DNA concentration measured in urine and in kidney significant and positive relationship was observed between *T. bryosalmonae* DNA in their urine than expected despite high concentrations of *T. bryosalmonae* DNA in their kidneys. It is noteworthy that a significant and positive relationship was observed between *T. bryosalmonae* DNA concentration measured in urine and in kidney for the site ORIAx in which no fish exhibited any PKD lesion (see inset in Fig. 5).

Table 2: Output of the best linear model explaining the quantity of *T. bryosalmonae* DNA excreted along with infected brown trout (*S. trutta*) urine. kDNA is the quantity of *T. bryosalmonae* DNA detected in fish kidney, Site is the fish site of origin, and Gross lesions represents the score of proliferative kidney disease gross lesions estimated through macroscopic examination of the fish. Adjusted $R^2 = 0.38$, P < 0.001

	df	F value	P value
kDNA	1	1.48	0.230
Site	3	8.14	< 0.001
Gross lesions score	3	1.55	0.212
kDNA × Gross lesions score	3	5.65	0.002





Figure 5: Relationship between the quantity of *T. bryosalmonae* DNA (parasite load) detected in urine (uDNA) and that detected in kidney (kDNA) for 63 infected individuals. Values are scaled to the mean for the different intensities of the proliferative kidney disease gross lesions scores. The different symbols represent different sites and the different colours are for different gross lesions scores. Mean values for each site are shown, along with their 95% confidence intervals (error bars). The inset shows the linear regression between uDNA and kDNA parasite load for the site ORIAx at the individual level.

Discussion

The aim of the present study was to develop a non-lethal method for monitoring endoparasite infection in wild host populations. Detection of endoparasite infection is usually performed through tissue sampling, which is often lethal for the host (e.g. Cilia et al., 2020; Waldner et al., 2020). However, the recent development of molecular techniques now permits to detect endoparasites DNA in the environment, faeces or other host fluids (Bohmann et al., 2014). Recently, Berger & Aubin-Horth (2018) developed an elegant non-lethal method to detect a large endoparasite using DNA shed by parasites via swabbing the host abdominal cavity and Jousseaume et al. (2021) used DNA from faecal samples to detect endoparasite infections in eels. Here, we followed this logic by hypothesizing that the brown trout microparasite *T. bryosalmonae*, which proliferates in fish kidney, could be non-lethally detected from fish urine excretion (Strepparava et al., 2018), therefore enlightening individual host infection

parameters. In line with our initial hypothesis, we successfully inferred parasite prevalence and occurrence from urine DNA ("uDNA") and we were able to determine individual fish parasite load, although this latter inference was strongly context dependent.

Based on individual infection status measured with a conventional (but lethal) approach as a reference (Hedrick et al., 1993; see Bruneaux et al., 2017), our uDNA diagnostic test correctly identified host infection status for 87 and 90% individuals with qPCR and ddPCR amplifications respectively, proving its efficiency for detecting *T. bryosalmonae* infection. Accordingly, test specificity was high and test sensitivity was slightly higher for ddPCR amplifications. Specifically, when an individual was not infected, the uDNA test was negative in 95% of the cases (specificity), and when an individual was infected, the uDNA test was positive in 83% and 87% cases (sensitivity) considering qPCR and ddPCR amplifications respectively. One limitation in the validation of the uDNA method is that our sampled populations showed extreme infection prevalence (either 0-5 or 100% of infected individuals), so that we could not validate the method for intermediate values of prevalence.

The overall patterns of individual infection and population prevalence were very similar when using the qPCR and ddPCR approaches. Moreover, parasite DNA concentrations found in urine with both amplification methods were strongly correlated (as in Koepfli et al., 2016; Mulero et al., 2020), suggesting that both qPCR and ddPCR can be reliably used to infer key epidemiological parameters from hosts' urine. Nevertheless, in agreement with recent studies (Koepfli et al., 2016; Wood et al., 2019), we found a higher sensitivity for ddPCR due to a lower percentage of false negative (i.e. fish found uninfected using uDNA while actually infected), but the difference between both methods was not significant (8% vs. 11% for qPCR). It is noteworthy that false negatives found with ddPCR were individuals with relatively low parasite load in their kidney (ranging between 1.27 10⁻⁶ and 1.45 10⁻³ copies of T. bryosalmonae 18S rDNA/one copy of S. trutta 12S rDNA), so that some of these false negatives could be attributed to a limit of detection, or to false positives of qPCR detection in the kidney. However, the uDNA diagnostic test was efficient even for very low parasite load in kidney (as low as 3.65 10⁻⁶ T. bryosalmonae DNA copy/S. trutta DNA copy), showing its high sensitivity, and when infected hosts showed no sign of PKD (i.e. asymptomatic infected fish). Identifying these asymptomatic infected individuals is particularly relevant as they can act as an invisible reservoir for potential future disease outbreaks. This is for instance the case when asymptomatic infected individuals move (or are moved by humans) into new locations with susceptible bryozoans. Besides, as the disease development depends -among other environmental factors- on water temperature, the presence of asymptomatic infected individuals can point out populations that have to be closely monitored if environmental conditions are changing as they would be in the frontline for developing PKD outbreaks (Okamura & Feist, 2011).

Overall, we found a positive relationship between the quantity of parasite DNA detected in urine and that detected in kidney, but this relationship was weak and strongly dependent upon fish gross PKD lesions score. The relationship between uDNA and kDNA was positive for fish showing none or moderate gross lesions and particularly strong in ORIAx, a site in which all fish were infected but not diseased (no PKD gross lesion detected). One potential explanation could be that parasite development and/or fish hosts immune responses (responsible for kidney hyperplasia in diseased fish) were likely less triggered in ORIAx compared to the other sites due to a lower water temperature or other local environmental factors (Okamura et al., 2011). By contrast, uDNA and kDNA were poorly correlated for fish with severe gross lesions, suggesting that high level of kidney damage may impair urine excretion and hence the quantification of parasite load in this fluid. Furthermore, we found that uDNA quantity varied among sampled sites. Specifically, mean uDNA quantity was significantly higher in fish from ARIPam compared to all other sites, while parasite load inferred from kDNA was similar among all sites. This illustrates that among-site variation in parasite load inferred from uDNA is yet complex to explain and must be carefully interpreted. Furthermore, another potential bias in our uDNA quantification approach is that we do not know the actual quantity of urine excreted by fish in the bags. Indeed, salmonid fish urine flow rate can be influenced by factors such as nutrition, health status, water quality and temperature, handling, hypoxic stress (Hunn, 1982). In the future, we propose using an "excretion control" such as urea concentration in the bag, to correct for the quantity of urine excreted. Future studies examining urine excretion may be necessary to refine this promising uDNA method for quantifying parasite load.

Finally, our results show that highly infected individuals do not release spores proportionally to their parasite load in the kidney, which suggests that kDNA may not necessarily be a good proxy for the infectious potential of fish. In other words, our results strongly suggest that fish with high concentration of T. bryosalmonae in the kidney do not substantially contribute to the excretion of spores in the open water, likely because of impaired kidney function and decreased excretion. These highly infected fish may therefore not be advantageous for the parasite life cycle and fitness. Our uDNA method actually provides the possibility to quantify what is excreted by fish in the open water, which can be used as a proxy of the number of released parasite spores. These two measures are thus complementary: kDNA informs on host parasite load, and uDNA could be used as a proxy of fish infectious potential, informing on the parasitic cycle dynamics. For example, in this study, the high uDNA excretion in fish from ARIPam (compared to other populations) seems to indicate a higher fitness of the parasite in this site. It could be explained by favourable environmental conditions for the parasite development, including the fish host's characteristics. The heterogeneity of uDNA release and thus of parasite spore shedding in this site could indeed highlight the presence of super-spreaders, i.e. infected fish hosts that highly contribute to the parasite cycle (Stein, 2011; Stephenson et al., 2017). This is particularly relevant as the current detection of T. bryosalmonae in streams through eDNA cannot discriminate between parasite

DNA stemming from fish spores and that from spores excreted from the primary host (bryozoans) (Fontes et al., 2017), so that uDNA gives access to new information on this infectious disease dynamics.

To conclude, we demonstrated the efficiency of a non-lethal method for endoparasite detection in the context of an emerging infectious disease in salmonid fish. The uDNA diagnostic test proved to be efficient in determining individual infection status and prevalence at population levels and provided encouraging results to estimate individual and population parasite loads. The ease and fast implementation of this new method opens fascinating perspectives for dissecting the eco-evolutionary dynamics of this host-parasite interaction considering large spatial and temporal scales. For instance, individual surveys along the infection course can be performed in the wild through capture-recapture to determine the ecological and evolutionary impacts of this emerging parasite on host populations. We thus encourage the future development of such non-invasive approaches based on host fluids for endoparasite detection to provide a better knowledge about wild animal population infection status and infectious potential, improve the surveillance of wildlife emerging diseases, and take appropriate decisions for management actions.

Author's contribution

SB, GL and LJ designed the method and coordinated the study. LG and AY conducted fish sampling. ED, SB, GL, LJ, EQ, CV and JC handled the fish and the experiment. AL performed fish dissections and lesions diagnostic of proliferative kidney disease. GL, CV, NP and ED performed laboratory work at EDB. ED ran the statistical analyses. ED, SB, EQ and GL interpreted the data. ED, SB, EQ, GL and LJ wrote the first draft of the manuscript. CV, NP, AL, LG, AY and JC read, commented and corrected the initial draft, and all authors gave final approval for publication.

Acknowledgments

We warmly thank the employees and volunteers from the Fédération Départementale de pêche de l'Ariège for the sampling, Frédéric Martins and Emeline Lhuillier from the Genotoul lab for their help with ddPCR at the Genotoul lab. We thank Hanna Hartikainen and an anonymous reviewer for their very relevant and detailed comments on the first version of this manuscript. This work was funded by the French Office for Biodiversity (OFB) and the French Water Agency (Agence de l'Eau Adour-Garonne AEAG). This work was carried out within the framework of the "Laboratoire d'Excellence" (LABEX) TULIP (ANR-10-LABX-41).

Ethics

Authorisation to collect brown trout were provided to the Fédération de l'Ariège de pêche et de protection du milieu aquatique by the « Arrêté préfectoral 2018-7 ».

Conflict of interest

The authors declare no conflict of interest.

Data archiving statement

Raw data from the main analyses are available on Figshare at the following link: https://figshare.com/articles/dataset/raw_data_uDNA_paper_txt/14605770.

Literature cited

- Abd Elfattah, A., Kumar, G., Soliman, H., & El Matbouli, M. (2014). Persistence of *Tetracapsuloides* bryosalmonae (Myxozoa) in chronically infected brown trout *Salmo trutta*. *Diseases of Aquatic* Organisms, 111(1), 41–49. https://doi.org/10.3354/da002768
- Akobeng, A. K. (2007). Understanding diagnostic tests 1: Sensitivity, specificity and predictive values. *Acta Paediatrica*, 96(3), 338–341. https://doi.org/10.1111/j.1651-2227.2006.00180.x
- Bailey, C., Holland, J. W., Secombes, C. J., & Tafalla, C. (2020). A portrait of the immune response to proliferative kidney disease (PKD) in rainbow trout. *Parasite Immunology*, 42(8). https://doi.org/10.1111/pim.12730
- Barton, K. (2019). MuMIn: Multi-Model Inference. R package version 1.43.15.
- Bass, D., Stentiford, G. D., Littlewood, D. T. J., & Hartikainen, H. (2015). Diverse Applications of Environmental DNA Methods in Parasitology. *Trends in Parasitology*, 31(10), 499–513. https://doi.org/10.1016/j.pt.2015.06.013
- Berger, C. S., & Aubin-Horth, N. (2018). An eDNA-qPCR assay to detect the presence of the parasite Schistocephalus solidus inside its threespine stickleback host. The Journal of Experimental Biology, 221(9). https://doi.org/10.1242/jeb.178137
- Bettge, K., Segner, H., Burki, R., Schmidt-Posthaus, H., & Wahli, T. (2009). Proliferative kidney disease (PKD) of rainbow trout: Temperature- and time-related changes of Tetracapsuloides bryosalmonae DNA in the kidney. 11.
- Bohmann, K., Evans, A., Gilbert, M. T. P., Carvalho, G. R., Creer, S., Knapp, M., Yu, D. W., & de Bruyn, M. (2014). Environmental DNA for wildlife biology and biodiversity monitoring. *Trends in Ecology & Evolution*, 29(6), 358–367. https://doi.org/10.1016/j.tree.2014.04.003
- Breed, A. C., Plowright, R. C., Hayman, D. T. S., Knobel, D. L., Molenaar, F. M., Garder-Roberts, D., Cleaveland, S., Haydon, D. T., Kock, R. A., Cunningham, A. A., Sainsbury, A. W., & Delahay, R. J. (2009). Disease Management in Endangered Mammals. In *Management of Disease in Wild Mammals* (Delahay R.J., Smith G.S., Hutchings M. R. (Eds.)). Springer Tokyo Berlin Heidelberg New York.
- Bruneaux, M., Visse, M., Gross, R., Pukk, L., Saks, L., & Vasemägi, A. (2017). Parasite infection and decreased thermal tolerance: Impact of proliferative kidney disease on a wild salmonid fish in the context of climate change. *Functional Ecology*, 31(1), 216–226. https://doi.org/10.1111/1365-2435.12701
- Burnham, K. P., & Anderson, D. R. (2002). *Model selection and multimodel inference: A practical information-theoretic approach* (2nd ed). Springer.
- Cilia, G., Bertelloni, F., Mignone, W., Spina, S., Berio, E., Razzuoli, E., Vencia, W., Franco, V., Cecchi, F., Bogi, S., Turchi, B., Cerri, D., & Fratini, F. (2020). Molecular detection of *Leptospira* spp. In wild boar (*Sus scrofa*) hunted in Liguria region (Italy). *Comparative Immunology, Microbiology and Infectious Diseases*, 68, 101410. https://doi.org/10.1016/j.cimid.2019.101410
- Curtis, B. J., & Wood, C. M. (1991). The function of the urinary bladder *in vivo* in the freshwater rainbow trout. *Journal of Experimental Biology*, 155, 567–583.
- Debes, P. V., Gross, R., & Vasemägi, A. (2017). Quantitative Genetic Variation in, and Environmental Effects on, Pathogen Resistance and Temperature-Dependent Disease Severity in a Wild Trout. *The American Naturalist*, *190*(2), 244–265. https://doi.org/10.1086/692536
- Doi, H., Takahara, T., Minamoto, T., Matsuhashi, S., Uchii, K., & Yamanaka, H. (2015). Droplet Digital Polymerase Chain Reaction (PCR) Outperforms Real-Time PCR in the Detection of Environmental DNA from an Invasive Fish Species. *Environmental Science & Technology*, 49(9), 5601–5608. https://doi.org/10.1021/acs.est.5b00253
- Etienne, L., Locatelli, S., Ayouba, A., Esteban, A., Butel, C., Liegeois, F., Aghokeng, A., Delaporte, E., Mpoudi Ngole, E., & Peeters, M. (2012). Noninvasive Follow-Up of Simian Immunodeficiency Virus Infection in Wild-Living Nonhabituated Western Lowland Gorillas in Cameroon. *Journal* of Virology, 86(18), 9760–9772. https://doi.org/10.1128/JVI.01186-12
- Fontes, I., Hartikainen, H., Holland, J., Secombes, C., & Okamura, B. (2017). Tetracapsuloides bryosalmonae abundance in river water. Diseases of Aquatic Organisms, 124(2), 145–157. https://doi.org/10.3354/dao03116

- Gallana, M., Ryser-Degiorgis, M.-P., Wahli, T., & Segner, H. (2013). Climate change and infectious diseases of wildlife: Altered interactions between pathogens, vectors and hosts. *Current Zoology*, 59(3), 427–437.
- Garmendia, L., & Lautraite, A. (2017). Fédération de l'Ariège de pêche et de protection du milieu aquatique. Cas d'une Tétracapsuloïdose sur l'axe Ariège, Rapport d'étude de la tétracapsuloïdose (« PKD ») infectant les truites fario dans le réseau hydrographique de la région d'Ax-Les-Thermes (Ariège, Oriège, Lauze).
- Gorgoglione, B., Wang, T., Secombes, C. J., & Holland, J. W. (2013). Immune gene expression profiling of Proliferative Kidney Disease in rainbow trout *Oncorhynchus mykiss* reveals a dominance of anti-inflammatory, antibody and T helper cell-like activities. *Veterinary Research*, 44(1), 55. https://doi.org/10.1186/1297-9716-44-55
- Hedrick, R. P., Baxa, D. V., De Kinkelin, P., & Okamura, B. (2004). Malacosporean-like spores in urine of rainbow trout react with antibody and DNA probes to *Tetracapsuloides bryosalmonae*. *Parasitology Research*, 92(1), 81–88. https://doi.org/10.1007/s00436-003-0986-3
- Hedrick, R. P., MacConnell, E., & de Kinkelin, P. (1993). Proliferative kidney disease of salmonid fish. Annual Review of Fish Diseases, 3, 277–290. https://doi.org/10.1016/0959-8030(93)90039-E
- Hervé, M. (2020). RVAideMemoire: Testing and Plotting Procedures for Biostatistics. R package version 0.9-75.
- Honma, H., Suyama, Y., & Nakai, Y. (2011). Detection of parasitizing coccidia and determination of host crane species, sex and genotype by faecal DNA analysis. *Molecular Ecology Resources*, 11(6), 1033–1044. https://doi.org/10.1111/j.1755-0998.2011.03048.x
- Hunn, J. B. (1982). Urine Flow Rate in Freshwater Salmonids: A Review. *The Progressive Fish-Culturist*, 44(3), 119–125. https://doi.org/10.1577/1548-8659(1982)44[119:UFRIFS]2.0.CO;2
- Hutchins, P. R., Sepulveda, A. J., Martin, R. M., & Hopper, L. R. (2018). A probe-based quantitative PCR assay for detecting <I>Tetracapsuloides bryosalmonae<:I> in fish tissue and environmental DNA water samples. *Conservation Genetics Resources*, 10(3), 317–319. https://doi.org/10.1007/s12686-017-0812-3
- Huver, J. R., Koprivnikar, J., Johnson, P. T. J., & Whyard, S. (2015). Development and application of an eDNA method to detect and quantify a pathogenic parasite in aquatic ecosystems. *Ecological Applications*, 25(4), 991–1002. https://doi.org/10.1890/14-1530.1
- Jerde, C. L., Mahon, A. R., Chadderton, W. L., & Lodge, D. M. (2011). "Sight-unseen" detection of rare aquatic species using environmental DNA. *Conservation Letters*, 4(2), 150–157. https://doi.org/10.1111/j.1755-263X.2010.00158.x
- Jousseaume, T., Roussel, J.-M., Beaulaton, L., Bardonnet, A., Faliex, E., Amilhat, E., Acou, A., Feunteun, E., & Launey, S. (2021). Molecular detection of the swim bladder parasite *Anguillicola crassus* (Nematoda) in fecal samples of the endangered European eel *Anguilla anguilla*. *Parasitology Research*, *120*(5), 1897–1902. https://doi.org/10.1007/s00436-021-07100-3
- Koepfli, C., Nguitragool, W., Hofmann, N. E., Robinson, L. J., Ome-Kaius, M., Sattabongkot, J., Felger, I., & Mueller, I. (2016). Sensitive and accurate quantification of human malaria parasites using droplet digital PCR (ddPCR). *Scientific Reports*, 6(1), 39183. https://doi.org/10.1038/srep39183
- Kumar, S., Kumara, H. N., Santhosh, K., & Sundararaj, P. (2019). Prevalence of gastrointestinal parasites in lion-tailed macaque *Macaca silenus* in central Western Ghats, India. *Primates*, 60(6), 537–546. https://doi.org/10.1007/s10329-019-00751-y
- Lafferty, K. D. (2009). The ecology of climate change and infectious diseases. *Ecology*, 90(4), 888–900. https://doi.org/10.1890/08-0079.1
- McAllister, C. T., Bursey, C. R., & Connior, M. B. (2016). New Host and Distributional Records for Helminth Parasites (Trematoda, Cestoda, Nematoda) from Amphibians (Caudata, Anura) and Reptiles (Testudines: Ophidia) of Oklahoma. *Proceedings of the Oklahoma Academy of Science*, 96, 76–82.
- Miotke, L., Lau, B. T., Rumma, R. T., & Ji, H. P. (2014). High Sensitivity Detection and Quantitation of DNA Copy Number and Single Nucleotide Variants with Single Color Droplet Digital PCR. *Analytical Chemistry*, 86(5), 2618–2624. https://doi.org/10.1021/ac403843j
- Morris, D. J., & Adams, A. (2006). Transmission of *Tetracapsuloides bryosalmonae* (Myxozoa: Malacosporea), the causative organism of salmonid proliferative kidney disease, to the

freshwater bryozoan *Fredericella sultana*. *Parasitology*, *133*(06), 701. https://doi.org/10.1017/S003118200600093X

- Mulero, S., Boissier, J., Allienne, J., Quilichini, Y., Foata, J., Pointier, J., & Rey, O. (2020). Environmental DNA for detecting *Bulinus truncatus*: A new environmental surveillance tool for schistosomiasis emergence risk assessment. *Environmental DNA*, 2(2), 161–174. https://doi.org/10.1002/edn3.53
- Okamura, B., & Feist, S. W. (2011). Emerging diseases in freshwater systems. *Freshwater Biology*, 56(4), 627–637. https://doi.org/10.1111/j.1365-2427.2011.02578.x
- Okamura, B., Hartikainen, H., Schmidt-Posthaus, H., & Wahli, T. (2011). Life cycle complexity, environmental change and the emerging status of salmonid proliferative kidney disease. *Freshwater Biology*, *56*(4), 735–753. https://doi.org/10.1111/j.1365-2427.2010.02465.x
- Pawlowski, J., Apothéloz-Perret-Gentil, L., & Altermatt, F. (2020). Environmental DNA: What's behind the term? Clarifying the terminology and recommendations for its future use in biomonitoring. *Molecular Ecology*, 29(22), 4258–4264. https://doi.org/10.1111/mec.15643
- R Core Team. (2020). R: A language and environment for statistical computing. *R Foundation for Statistical Computing, Vienna, Austria*. https://www.R-project.org/
- Råberg, L., Sim, D., & Read, A. F. (2007). Disentangling Genetic Variation for Resistance and Tolerance to Infectious Diseases in Animals. *Science*, 318(5851), 812–814. https://doi.org/10.1126/science.1148526
- Raffard, A., Cucherousset, J., Prunier, J. G., Loot, G., Santoul, F., & Blanchet, S. (2019). Variability of functional traits and their syndromes in a freshwater fish species (*Phoxinus phoxinus*): The role of adaptive and nonadaptive processes. *Ecology and Evolution*, 9(5), 2833–2846. https://doi.org/10.1002/ece3.4961
- Riepe, T., Calhoun, D., & Johnson, P. (2019). Comparison of direct and indirect techniques for evaluating endoparasite infections in wild-caught newts (*Taricha torosa* and *T. granulosa*). *Diseases of Aquatic Organisms*, 134(2), 137–146. https://doi.org/10.3354/dao03365
- Rusch, J. C., Hansen, H., Strand, D. A., Markussen, T., Hytterød, S., & Vrålstad, T. (2018). Catching the fish with the worm: A case study on eDNA detection of the monogenean parasite *Gyrodactylus salaris* and two of its hosts, Atlantic salmon (*Salmo salar*) and rainbow trout (*Oncorhynchus mykiss*). *Parasites & Vectors*, 11(1), 333. https://doi.org/10.1186/s13071-018-2916-3
- Ryser-Degiorgis, M.-P. (2013). Wildlife health investigations: Needs, challenges and recommendations. BMC Veterinary Research, 9(1), 223. https://doi.org/10.1186/1746-6148-9-223
- Schrag, S. J., & Wiener, P. (1995). Emerging infectious disease: What are the relative roles of ecology and evolution? *Trends in Ecology & Evolution*, 10(8), 319–324. https://doi.org/10.1016/S0169-5347(00)89118-1
- Sengupta, M. E., Hellström, M., Kariuki, H. C., Olsen, A., Thomsen, P. F., Mejer, H., Willerslev, E., Mwanje, M. T., Madsen, H., Kristensen, T. K., Stensgaard, A.-S., & Vennervald, B. J. (2019). Environmental DNA for improved detection and environmental surveillance of schistosomiasis. *Proceedings of the National Academy of Sciences*, *116*(18), 8931–8940. https://doi.org/10.1073/pnas.1815046116
- Shokralla, S., Spall, J. L., Gibson, J. F., & Hajibabaei, M. (2012). Next-generation sequencing technologies for environmental DNA research. *Molecular Ecology*, 21(8), 1794–1805. https://doi.org/10.1111/j.1365-294X.2012.05538.x
- Sjöberg, N. B., Petersson, E., Wickström, H., & Hansson, S. (2009). Effects of the swimbladder parasite *Anguillicola crassus* on the migration of European silver eels *Anguilla anguilla* in the Baltic Sea. *Journal of Fish Biology*, 74(9), 2158–2170. https://doi.org/10.1111/j.1095-8649.2009.02296.x
- Smith, K. F., Sax, D. F., & Lafferty, K. D. (2006). Evidence for the Role of Infectious Disease in Species Extinction and Endangerment. *Conservation Biology*, 20(5), 1349–1357. https://doi.org/10.1111/j.1523-1739.2006.00524.x
- Soliman, H., Kumar, G., & El-Matbouli, M. (2018). *Tetracapsuloides bryosalmonae* persists in brown trout *Salmo trutta* for five years post exposure. *Diseases of Aquatic Organisms*, 127(2), 151–156. https://doi.org/10.3354/dao03200
- Stein, R. A. (2011). Super-spreaders in infectious diseases. *International Journal of Infectious Diseases*, 15(8), e510–e513. https://doi.org/10.1016/j.ijid.2010.06.020
- Stephenson, J. F., Young, K. A., Fox, J., Jokela, J., Cable, J., & Perkins, S. E. (2017). Host heterogeneity affects both parasite transmission to and fitness on subsequent hosts. *Philosophical Transactions of the Royal Society B: Biological Sciences*, 372(1719), 20160093. https://doi.org/10.1098/rstb.2016.0093
- Strepparava, N., Segner, H., Ros, A., Hartikainen, H., Schmidt-Posthaus, H., & Wahli, T. (2018). Temperature-related parasite infection dynamics: The case of proliferative kidney disease of brown trout. *Parasitology*, 145(3), 281–291. https://doi.org/10.1017/S0031182017001482
- Villéger, S., Grenouillet, G., Suc, V., & Brosse, S. (2012). Intra- and interspecific differences in nutrient recycling by European freshwater fish. *Freshwater Biology*, 57(11), 2330–2341. https://doi.org/10.1111/fwb.12009
- Waldner, K., Bechter, T., Auer, S., Borgwardt, F., El-Matbouli, M., & Unfer, G. (2020). A brown trout (*Salmo trutta*) population faces devastating consequences due to proliferative kidney disease and temperature increase: A case study from Austria. *Ecology of Freshwater Fish*, 29(3), 465– 476. https://doi.org/10.1111/eff.12528
- Wood, S. A., Pochon, X., Laroche, O., Ammon, U., Adamson, J., & Zaiko, A. (2019). A comparison of droplet digital polymerase chain reaction (PCR), quantitative PCR and metabarcoding for species-specific detection in environmental DNA. *Molecular Ecology Resources*, 19(6), 1407– 1419. https://doi.org/10.1111/1755-0998.13055



Supporting information

Figure S1: Proliferative kidney disease gross lesions score and *T. bryosalmonae* infection prevalence according to the sampled sites. Infection prevalence (%) measured in kidney (kDNA) is indicated in italic at the bottom and is increasing from left to right. Sample sizes are indicated above bars.



Figure S2. Relationship between the quantity of *T. bryosalmonae* DNA detected in urine (uDNA) with qPCR and ddPCR for infected individuals (with *T. bryosalmonae* DNA detected in the kidney).

Table S1: Output of the comparison of linear models explaining the quantity of *T. bryosalmonae* DNA found in brown trout (*S. trutta*) urine based on the Akaike Information Criterion. The full model (M12) includes parasite load inferred from trout kidney (kDNA), proliferative kidney disease gross lesions score, fish site origin, and the interaction between kDNA and gross lesions score as well as that between kDNA and site origin.

Model	kDNA	Site	Gross lesions score	kDNA × Site	kDNA × Gross lesions score	df	AICc	Δ_{AICc}	W
M11	х	х	х		х	12	-8.4	0	0.72
M2		х				5	-5.34	3.06	0.16
M3	Х	х				6	-3.25	5.15	0.05
M12	Х	Х	Х	х	Х	15	-2.55	5.85	0.04
M6		Х	Х			8	-1.37	7.02	0.02
M7	х	х	х			9	0.53	8.92	0.01
M10	Х		Х		Х	9	3.31	11.71	0
M4			Х			5	3.72	12.12	0
M8	Х	х		х		9	3.81	12.2	0
M5	Х		Х			6	5.48	13.88	0
M0						2	6.44	14.84	0
M1	Х					3	7.71	16.1	0
M9	Х	Х	Х	Х		12	8.48	16.88	0

df: degrees of freedom, AICc: corrected Akaike Information Criterion, Δ AICc: difference in AICc compared to the smallest, W_i : model Akaike weight.

Chapter 2. Investigating the environmental conditions shaping parasite distribution and infection prevalence in host populations: an integrative approach using eDNA.

Eloïse Duval^{1,3}, Simon Blanchet^{1,2}, Erwan Quéméré³, Lisa Jacquin^{2,4}, Charlotte Veyssière² & Géraldine Loot^{2,4}.

¹ CNRS, Université Toulouse III Paul Sabatier, Station d'Écologie Théorique et Expérimentale du CNRS à Moulis, UMR-5321, 2 route du CNRS, F-09200 Moulis, France.

² Université Toulouse III Paul Sabatier, CNRS, IRD, UMR-5174 EDB (Laboratoire Evolution & Diversité Biologique), 118 route de Narbonne, F-31062, Toulouse, France.

³ INRA, Agrocampus Ouest, UMR-985 ESE (Ecologie et Santé des Ecosystèmes), 65 rue de Saint Brieuc F-35042 Rennes, France

⁴ Institut Universitaire de France, Paris, France.

Abstract

Infectious diseases result from the interaction between hosts, parasites, and the environment. Understanding the environmental conditions (abiotic and biotic) triggering disease development is critical to anticipate the decline of host populations. The abiotic environment may act at different levels of the interaction: on the parasite within the host, on the parasite out of the host as free-living stages, and on the host physiology. Biotic conditions, including the density of susceptible hosts and the density of parasite propagules, are also influenced by the abiotic conditions. Acting synergistically at these different levels, the environmental conditions shape the parasite distribution in the milieu and within its hosts, as well as the infection outcomes. It is therefore important to apprehend parasite distribution as free-living stages and within its hosts together with the underlying environmental factors to successfully forecast disease outbreaks. These two parts of the parasite life cycle are rarely investigated conjointly, mostly because of detection issues of the propagules in the open environment. Here, we aimed at identifying the environmental drivers of T. bryosalmonae propagules' distribution in the milieu and of the infection prevalence in brown trout Salmo trutta host populations to provide a general and mechanistic understanding of the emergence of proliferative kidney disease in natural ecosystems. We used environmental DNA (eDNA) to detect and quantify the heteroxeneous freshwater parasite, together with its salmonid and bryozoan hosts. We measured environmental parameters and used available databases to identify the underlying abiotic environmental factors. Our results demonstrated that the abundance of the parasite in the water was tightly linked to the abundance of its hosts but poorly to abiotic factors, whereas the infection prevalence in fish was tightly linked to abiotic factors related to water quality and weakly to the abundance of parasites in the water. This study further shows the importance of the abiotic context in shaping the outcomes of biotic interactions and refines the understanding of the dynamics of a wild emerging infectious disease.

Keywords: parasite distribution, environmental DNA, environmental drivers, host-parasite interactions,

wildlife infectious disease

Introduction

The outcomes of host-parasite interactions strongly depend on the surrounding environmental conditions (Wolinska & King, 2009). Rapid and drastic changes in environmental conditions can affect key characteristics of either hosts or parasites, such as parasite virulence and transmission rate, host susceptibility to infection or their geographic range (Budria & Candolin, 2014). These environmental changes, coupled with human-assisted dispersal, can favour the expansion of diseases outside of their native range (e.g., malaria; Tatem et al., 2006; Chaves & Koenraadt, 2010). These changes can also destabilise "benign" host-parasite dynamics that are endemic to a particular area -i.e., interactions in which infection causes non-noticeable damages to the hosts-, which can turn into the emergence of infectious diseases with major deleterious impacts on host populations (Schrag & Wiener, 1995; Lafferty, 2009; Gallana et al., 2013; Altizer et al., 2013). Understanding under which environmental conditions parasite infections become excessively harmful to the hosts is critical to anticipate host population health issues and demographic declines.

The environmental conditions driving host-parasite dynamics consist of abiotic conditions acting on the hosts, on the parasite inside its hosts (especially when the hosts are ectotherms), and on the parasite outside its hosts as free-living stages. They also include biotic conditions such as the density of parasite propagules and the density of hosts in the environment (Pietrock & Marcogliese, 2003; Lagrue & Poulin, 2015). For instance, we might expect that the higher the density of parasite infective stages in the milieu, the more likely the infection of the hosts, and reciprocally the higher the host density, the more efficient the parasite life cycle (Arneberg et al., 1998; Hallett et al., 2012). Acting synergistically, these multi-level environmental conditions shape the parasite distribution in the milieu and in its hosts, as well as the impact of the infection on the host populations (Turner et al., 2021). A (subtle) change in one of these environmental conditions can lead to an increase in infection prevalence by favouring parasite development and transmission, by decreasing the host capacity to control the parasite infection, and/or by increasing parasite pathogenicity (Martin et al., 2010; Budria & Candolin, 2014; Cable et al., 2017). As an example, Johnson et al. (2007) identified cascading effects of eutrophication on the outcomes of the trematode parasite *Ribeiroia ondatrae* infection in the amphibian Rana clamitans. Eutrophication promoted algal development, increasing the density of snails Planorbella trivolvis, the intermediate host of R. ondatrae, that in turn produced more infective stages of the parasite leading to higher propagule pressure in the open environment and increased infection intensity within the amphibian larvae. Furthermore, other studies found that an increase in water temperature negatively affected the immune capacity of amphibian hosts, which increased their susceptibility to infection by the fungus Batrachochytrium dendrobatidis, and ultimately accelerated their worldwide decline (Raffel et al., 2006; Rohr & Raffel, 2010). The increase in B. dendrobatidis free-living zoospore density is also a factor driving host mortality through increased effective parasite

load in the hosts (Fisher & Garner, 2020). To understand when and how apparently benign host-parasite interactions may lead to disease outbreaks, it is thus important to disentangle the impacts of the environment on both the parasite exposure and the host susceptibility (James et al., 2015; Stewart Merrill et al., 2021).

The acquisition of knowledge about the distribution of the parasite propagules and its underlying environmental factors is therefore substantial to successfully understand and forecast disease outbreaks (Marcogliese, 2008; Okamura & Feist, 2011; Cable et al., 2017). In most studies however, parasites are quantified within their host organisms (as prevalence or intensity estimates), but rarely as free-living propagules in the environment, so that there is a lack of information about the host exposure to infective propagules. The main reason is that free-living propagules are difficult to detect because of they are microscopic and highly diluted in the environment, which complicates their detection and quantification without appropriate tools. The development of molecular detection techniques related to environmental DNA (eDNA) have revolutionised the assessment of the presence of rare and cryptic species, as well as the early detection of invasive species (Bohmann et al., 2014; Rees et al., 2014). Recent improvements in the accuracy of eDNA detection and quantification enable to quantify the abundance (or relative abundance) of the target species (Lodge et al., 2012; Doi et al., 2015; Seymour, 2019). Accordingly, eDNA has become an important tool in parasitology to enhance the detection of otherwise invisible pathogen agents (Huver et al., 2015; Bass et al., 2015). For instance, Carraro et al. (2017, 2018) used eDNA to unravel patterns of abundance and distribution of Tetracapsuloides bryosalmonae, an emerging myxozoan parasite of salmonid fish, in a Swiss river. Parasite DNA that is detected in freshwaters may therefore be used as a proxy for the quantification of the host exposure to parasite propagules, an important factor modulating the infection success of parasites.

Here, we aimed at identifying the environmental drivers of parasite propagules in the milieu (①, Fig. 1) and parasite prevalence in host populations (②, Fig. 1) to provide a general and mechanistic understanding of the emergence of an infectious disease in natural ecosystems. We notably tested the prediction that the probability for disease emergence increases with increasing parasite propagules pressure in the milieu and with higher levels of environmental stressors acting on the hosts. We used eDNA for estimating parasite free-living propagules distribution and abundance, and we investigated their environmental determinants (abiotic and biotic through host abundances). *T. bryosalmonae* is a parasite species, so that its survival in an ecosystem depends on the presence of its hosts. However, the abiotic environmental conditions may have a role in its survival in the open environment during the between-hosts transmission (Pietrock & Marcogliese, 2003). We compared models including biotic, abiotic factors) and the abiotic factors. We then tested the relative role of the parasite exposure (quantity of propagules in the milieu) and the abiotic stressors (that impact host susceptibility) on the prevalence

of a wildlife emerging infectious disease. We focused on the interaction between *T. bryosalmonae* and the brown trout *Salmo trutta*. *T. bryosalmonae* is a myxozoan parasite with a complex life cycle involving a salmonid fish and a bryozoan, that is responsible for the salmonids' proliferative kidney disease (PKD), causing high mortality especially when associated with elevated temperatures (a major source of environmental stress for salmonids, Okamura et al., 2011). The intertwined mechanisms sustaining this temperature-associated mortality are still poorly understood, especially in the wild. In particular, it is not clear yet whether increased water temperatures trigger disease development by favouring the abundance of free-living infective stages (indirect impact of the abiotic environmental conditions through biotic interaction), and/or by destabilising the immune and physiological capacity of the fish host (direct impact of the abiotic environmental conditions, see Fig. 1 for a visual representation). Therefore, we compared models including the biotic, abiotic conditions or both to assess their relative importance in explaining infection prevalence within the fish hosts.



Figure 1. Visual representation of the paper's aims. First (1), we investigated the abiotic and biotic factors responsible for *T. bryosalmonae*'s distribution (occurrence and abundance) in the milieu. We also investigated which of the abiotic (as a whole) and biotic factors (as a whole) are the most important for the parasite distribution (represented by the dashed double-arrow determining the blue arrows width). In a second part (2), we investigated in the same way the abiotic and biotic (here the parasite propagules abundance) factors responsible for the infection prevalence in brown trout. We also investigated which of the abiotic (as a whole) and biotic factors are the most important for the infection prevalence in fish (represented by the dashed double-arrow determining the yellow arrows width). We can see from this representation that the abiotic factors may act directly on fish infection prevalence by acting on the fish physiology, but also indirectly by determining the parasite propagules abundance. The dashed-grey arrow represents the role of the abiotic factors in shaping the hosts' distributions, that were investigated in Appendix 5.

Methods

Studied host-parasite system

T. bryosalmonae alternates between two hosts to complete its life cycle: a bryozoan (definitive host, here Fredericella sultana, its main and most widespread bryozoan host in our study area, Schmidt-Posthaus et al., 2021, Hartikainen, pers. comm.) and a salmonid fish (intermediate host, here S. trutta) (Okamura et al., 2011). The transitions between its life stages are temperature dependent. Parasite release in the river by the bryozoans occurs in spring when the water temperature reaches 9°C (Gay et al., 2001), and shows another peak during autumn (Tops et al., 2009). The released parasite propagules infect brown trout by entering through gills and skin, circulate through the blood until reaching the kidney where they develop, potentially triggering an exaggerated immune reaction of the fish host when water temperature exceeds 15°C, leading to disease development (Hedrick et al., 1993). The disease may develop at the first infection of naive fish, and if they survive, they acquire immunity upon reinfection, so that young-of-the-year fish are the most impacted (Feist & Longshaw, 2006). The severity of PKD development following T. bryosalmonae infection in brown trout, also dependent on water temperature, modulates brown trout thermal tolerance and metabolic rate (Okamura et al., 2011; Bruneaux et al., 2017). On the other hand, brown trout is a cold-water species, and as such, its physiology is impacted by water temperature (Elliott & Elliott, 2010). The development of the parasite and its bryozoan host further depend on the water quality and the quantity of nutrient available in the stream, while brown trout physiology may also be negatively affected by decreased water quality (Hartikainen et al., 2009; Bailey et al., 2018; Rubin et al., 2019).

We first jointly quantified the abundance of *T. bryosalmonae*'s hosts (brown trout and bryozoan *Fredericella sultana*) and measured a wide range of abiotic factors to investigate their respective impacts on *T. bryosalmonae*'s free life stages distribution. Then, we disentangled the effects of the abundance of parasite propagules in the stream from the effects of the abiotic environmental factors on the infection prevalence in young-of-the-year fish at their first encounter with the parasite.

eDNA sampling

The study covered a wide area in Southern France, with 83 sites sampled on a total of 54 streams (Fig. 2). We sampled eDNA between the 30th of July and the 14th of August 2020.



Figure 2. Map of the 83 sampled sites. The presence or absence of detection of the parasite (T. *bryosalmonae*) and its bryozoan host (F. *sultana*) is represented by circles, and the fish infection prevalence indicated by fishes. Inset indicates the location of the studied area, at the South of France. The dotted ellipse shows three sites sampled on the Neste river, described as an example in the discussion.

At each site, we filtered up to 12L of upstream water onto 1.2 μ m cellulose nitrate Sartorius® filters (Ø 50mm) with a Vampire sampler (Bürkle®) and Sartorius® filter holders as follows: we used 8 filters per site and filtered a maximum of 1.5L per filter, less when the turbidity of the water prevented it, and we measured the volume of water filtered per filter. We put the filters in pairs in 5mL Eppendorf® tubes, to get 4 field replicates per site that were stored in a cooler on the field and then at -80°C until DNA extraction.

DNA extraction and amplification

We performed DNA extraction directly on filters using QIAGEN® PowerSoil pro kit following manufacturer recommendations and under strict laboratory environment required for eDNA extractions.

We used the primers and probes designed by Carraro et al. (2018) and Carim et al. (2016) to amplify a 71bp fragment of *F. sultana* 16S SSU rDNA sequence, a 102bp fragment of *T. bryosalmonae* COI DNA and a 108 bp fragment of *S. trutta* cytochrome B DNA (Table S1). Target DNA was amplified with duplex droplet digital PCR (ddPCR) using a BioRad QX200 Droplet Digital PCR systemTM (Bio-Rad, Temse, Belgium), with the following thermal conditions: 10min at 95°C, then 40 cycles encompassing 30s at 94°C and 1min at 60°C, followed by 10min at 98°C and 30min at 4°C. The PCR reactions were performed on a total volume of 22µL including 11µL of EvaGreen digital PCR Supermix,

2.4 μ L of sample DNA and 8.6 μ L of primer mix (including 1.9 μ L of each primer and 0.5 μ L of each probe, 10 μ M). Each 96-well run included 4 PCR negative controls with water only, and 1 PCR positive control consisting of *F. sultana* tissue infected by *T. bryosalmonae*. The baseline threshold for separating positive and negative droplets was manually chosen for each ddPCR run, according to the distribution of the droplets from the negative and positive control wells.

We run 2 ddPCRs per sample: one with the primers and probes amplifying *F. sultana* and *T. bryosalmonae* and one with the primers and probes amplifying *S. trutta* and *T. bryosalmonae*. We targeted *T. bryosalmonae* twice to maximise the chances of detection of this species for which we expected low eDNA concentrations in the water (Sieber et al., 2020).

Environmental data

A wide range of environmental factors were measured or extracted from available databases for each sampling site to assess their impact on T. bryosalmonae distribution, abundance, and on the infection prevalence in fish. We focused on factors related to water temperature and quality because they are relevant to the establishment of the bryozoan, the parasite and the disease, but also to brown trout physiology (see above). Upon eDNA sampling, we used an In-Situ® Aqua TROLL 500 Multiparameter Sonde to measure water temperature, pH, specific conductivity and O₂ concentration. We used QGIS software (2022) to get information on the land use with the CORINE Land Cover 2018 dataset (European Environment Agency) on a 2km buffer around each site and collected the percentage of forest, urban and agricultural land, as land use may impact water quality through chemical pollution or agricultural inputs (Tong & Chen, 2002). We used the Réseau Hydrographique Théorique (RHT, Pella et al., 2012) to get information on the mean flow (module), the sediment size (phi: the higher the value, the finer the sediment), the river width and depth. The mean slope (%) of the upstream 2kms was computed. The cumulative height of dams and weirs as well as the cumulative nominal capacity of the Wastewater Treatment Plants (WWTP) 2kms upstream of the sampled sites were computed using the SANDRE (Service d'Administration Nationale des Données et Référentiels sur l'Eau) database, as these facilities may act on water temperature and quality (Carey & Migliaccio, 2009; Zaidel et al., 2021). The mean flow, the cumulative height of dams and the cumulative nominal capacity of WWTP were logged to homogenise their distribution.

We checked for correlation between the environmental variables and removed those that had a correlation coefficient > |0.7|, that is the percentage of forested area (we kept the percentage of agricultural area), and the river width and depth which were highly correlated with and thus represented by the mean water flow.

Infection prevalence

Infection prevalence in juvenile brown trout was estimated at 46 out of the 83 sites. We could not cover all the sites because electro-fishing is time-consuming and mobilises a consequent team on the field. For each of these 46 sites, we sampled up to 20 individuals (according to local abundances that were sometimes lower). We targeted juvenile trout (mean size (SD) 78mm (16)), corresponding mainly to young-of-the-year (0+) fish because it is the most sensitive stage to environmental stressors and parasite infection, and also the most abundant cohort. We used a non-lethal method (uDNA for urine DNA) based on eDNA detection and the excretion of T. bryosalmonae spores by infected fish through urine excretion to infer each fish infection status. Details about the uDNA method are available from previous studies (Duval et al., 2021; 2022). The infection prevalence at each site was measured as the number of fish infected divided by the number of tested fish. Fish were then released alive at their sites of sampling. Authorisations to sample brown trout were provided by the Directions Départementales des Territoires of Ariège, Haute-Garonne and Hautes-Pyrénées, respectively. Prevalence was estimated for 31 sites in 2019 and 15 sites in 2020. For these 15 latter sites, the prevalence was also assessed in 2019 and shows that there was a high temporal congruency in the infection prevalence measured in both years (r = 0.89, n = 15, P < 0.001). We chose to use prevalence data from 2020 when available to be the closest in time to the eDNA sampling in the rivers.

Statistical analyses

The statistical analyses were conducted in the R environment (R 4.0.3; R Core Team 2020).

We divided the raw eDNA concentration of the three species by the number of liters filtered on the field, and averaged the concentrations found for the 4 field replicates of each site (8 replicates for *T*. *bryosalmonae* because the 4 field replicates were amplified twice, see above). The concentrations of *S*. *trutta* were multiplied by 100 and the concentrations of *T*. *bryosalmonae* and *F*. *sultana* by 10000 to assimilate concentrations to count data and ease modelling. The species concentrations were log-transformed when used as explanatory variables of the different models.

We used hurdle linear models with negative binomial distribution to account for the excess of zeros in the distribution of *T. bryosalmonae* eDNA concentration (aim 1, Fig. 1). These models analyse the relationship between the eDNA concentration and the environmental variables in two parts: a binary part modelling the occurrence (i.e., presence/absence), and a negative-binomial part modelling the abundance when the species is present. After visual exploration of the dataset, we included a polynomial term for the effect of water temperature in the models.

Due to the high number of environmental variables, we used a model selection procedure based on the small-sample size corrected Akaike Information Criterion (*AICc*, Burnham & Anderson, 2002; Plowright et al., 2008) with the *MuMIn* package (Barton, 2020) to identify the most impacting variables for *T. bryosalmonae* concentration. We kept models with $\Delta AICc < 4$ relative to the best model and computed the relative importance (*RI*) of each variable as the cumulative weight of each model in which it appears (Burnham & Anderson, 2002). The cumulative weight of the model selection with $\Delta AICc < 4$ was standardised so that the *RI* of each term varied between 0 and 1. We considered that a variable was relevant when *RI* > 0.5 (De Kort et al., 2021). We then used a model averaging procedure to compute the mean estimate of each relevant variable, averaging the estimates of the models in which it appeared (i.e., with the subset method).

We used negative binomial linear models to assess the relative role of the environmental variables and of the abundance of parasite spores in the water on the fish infection prevalence (aim 2, Fig. 1). We assessed the relative importance of each variable by using the same framework as for *T*. *bryosalmonae* eDNA concentration in the water, i.e., model selection and model averaging on the variables with a *RI* > 0.5.

To assess the relative roles of the abiotic and biotic factors in determining the parasite distribution and the infection prevalence within fish, we compared models including either only abiotic environmental variables, only biotic variables, or both (among the variables with a RI > 0.5) with likelihood ratio tests.

Results

As expected, *S. trutta* eDNA was detected at all sampled sites, supporting an efficient eDNA sampling and conservation protocol. The mapping of *T. bryosalmonae* and *F. sultana* occurrence revealed that most of the time, *T. bryosalmonae* was detected together with its bryozoan host (red dots, Fig. 2), except for four sites where it was detected alone (orange dots, Fig. 2). We found *T. bryosalmonae* in the water for 21 out of 27 sites with infected fish. Some discrepancies appear where we detected the parasite in fish hosts but not in the river and *vice-versa* (red fish with green or yellow dots, or green fish with orange or red dots, Fig. 2). We did not detect neither the bryozoan nor the parasite, either through river eDNA or within the fish host in 29 out of the 83 sampled sites (green dots associated with green fish and green dots alone, Fig. 2).

T. bryosalmonae distribution

The model selection procedure with the complete set of environmental variables (abiotic and biotic) revealed that the abundance of the two hosts, the water conductivity and the cumulative height of the

upstream dams were the most likely variables for explaining the distribution of *T. bryosalmonae* DNA in the water (all RI > 0.75, Fig. 3a). The DNA abundances of trout and bryozoan in the water had the highest *RI* for explaining both the occurrence and abundance of *T. bryosalmonae* (Fig. 3a). The probability of occurrence of *T. bryosalmonae* in a site increased as the DNA abundance of the two hosts increased, and –once settled in a site- its abundance increased as the DNA abundance of the two hosts increased (Fig. 3b).

Comparison models including or excluding abiotic and biotic variables respectively revealed that the model including the two hosts abundances together with abiotic environmental variables explained significantly more variance in *T. bryosalmonae* distribution than the models with either abiotic information or biotic information alone (likelihood ratio tests; abiotic *vs* abiotic + biotic, $\chi^2 = 62.17$, *df* =1, *P* < 0.0001; biotic *vs* abiotic + biotic, $\chi^2 = 12.34$, *df* = 2, *P* = 0.002; Table 1). Interestingly, the model including only the host abundances explained a large amount of variance in the distribution of *T. bryosalmonae*, whereas the model including only the best environmental predictors explained a very low amount of variance in the distribution of *T. bryosalmonae* (Table 1).

Overall, this indicates that the distribution of *T. bryosalmonae* DNA in the water (a proxy for the parasite propagules pressure) was mainly driven by the abundance of its two hosts in the milieu, and poorly by the surrounding environment.

Table 1. Log-likelihood and pseudo- R^2 of the different models built after model selection including abiotic and/or biotic variables (respectively the host DNA abundances for *T. bryosalmonae* distribution and the propagule DNA abundance for the infection prevalence in fish). The three models (abiotic, biotic and abiotic + biotic) were compared through likelihood ratio tests and the associated p-value is indicated by the stars.

Models containing variables with <i>RI</i> > 0.5 after individual model selections	Log-likelihood (R²)	Df		
<i>T. bryosalmonae</i> distribution (hurdle models)				_
eDNA T.b. ~ abiotic environment	-312 (0.06)	8	←*** ←	
eDNA T.b. ~ biotic environment	-287 (0.55)	7	\leftarrow	***
eDNA T.b. ~ abiotic environment + biotic environment	-281 (0.59)	9	$\boldsymbol{\rho}^{**} \boldsymbol{\rho}$	
Infection prevalence (binomial models)				
Prevalence ~ abiotic environment	-10.3 (0.78)	3	∽*** 6	
Prevalence ~ propagule abundance	-23.8 (0.31)	2	\leftrightarrow	•
Prevalence ~ abiotic environment + propagule abundance	-7.9 (0.87)	5		_

. $P \le 0.1$, ** $P \le 0.01$, *** $P \le 0.001$. $R^2 = (cor(obs, fitted))^2$ is also given for information. Df: degrees of freedom.



76

Figure 3. (a) Relative importance of the environmental factors for the modelling of the abundance (concentration) and occurrence of *T. bryosalmonae* in the milieu. The relative importance is the standardized (between 0 and 1) cumulative weight of each model in which it appears. The dashed grey line indicates the 0.5 threshold. (b) Mean estimates of the effects of the relevant variables on *T. bryosalmonae* abundance and occurrence. The 95% confidence intervals are indicated. The dashed black line represents a null effect.

Infection prevalence

The model selection procedure with the complete set of environmental variables (abiotic and parasite propagules abundance) revealed that the sediment size, the water temperature, the abundance of *T*. *bryosalmonae* and the percentage of agricultural land were the most likely variables for explaining the infection prevalence within fish (RI > 0.5, Fig. 4a), and that they were all positively linked to the infection prevalence (Fig. 4b).



Figure 4. (a) Relative importance of the environmental factors for the modelling of fish infection prevalence by *T. bryosalmonae*. The relative importance is the standardized (between 0 and 1) cumulative weight of each model in which it appears. The dashed grey line indicates the 0.5 threshold. (b) Mean estimates of the effects of the relevant variables on fish infection prevalence by *T. bryosalmonae*. The 95% confidence intervals are indicated. The dashed black line represents a null effect.

Comparison models including or excluding abiotic and biotic variables respectively revealed that the model including the abiotic environmental variables only was the most parsimonious to explain the infection prevalence (likelihood ratio tests; abiotic *vs* propagules + abiotic, $\chi^2 = 4.86$, df = 2, P = 0.09; propagules *vs* abiotic + propagules, $\chi^2 = 31.84$, df = 3, P < 0.0001; abiotic vs propagules, $\chi^2 = 31.84$, df = 3, P < 0.0001; abiotic vs propagules, $\chi^2 = 31.84$, df = 3, P < 0.0001; abiotic vs propagules, $\chi^2 = 31.84$, df = 3, P < 0.0001; abiotic vs propagules, $\chi^2 = 31.84$, df = 3, P < 0.0001; abiotic vs propagules, $\chi^2 = 31.84$, df = 3, P < 0.0001; abiotic vs propagules, $\chi^2 = 31.84$, df = 3, P < 0.0001; abiotic vs propagules, $\chi^2 = 31.84$, df = 3, P < 0.0001; abiotic vs propagules, $\chi^2 = 31.84$, df = 3, P < 0.0001; abiotic vs propagules, $\chi^2 = 31.84$, df = 3, P < 0.0001; abiotic vs propagules, $\chi^2 = 31.84$, df = 3, P < 0.0001; abiotic vs propagules, $\chi^2 = 31.84$, df = 3, P < 0.0001; abiotic vs propagules, $\chi^2 = 31.84$, df = 3, P < 0.0001; abiotic vs propagules, $\chi^2 = 31.84$, df = 3, P < 0.0001; abiotic vs propagules, $\chi^2 = 31.84$, df = 3, P < 0.0001; abiotic vs propagules, $\chi^2 = 31.84$, df = 3, P < 0.0001; abiotic vs propagules, $\chi^2 = 31.84$, df = 3, P < 0.0001; abiotic vs propagules, $\chi^2 = 31.84$, df = 3, P < 0.0001; abiotic vs propagules, $\chi^2 = 31.84$, df = 3, P < 0.0001; abiotic vs propagules, $\chi^2 = 31.84$, df = 3, P < 0.0001; abiotic vs propagules, $\chi^2 = 31.84$, df = 3, P < 0.0001; abiotic vs propagules, $\chi^2 = 31.84$, df = 3, P < 0.0001; abiotic vs propagules, $\chi^2 = 31.84$, $\chi^2 = 31.84$

Overall, this indicates that the infection prevalence in brown trout was most likely driven by abiotic environmental stressors acting directly on the hosts, independently of the abundance of the parasite propagules in the water.

Discussion

In this study, we investigated the relative roles of the abiotic and biotic environment in shaping a myxozoan parasite distribution and the infection prevalence in its fish host. Our results indicated that the abundance of the hosts was the most important driving factor for *T. brysalmonae*'s distribution, even though the addition of abiotic variables contributed further. On the other hand, the infection prevalence measured in brown trout populations was mostly driven by abiotic environmental variables and the influence of the abundance of parasite propagules in the water was not significant.

The mapping of *T. bryosalmonae* and its bryozoan host occurrences highlights sites where future PKD outbreaks might occur in fish, that is sites where either the bryozoan, the parasite or both are detected in the water but where infection is not detected in the fish urine. For instance, in the Neste river (sites included in the dotted ellipse of Fig. 2), the parasite was detected at all the three sampled sites, but we did not detect the infection in the fish urine at the upstream site. A weak infection prevalence was also found within juveniles' kidney tested in-between our two sampled fish populations (Lautraite, pers. comm.), confirming that what we measure in fish urine is a good proxy of the actual infection prevalence within fish kidney (Duval et al., 2021). Furthermore, this example illustrates the influence of the gradient of environmental conditions from the upstream to the downstream parts of a river on the infection prevalence within fish, even when the parasite is present at all sites. Hence, the upstream sites with parasite detected in the water but not in fish could reveal an impact of the abiotic environment on the ability of the parasite to infect its fish hosts. There could be a mis-optimisation for the parasite between its release from bryozoans and its transmission to the fish. The abiotic environmental conditions may either cause an increased mortality of parasite spores that may prevent fish infection, and/or enhance parasite avoidance or clearance by fish hosts (Pietrock & Marcogliese, 2003; Vale et al., 2011). However, at these sites, the infection prevalence in fish was tested in 2019, and the eDNA sampling occurred in 2020, so that the infection prevalence within fish may have increased. On the other hand, there are sites in which we detect the infection within the fish hosts, but not through eDNA detection in the water. These discrepancies may be due to a difficulty in detecting parasite eDNA that has already been reported in an experimental study (Sieber et al., 2020). Besides, we previously showed that diseased fish may excrete a reduced quantity of parasite spores due to impaired kidney functions (Duval et al., 2021), so that it might be more difficult to find it in the open environment if populations are diseased. Alternatively, it could reveal a negative impact of abiotic environmental factors such as water temperature on the spore survival (Pietrock & Marcogliese, 2003) or on DNA degradation (Strickler et al., 2015). Indeed, even though we were able to detect brown trout DNA at all sites, the efficiency of DNA detection might be species-specific (Breton et al., 2022), notably depending on the primers and the amplified sequence (Beng & Corlett, 2020). The mapping of T. bryosalmonae's distribution further

revealed that a lot of sites in our study area are for now exempt of any trace of the parasite cycle, because neither *T. bryosalmonae* nor *F. sultana* were detected in the water, nor *T. bryosalmonae* within the fish.

The distribution of parasite DNA in the water, used as a proxy of the parasite propagules distribution, was mainly and positively driven by the abundance of its bryozoan and fish hosts. The association with the bryozoan abundance was especially expected as it is *T. bryosalmonae*'s definitive host (Okamura et al., 2011). The strong association with brown trout abundance was more surprising as previous studies suggested that the parasite eDNA in the river may mostly originate from bryozoan release (Carraro et al., 2017, 2018), though they actually did not estimate brown trout abundance. Biotic interactions may indeed prevail over abiotic factors in shaping the distribution of a species, especially for parasites which depend on the presence of hosts for survival and reproduction (Staniczenko et al., 2017; Facon et al., 2021). The height of upstream dams and water conductivity were also positively linked to the occurrence of the parasite in the river water. This could be explained by the fact that these two environmental conditions represent favourable environment for bryozoan growth and parasite release (i.e., warmer water coming from the upstream reservoir, and higher nutrient concentration; Hartikainen et al., 2009; Ros et al., 2022).

On the contrary, the infection prevalence in fish was mostly driven by abiotic environmental conditions, although we also revealed a potential positive influence of the abundance of parasite propagules. This corroborates a recent experimental study that found no difference in fish infection intensity (parasite load in the kidney) between fish exposed once to either low or high parasite spores concentrations, or fish exposed repeatedly to low spore concentrations (Strepparava et al., 2020). In this latter study however, the infection prevalence (% of individual infected) was lower in fish exposed once to a lower concentration in propagules, but this situation is unlikely in the wild where the release of infective spores is most likely diffuse in time. This experiment was furthermore lead under controlled conditions, with a constant temperature of 15°C, so that they did not investigate the influence of fluctuating abiotic environmental conditions. Even if the parasite is present in the environment (and here, although eDNA does not differentiate between spores released by bryozoan or by fish, we may expect that spores infective to the fish are present, at least at sites where the bryozoan is also detected), it is only when the abiotic conditions deteriorate for the fish host that the infection prevalence increases, most probably because of impaired fish physiology, and/or of increased parasite proliferation within the fish host (Bruneaux et al., 2017; Lauringson et al., 2021).

In agreement, our study highlights the importance of water temperature and sediment size in driving the infection prevalence. The water temperature is a major driver of brown trout physiology, and its metabolic and immunologic aptitudes have been shown to deteriorate in warm conditions, especially when combined with *T. bryosalmonae*'s infection (Bruneaux et al., 2017; Bailey et al., 2017; Borgwardt et al., 2020; Waldner et al., 2021). The increased infection prevalence with finer sediments is to take

more cautiously because this variable could represent other patterns of the downstream variation in the river. However, the correlation between sediment size and the distance from the river source was only moderate (r = -0.29, P = 0.01), and it is possible that finer sediment size could better retain parasite free-living spores and favour their contact with juvenile fish that on the other hand preferably use coarser substrate when available (Heggenes et al., 1999; Droppo et al., 2006). In that sense, Mathieu-Bégné et al. (2021) found a high influence of the microhabitat on the infection of the rostrum dace *Leuciscus burdigalensis* by the crustacean ectoparasite *Tracheliast polycopus*, including of the riverbed substrate. Such hotspots of infection may also exist for *T. bryosalmonae*, so that greater fine-sediment patches on the riverbed could be associated with higher infection prevalence in fish. Overall, these results corroborate a recent review showing a stronger impact of negative biotic interactions when closer to the species warm limit range (Paquette & Hargreaves, 2021).

Here, we highlighted the importance of the hosts abundance in determining a parasite distribution. The identification of sites containing the basement for disease establishment in fish (i.e., with the detection of either the parasite, the bryozoan or both) via eDNA could help to prioritise populations in management plans (Amarasiri et al., 2021). However, the presence of the parasite itself does not explain the infection prevalence in its vertebrate host, which is rather triggered by the impact of environmental conditions on host physiology and/or parasite multiplication inside the host. This study further shows the importance of the abiotic context in shaping the outcomes of biotic interactions, and refines the understanding of the dynamics of a wild emerging infectious disease.

Literature cited

- Altizer, S., Ostfeld, R. S., Johnson, P. T. J., Kutz, S., & Harvell, C. D. (2013). Climate Change and Infectious Diseases: From Evidence to a Predictive Framework. *Science*, 341(6145), 514–519. https://doi.org/10.1126/science.1239401
- Amarasiri, M., Furukawa, T., Nakajima, F., & Sei, K. (2021). Pathogens and disease vectors/hosts monitoring in aquatic environments: Potential of using eDNA/eRNA based approach. *Science* of The Total Environment, 796, 148810. https://doi.org/10.1016/j.scitotenv.2021.148810
- Arneberg, P., Skorping, A., Grenfell, B., & Read, A. F. (1998). Host densities as determinants of abundance in parasite communities. *Proceedings of the Royal Society of London. Series B: Biological Sciences*, 265(1403), 1283–1289. https://doi.org/10.1098/rspb.1998.0431
- Bailey, C., Rubin, A., Strepparava, N., Segner, H., Rubin, J.-F., & Wahli, T. (2018). Do fish get wasted? Assessing the influence of effluents on parasitic infection of wild fish. *PeerJ*, 6, e5956. https://doi.org/10.7717/peerj.5956
- Bailey, C., Segner, H., Casanova-Nakayama, A., & Wahli, T. (2017). Who needs the hotspot? The effect of temperature on the fish host immune response to *Tetracapsuloides bryosalmonae* the causative agent of proliferative kidney disease. *Fish & Shellfish Immunology*, 63, 424–437. https://doi.org/10.1016/j.fsi.2017.02.039
- Barton, K. (2020). *MuMIn: Multi-Model Inference. R package version 1.43.17.* https://CRAN.R-project.org/package=MuMIn
- Bass, D., Stentiford, G. D., Littlewood, D. T. J., & Hartikainen, H. (2015). Diverse Applications of Environmental DNA Methods in Parasitology. *Trends in Parasitology*, 31(10), 499–513. https://doi.org/10.1016/j.pt.2015.06.013
- Beng, K. C., & Corlett, R. T. (2020). Applications of environmental DNA (eDNA) in ecology and conservation: Opportunities, challenges and prospects. *Biodiversity and Conservation*, 29(7), 2089–2121. https://doi.org/10.1007/s10531-020-01980-0
- Bohmann, K., Evans, A., Gilbert, M. T. P., Carvalho, G. R., Creer, S., Knapp, M., Yu, D. W., & de Bruyn, M. (2014). Environmental DNA for wildlife biology and biodiversity monitoring. *Trends in Ecology & Evolution*, 29(6), 358–367. https://doi.org/10.1016/j.tree.2014.04.003
- Borgwardt, F., Unfer, G., Auer, S., Waldner, K., El-Matbouli, M., & Bechter, T. (2020). Direct and Indirect Climate Change Impacts on Brown Trout in Central Europe: How Thermal Regimes Reinforce Physiological Stress and Support the Emergence of Diseases. *Frontiers in Environmental Science*, 8, 59. https://doi.org/10.3389/fenvs.2020.00059
- Breton, B. A., Beaty, L., Bennett, A. M., Kyle, C. J., Lesbarrères, D., Vilaça, S. T., Wikston, M. J. H., Wilson, C. C., & Murray, D. L. (2022). Testing the effectiveness of environmental DNA (eDNA) to quantify larval amphibian abundance. *Environmental DNA*, edn3.332. https://doi.org/10.1002/edn3.332
- Bruneaux, M., Visse, M., Gross, R., Pukk, L., Saks, L., & Vasemägi, A. (2017). Parasite infection and decreased thermal tolerance: Impact of proliferative kidney disease on a wild salmonid fish in the context of climate change. *Functional Ecology*, 31(1), 216–226. https://doi.org/10.1111/1365-2435.12701
- Budria, A., & Candolin, U. (2014). How does human-induced environmental change influence hostparasite interactions? *Parasitology*, 141(4), 462–474. https://doi.org/10.1017/S0031182013001881
- Burnham, K. P., & Anderson, D. R. (2002). *Model selection and multimodel inference: A practical information-theoretic approach* (2nd ed). Springer.
- Cable, J., Barber, I., Boag, B., Ellison, A. R., Morgan, E. R., Murray, K., Pascoe, E. L., Sait, S. M., Wilson, A. J., & Booth, M. (2017). Global change, parasite transmission and disease control: Lessons from ecology. *Philosophical Transactions of the Royal Society B: Biological Sciences*, 372(1719), 20160088. https://doi.org/10.1098/rstb.2016.0088
- Carey, R. O., & Migliaccio, K. W. (2009). Contribution of Wastewater Treatment Plant Effluents to Nutrient Dynamics in Aquatic Systems: A Review. *Environmental Management*, 44(2), 205– 217. https://doi.org/10.1007/s00267-009-9309-5
- Carim, K. J., Wilcox, T. M., Anderson, M., Lawrence, D. J., Young, M. K., McKelvey, K. S., & Schwartz, M. K. (2016). An environmental DNA marker for detecting nonnative brown trout

(Salmo trutta). Conservation Genetics Resources, 8(3), 259–261. https://doi.org/10.1007/s12686-016-0548-5

- Carraro, L., Bertuzzo, E., Mari, L., Fontes, I., Hartikainen, H., Strepparava, N., Schmidt-Posthaus, H., Wahli, T., Jokela, J., Gatto, M., & Rinaldo, A. (2017). Integrated field, laboratory, and theoretical study of PKD spread in a Swiss prealpine river. *Proceedings of the National Academy of Sciences*, 114(45), 11992–11997. https://doi.org/10.1073/pnas.1713691114
- Carraro, L., Hartikainen, H., Jokela, J., Bertuzzo, E., & Rinaldo, A. (2018). Estimating species distribution and abundance in river networks using environmental DNA. *Proceedings of the National Academy of Sciences*, 201813843. https://doi.org/10.1073/pnas.1813843115
- Chaves, L. F., & Koenraadt, C. J. M. (2010). Climate Change and Highland Malaria: Fresh Air for a Hot Debate. *The Quarterly Review of Biology*, 85(1), 27–55. https://doi.org/10.1086/650284
- De Kort, H., Prunier, J. G., Ducatez, S., Honnay, O., Baguette, M., Stevens, V. M., & Blanchet, S. (2021). Life history, climate and biogeography interactively affect worldwide genetic diversity of plant and animal populations. *Nature Communications*, *12*(1), 516. https://doi.org/10.1038/s41467-021-20958-2
- Doi, H., Uchii, K., Takahara, T., Matsuhashi, S., Yamanaka, H., & Minamoto, T. (2015). Use of Droplet Digital PCR for Estimation of Fish Abundance and Biomass in Environmental DNA Surveys. *PLOS ONE*, 10(3), e0122763. https://doi.org/10.1371/journal.pone.0122763
- Droppo, I. G., Liss, S. N., Williams, D., & Leppard, G. G. (2006). River sediment/pathogen interactions: Importance for policy development on safe water practices. In J. S. Rowan, R. W. Duck, & A. Werritty, *Sediment Dynamics and the Hydromorphology of Fluvial Systems* (IAHS Publication 306, pp. 314–321).
- Duval, E., Blanchet, S., Quéméré, E., Jacquin, L., Veyssière, C., Lautraite, A., Garmendia, L., Yotte, A., Parthuisot, N., Côte, J., & Loot, G. (2021). Urine DNA (uDNA) as a non-lethal method for endoparasite biomonitoring: Development and validation. *Environmental DNA*, edn3.228. https://doi.org/10.1002/edn3.228
- Duval, E., Quéméré, E., Loot, G., Jacquin, L., Veyssière, C., & Blanchet, S. (2022). A multifaceted index of population health to detect risk-prone populations and underlying stressors in wildlife. *Biological Conservation*, 274, https://doi.org/10.1016/j.biocon.2022.109706
- Elliott, J. M., & Elliott, J. A. (2010). Temperature requirements of Atlantic salmon *Salmo salar*, brown trout *Salmo trutta* and Arctic charr *Salvelinus alpinus*: Predicting the effects of climate change. *Journal of Fish Biology*, 25.
- Facon, B., Hafsi, A., Dubart, M., Chiquet, J., Frago, E., Chiroleu, F., & Ravigné, V. (2021). Joint species distributions reveal the combined effects of host plants, abiotic factors and species competition as drivers of species abundances in fruit flies. *Ecology Letters*, 45.
- Feist, S. W., & Longshaw, M. (2006). Phylum Myxozoa. In P. T. K. Woo, *Fish diseases and disorders* (CAB International, pp. 230–296).
- Fisher, M. C., & Garner, T. W. J. (2020). Chytrid fungi and global amphibian declines. *Nature Reviews Microbiology*, *18*(6), 332–343. https://doi.org/10.1038/s41579-020-0335-x
- Gallana, M., Ryser-Degiorgis, M.-P., Wahli, T., & Segner, H. (2013). Climate change and infectious diseases of wildlife: Altered interactions between pathogens, vectors and hosts. *Current Zoology*, 59(3), 427–437.
- Gay, M., Okamura, B., & de Kinkelin, P. (2001). Evidence that infectious stages of *Tetracapsula* bryosalmonae for rainbow trout *Oncorhynchus mykiss* are present throughout the year. *Diseases* of Aquatic Organisms, 46, 31–40. https://doi.org/10.3354/dao046031
- Hallett, S. L., Ray, R. A., Hurst, C. N., Holt, R. A., Buckles, G. R., Atkinson, S. D., & Bartholomew, J. L. (2012). Density of the Waterborne Parasite *Ceratomyxa shasta* and Its Biological Effects on Salmon. *Applied and Environmental Microbiology*, 78(10), 3724–3731. https://doi.org/10.1128/AEM.07801-11
- Hartikainen, H., Johnes, P., Moncrieff, C., & Okamura, B. (2009). Bryozoan populations reflect nutrient enrichment and productivity gradients in rivers. *Freshwater Biology*, 54(11), 2320–2334. https://doi.org/10.1111/j.1365-2427.2009.02262.x
- Hedrick, R. P., MacConnell, E., & de Kinkelin, P. (1993). Proliferative kidney disease of salmonid fish. *Annual Review of Fish Diseases*, *3*, 277–290. https://doi.org/10.1016/0959-8030(93)90039-E

- Heggenes, J., Bagliniere, J. L., & Cunjak, R. A. (1999). Spatial niche variability for young Atlantic salmon (*Salmo salar*) and brown trout (*S. trutta*) in heterogeneous streams. *Ecology of Freshwater Fish*, 8(1), 1–21. https://doi.org/10.1111/j.1600-0633.1999.tb00048.x
- Huver, J. R., Koprivnikar, J., Johnson, P. T. J., & Whyard, S. (2015). Development and application of an eDNA method to detect and quantify a pathogenic parasite in aquatic ecosystems. *Ecological Applications*, 25(4), 991–1002. https://doi.org/10.1890/14-1530.1
- James, T. Y., Toledo, L. F., Rödder, D., Silva Leite, D., Belasen, A. M., Betancourt-Román, C. M., Jenkinson, T. S., Soto-Azat, C., Lambertini, C., Longo, A. V., Ruggeri, J., Collins, J. P., Burrowes, P. A., Lips, K. R., Zamudio, K. R., & Longcore, J. E. (2015). Disentangling host, pathogen, and environmental determinants of a recently emerged wildlife disease: Lessons from the first 15 years of amphibian chytridiomycosis research. *Ecology and Evolution*, 5(18), 4079– 4097. https://doi.org/10.1002/ece3.1672
- Johnson, P. T. J., Chase, J. M., Dosch, K. L., Hartson, R. B., Gross, J. A., Larson, D. J., Sutherland, D. R., & Carpenter, S. R. (2007). Aquatic eutrophication promotes pathogenic infection in amphibians. *Proceedings of the National Academy of Sciences*, 104(40), 15781–15786. https://doi.org/10.1073/pnas.0707763104
- Lafferty, K. D. (2009). The ecology of climate change and infectious diseases. *Ecology*, 90(4), 888–900. https://doi.org/10.1890/08-0079.1
- Lagrue, C., & Poulin, R. (2015). Bottom-up regulation of parasite population densities in freshwater ecosystems. *Oikos*, *124*(12), 1639–1647. https://doi.org/10.1111/oik.02164
- Lauringson, M., Nousiainen, I., Kahar, S., Burimski, O., Gross, R., Kaart, T., & Vasemägi, A. (2021). Climate change-driven disease in sympatric hosts: Temporal dynamics of parasite burden and proliferative kidney disease in wild brown trout and Atlantic salmon. *Journal of Fish Diseases*, 44(6), 689–699. https://doi.org/10.1111/jfd.13330
- Lodge, D. M., Turner, C. R., Jerde, C. L., Barnes, M. A., Chadderton, L., Egan, S. P., Feder, J. L., Mahon, A. R., & Pfrender, M. E. (2012). Conservation in a cup of water: Estimating biodiversity and population abundance from environmental DNA. *Molecular Ecology*, *21*(11), 2555–2558. https://doi.org/10.1111/j.1365-294X.2012.05600.x
- Marcogliese, D. J. (2008). The impact of climate change on the parasites and infectious diseases of aquatic animals. *Rev. Sci. Tech. Off. Int. Epiz.*, 27(2), 18.
- Martin, L. B., Hopkins, W. A., Mydlarz, L. D., & Rohr, J. R. (2010). The effects of anthropogenic global changes on immune functions and disease resistance: Ecoimmunology and global change. *Annals of the New York Academy of Sciences*, *1195*(1), 129–148. https://doi.org/10.1111/j.1749-6632.2010.05454.x
- Mathieu-Bégné, E., Blanchet, S., Rey, O., Scelsi, O., Poesy, C., Marselli, G., & Loot, G. (2021). A finescale analysis reveals microgeographic hotspots maximizing infection rate between a parasite and its fish host. *Functional Ecology*, *36*(2), 380–391. https://doi.org/10.1111/1365-2435.13967
- Okamura, B., & Feist, S. W. (2011). Emerging diseases in freshwater systems. *Freshwater Biology*, 56(4), 627–637. https://doi.org/10.1111/j.1365-2427.2011.02578.x
- Okamura, B., Hartikainen, H., Schmidt-Posthaus, H., & Wahli, T. (2011). Life cycle complexity, environmental change and the emerging status of salmonid proliferative kidney disease. *Freshwater Biology*, *56*(4), 735–753. https://doi.org/10.1111/j.1365-2427.2010.02465.x
- Paquette, A., & Hargreaves, A. L. (2021). Biotic interactions are more often important at species' warm versus cool range edges. *Ecology Letters*, 24(11), 2427–2438. https://doi.org/10.1111/ele.13864
- Pella, H., Lejot, J., Lamouroux, N., & Snelder, T. (2012). Le réseau hydrographique théorique (RHT) français et ses attributs environnementaux. Géomorphologie : relief, processus, environnement, 18(3), 317–336. https://doi.org/10.4000/geomorphologie.9933
- Pietrock, M., & Marcogliese, D. J. (2003). Free-living endohelminth stages: At the mercy of environmental conditions. *Trends in Parasitology*, *19*(7), 293–299. https://doi.org/10.1016/S1471-4922(03)00117-X
- Plowright, R. K., Sokolow, S. H., Gorman, M. E., Daszak, P., & Foley, J. E. (2008). Causal inference in disease ecology: Investigating ecological drivers of disease emergence. *Frontiers in Ecology* and the Environment, 6(8), 420–429. https://doi.org/10.1890/070086
- QGIS Development Team. (2022). QGIS Geographic Information System. https://www.qgis.org

- R Core Team. (2020). R: A language and environment for statistical computing. *R Foundation for Statistical Computing*, Vienna, Austria. https://www.R-project.org/
- Raffel, T. R., Rohr, J. R., Kiesecker, J. M., & Hudson, P. J. (2006). Negative effects of changing temperature on amphibian immunity under field conditions. *Functional Ecology*, 20(5), 819– 828. https://doi.org/10.1111/j.1365-2435.2006.01159.x
- Rees, H. C., Maddison, B. C., Middleditch, D. J., Patmore, J. R. M., & Gough, K. C. (2014). REVIEW: The detection of aquatic animal species using environmental DNA - a review of eDNA as a survey tool in ecology. *Journal of Applied Ecology*, 51(5), 1450–1459. https://doi.org/10.1111/1365-2664.12306
- Rohr, J. R., & Raffel, T. R. (2010). Linking global climate and temperature variability to widespread amphibian declines putatively caused by disease. *Proceedings of the National Academy of Sciences*, 107(18), 8269–8274. https://doi.org/10.1073/pnas.0912883107
- Ros, A., Schmidt-Posthaus, H., & Brinker, A. (2022). Mitigating human impacts including climate change on proliferative kidney disease in salmonids of running waters. *Journal of Fish Diseases*, 45(4), 497–521. https://doi.org/10.1111/jfd.13585
- Rubin, A., de Coulon, P., Bailey, C., Segner, H., Wahli, T., & Rubin, J.-F. (2019). Keeping an Eye on Wild Brown Trout (*Salmo trutta*) Populations: Correlation Between Temperature, Environmental Parameters, and Proliferative Kidney Disease. *Frontiers in Veterinary Science*, 6, 281. https://doi.org/10.3389/fvets.2019.00281
- Schmidt-Posthaus, H., Schneider, E., Schölzel, N., Hirschi, R., Stelzer, M., & Peter, A. (2021). The role of migration barriers for dispersion of Proliferative Kidney Disease—Balance between disease emergence and habitat connectivity. *PLOS ONE*, 16(3), e0247482. https://doi.org/10.1371/journal.pone.0247482
- Schrag, S. J., & Wiener, P. (1995). Emerging infectious disease: What are the relative roles of ecology and evolution? *Trends in Ecology & Evolution*, 10(8), 319–324. https://doi.org/10.1016/S0169-5347(00)89118-1
- Seymour, M. (2019). Rapid progression and future of environmental DNA research. *Communications Biology*, 2(1), 80. https://doi.org/10.1038/s42003-019-0330-9
- Sieber, N., Hartikainen, H., & Vorburger, C. (2020). Validation of an eDNA-based method for the detection of wildlife pathogens in water. *Diseases of Aquatic Organisms*, 141, 171–184. https://doi.org/10.3354/dao03524
- Staniczenko, P. P. A., Sivasubramaniam, P., Suttle, K. B., & Pearson, R. G. (2017). Linking macroecology and community ecology: Refining predictions of species distributions using biotic interaction networks. *Ecology Letters*, 20(6), 693–707. https://doi.org/10.1111/ele.12770
- Stewart Merrill, T. E., Hall, S. R., & Cáceres, C. E. (2021). Parasite exposure and host susceptibility jointly drive the emergence of epidemics. *Ecology*, *102*(2). https://doi.org/10.1002/ecy.3245
- Strepparava, N., Ros, A., Hartikainen, H., Schmidt-Posthaus, H., Wahli, T., Segner, H., & Bailey, C. (2020). Effects of parasite concentrations on infection dynamics and proliferative kidney disease pathogenesis in brown trout (*Salmo trutta*). *Transboundary and Emerging Diseases*, tbed.13615. https://doi.org/10.1111/tbed.13615
- Strickler, K. M., Fremier, A. K., & Goldberg, C. S. (2015). Quantifying effects of UV-B, temperature, and pH on eDNA degradation in aquatic microcosms. *Biological Conservation*, *183*, 85–92. https://doi.org/10.1016/j.biocon.2014.11.038
- Tatem, A. J., Hay, S. I., & Rogers, D. J. (2006). Global traffic and disease vector dispersal. *Proceedings* of the National Academy of Sciences, 103(16), 6242–6247. https://doi.org/10.1073/pnas.0508391103
- Tong, S. T. Y., & Chen, W. (2002). Modeling the relationship between land use and surface water quality. *Journal of Environmental Management*, 66(4), 377–393. https://doi.org/10.1006/jema.2002.0593
- Tops, S., Hartikainen, H.-L., & Okamura, B. (2009). The effects of infection by *Tetracapsuloides bryosalmonae* (Myxozoa) and temperature on *Fredericella sultana* (Bryozoa). *International Journal for Parasitology*, 39(9), 1003–1010. https://doi.org/10.1016/j.ijpara.2009.01.007
- Turner, W. C., Kamath, P. L., van Heerden, H., Huang, Y.-H., Barandongo, Z. R., Bruce, S. A., & Kausrud, K. (2021). The roles of environmental variation and parasite survival in virulence–

transmission relationships. *Royal Society Open Science*, 8(6), 210088. https://doi.org/10.1098/rsos.210088

- Vale, P. F., Wilson, A. J., Best, A., Boots, M., & Little, T. J. (2011). Epidemiological, Evolutionary, and Coevolutionary Implications of Context-Dependent Parasitism. *The American Naturalist*, 177(4), 510–521. https://doi.org/10.1086/659002
- Waldner, K., Borkovec, M., Borgwardt, F., Unfer, G., & El-Matbouli, M. (2021). Effect of water temperature on the morbidity of *Tetracapsuloides bryosalmonae* (Myxozoa) to brown trout (*Salmo trutta*) under laboratory conditions. *Journal of Fish Diseases*, 44(7), 1005–1013. https://doi.org/10.1111/jfd.13361
- Wolinska, J., & King, K. C. (2009). Environment can alter selection in host–parasite interactions. *Trends in Parasitology*, 25(5), 236–244. https://doi.org/10.1016/j.pt.2009.02.004
- Zaidel, P. A., Roy, A. H., Houle, K. M., Lambert, B., Letcher, B. H., Nislow, K. H., & Smith, C. (2021). Impacts of small dams on stream temperature. *Ecological Indicators*, *120*, 106878. https://doi.org/10.1016/j.ecolind.2020.106878

Supporting information

Table S1. Primers and probes sequences used to amplify *F. sultana*, *T. bryosalmonae* and *S. trutta* eDNA.

Species	Primers and probes	Sequences (5'-3')	Size and fragment nature	Reference	
Fredericella sultana	Fs_16S_F1q	CATTGAGCTTCGGGAATGTT			
(bruozoan bost)	Fs_16S_R1q	Fs_16S_R1q ATGAAACCTCGTCCCTTGTG		Carraro et al. 2018	
(bryozdan nost)	Fs_probe_16S Cy5-GGGGTCAGGTTGCTAAGCCATGABHQ-2				
Totracansulaidas bruasalmanaa	Tb_COI_F1q	GGTTGTTTAGTTTGGGCTCACC			
(myyozoon porosito)	Tb_COI_R1q	D_COI_R1q TCCCTGTAGGGACAGCTATTG		Carraro et al. 2018	
(myxozoan parasite)	Tb_probe_COI	FAM-CAAGATCTTATTTTATGGCTGCCAC-BHQ-1			
Salma trutta	Forward	CGCCCGAGGACTCTACTATGGT			
(brown trout boot)	Reverse	GGAAGAACGTAGCCCACGAA	108bp, cytochrome b	Carim et al. 2016	
(brown trout nost)	Probe	FAM-CGGAGTCGTACTGCTAC-MGBNFQ			

Chapter 3. Eco-evolutionary response of cold-water fish populations to the combined effect of an emerging parasite and temperature.

Eloïse Duval^{1,3}, Simon Blanchet^{1,2}, Lisa Jacquin^{2,4}, Charlotte Veyssière², Géraldine Loot^{2,4} & Erwan Quéméré³.

¹ CNRS, Université Toulouse III Paul Sabatier, Station d'Écologie Théorique et Expérimentale du CNRS à Moulis, UMR-5321, 2 route du CNRS, F-09200 Moulis, France.

² Université Toulouse III Paul Sabatier, CNRS, IRD, UMR-5174 EDB (Laboratoire Evolution & Diversité Biologique), 118 route de Narbonne, F-31062, Toulouse, France.

³ INRA, Agrocampus Ouest, UMR-985 ESE (Ecologie et Santé des Ecosystèmes), 65 rue de Saint Brieuc F-35042 Rennes, France

⁴Institut Universitaire de France, Paris, France.

Abstract

The intraspecific variation of wildlife populations, composed of genetic and phenotypic diversity, is challenged by changes in environmental conditions and their associated mortality events. Genetic diversity may be altered through the random loss of individuals due to the demographic decline, and/or from the loss of maladapted individuals (directional selection). Meanwhile, phenotypic variation may also shift, either via the selection of the fittest individuals (viability selection), or via a trade-off between maintenance functions and the considered traits (plasticity). Emerging infectious diseases represent a change in biotic conditions where a parasite infection might cause high mortality in host populations. The impact of the infection may furthermore be triggered by environmental stressors. Here, we investigated the combined impact of water temperature and infection by Tetracapsuloides bryosalmonae, a myxozoan parasite causing the salmonid proliferative kidney disease (PKD), a temperature-dependent disease, on wild brown trout Salmo trutta populations. We sampled juveniles before and after summer (propitious period for PKD development), along gradients of infection prevalence and water temperature, and compared population density and intraspecific variation between the two sampling sessions. Population density decreased in naive populations after infection, but not in populations already heavily infected the year before. High infection prevalence reduced diversity of a immune-related gene, but not of neutral loci. This suggests directional selection acting at this adaptive locus. We further revealed a negative effect of temperature and infection on juvenile body condition and interactive effects of both stressors on carotenoid-based coloration. Together, our results suggest that the initial mortality caused by the disease outbreak might be followed by a rapid micro-evolution of the populations' immunity. Moreover, we observed phenotypic responses likely resulting from a trade-off between fitness-related traits, illustrating a short-term plastic response that could contribute to the persistence of the juvenile cohort. However, the adaptive effects of these genetic and plastic phenotypic responses on long term fitness remain to be tested.

Keywords: multiple stressors, emerging infectious disease, salmonids, selection, micro-evolution

Introduction

Intraspecific variation of phenotypes and genotypes is a major component of biodiversity, at the foundation of many ecological and evolutionary processes promoting populations' dynamics, species interactions and ecosystems' functions (Mimura et al., 2017). In the context of global change, species are increasingly confronted to rapid changes in environmental conditions and exposure to multiple stressors (Easterling et al., 2000). This may lead to large declines in wild populations and decrease in intraspecific variation depending on the magnitude and combinations of stressors and the type of response to selection (i.e. plastic, genetic or range shift changes) (Canale & Henry, 2010; Pauls et al., 2013; Grant et al., 2017). Deciphering the ecological and evolutionary mechanisms involved in the rapid adaptation of wild populations is thus critical for predicting their short- and long-term persistence and guide management decisions (Fox et al., 2019). However, few studies have investigated the impacts of changes in environmental conditions by comparing the patterns of intraspecific variation of wildlife populations before and after a sudden change in biotic or abiotic conditions. In some cases, extreme disturbances such as storms or floods could have marked effects on phenotypic and genetic variation through changes in natural selection (Vignieri, 2010; Banks et al., 2013; Campbell-Staton et al., 2017; Donihue et al., 2018). In other cases, sudden changes in environmental conditions may result in weak changes in intraspecific variation (Blondel et al., 2021), depending on plastic responses and changes in intraspecific diversity in phenotypes and genotypes, indicating that general conclusions are still hard to draw.

Emerging infectious diseases (i.e., diseases that recently increased in incidence or that infected new host populations; Schrag & Wiener, 1995) represent a special case of sudden change in biotic conditions, and large disease outbreaks may cause severe mortality in wild host populations (Gallana et al., 2013; Fisher & Garner, 2020; Hoyt et al., 2021). When such mortality events occur, several mutually non-exclusive mechanisms can operate in synergy driving shifts in genotypes and/or phenotypes. First, changes in genotypes following disease outbreaks may occur due to demographic crashes leading to an erosion of the global genetic diversity (genetic bottleneck; Nei et al., 1975; Trudeau et al., 2004; Albert et al., 2015). Concomitantly, a shift in genotypes in the surviving population may also arise from the directional selection of genotypes providing better resistance or tolerance to the infection through differential mortality (Epstein et al., 2016; Wright et al., 2017; Auteri & Knowles, 2020). Under parasitemediated selection, a higher change in genetic diversity is expected at non-neutral (adaptive) loci involved in pathogen resistance/tolerance compared to neutral loci (e.g. in Westerdahl et al., 2004; Savage & Zamudio, 2016). Consistently, several studies showed genotypic shifts after a disease outbreak at putative loci for disease resistance, e.g. in sea stars for wasting disease (Schiebelhut et al., 2018), in bats for white-nose syndrome (Gignoux-Wolfsohn et al., 2021) or in Tasmanian devils for facial tumour disease (Epstein et al., 2016).

Second, phenotypic changes are also frequently observed after a sudden disruption of environmental conditions such as disease outbreaks (Poulin & Thomas, 1999; Altizer et al., 2003). Parasite infection may favour shifts in fitness-related traits such as body mass or condition (Wilcoxen et al., 2010; Vander Wal et al., 2014), but also indirectly affect traits linked to individual quality and health such as ornamental traits (Siva–Jothy, 2000; Martínez-Padilla et al., 2007; Ezenwa & Jolles, 2008; Ducrest et al., 2008). Two main mechanisms are often invoked. First, when a trait is positively correlated with survival, viability (directional) selection may lead to a shift in the mean of the considered trait after the infection event (viability selection hypothesis) (Hoekstra et al., 2001; Hadfield, 2008). For instance, house finches (Carpodacus mexicanus) surviving Mycoplasma gallisepticum infection responsible for mycoplasma conjunctivitis had a smaller mean size compared to individuals before infection, and male survivors were redder than the deceased males (Nolan et al., 1998). Alternatively, plastic responses (i.e., the ability of a genotype to express different phenotypes according to the environmental conditions; Fox et al., 2019) could lead to changes in phenotypic traits that could enhance fitness, and to trade-offs between traits involved in stress resistance and performance (plasticity hypothesis). Indeed, the cost of the immune response for parasite control during the infection may decrease the amount of resources allocated to costly traits such as body condition or ornamental traits (Folstad & Karter, 1992; Sheldon & Verhulst, 1996), leading to a shift towards lower mean values after the infection. For instance, infection leads to lower horn growth in bighorn sheep (Martin et al., 2022) and lower coloration signals in blackbirds (Faivre et al., 2003; Baeta et al., 2008). The joint study of demography, genetic and phenotypic variation provides a better understanding of host populations responses to parasitism at different levels, and identifies potential selection on phenotypes and genotypes, as well as phenotypic plasticity (Hendry & Kinnison, 1999; Linnen & Hoekstra, 2009).

Furthermore, the impact of pathogen infection on wild populations may be exacerbated by specific environmental conditions and concomitant exposure to other stressors (Wolinska & King, 2009; Crozier & Hutchings, 2014). Indeed, several environmental conditions may influence host immunocompetence (Sandland & Minchella, 2003), as well as pathogen life cycles (Brown et al., 2003; Mitchell et al., 2005), therefore altering disease outbreaks (Johnson et al., 2010; Marcogliese & Pietrock, 2011; Gehman et al., 2018). Most of the studies investigating the impact of an extreme event on intraspecific variation focus on a single environmental stressor, but in the case of an emerging disease, the parasite and the environment could have synergetic effects on intraspecific variation, strengthening the overall selective pressure exerted on the host population (Johnson & Penaluna, 2019). Accordingly, some studies have shown how environmental stressors can exacerbate the infection outcomes in the host population, resulting in more severe disease (Harvell et al., 2002; Altizer et al., 2013). For instance, the proliferative kidney disease (PKD) of salmonids, caused by the myxozoan parasite *Tetracapsuloides bryosalmonae*, is known for its temperature-dependent severity (Okamura et al., 2011; Bailey et al., 2017; Waldner et al., 2021), and fish become diseased during the warmest days of the summer (Carraro

et al., 2016). Water temperature is also a major determinant of salmonids' ecological niche, so that a high temperature may have a negative impact on salmonids intraspecific variation even in uninfected populations (Elliott & Elliott, 2010). Conversely, the parasite might have negligible impact on host health at lower temperatures (Bailey et al., 2017). This illustrates the importance of investigating the impacts of multiple stressors in heterogeneous populations to better predict and anticipate the effects of emerging diseases on wildlife health and population persistence in the context of global changes (Altizer et al., 2013; Merilä & Hendry, 2014; Reid et al., 2019).

In this study, we investigated the combined effect of the infection by T. bryosalmonae and water temperature on the genotypic and phenotypic variation of wild populations of brown trout (Salmo trutta). We studied 15 populations differently exposed to gradients of water temperature and infection prevalence and tested whether the parasite-temperature-mediated selection led to significant and directional shifts in abundance, genotypic diversity and phenotypes during the summer (before/after PKD outbreak). We used Single Nucleotide Polymorphism (SNPs) and a locus from the Major Histocompatibility Complex (MHC, a highly polymorphic complex of genes implicated in pathogen recognition as part of the acquired immune system, Bernatchez & Landry, 2003) to assess populations genetic diversity at neutral and adaptive loci respectively. We measured fish body condition, together with melanin and carotenoid-based colouration as phenotypic traits related to individual fitness (Robinson et al., 2008; Ducrest et al., 2008; Price et al., 2008). As we expected a cumulative and amplifying effect of the temperature and the infection, we predicted a decrease in population density due to high mortality at sites showing both high temperature and high disease prevalence (Waldner et al., 2021; Ros et al., 2022). In turn, we expected that this demographic loss would be associated with a loss in genetic diversity both at the SNPs and MHC loci but with different patterns among neutral and immunogenetic diversity (Hoffmann & Willi, 2008; Banks et al., 2013). Similarly, we expected to find differences in the mean phenotypes of fitness-related traits for the warmer sites with higher prevalence. If the individuals surviving the summer are the fittest with higher body condition and more pronounced ornamental traits (i.e., viability selection hypothesis), we expect an increase in these traits mean at the population level (Nolan et al., 1998). Alternatively, if surviving individuals in these warm and highly prevalent sites suffer from pathogen control and have limited resources to allocate to their growth or ornamental traits, phenotypic plasticity may lead to a decrease in these traits means at the population level through a resource trade-off, in line with the plasticity hypothesis (Wernicke von Siebenthal et al., 2018; Masó et al., 2022).

Methods

Studied species

The brown trout is a salmonid species known for its very high degree of polymorphism, especially for its diversity of life history strategies (Cucherousset et al., 2005). This species shows high genetic diversity and phenotypic plasticity, reflected by high levels of genetic and phenotypic divergence between populations even at a small geographical scale without geographic barriers (Stelkens et al., 2012). This high level of intraspecific variability has enabled this species to establish in a wide geographical area outside of its natural range (Elliott, 1994). The main determining factor for brown trout establishment is water temperature, with optimum temperature at around 14°C and a physiological stress induced above 19.5°C at the adult stage (Elliott, 1994). Warming water can induce physiological stress, resulting in decreased growth and impaired immune function (Borgwardt et al., 2020). In addition, increase in water temperature favours the development of T. bryosalmonae, while decreasing brown trout tolerance to infection and enhancing PKD symptoms and mortality (Okamura et al., 2011; Bruneaux et al., 2017; Waldner et al., 2021). PKD outbreaks are reported during the summer (corresponding to the warmest days), and mean summer temperature is expected to influence the severity of the outbreaks (Bruneaux et al., 2017). PKD may develop upon the first infection (i.e., in naive fish), and fish surviving the infection likely develop an adaptive immune response preventing disease development upon reinfection, so that young-of-the-year fish represent the most sensitive stage (Foott & Hedrick, 1987; Feist & Longshaw, 2006; Okamura et al., 2011). We therefore expected that parasite infection in colder sites would have few effects on brown trout populations, whereas high infection prevalence associated with high water temperature would act synergistically on juvenile brown trout intraspecific variation during the summer.

Brown trout sampling

We sampled brown trout populations in 15 sites located at the foothills of the Pyrenean mountains in southern France (Fig. 1). These sites were chosen based upon an extensive field study conducted in 2019, at first aiming at a balanced sampling design with cold, warm, infected and non-infected sites. In 2020, most of the 2019 non-infected (i.e., naive) sites became infected, but we ended up with a fair gradient of *T. bryosalmonae* infection prevalence from 0 to 100%, and a gradient of mean summer temperature from 14.5°C to 18.2°C (Table 1, Fig. 1, Fig. S1). These gradients were not significantly correlated (Spearman correlation test, *rho* = 0.37, *P* = 0.18). At each site, we electro-fished juvenile brown trout before and after the summer, once at the end of June 2020 (S1), and once at the end of September 2020 (S2). Between the two sessions, water temperature was recorded every 4h with HOBO® loggers. This period corresponds to the warmest days of the year that are most favourable to

PKD development (Fig. S2, Okamura et al., 2011; Waldner et al., 2021), and therefore represents the period at which the pressure imposed by the two stressors should be the strongest. We aimed at sampling as many fish as possible at each site and session, resulting in a total of 1046 brown trout (12 to 52 individuals per session and per site according to local abundances, Table S1). We targeted small juvenile trout (mean = 66mm, range = 35-95mm for S1 and mean = 90mm, range = 50–136mm for S2, Appendix S1), corresponding to young-of-the-year (0+) because this is the age at which brown trout are the most susceptible to PKD and water temperature (Elliott & Elliott, 2010; Okamura et al., 2011, see above). Juvenile brown trout density was estimated as the number of trout caught per minute using single-pass electrofishing surveys ("catch-per-unit-effort", CPUE; Hanks et al., 2018; Kruse et al., 1998; Table 1).

After all measures (excretion, anaesthesia, pictures, morphometry, and fin-clip, see hereafter), all fish were put in a bucket with stream water and air pumps to recover, before being released at the site of sampling.

Morphometric measures and fin-clip

We anaesthetised each fish with benzocaine before length and weight measurements to the nearest mm and the nearest 0.01g, respectively. We calculated fish body condition (K) according to equation (1) (Fulton, 1904), where W is fish mass in g, and L the fish length in cm (Table 1).

(1)
$$K = \frac{W}{L^3} \times 100$$

Infection prevalence

The infection prevalence by *T. bryosalmonae* at each site was assessed by examining the infection status of 20 randomly selected fish (or less when less individuals were available). The infection status was determined using the non-lethal urine DNA (uDNA) method developed in Duval et al. (2021) that quantifies parasite DNA excreted in fish urine. Briefly, after recovering for about 30 minutes, fish were placed individually into plastic bottles with commercial mineral water for 1 hour to let time for the infected fish to excrete pathogen spores or DNA through urine. We then filtered the water using a peristatic pump and extracted DNA from filters. Lastly, we used specific droplet digital PCRs (ddPCRs) to quantify *T. bryosalmonae* DNA (see Duval et al., 2021; 2022 for details). Population infection prevalence corresponds to the number of positive individuals for *T. bryosalmonae* DNA detection divided by the number of sampled individuals. We used uDNA to detect fish infection in September during S2 (i.e. after summer) to maximise the number of infected fish actually shedding parasite spores (Okamura et al., 2011; Ros et al., 2022).

Site	Infection prevalence (%)	Mean summer temperature (°C)	Density (N/min)	Δ _{density}	N _{SNPs}	$\Delta_{N SNPs}$	N _{MHC}	∆ _{N MHC}	H _{e-SNPs}	∆ _{He-SNPs}	Н _{е-МНС}	Δ _{<i>He-</i> MHC}	Mean <i>K</i>	Δκ	Mean carotenoid area (%)	∆ _{carotenoids}	Mean melanin area (%)	Δ _{melanin}
BOUSer	100	17.75	1.43	-26	262	-1.9	12	0	0.229	-0.9	0.820	-3.9	1.27	-8	0.9	5	37	23
			1.00		257		12		0.227		0.766		1.17		1.0		40	
ARRGou	100	16.63	0.74	-32	222	-2.7	16	-25	0.166	-1.2	0.866	-3.9	1.23	-4	1.6	58	47	-9
			0.50		216		12		0.164		0.832		1.17		2.5		43	
JOBEnc	90	16.49	0.63	15 234	234	0.4	19	-11	0.19	1.6	0.87	3.5	1.22	-0.78	1.6	39	43	9
			0.72		235		17		0.19		0.90		1.21		2.3		47	
TORTuz	89	17.44	0.69	-56	229 2	2.6	17	-18	0.20	1.5	0.89	-5.1	1.27	-13.18	0.8	210	41	33
			0.30		235		14		0.20		0.85		1.11	2.6		55		
ARZDur	85	17.41	1.13	-73 224	224	-2.7	9	-11	0.17	-1.2	0.82	-3.2	1.19	4.32	1.7	27	40	2
			0.31		218		8		0.17		0.80		1.24		2.2		41	
GARVil	78	17.21	0.36	-53	230	-2.6	15	-13	0.20	1.0	0.88	23	1.21	3.11	0.7	251	41	-4
			0.17		224		13		0.20		0.90	2.0	1.25		2.4		40	
ARIVar	67	17.33	0.53	-58	264	-23	16	0	0.23	-0.9	0.88	2.5	1.22	1.19	1.0	321	51	-17
			0.23		258	2.0	16		0.23	0.0	0.90	2.0	1.24	1.10	4.4	521	42	
SAL Tau	53	17.62	0.63	-72	227	1.8	17	-41	0.20	4.0	0.86	20	1.20	2 37	0.7	325	45	-14
SALTAU	55		0.18	-12	231	1.0	10	-41	0.21	4.0	0.88	2.0	1.23	2.07	2.8		39	-14
	50	18.23	0.70	45	235	-26	19	11	0.19	-1.0	0.90	2.3	1.25	-4 51	3.3	33	42	5
ANDIVIAII	50		0.38	-40	229	-2.0	21		0.19		0.92	2.5	1.19 -4.51	4.4		44	5	
CERRai	40	17.84	0.30	10	233	-1.7 21 17	21	-19	0.20	-2.9	0.93	1.4	1.17 1.50	1.9	101	44	10	
GERFOI	42	17.04	0.24	-19	229		17		0.20		0.92	-1.4	1.19	1.50	3.7	101	39	-12
ADDDor	22	15.29	0.97	40	230	1.2	10	50	0.184	E 4	0.819	4.5	1.23	c	0.5	402	47	0
ARBBar	22		0.56	-42	227	-1.3	15	50	0.194	5.4	0.856	4.5	1.30	0	2.6	403	51	0
ADOEN	00	17.00	1.04	0	245	0.0	18	47	0.22	0.0	0.891	0.0	1.10	0	0.8	460	48	40
ARGFO	20		1.10	250	250	2.0	21	17	0.21	-3.2	0.923	3.6	1.13	3	4.7	469	43	-10
4000	5	15.79	0.50	228	228		14	00	0.181	7.0	0.903	10	1.14		1.2		41	
ARGPon			0.35	-29	234 2.6	2.6	18	29	0.194	7.2	0.886	-1.9		11	11.4	834	54	32
ARZPon	-	16.76	0.88	217		8	8	0.154		0.699		1.21		1.5		45		
	5		0.58	-34	219	219 0.92	10	25	0.158	2.6	0.782	11.9	1.20	-1	6.0	297	46	2
GERAsp			2.17 1.95 -10		236 232 -1		15		0.187		0.859	-3.1	1.13		0.2		52	
	0	14.51		-10		-1.7	12	-20	0.181	-3.2	0.832		1.29	14	3.3	1286	50	-2

Table 1. Sampled sites and their measured parameters for the two sampling sessions, ranked by decreasing infection prevalence.

The infection prevalence was measured once at the second sampling session.

N_{SNPs}, N_{MHC}: Total number of alleles at the SNPs and MHC loci respectively.

H_{e-SNPs}, *H_{e-MHC}*: Expected heterozygosity at the SNPs and MHC loci respectively.

K: body condition factor.

94

 Δ : difference in % between the parameter measured in S2 and that measured in S1, see formula (2).



Figure 1. Map showing the 15 sampled sites and their infection prevalence by *T. bryosalmonae* as measured during the second sampling session in September 2020. The top-corner inset shows the location of the study area, in Southern France. Mean summer temperature (°C) is indicated in brackets.

Colouration measurement

We adapted a standardised protocol to measure brown trout carotenoid and melanin-based colouration based on previous studies (Wedekind et al., 2008; Jacquin et al., 2017). Briefly, we took a picture of the left side of each fish with a Canon® PowerShot G16 camera, under a white light cage to standardise light conditions. Brightness values of each image were then calibrated using a color checker of known standard values (X-Rite® ColorChecker Passport) to correct for potential differences in brightness between pictures using GIMP 2.10.8 (The GIMP Development Team, 2019). We then treated each image with ImageJ 1.52a (Schneider et al., 2012), defining a zone excluding the head and fins, and calculated the percentage of fish skin covered by black colouration (melanin-based colouration) and red colouration (carotenoid-based colouration, Table 1).

Neutral and adaptive genetic diversity

A small piece of pelvic fin was sampled and stored in 70% ethanol for individual genotyping. Individuals were genotyped using two marker types: (1) a set of 173 neutral SNPs evenly spread on the brown trout genome (Saint-Pé et al., 2019) and (2) the DAB-MHC exon 2 locus, a highly polymorphic gene involved in pathogen resistance in vertebrates including Salmonids (Dionne et al., 2007).

SNPs

After DNA extraction, SNP genotyping was performed using the KASPar allele-specific fluorescent genotyping system provided by the LGC® Group (UK). To identify potential genotyping errors, we ran tests for Hardy-Weinberg equilibrium (Hosking et al., 2004) with the *genepop* 1.1.7 package (Rousset, 2008) and removed from the analyses loci that departed from the equilibrium in all the populations in which they were polymorphs. In the end, we kept 144 polymorph SNPs loci (Appendix S2). Because at least one of the populations (GARVil) could be affected by hatchery fish stocking, we used *Structure* 2.3.4 (Pritchard et al., 2000) to compare each population with the genotype of individuals from neighbouring hatcheries to identify and remove stocked individuals from the dataset (representing a total of 31 individuals, Appendix S3). Neutral genetic diversity at each site in S1 and S2 was evaluated by computing the total number of alleles (N_{SNP}) and the expected heterozygosity (H_{e-SNP}) with the *poppr* package (Kamvar et al., 2014; Table 1). We used the number of alleles in a population rather than the allelic richness because it directly represents the extinction or apparition of genetic variants (Hoban et al., 2014).

МНС

We amplified and sequenced the second exon of the DAB-MHC locus (255 pb), encoding the ligandbinding domain of the protein. A single DAB locus has been reported salmonids (Stet et al., 2002). We used the primers TVS4501 5'-TCTGTATTATGTTTTCCTTCC-3' (Langefors et al., 2000) and ÅL1002 3'-CACCTGTCTTGTCCAGTATG-5' (Olsén et al., 1998), with the addition of a partial overhang Illumina sequencing primers in 5'-end. These primers were first designed for Atlantic salmon (Salmo salar) but have been successfully used for brown trout in previous studies (Forsberg et al., 2007; Jacob et al., 2010; Monzón-Argüello et al., 2014; Schenekar & Weiss, 2017). We used a two-step PCR approach with a dual-index paired-end sequencing approach (see Galan et al., 2018; Portanier et al., 2019) following the procedure detailed in Appendix S2. We used *obitools* (Boyer et al., 2016) to sort sequences, identify the haplotypes and discard artefactual variants, and genotype the individuals. We checked for the presence of codon sites affected by positive selection with the help of a maximumlikelihood analysis by using the programme codeML implemented in pamIX 1.3.1 software (Xu & Yang, 2013) as detailed in Schwensow et al. (2007). The analysis, run with our allele sequences aligned with 100 sequences retrieved on GenBank from previous studies (Shum et al., 2001; Forsberg et al., 2007; Jacob et al., 2010; Monzón-Argüello et al., 2014; Schenekar & Weiss, 2017), identified 22 positively selected codon sites that were used to define a total of 118 MHC alleles in our dataset. As for the SNPs, we computed the number of alleles (N_{MHC}) and the expected heterozygosity (H_{e-MHC}) at each site in S1 and S2 with the *poppr* package (Kamvar et al., 2014; Table 1).

Statistical analyses

To evaluate the variation of each parameter (body condition, melanin and carotenoid-based colouration, number of alleles and H_e for SNPs and MHC) between the two sampling sessions (S₁ and S₂) at each site, we used a delta statistic computed as follows:

(2)
$$\Delta_{parameter} = \frac{parameter S_2 - parameter S_1}{parameter S_1} \times 100$$

A negative delta value indicates a decrease in the parameter during the summer whereas a positive delta value indicates an increase. This metric allows to compare populations while taking into account their own initial values.

Then, to investigate the potential impacts of water temperature, infection prevalence and both stressors on these deltas, we used linear models using the following formula:

(3) $\Delta_{parameter} \sim temperature + prevalence + temperature: prevalence$

For each parameter, we then used a model selection procedure based on the small-sample size corrected Akaike Information Criterion (*AICc*, Burnham & Anderson, 2002) with the *MuMIn* package (Barton, 2020), to reveal potential effects that might be overriding because of the interaction effect and collinearity (even though the correlation between infection prevalence and water temperature was not significant, see above). We examined models with $\Delta AICc < 4$ relative to the best model and computed the relative importance (*RI*) of each variable as the cumulative weight of each model in which it appears (Burnham & Anderson, 2002). The cumulative weight of the model selection with $\Delta AICc < 4$ was standardised so that the *RI* of each term varied between 0 and 1. We considered that a variable was relevant when *RI* > 0.5, and corresponding variables were thereafter included in the final model used to infer their estimates (De Kort et al., 2021). All statistical analyses were conducted in the R environment (R 4.1.2; R Core Team, 2021).

Results

Variation in population density

The variation in population density between the two sampling sessions was poorly explained by the mean summer water temperature and the infection prevalence (RI < 0.5 for both variables, Table 2). However, three highly infected populations were identified as outliers in this relationship, and they corresponded to already highly infected populations the previous year (infection prevalence > 90% in 2019, see Fig. S1, Fig. 2a). After removing these three populations, the final model showed a marked negative effect of the infection prevalence on the variation in density (RI = 1, Table 2; $R^2_{adj} = 0.52$, P =




Figure 2. Variation in brown trout density between the two sampling sessions according to the infection prevalence in the different sites (**a**) including all the sampled sites and (**b**) after removing 3 sites (red points in (**a**)) that were already infected with a high prevalence in 2019 (> 90%, Fig. S1).

Delta	Infection prevalence	Water temperature	Infection prevalence : Water temperature		
Density	0.18	0.31	0.00		
Density (12 sites) ^a	1.00	0.00	0.00		
Number of SNP alleles	0.31	0.12	0.00		
Number of MHC alleles	0.63	0.20	0.00		
SNP heterozygosity	0.22	0.19	0.00		
MHC heterozygosity	0.63	0.38	0.23		
Body condition	0.67	0.73	0.00		
Carotenoid area	1.00	1.00	0.80		
Melanin area	0.15	0.16	0.00		

Table 2. Relative importance of the variables in the model selection $\Delta AICc < 4$.

^a Density delta after removing 3 populations with very high infection prevalence in 2019. Terms with relative importance > 0.50 indicated in bold were used in the final

Variation in genetic diversity

We did not observe any effect of the infection prevalence, the water temperature and their interaction on the variation in neutral genetic diversity across sessions (i.e., the number of alleles or the expected heterozygosity at the SNPs loci; RI < 0.5, Table 2, Fig. S3).

By contrast, we found relationships between the variation in the number of alleles and the expected heterozygosity at the MHC (indices of adaptive genetic diversity) and the infection prevalence (RI = 0.63 for both N_{MHC} and H_{e-MHC} , Table 2). There was a significant decrease in the number of alleles at the MHC with infection prevalence (Fig.3a, $R^2_{adj} = 0.21$, P = 0.048). The MHC heterozygosity also tended to diminish when the infection prevalence increased (Fig. 3b, $R^2_{adj} = 0.13$, P = 0.098).



Figure 3. Variation in the genetic diversity at the MHC locus according to the infection prevalence and the water temperature. (a) Variation in the number of alleles. (b) Variation in expected heterozygosity.

Variation in body condition and colouration

Variation in body condition between sessions was related to the infection prevalence and the mean summer water temperature (RI = 0.67 and 0.73 respectively, Table 2, $R^2_{adj} = 0.53$, P = 0.004). Fish body condition decreased in sites with a high infection prevalence and/or a high water temperature (Fig. 4a, Fig. 4b). Carotenoid-based colouration was affected by infection prevalence, mean summer water temperature and their interaction (RI > 0.5 for the 3 variables, Table 2, $R^2_{adj} = 0.81$, P < 0.001). More specifically, fish carotenoid-based colouration at cold and non-infected sites sharply increased during the summer, while non-infected warmer sites showed a smaller increase in carotenoid-based colouration. However, the difference in carotenoid-based colouration enrichment during summer between cold and warm sites was erased with increasing infection prevalence (Fig. 4c). In contrast, melanin-based colouration was not affected by either stressor (Table 2, Fig. S3).



Figure 4. Variation in phenotypic traits related to the individual fitness. (a) Variation in the mean body condition according to the infection prevalence (with fixed value for water temperature) and (b) to the water temperature (with fixed value for infection prevalence). (a) and (b) illustrate the same model output ($\Delta_{body \ condition} \sim infection \ prevalence + water \ temperature, R^2adj = 0.53, P = 0.004$). (c) Variation in the mean area of carotenoids according to the infection prevalence and its interaction with the water temperature.

Discussion

Most studies investigating the evolution of intra-specific variation of wild populations before and after their exposure to stressful conditions focus on either the genetic or the phenotypic level but rarely investigate both levels in parallel. Furthermore, multi-stressor approaches are still scarce, although stressors often interact in complex and sometimes unexpected ways (Spears et al., 2021). In this study, we investigated the combined impact of summer water temperature and parasite infection on wild brown trout populations density and intraspecific variation at genetic and phenotypic levels. Brown trout

density decreased with increasing *T. bryosalmonae* parasite prevalence, except for populations that were already highly infected the year before. We also found a decline of genetic diversity at a MHC gene involved in adaptive immune response in infected populations, and highlighted a combined effect of infection and temperature on the alteration of phenotypic traits related to the fitness.

Impacts of temperature and infection on brown trout density

As expected, we found a stronger decrease in brown trout density with increasing prevalence, except for three highly infected populations whose densities only slightly varied over the summer. These three populations were already highly infected in 2019 (infection prevalence > 90%, Fig. S1), suggesting that the parasite infection might have a greater impact in "naive" populations. This is an important result suggesting that infected populations may suffer large population declines (up to 90% young-of-the-year (YOY) mortality reported in wild populations; Schager et al., 2007), but that an attenuation of population declines may occur in the years following first high infection, therefore exhibiting transient dynamics, that is, dynamics (here high declines) that are different from the long-term dynamics (e.g., stabilisation likely due to micro-evolution and adaptation, see below) (Hastings, 2004; Briggs et al., 2010; Wells et al., 2019). Two non-exclusive mechanisms could explain the reduced mortality in populations that had already suffered a major PKD outbreak the summer before. Here, we worked on YOY individuals at their first encounter with the parasite. First, a strong directional selection might have already operated on breeders, leaving only the most resistant or tolerant individuals for reproduction. This would result in a higher resistance/tolerance in their offspring if these traits are genetically determined and heritable (McKnight et al., 2017). It has indeed been suggested that when a parasite is highly pathogenic, the disease dynamics could be mainly driven by host characteristics rather than parasite virulence, likely through the selection of adaptive host genotypes and the rapid micro-evolution of host resistance or tolerance (Voyles et al., 2018). A second non-exclusive mechanism could be an induction of immune response in juveniles which could be facilitated by epigenetic responses or by maternal transfer of immunity (Gasparini et al., 2002; Hasselquist & Nilsson, 2009), which is a common mechanism in fish (e.g., in rainbow trout (Oncorhynchus mykiss), Oshima et al., 1996; and tilapias (Oreochromis niloticus), Nurani et al., 2020). For this mechanism to be efficient in protecting a population from an outbreak, a large proportion of the breeding females would have to be infected (Boulinier & Staszewski, 2008), which could potentially explain why populations previously infected but at a low prevalence declined. Such rapid micro-evolutionary response could be favoured by the homing behavior in brown trout (Hansen et al., 2002). Further studies on the underpinning mechanisms are now necessary to conclude on the relative importance of the genetic and maternal source of heritability of pathogen resistance/tolerance.

Nonetheless, part of these results could also translate juvenile movements during summer, i.e., emigration or immigration of fish individuals in the studied populations. Indeed, rivers are dendritic ecological networks where fish populations are connected by migration (Paz-Vinas & Blanchet, 2015). Brown trout juveniles are known to disperse downstream during summer over short distances (~200m) (Jenkins et al., 1999; Vøllestad et al., 2012), as illustrated by the appearance of alleles in some of this study's populations after summer (positive delta for the number of alleles). Therefore, potential mortality due to water temperature/and or parasite infection may be partly offset by the colonisation of new individuals from neighbouring populations (e.g., Hanski, 1998; Carlson et al., 2014). In this case, immigrating individuals entering the population could potentially compensate mortality and increase population viability, resulting in little net change in density over time. Further studies quantifying migration between connected populations are thus necessary to better understand the role of dispersal in the eco-evolutionary rescue from an emerging parasite threat (Thrall & Burdon, 1997; Jiao et al., 2020).

Impacts of temperature and infection on genetic variation

In line with a potential compensation of mortality through immigration, we did not detect any erosion of the neutral genetic diversity at the sites under high stressor pressure. The neutral genetic variation measured either through alleles number or expected heterozygosity remained stable across the summer for all the sampled populations. Accordingly, other studies in the wild have found that population declines were not systematically accompanied by declines in genetic diversity (Lachish et al., 2011; Vazquez et al., 2019).

However, we observed a different pattern for immunity-related genetic variation with a decrease in allele number at the MHC locus in highly infected populations. This result may illustrate a potential directional selection of advantageous alleles that could in turn lead to the observed decrease of the expected number of heterozygous individuals (Nielsen, 2005). This result is consistent with previous studies showing rapid and adaptive evolution of MHC genes in response to pathogen-mediated selective pressures (Eizaguirre et al., 2012; Phillips et al., 2018), including in salmonids (*Oncorhynchus mykiss*) showing rapid natural selection for resistance to *Myxobolus cerebralis*, another myxozoan parasite (Miller & Vincent, 2008), and wild brown trout exposed to *T. bryosalmonae* (Debes et al., 2017).

Impacts of stressors on phenotypic variation: body condition

A major result of this study was that both infection prevalence and elevated summer water temperature had independent negative effects on fish body condition. Summer is an important period for food intake and growth in brown trout (Kelly-Quinn & Bracken, 1990; Marsh et al., 2022). Elevated summer water temperature could decrease feeding efficiency by increasing the portion of time spent above the optimal

temperature for feeding (Elliott & Elliott, 2010; Parra et al., 2012). Brown trout adults stop feeding at 19.5°C, and small individuals are known to be more sensitive to temperature (Elliott & Elliott, 2010). The temperature of our sampled sites often reached 19.5°C (Fig. S2), and the time spent above this threshold was indeed highly correlated with the mean temperature (Fig. S2, Fig. S4).

In addition, we found a negative effect of infection on body condition, which could potentially be explained by the cost of immunity and a trade-off with other costly functions such as maintenance and growth in infected individuals (Rauw, 2012). This would validate the phenotypic plasticity hypothesis modulating this underpinning resource trade-off. Accordingly, a negative impact of *T. bryosalmonae* infection was found on the variation in body condition throughout summer in farmed Atlantic salmon (Cauwelier et al., 2010).

These negative effects of temperature and infection on body condition could have important effects on individual fitness later in life. Indeed, individuals experiencing good environmental conditions in their early life may have a higher fitness later in life (reproduction and survival) than those experiencing poor conditions (illustrating the silver spoon effect; Grafen, 1988; e.g., in Vasilieva & Tchabovsky, 2020). Negative effects of high infection and/or high water temperature on body condition and growth during the first summer of juvenile trout may thus have long-term effect on over-winter survival, size at all age and adult future reproduction (Biro et al., 2004; Parra et al., 2009; Ros et al., 2022).

Impacts of stressors on phenotypic variation: colouration

Interestingly, both stressors (infection prevalence and water temperature) had interacting effects on the carotenoid-based skin colouration. Carotenoids cannot be synthetised by animals and are acquired through the diet (Price et al., 2008). Generally, carotenoid availability is expected to increase during summer due to enhanced river productivity, thereby enhancing brown trout carotenoid coloration. Accordingly, the coldest non-infected sites showed a great increase in carotenoid-based colouration during the summer. At the warmest sites, the carotenoid-based colouration remained stable during summer, whatever the infection prevalence (i.e., the water temperature stressor alone may have limited the gain in carotenoid-based colouration enrichment during summer between cold and warm sites decreased with increasing infection prevalence. This suggests that the use of carotenoid pigments for colouration is already limited by temperature at warm sites, and that infection prevalence plays a more important role in carotenoid-based colouration (and/or that infection prevalence suppresses the effects of infection on colouration (and/or that infection prevalence suppresses the benefiting effect of cold temperature). This could be explained by the fact that carotenoid pigments are involved both in immunity and ornamentation, and there is a presumed trade-off between carotenoid

colouration and immunity (Lozano, 1994; Clotfelter et al., 2007; Baeta et al., 2008). Infected individuals might thus lack of carotenoid pigments to allocate to their pigmentation (Blount, 2004). In addition, the interaction between infection and temperature, and the suppressed infection effect at high temperature, suggests antagonistic interaction between both stressors (i.e., one stressor decreases the effect of another stressor, Jackson et al., 2016; Johnson & Penaluna, 2019). Higher food availability at warmer sites could offset the effects of infection prevalence on colouration if individuals are able to compensate for the cost of immunity by increasing food intake (Sheldon & Verhulst, 1996). This result would thus also corroborate the phenotypic plasticity hypothesis.

In contrast, we did not find any relationship between the changes in melanin-based coloration during the summer and the two environmental stressors. This could be explained by the fact that skin coloration in melanin is more genetically determined and less condition-dependent than the coloration in carotenoids (e.g. in Hill & Brawner, 1998; Roulin, 2016).

Conclusion and perspectives

Altogether, our results suggest that *T. bryosalmonae* may have a severe impact on wild brown trout populations with consequences on density in naive populations and on intraspecific variation at both genetic and phenotypic levels. Results also suggest an attenuation of the infection effects on population density in the years following high infection prevalence. The mechanisms involved in this apparent rapid adaptation remain to be clarified but our study emphasises the importance of standing genetic variation, especially at adaptive loci (Eizaguirre et al., 2012; Phillips et al., 2018) and/or of the potential beneficial effect of migration from neighbouring population bringing new individuals and genes (genetic rescue; Tallmon et al., 2004).

In addition, water temperature and infection prevalence both had important effects on phenotypic intraspecific variation in body condition and carotenoid-based coloration, which suggests a plastic response and a reallocation of resources between fitness-related traits (maintenance, immunity and coloration) under adverse environmental conditions. Besides, we found interactive effects of both stressors on carotenoid-based colouration, suggesting a complex antagonistic combined effect. These early effects on juvenile phenotype may have long-lasting effects on individual fitness including future reproduction and survival to other stressors (Lindström, 1999; Pigeon & Pelletier, 2018). These results suggest a directional parasite-mediated selection operating at an adaptive locus involved in immune response, and a plastic response to multiple stressors of phenotypes probably linked to resource trade-offs. A population monitoring before and after disease outbreaks for several consecutive years, together with the investigation of small-scale dispersal, may help to confirm possible rapid micro-evolution in genetic and phenotypic variation and their long-term effects (Royle et al., 2018; Vredenburg et al., 2019).

Literature cited

- Albert, E. M., Fernández-Beaskoetxea, S., Godoy, J. A., Tobler, U., Schmidt, B. R., & Bosch, J. (2015). Genetic management of an amphibian population after a chytridiomycosis outbreak. *Conservation Genetics*, 16(1), 103–111. https://doi.org/10.1007/s10592-014-0644-6
- Altizer, S., Harvell, D., & Friedle, E. (2003). Rapid evolutionary dynamics and disease threats to biodiversity. *Trends in Ecology & Evolution*, 18(11), 589–596. https://doi.org/10.1016/j.tree.2003.08.013
- Altizer, S., Ostfeld, R. S., Johnson, P. T. J., Kutz, S., & Harvell, C. D. (2013). Climate Change and Infectious Diseases: From Evidence to a Predictive Framework. *Science*, 341(6145), 514–519. https://doi.org/10.1126/science.1239401
- Auteri, G. G., & Knowles, L. L. (2020). Decimated little brown bats show potential for adaptive change. *Scientific Reports*, 10(1), 3023. https://doi.org/10.1038/s41598-020-59797-4
- Baeta, R., Faivre, B., Motreuil, S., Gaillard, M., & Moreau, J. (2008). Carotenoid trade-off between parasitic resistance and sexual display: An experimental study in the blackbird (*Turdus merula*). *Proceedings of the Royal Society B: Biological Sciences*, 275(1633), 427–434. https://doi.org/10.1098/rspb.2007.1383
- Bailey, C., Segner, H., Casanova-Nakayama, A., & Wahli, T. (2017). Who needs the hotspot? The effect of temperature on the fish host immune response to *Tetracapsuloides bryosalmonae* the causative agent of proliferative kidney disease. *Fish & Shellfish Immunology*, 63, 424–437. https://doi.org/10.1016/j.fsi.2017.02.039
- Banks, S. C., Cary, G. J., Smith, A. L., Davies, I. D., Driscoll, D. A., Gill, A. M., Lindenmayer, D. B., & Peakall, R. (2013). How does ecological disturbance influence genetic diversity? *Trends in Ecology & Evolution*, 28(11), 670–679. https://doi.org/10.1016/j.tree.2013.08.005
- Barton, K. (2020). *MuMIn: Multi-Model Inference. R package version 1.43.17.* https://CRAN.R-project.org/package=MuMIn
- Bernatchez, L., & Landry, C. (2003). MHC studies in nonmodel vertebrates: What have we learned about natural selection in 15 years?: MHC studies in nonmodel vertebrates. *Journal of Evolutionary Biology*, 16(3), 363–377. https://doi.org/10.1046/j.1420-9101.2003.00531.x
- Biro, P. A., Morton, A. E., Post, J. R., & Parkinson, E. A. (2004). Over-winter lipid depletion and mortality of age-0 rainbow trout (*Oncorhynchus mykiss*). *Canadian Journal of Fisheries and Aquatic Sciences*, 61, 7.
- Blondel, L., Paterson, I. G., Bentzen, P., & Hendry, A. P. (2021). Resistance and resilience of genetic and phenotypic diversity to "black swan" flood events: A retrospective analysis with historical samples of guppies. *Molecular Ecology*, 30(4), 1017–1028. https://doi.org/10.1111/mec.15782
- Blount, J. D. (2004). Carotenoids and life-history evolution in animals. *Archives of Biochemistry and Biophysics*, 430(1), 10–15. https://doi.org/10.1016/j.abb.2004.03.039
- Borgwardt, F., Unfer, G., Auer, S., Waldner, K., El-Matbouli, M., & Bechter, T. (2020). Direct and Indirect Climate Change Impacts on Brown Trout in Central Europe: How Thermal Regimes Reinforce Physiological Stress and Support the Emergence of Diseases. *Frontiers in Environmental Science*, 8, 59. https://doi.org/10.3389/fenvs.2020.00059
- Boulinier, T., & Staszewski, V. (2008). Maternal transfer of antibodies: Raising immuno-ecology issues. *Trends in Ecology & Evolution*, 23(5), 282–288. https://doi.org/10.1016/j.tree.2007.12.006
- Boyer, F., Mercier, C., Bonin, A., Le Bras, Y., Taberlet, P., & Coissac, E. (2016). OBITOOLS: a UNIXinspired software package for DNA metabarcoding. *Molecular Ecology Resources*, 16, 176– 182.
- Briggs, C. J., Knapp, R. A., & Vredenburg, V. T. (2010). Enzootic and epizootic dynamics of the chytrid fungal pathogen of amphibians. *Proceedings of the National Academy of Sciences*, 107(21), 9695–9700. https://doi.org/10.1073/pnas.0912886107
- Brown, M. J. F., Schmid-Hempel, R., & Schmid-Hempel, P. (2003). Strong context-dependent virulence in a host–parasite system: Reconciling genetic evidence with theory. *Journal of Animal Ecology*, 72, 994–1002.
- Bruneaux, M., Visse, M., Gross, R., Pukk, L., Saks, L., & Vasemägi, A. (2017). Parasite infection and decreased thermal tolerance: Impact of proliferative kidney disease on a wild salmonid fish in

the context of climate change. *Functional Ecology*, *31*(1), 216–226. https://doi.org/10.1111/1365-2435.12701

- Burnham, K. P., & Anderson, D. R. (2002). *Model selection and multimodel inference: A practical information-theoretic approach* (2nd ed). Springer.
- Campbell-Staton, S. C., Cheviron, Z. A., Rochette, N., Catchen, J., Losos, J. B., & Edwards, S. V. (2017). Winter storms drive rapid phenotypic, regulatory, and genomic shifts in the green anole lizard. *Science*, 357(6350), 495–498. https://doi.org/10.1126/science.aam5512
- Canale, C., & Henry, P. (2010). Adaptive phenotypic plasticity and resilience of vertebrates to increasing climatic unpredictability. *Climate Research*, *43*(1), 135–147. https://doi.org/10.3354/cr00897
- Carlson, S. M., Cunningham, C. J., & Westley, P. A. H. (2014). Evolutionary rescue in a changing world. *Trends in Ecology & Evolution*, 29(9), 521–530. https://doi.org/10.1016/j.tree.2014.06.005
- Carraro, L., Mari, L., Hartikainen, H., Strepparava, N., Wahli, T., Jokela, J., Gatto, M., Rinaldo, A., & Bertuzzo, E. (2016). An epidemiological model for proliferative kidney disease in salmonid populations. *Parasites & Vectors*, 9(1). https://doi.org/10.1186/s13071-016-1759-z
- Cauwelier, E., Gilbey, J., Jones, C., Noble, L., & Verspoor, E. (2010). Genotypic and phenotypic correlates with proliferative kidney disease-induced mortality in Atlantic salmon. *Diseases of Aquatic Organisms*, 89, 125–135. https://doi.org/10.3354/dao02191
- Clotfelter, E. D., Ardia, D. R., & McGraw, K. J. (2007). Red fish, blue fish: Trade-offs between pigmentation and immunity in Betta splendens. *Behavioral Ecology*, *18*(6), 1139–1145. https://doi.org/10.1093/beheco/arm090
- Crozier, L. G., & Hutchings, J. A. (2014). Plastic and evolutionary responses to climate change in fish. *Evolutionary Applications*, 7(1), 68–87. https://doi.org/10.1111/eva.12135
- Cucherousset, J., Ombredane, D., Charles, K., Marchand, F., & Baglinière, J.-L. (2005). A continuum of life history tactics in a brown trout (*Salmo trutta*) population. *Canadian Journal of Fisheries and Aquatic Sciences*, 62(7), 1600–1610. https://doi.org/10.1139/f05-057
- De Kort, H., Prunier, J. G., Ducatez, S., Honnay, O., Baguette, M., Stevens, V. M., & Blanchet, S. (2021). Life history, climate and biogeography interactively affect worldwide genetic diversity of plant and animal populations. *Nature Communications*, *12*(1), 516. https://doi.org/10.1038/s41467-021-20958-2
- Debes, P. V., Gross, R., & Vasemägi, A. (2017). Quantitative Genetic Variation in, and Environmental Effects on, Pathogen Resistance and Temperature-Dependent Disease Severity in a Wild Trout. *The American Naturalist*, *190*(2), 244–265. https://doi.org/10.1086/692536
- Dionne, M., Miller, K. M., Dodson, J. J., Caron, F., & Bernatchez, L. (2007). Clinal variation in MHC diversity with temperature: Evidence for the role of host? Pathogen interaction on local adaptation in Atlantic salmon. *Evolution*, *61*(9), 2154–2164. https://doi.org/10.1111/j.1558-5646.2007.00178.x
- Donihue, C. M., Herrel, A., Fabre, A.-C., Kamath, A., Geneva, A. J., Schoener, T. W., Kolbe, J. J., & Losos, J. B. (2018). Hurricane-induced selection on the morphology of an island lizard. *Nature*, *560*(7716), 88–91. https://doi.org/10.1038/s41586-018-0352-3
- Ducrest, A., Keller, L., & Roulin, A. (2008). Pleiotropy in the melanocortin system, coloration and behavioural syndromes. *Trends in Ecology & Evolution*, 23(9), 502–510. https://doi.org/10.1016/j.tree.2008.06.001
- Duval, E., Blanchet, S., Quéméré, E., Jacquin, L., Veyssière, C., Lautraite, A., Garmendia, L., Yotte, A., Parthuisot, N., Côte, J., & Loot, G. (2021). Urine DNA (uDNA) as a non-lethal method for endoparasite biomonitoring: Development and validation. *Environmental DNA*, edn3.228. https://doi.org/10.1002/edn3.228
- Duval, E., Quéméré, E., Loot, G., Jacquin, L., Veyssière, C., & Blanchet, S. (2022). A multifaceted index of population health to detect risk-prone populations and underlying stressors in wildlife. *Biological Conservation*, 274, https://doi.org/10.1016/j.biocon.2022.109706
- Easterling, D. R., Meehl, G. A., Parmesan, C., Changnon, S. A., Karl, T. R., & Mearns, L. O. (2000). Climate Extremes: Observations, Modeling, and Impacts. *Science*, 289(5487), 2068–2074. https://doi.org/10.1126/science.289.5487.2068

- Eizaguirre, C., Lenz, T. L., Kalbe, M., & Milinski, M. (2012). Rapid and adaptive evolution of MHC genes under parasite selection in experimental vertebrate populations. *Nature Communications*, *3*(1), 621. https://doi.org/10.1038/ncomms1632
- Elliott, J. M. (1994). Quantitative Ecology and the Brown Trout (Oxford University Press).
- Elliott, J. M., & Elliott, J. A. (2010). Temperature requirements of Atlantic salmon *Salmo salar*, brown trout *Salmo trutta* and Arctic charr *Salvelinus alpinus*: Predicting the effects of climate change. *Journal of Fish Biology*, 25.
- Epstein, B., Jones, M., Hamede, R., Hendricks, S., McCallum, H., Murchison, E. P., Schönfeld, B., Wiench, C., Hohenlohe, P., & Storfer, A. (2016). Rapid evolutionary response to a transmissible cancer in Tasmanian devils. *Nature Communications*, 7(1), 12684. https://doi.org/10.1038/ncomms12684
- Ezenwa, V. O., & Jolles, A. E. (2008). Horns honestly advertise parasite infection in male and female African buffalo. *Animal Behaviour*, 75(6), 2013–2021. https://doi.org/10.1016/j.anbehav.2007.12.013
- Faivre, B., Grégoire, A., Préault, M., Cézilly, F., & Sorci, G. (2003). Immune Activation Rapidly Mirrored in a Secondary Sexual Trait. Science, 300(5616), 103–103. https://doi.org/10.1126/science.1081802
- Feist, S. W., & Longshaw, M. (2006). Phylum Myxozoa. In P. T. K. Woo, *Fish diseases and disorders* (CAB International, pp. 230–296).
- Fisher, M. C., & Garner, T. W. J. (2020). Chytrid fungi and global amphibian declines. *Nature Reviews Microbiology*, *18*(6), 332–343. https://doi.org/10.1038/s41579-020-0335-x
- Folstad, I., & Karter, A. J. (1992). Parasites, Bright Males, and the Immunocompetence Handicap. *The American Naturalist*, *139*(3), 603–622. https://doi.org/10.1086/285346
- Foott, J. S., & Hedrick, R. P. (1987). Seasonal occurrence of the infectious stage of proliferative kidney disease (PKD) and resistance of rainbow trout, *Salmo gairdneri* Richardson, to reinfection. *Journal of Fish Biology*, 30(4), 477–483. https://doi.org/10.1111/j.1095-8649.1987.tb05771.x
- Forsberg, L. A., Dannewitz, J., Petersson, E., & Grahn, M. (2007). Influence of genetic dissimilarity in the reproductive success and mate choice of brown trout—Females fishing for optimal MHC dissimilarity. *Journal of Evolutionary Biology*, 20(5), 1859–1869. https://doi.org/10.1111/j.1420-9101.2007.01380.x
- Fox, R. J., Donelson, J. M., Schunter, C., Ravasi, T., & Gaitán-Espitia, J. D. (2019). Beyond buying time: The role of plasticity in phenotypic adaptation to rapid environmental change. *Philosophical Transactions of the Royal Society B: Biological Sciences*, 374(1768), 20180174. https://doi.org/10.1098/rstb.2018.0174
- Fulton, T. W. (1904). The rate of growth of fishes. Twenty-Second Annual Report, pp 141-241.
- Galan, M., Pons, J.-B., Tournayre, O., Pierre, É., Leuchtmann, M., Pontier, D., & Charbonnel, N. (2018).
 Metabarcoding for the parallel identification of several hundred predators and their prey: Application to bat species diet analysis. *Molecular Ecology Resources*, 18(3), 474–489. https://doi.org/10.1111/1755-0998.12749
- Gallana, M., Ryser-Degiorgis, M.-P., Wahli, T., & Segner, H. (2013). Climate change and infectious diseases of wildlife: Altered interactions between pathogens, vectors and hosts. *Current Zoology*, 59(3), 427–437.
- Gasparini, J., McCoy, K. D., Tveraa, T., & Boulinier, T. (2002). Related concentrations of specific immunoglobulins against the Lyme disease agent *Borrelia burgdorferi* sensu lato in eggs, young and adults of the kittiwake (*Rissa tridactyla*). *Ecology Letters*, 5(4), 519–524. https://doi.org/10.1046/j.1461-0248.2002.00345.x
- Gehman, A.-L. M., Hall, R. J., & Byers, J. E. (2018). Host and parasite thermal ecology jointly determine the effect of climate warming on epidemic dynamics. *Proceedings of the National Academy of Sciences*, *115*(4), 744–749. https://doi.org/10.1073/pnas.1705067115
- Gignoux-Wolfsohn, S. A., Pinsky, M. L., Kerwin, K., Herzog, C., Hall, M., Bennett, A. B., Fefferman, N. H., & Maslo, B. (2021). Genomic signatures of selection in bats surviving white-nose syndrome. *Molecular Ecology*, 30(22), 5643–5657. https://doi.org/10.1111/mec.15813
- Grafen, A. (1988). On the Uses of Data on Lifetime Reproductive Success. In T. H. Clutton-Brock, *Reproductive success* (University of Chicago Press, pp. 454–471).

- Grant, P. R., Grant, B. R., Huey, R. B., Johnson, M. T. J., Knoll, A. H., & Schmitt, J. (2017). Evolution caused by extreme events. *Philosophical Transactions of the Royal Society B: Biological Sciences*, 372(1723), 20160146. https://doi.org/10.1098/rstb.2016.0146
- Hadfield, J. D. (2008). Estimating evolutionary parameters when viability selection is operating. *Proceedings of the Royal Society B: Biological Sciences*, 275(1635), 723–734. https://doi.org/10.1098/rspb.2007.1013
- Hanks, R. D., Kanno, Y., & Rash, J. M. (2018). Can Single-Pass Electrofishing Replace Three-Pass Depletion for Population Trend Detection? *Transactions of the American Fisheries Society*, 147(4), 729–739. https://doi.org/10.1002/tafs.10061
- Hansen, M. M., Ruzzante, D. E., Nielsen, E. E., Bekkevold, D., & Mensberg, K.-L. D. (2002). Longterm effective population sizes, temporal stability of genetic composition and potential for local adaptation in anadromous brown trout (*Salmo trutta*) populations. *Molecular Ecology*, 11, 2523–2535.
- Hanski, I. (1998). Metapopulation dynamics. Nature, 396(396), 41-49.
- Harvell, C. D., Mitchell, C. E., Ward, J. R., Altizer, S., Dobson, A. P., Ostfeld, R. S., & Samuel, M. D. (2002). Climate Warming and Disease Risks for Terrestrial and Marine Biota. *Science*, 296(5576), 2158–2162. https://doi.org/10.1126/science.1063699
- Hasselquist, D., & Nilsson, J.-Å. (2009). Maternal transfer of antibodies in vertebrates: Transgenerational effects on offspring immunity. *Philosophical Transactions of the Royal Society B: Biological Sciences*, 364(1513), 51–60. https://doi.org/10.1098/rstb.2008.0137
- Hastings, A. (2004). Transients: The key to long-term ecological understanding? *Trends in Ecology & Evolution*, 19(1), 39–45. https://doi.org/10.1016/j.tree.2003.09.007
- Hendry, A. P., & Kinnison, M. T. (1999). Perspective: The pace of modern life: Measuring rates of contemporary microevolution. *Evolution*, 53(6), 1637–1653. https://doi.org/10.1111/j.1558-5646.1999.tb04550.x
- Hill, G. E., & Brawner, W. R. (1998). Melanin-based plumage coloration in the house finch is unaffected by coccidial infection. *Proceedings of the Royal Society of London. Series B: Biological Sciences*, 265(1401), 1105–1109. https://doi.org/10.1098/rspb.1998.0405
- Hoban, S., Arntzen, J. A., Bruford, M. W., Godoy, J. A., Rus Hoelzel, A., Segelbacher, G., Vilà, C., & Bertorelle, G. (2014). Comparative evaluation of potential indicators and temporal sampling protocols for monitoring genetic erosion. *Evolutionary Applications*, 7(9), 984–998. https://doi.org/10.1111/eva.12197
- Hoekstra, H. E., Hoekstra, J. M., Berrigan, D., Vignieri, S. N., Hoang, A., Hill, C. E., Beerli, P., & Kingsolver, J. G. (2001). Strength and tempo of directional selection in the wild. *Proceedings* of the National Academy of Sciences, 98(16), 9157–9160. https://doi.org/10.1073/pnas.161281098
- Hoffmann, A. A., & Willi, Y. (2008). Detecting genetic responses to environmental change. *Nature Reviews Genetics*, 9(6), 421–432. https://doi.org/10.1038/nrg2339
- Hosking, L., Lumsden, S., Lewis, K., Yeo, A., McCarthy, L., Bansal, A., Riley, J., Purvis, I., & Xu, C.-F. (2004). Detection of genotyping errors by Hardy–Weinberg equilibrium testing. *European Journal of Human Genetics*, 12(5), 395–399. https://doi.org/10.1038/sj.ejhg.5201164
- Hoyt, J. R., Kilpatrick, A. M., & Langwig, K. E. (2021). Ecology and impacts of white-nose syndrome on bats. *Nature Reviews Microbiology*, 19(3), 196–210. https://doi.org/10.1038/s41579-020-00493-5
- Jackson, M. C., Loewen, C. J. G., Vinebrooke, R. D., & Chimimba, C. T. (2016). Net effects of multiple stressors in frehwater ecosystems: A meta-analysis. *Global Change Biology*, 22, 180–189.
- Jacob, A., Evanno, G., Von Siebenthal, B. A., Grossen, C., & Wedekind, C. (2010). Effects of different mating scenarios on embryo viability in brown trout. *Molecular Ecology*, 19(23), 5296–5307. https://doi.org/10.1111/j.1365-294X.2010.04884.x
- Jacquin, L., Gauthey, Z., Roussille, V., Le Hénaff, M., Tentelier, C., & Labonne, J. (2017). Melanin in a changing world: Brown trout coloration reflects alternative reproductive strategies in variable environments. *Behavioral Ecology*, 28(6), 1423–1434. https://doi.org/10.1093/beheco/arx102
- Jenkins, T. M., Diehl, S., Kratz, K. W., & Cooper, S. D. (1999). Effects of population density on individual growth of brown trout in streams. *Ecology*, 80(3), 941–956. https://doi.org/10.1890/0012-9658(1999)080[0941:EOPDOI]2.0.CO;2

- Jiao, J., Gilchrist, M. A., & Fefferman, Nina. H. (2020). The impact of host metapopulation structure on short-term evolutionary rescue in the face of a novel pathogenic threat. *Global Ecology and Conservation*, 23, e01174. https://doi.org/10.1016/j.gecco.2020.e01174
- Johnson, P. T. J., Townsend, A. R., Cleveland, C. C., Glibert, P. M., Howarth, R. W., McKenzie, V. J., Rejmankova, E., & Ward, M. H. (2010). Linking environmental nutrient enrichment and disease emergence in humans and wildlife. *Ecological Applications*, 20(1), 16–29. https://doi.org/10.1890/08-0633.1
- Johnson, S. L., & Penaluna, B. E. (2019). Climate Change and Interactions With Multiple Stressors in Rivers. In *Multiple Stressors in River Ecosystems* (pp. 23–44). Elsevier. https://doi.org/10.1016/B978-0-12-811713-2.00002-9
- Kamvar, Z. N., Tabima, J. F., & Grünwald, N. J. (2014). Poppr: An R package for genetic analysis of populations with clonal, partially clonal, and/or sexual reproduction. *PeerJ*, 2:e281. https://doi.org/10.7717/peerj.281
- Kelly-Quinn, M., & Bracken, J. J. (1990). A seasonal analysis of the diet and feeding dynamics of brown trout, Salmo trutta L., in a small nursery system. Aquaculture and Fisheries Management, 21, 107–124.
- Kruse, C. G., Hubert, W. A., & Rahel, F. J. (1998). Single-Pass Electrofishing Predicts Trout Abundance in Mountain Streams with Sparse Habitat. North American Journal of Fisheries Management, 18, 940–946.
- Lachish, S., Miller, K. J., Storfer, A., Goldizen, A. W., & Jones, M. E. (2011). Evidence that diseaseinduced population decline changes genetic structure and alters dispersal patterns in the Tasmanian devil. *Heredity*, 106(1), 172–182. https://doi.org/10.1038/hdy.2010.17
- Langefors, A., Lohm, J., von Schantz, T., & Grahn, M. (2000). Screening of Mhc variation in Atlantic salmon (Salmo salar): A comparison of restriction fragment length polymorphism (RFLP), denaturing gradient gel electrophoresis (DGGE) and sequencing. *Molecular Ecology*, 9(2), 215– 219. https://doi.org/10.1046/j.1365-294x.2000.00838.x
- Lindström, J. (1999). Early development and fitness in birds and mammals. *Trends in Ecology & Evolution*, 14(9), 343–348. https://doi.org/10.1016/S0169-5347(99)01639-0
- Linnen, C. R., & Hoekstra, H. E. (2009). Measuring Natural Selection on Genotypes and Phenotypes in the Wild. Cold Spring Harbor Symposia on Quantitative Biology, 74(0), 155–168. https://doi.org/10.1101/sqb.2009.74.045
- Lozano, G. A. (1994). Carotenoids, Parasites, and Sexual Selection. *Oikos*, 70(2), 309. https://doi.org/10.2307/3545643
- Marcogliese, D. J., & Pietrock, M. (2011). Combined effects of parasites and contaminants on animal health: Parasites do matter. *Trends in Parasitology*, 27(3), 123–130. https://doi.org/10.1016/j.pt.2010.11.002
- Marsh, J. E., Lauridsen, R. B., Gregory, S. D., Kratina, P., Cooling, D., Jones, J. I., & Scott, L. J. (2022). High summer macrophyte cover increases abundance, growth, and feeding of juvenile Atlantic salmon. *Ecological Applications*, 32(2), 13.
- Martin, A. M., Hogg, J. T., Manlove, K. R., LaSharr, T. N., Shannon, J. M., McWhirter, D. E., Miyasaki, H., Monteith, K. L., & Cross, P. C. (2022). Disease and secondary sexual traits: Effects of pneumonia on horn size of bighorn sheep. *The Journal of Wildlife Management*, 86(1). https://doi.org/10.1002/jwmg.22154
- Martínez-Padilla, J., Mougeot, F., Pérez-Rodríguez, L., & Bortolotti, G. R. (2007). Nematode parasites reduce carotenoid-based signalling in male red grouse. *Biology Letters*, 3(2), 161–164. https://doi.org/10.1098/rsbl.2006.0593
- Masó, G., Vicente-Sastre, D., & Fitze, P. S. (2022). Intrinsic climatic predictability affects ornamental coloration of adult males: Evidence for compensation among carotenoid- and melanin-based coloration. *Functional Ecology*, 36(5), 1087–1097. https://doi.org/10.1111/1365-2435.14021
- McKnight, D. T., Schwarzkopf, L., Alford, R. A., Bower, D. S., & Zenger, K. R. (2017). Effects of emerging infectious diseases on host population genetics: A review. *Conservation Genetics*, 18(6), 1235–1245. https://doi.org/10.1007/s10592-017-0974-2
- Merilä, J., & Hendry, A. P. (2014). Climate change, adaptation, and phenotypic plasticity: The problem and the evidence. *Evolutionary Applications*, 7(1), 1–14. https://doi.org/10.1111/eva.12137

- Miller, M. P., & Vincent, E. R. (2008). Rapid natural selection for resistance to an introduced parasite of rainbow trout: Rainbow trout parasite resistance. *Evolutionary Applications*, 1(2), 336–341. https://doi.org/10.1111/j.1752-4571.2008.00018.x
- Mimura, M., Yahara, T., Faith, D. P., Vázquez-Domínguez, E., Colautti, R. I., Araki, H., Javadi, F., Núñez-Farfán, J., Mori, A. S., Zhou, S., Hollingsworth, P. M., Neaves, L. E., Fukano, Y., Smith, G. F., Sato, Y.-I., Tachida, H., & Hendry, A. P. (2017). Understanding and monitoring the consequences of human impacts on intraspecific variation. *Evolutionary Applications*, 10(2), 121–139. https://doi.org/10.1111/eva.12436
- Mitchell, S. E., Rogers, E. S., Little, T. J., & Read, A. F. (2005). Host-parasite and genotype-byenvironment interactions: Temperature modifies potential for selection by a sterilizing pathogen. *Evolution*, 59(1), 70–80. https://doi.org/10.1111/j.0014-3820.2005.tb00895.x
- Monzón-Argüello, C., Garcia de Leaniz, C., Gajardo, G., & Consuegra, S. (2014). Eco-immunology of fish invasions: The role of MHC variation. *Immunogenetics*, 66(6), 393–402. https://doi.org/10.1007/s00251-014-0771-8
- Nei, M., Maruyama, T., & Chakraborty, R. (1975). The Bottleneck Effect and Genetic Variability in Populations. *Evolution*, 11.
- Nielsen, R. (2005). Molecular Signatures of Natural Selection. *Annual Review of Genetics*, 39(1), 197–218. https://doi.org/10.1146/annurev.genet.39.073003.112420
- Nolan, P. M., Hill, G. E., & Stoehr, A. M. (1998). Sex, size, and plumage redness predict house finch survival in an epidemic. *Proceedings of the Royal Society of London. Series B: Biological Sciences*, 265(1400), 961–965. https://doi.org/10.1098/rspb.1998.0384
- Nurani, F. S., Sukenda, S., & Nuryati, S. (2020). Maternal immunity of tilapia broodstock vaccinated with polyvalent vaccine and resistance of their offspring against *Streptococcus agalactiae*. *Aquaculture Research*, *51*(4), 1513–1522. https://doi.org/10.1111/are.14499
- Okamura, B., Hartikainen, H., Schmidt-Posthaus, H., & Wahli, T. (2011). Life cycle complexity, environmental change and the emerging status of salmonid proliferative kidney disease. *Freshwater Biology*, *56*(4), 735–753. https://doi.org/10.1111/j.1365-2427.2010.02465.x
- Olsén, K. H., Grahn, M., Lohm, J., & Langefors, Å. (1998). MHC and kin discrimination in juvenile Arctic charr, *Salvelinus alpinus* (L.). *Animal Behaviour*, 56(2), 319–327. https://doi.org/10.1006/anbe.1998.0837
- Oshima, S. -i., Hata, J. -i., Segawa, C., & Yamashita, S. (1996). Mother to fry, successful transfer of immunity against infectious haematopoietic necrosis virus infection in rainbow trout. *Journal of General Virology*, 77(10), 2441–2445. https://doi.org/10.1099/0022-1317-77-10-2441
- Parra, I., Almodóvar, A., Ayllón, D., Nicola, G. G., & Elvira, B. (2012). Unravelling the effects of water temperature and density dependence on the spatial variation of brown trout (*Salmo trutta*) body size. *Canadian Journal of Fisheries and Aquatic Sciences*, 69(5), 821–832. https://doi.org/10.1139/f2012-025
- Parra, I., Almodóvar, A., Nicola, G. G., & Elvira, B. (2009). Latitudinal and altitudinal growth patterns of brown trout *Salmo trutta* at different spatial scales. *Journal of Fish Biology*, 74(10), 2355–2373. https://doi.org/10.1111/j.1095-8649.2009.02249.x
- Pauls, S. U., Nowak, C., Bálint, M., & Pfenninger, M. (2013). The impact of global climate change on genetic diversity within populations and species. *Molecular Ecology*, 22(4), 925–946. https://doi.org/10.1111/mec.12152
- Paz-Vinas, I., & Blanchet, S. (2015). Dendritic connectivity shapes spatial patterns of genetic diversity: A simulation-based study. *Journal of Evolutionary Biology*, 28(4), 986–994.
- Phillips, K. P., Cable, J., Mohammed, R. S., Herdegen-Radwan, M., Raubic, J., Przesmycka, K. J., van Oosterhout, C., & Radwan, J. (2018). Immunogenetic novelty confers a selective advantage in host–pathogen coevolution. *Proceedings of the National Academy of Sciences*, 115(7), 1552– 1557. https://doi.org/10.1073/pnas.1708597115
- Pigeon, G., & Pelletier, F. (2018). Direct and indirect effects of early-life environment on lifetime fitness of bighorn ewes. *Proceedings of the Royal Society B: Biological Sciences*, 285(1870), 20171935. https://doi.org/10.1098/rspb.2017.1935
- Portanier, E., Garel, M., Devillard, S., Maillard, D., Poissant, J., Galan, M., Benabed, S., Poirel, M.-T., Duhayer, J., Itty, C., & Bourgoin, G. (2019). Both candidate gene and neutral genetic diversity

correlate with parasite resistance in female Mediterranean mouflon. *BMC Ecology*, *19*(1), 12. https://doi.org/10.1186/s12898-019-0228-x

- Poulin, R., & Thomas, F. (1999). Phenotypic Variability Induced by Parasites: Extent and Evolutionary Implications. *Parasitology Today*, 15(1), 28–32. https://doi.org/10.1016/S0169-4758(98)01357-X
- Price, A. C., Weadick, C. J., Shim, J., & Rodd, F. H. (2008). Pigments, Patterns, and Fish Behavior. Zebrafish, 5(4), 297–307. https://doi.org/10.1089/zeb.2008.0551
- Pritchard, J. K., Stephens, M., & Donnelly, P. (2000). Inference of Population Structure Using Multilocus Genotype Data. *Genetics*, 155(2), 945–959. https://doi.org/10.1093/genetics/155.2.945
- R Core Team. (2021). R: A language for statistical computing. *R Foundation for Statistical Computing, Vienna, Austria*. https://www.R-project.org/
- Rauw, W. M. (2012). Immune response from a resource allocation perspective. *Frontiers in Genetics*, 3. https://doi.org/10.3389/fgene.2012.00267
- Reid, A. J., Carlson, A. K., Creed, I. F., Eliason, E. J., Gell, P. A., Johnson, P. T. J., Kidd, K. A., MacCormack, T. J., Olden, J. D., Ormerod, S. J., Smol, J. P., Taylor, W. W., Tockner, K., Vermaire, J. C., Dudgeon, D., & Cooke, S. J. (2019). Emerging threats and persistent conservation challenges for freshwater biodiversity. *Biological Reviews*, 94(3), 849–873. https://doi.org/10.1111/brv.12480
- Robinson, M. L., Gomez-Raya, L., Rauw, W. M., & Peacock, M. M. (2008). Fulton's body condition factor K correlates with survival time in a thermal challenge experiment in juvenile Lahontan cutthroat trout (*Oncorhynchus clarki henshawi*). *Journal of Thermal Biology*, 33(6), 363–368. https://doi.org/10.1016/j.jtherbio.2008.05.004
- Ros, A., Schmidt-Posthaus, H., & Brinker, A. (2022). Mitigating human impacts including climate change on proliferative kidney disease in salmonids of running waters. *Journal of Fish Diseases*, 45(4), 497–521. https://doi.org/10.1111/jfd.13585
- Roulin, A. (2016). Condition-dependence, pleiotropy and the handicap principle of sexual selection in melanin-based colouration: Melanin and sexual selection. *Biological Reviews*, 91(2), 328–348. https://doi.org/10.1111/brv.12171
- Rousset, F. (2008). genepop'007: A complete re-implementation of the genepop software for Windows and Linux. *Molecular Ecology Resources*, 8(1), 103–106. https://doi.org/10.1111/j.1471-8286.2007.01931.x
- Royle, J. A., Fuller, A. K., & Sutherland, C. (2018). Unifying population and landscape ecology with spatial capture-recapture. *Ecography*, *41*(3), 444–456. https://doi.org/10.1111/ecog.03170
- Saint-Pé, K., Leitwein, M., Tissot, L., Poulet, N., Guinand, B., Berrebi, P., Marselli, G., Lascaux, J.-M., Gagnaire, P.-A., & Blanchet, S. (2019). Development of a large SNPs resource and a lowdensity SNP array for brown trout (*Salmo trutta*) population genetics. *BMC Genomics*, 13.
- Sandland, G. J., & Minchella, D. J. (2003). Costs of immune defense: An enigma wrapped in an environmental cloak? *Trends in Parasitology*, *19*(12), 571–574. https://doi.org/10.1016/j.pt.2003.10.006
- Savage, A. E., & Zamudio, K. R. (2016). Adaptive tolerance to a pathogenic fungus drives major histocompatibility complex evolution in natural amphibian populations. *Proceedings of the Royal Society B: Biological Sciences*, 283(1827), 20153115. https://doi.org/10.1098/rspb.2015.3115
- Schager, E., Peter, A., & Burkhardt-Holm, P. (2007). Status of young-of-the-year brown trout (Salmo trutta fario) in Swiss streams: Factors influencing YOY trout recruitment. Aquatic Sciences, 69(1), 41–50. https://doi.org/10.1007/s00027-007-0845-x
- Schenekar, T., & Weiss, S. (2017). Selection and genetic drift in captive versus wild populations: An assessment of neutral and adaptive (MHC-linked) genetic variation in wild and hatchery brown trout (*Salmo trutta*) populations. *Conservation Genetics*, 18(5), 1011–1022. https://doi.org/10.1007/s10592-017-0949-3
- Schiebelhut, L. M., Puritz, J. B., & Dawson, M. N. (2018). Decimation by sea star wasting disease and rapid genetic change in a keystone species, *Pisaster ochraceus*. *Proceedings of the National Academy of Sciences*, 115(27), 7069–7074. https://doi.org/10.1073/pnas.1800285115

- Schneider, C. A., Rasband, W. S., & Eliceiri, K. W. (2012). NIH Image to ImageJ: 25 years of image analysis. *Nature Methods*, 9(7), 671–675. https://doi.org/10.1038/nmeth.2089
- Schrag, S. J., & Wiener, P. (1995). Emerging infectious disease: What are the relative roles of ecology and evolution? *Trends in Ecology & Evolution*, 10(8), 319–324. https://doi.org/10.1016/S0169-5347(00)89118-1
- Schwensow, N., Fietz, J., Dausmann, K. H., & Sommer, S. (2007). Neutral versus adaptive genetic variation in parasite resistance: Importance of major histocompatibility complex supertypes in a free-ranging primate. *Heredity*, 99(3), 265–277. https://doi.org/10.1038/sj.hdy.6800993
- Sheldon, B. C., & Verhulst, S. (1996). Ecological immunology: Costly parasite defences and trade-offs in evolutionary ecology. *Trends in Ecology & Evolution*, 11(8), 317–321. https://doi.org/10.1016/0169-5347(96)10039-2
- Shum, B. P., Guethlein, L., Flodin, L. R., Adkison, M. A., Hedrick, R. P., Nehring, R. B., Stet, R. J. M., Secombes, C., & Parham, P. (2001). Modes of Salmonid MHC Class I and II Evolution Differ from the Primate Paradigm. *The Journal of Immunology*, 166(5), 3297–3308. https://doi.org/10.4049/jimmunol.166.5.3297
- Siva–Jothy, M. T. (2000). A mechanistic link between parasite resistance and expression of a sexually selected trait in a damselfly. *Proceedings of the Royal Society of London. Series B: Biological Sciences*, 267(1461), 2523–2527. https://doi.org/10.1098/rspb.2000.1315
- Spears, B. M., Chapman, D. S., Carvalho, L., Feld, C. K., Gessner, M. O., Piggott, J. J., Banin, L. F., Gutiérrez-Cánovas, C., Solheim, A. L., Richardson, J. A., Schinegger, R., Segurado, P., Thackeray, S. J., & Birk, S. (2021). Making waves. Bridging theory and practice towards multiple stressor management in freshwater ecosystems. *Water Research*, 196, 116981. https://doi.org/10.1016/j.watres.2021.116981
- Stelkens, R. B., Jaffuel, G., Escher, M., & Wedekind, C. (2012). Genetic and phenotypic population divergence on a microgeographic scale in brown trout. *Molecular Ecology*, 21(12), 2896–2915. https://doi.org/10.1111/j.1365-294X.2012.05581.x
- Stet, R., de Vries, B., Mudde, K., Hermsen, T., van Heerwaarden, J., Shum, B., & Grimholt, U. (2002). Unique haplotypes of co-segregating major histocompatibility class II A and class II B alleles in Atlantic salmon (*Salmo salar*) give rise to diverse class II genotypes. *Immunogenetics*, 54(5), 320–331. https://doi.org/10.1007/s00251-002-0477-1
- Tallmon, D., Luikart, G., & Waples, R. (2004). The alluring simplicity and complex reality of genetic rescue. Trends in Ecology & Evolution, 19(9), 489–496. https://doi.org/10.1016/j.tree.2004.07.003
- The GIMP Development Team. (2019). GIMP. https://www.gimp.org
- Thrall, P. H., & Burdon, J. J. (1997). Host-Pathogen Dynamics in a Metapopulation Context: The Ecological and Evolutionary Consequences of Being Spatial. *The Journal of Ecology*, 85(6), 743. https://doi.org/10.2307/2960598
- Trudeau, K. M., Britten, H. B., & Restani, M. (2004). Sylvatic plague reduces genetic variability in black-tailed prairie dogs. *Journal of Wildlife Diseases*, 40(2), 205–211. https://doi.org/10.7589/0090-3558-40.2.205
- Vander Wal, E., Garant, D., & Pelletier, F. (2014). Evolutionary perspectives on wildlife disease: Concepts and applications. *Evolutionary Applications*, 7(7), 715–722. https://doi.org/10.1111/eva.12179
- Vasilieva, N. A., & Tchabovsky, A. V. (2020). Early predictors of female lifetime reproductive success in a solitary hibernator: Evidence for "silver spoon" effect. *Oecologia*, 193(1), 77–87. https://doi.org/10.1007/s00442-020-04649-1
- Vazquez, A., Gustafson, K. D., Harmeling, B., & Ernest, H. B. (2019). Genetic diversity of yellowbilled magpies (*Pica nutalli*) before and after a west Nile virus epidemic. *Journal of Wildlife Diseases*, 55(2), 316. https://doi.org/10.7589/2018-01-023
- Vignieri, S. N. (2010). The Genetic Effects of Ecological Disturbance: Flooding in Jumping Mice. *The American Naturalist*, 175(1), 126–135. https://doi.org/10.1086/648606
- Vøllestad, L. A., Serbezov, D., Bass, A., Bernatchez, L., Olsen, E. M., & Taugbøl, A. (2012). Smallscale dispersal and population structure in stream-living brown trout (*Salmo trutta*) inferred by mark–recapture, pedigree reconstruction, and population genetics. *Canadian Journal of Fisheries and Aquatic Sciences*, 69(9), 1513–1524. https://doi.org/10.1139/f2012-073

- Voyles, J., Woodhams, D. C., Saenz, V., Byrne, A. Q., Perez, R., Rios-Sotelo, G., Ryan, M. J., Bletz, M. C., Sobell, F. A., McLetchie, S., Reinert, L., Rosenblum, E. B., Rollins-Smith, L. A., Ibáñez, R., Ray, J. M., Griffith, E. J., Ross, H., & Richards-Zawacki, C. L. (2018). Shifts in disease dynamics in a tropical amphibian assemblage are not due to pathogen attenuation. *Science*, 359(6383), 1517–1519. https://doi.org/10.1126/science.aao4806
- Vredenburg, V. T., McNally, S. V. G., Sulaeman, H., Butler, H. M., Yap, T., Koo, M. S., Schmeller, D. S., Dodge, C., Cheng, T., Lau, G., & Briggs, C. J. (2019). Pathogen invasion history elucidates contemporary host pathogen dynamics. *PLOS ONE*, 14(9), e0219981. https://doi.org/10.1371/journal.pone.0219981
- Waldner, K., Borkovec, M., Borgwardt, F., Unfer, G., & El-Matbouli, M. (2021). Effect of water temperature on the morbidity of *Tetracapsuloides bryosalmonae* (Myxozoa) to brown trout (*Salmo trutta*) under laboratory conditions. *Journal of Fish Diseases*, 44(7), 1005–1013. https://doi.org/10.1111/jfd.13361
- Wedekind, C., Jacob, A., Evanno, G., Nusslé, S., & Müller, R. (2008). Viability of brown trout embryos positively linked to melanin-based but negatively to carotenoid-based colours of their fathers. *Proceedings of the Royal Society B: Biological Sciences*, 275(1644), 1737–1744. https://doi.org/10.1098/rspb.2008.0072
- Wells, K., Hamede, R. K., Jones, M. E., Hohenlohe, P. A., Storfer, A., & McCallum, H. I. (2019). Individual and temporal variation in pathogen load predicts long-term impacts of an emerging infectious disease. *Ecology*, 100(3).
- Wernicke von Siebenthal, E., Rehberger, K., Bailey, C., Ros, A., Herzog, E., & Segner, H. (2018). Trade-Offs Underwater: Physiological Plasticity of Rainbow Trout (*Oncorhynchus mykiss*) Confronted by Multiple Stressors. *Fishes*, 3(4), 49. https://doi.org/10.3390/fishes3040049
- Westerdahl, H., Hansson, B., Bensch, S., & Hasselquist, D. (2004). Between-year variation of MHC allele frequencies in great reed warblers: Selection or drift?: Temporal variation of MHC allele frequencies. *Journal of Evolutionary Biology*, 17(3), 485–492. https://doi.org/10.1111/j.1420-9101.2004.00711.x
- Wilcoxen, T. E., Boughton, R. K., & Schoech, S. J. (2010). Selection on innate immunity and body condition in Florida scrub-jays throughout an epidemic. *Biology Letters*, 6(4), 552–554. https://doi.org/10.1098/rsbl.2009.1078
- Wolinska, J., & King, K. C. (2009). Environment can alter selection in host–parasite interactions. *Trends in Parasitology*, 25(5), 236–244. https://doi.org/10.1016/j.pt.2009.02.004
- Wright, B., Willet, C. E., Hamede, R., Jones, M., Belov, K., & Wade, C. M. (2017). Variants in the host genome may inhibit tumour growth in devil facial tumours: Evidence from genome-wide association. *Scientific Reports*, 7(1), 423. https://doi.org/10.1038/s41598-017-00439-7
- Xu, B., & Yang, Z. (2013). PAMLX: A Graphical User Interface for PAML. *Molecular Biology and Evolution*, 30(12), 2723–2724. https://doi.org/10.1093/molbev/mst179

Supporting information



Figure S1. (a) Infection prevalence in 2020 according to the infection prevalence in 2019, and **(b)** Water temperature in 2020 according to water temperature in 2019. The dashed line represents a 1:1 relation, dots above and below the line represent respectively an increase and a decrease in prevalence **(a)** or in temperature **(b)**. Water temperature remained stable across the two years, while most of the sites showed an increase in infection prevalence.



Figure S2. Continuous recording of water temperature at the 15 sampled sites. Sites are ranked by their density delta (disparition rate during the summer, D) and their infection prevalence is indicated (P). Five sites are represented per panel for visualisation purpose. Temperature thresholds for the release of *T. bryosalmone* infective spores from bryozoans (9°C, Gay et al. 2001), the initiation of proliferative kidney disease symptoms (15°C, Hedrick et al. 1993), and the limit for brown trout feeding (19.5°C, Elliott & Elliott 2010) are indicated by the dashed horizontal lines.



Figure S3. Supplementary biplots. Difference in the number of SNPs (delta) before and after the summer according to infection prevalence (**a**) and water temperature (**b**). Delta He according to infection prevalence (**b**) and water temperature (**c**). Delta in melanin area according to infection prevalence (**d**) and water temperature (**e**).





Figure S4. Percentage of time recorded between the 3^{rd} of July and the 14^{th} of September with a temperature > 19.5°C, the upper limit for brown trout feeding, according to the mean summer water temperature.

Site	Sampling date	N total	N 0+ wild	N Body condition	N Coloration	N SNPs	N MHC	N Prevalence
ARBBar	29/06/2020	50	34	34	34	34	18	
	15/09/2020	32	31	31	31	25	19	20
ARBMan	29/06/2020	34	33	33	33	33	30	
	21/09/2020	41	39	39	39	38	37	19
ARGFoi	22/06/2020	52	52	52	52	52	44	
	18/09/2020	45	45	45	45	44	45	20
ARGPon	03/07/2020	30	30	30	30	30	29	
	17/09/2020	32	28	28	28	28	28	20
ARIVar	03/07/2020	34	32	31	32	32	29	
	18/09/2020	20	18	18	18	17	16	18
ARRGou	01/07/2020	40	37	37	36	37	36	
	23/09/2020	27	27	27	27	26	24	20
ARZDur	22/06/2020	36	36	36	36	36	36	
	16/09/2020	22	20	20	20	20	20	20
ARZPon	03/07/2020	45	44	44	44	44	43	
	16/09/2020	37	35	35	35	35	35	20
POLISor	01/07/2020	43	43	43	43	43	33	
BOOSer	23/09/2020	36	36	36	36	36	27	20
GARVil	29/06/2020	31	20	20	19	20	20	
	25/09/2020	15	12	6	12	11	11	9
GERAsp	24/06/2020	52	52	51	52	52	38	
	14/09/2020	45	43	43	43	40	37	19
GERRoi	24/06/2020	27	21	21	21	21	21	
GERPOI	29/09/2020	25	22	22	22	22	20	19
JOBEnc	24/06/2020	45	44	44	44	44	43	
	22/09/2020	42	39	39	39	35	31	20
CALT	02/07/2020	35	33	33	33	33	31	
SALIAU	21/09/2020	16	15	15	15	15	11	15
TOPT	01/07/2020	32	31	30	31	29	27	
TOKTUZ	24/09/2020	25	23	23	23	22	19	18

Table S1. Number of brown trout individuals at each sampling session, for each measured parameter.

Appendix S1. Discrimination between 0+ and 1+ individuals in our dataset.

In this study, we were interested in studying fish response to the infection at their first encounter with the parasite. Therefore, we aimed at sampling 0+ individuals only, but the discrimination between 0+ and 1+ fish is not obvious on the field. To discriminate these two age classes in the dataset, we used size distributions at each site and each session. The individuals were suspected to be 1+ when they were outside the 0.95 confidence interval for the associated normal distribution plus the difference between the lower-tail of the normal distribution and the minimum size of that site and session. We double-checked with recapture data (obtained with the SNPs genotyping and the *allelematch* R package): if an individual was part of the 0+ distribution in S1 but was out of the distribution in S2, it was still considered as a 0+ and *vice-versa*.

Appendix S2. Two-step PCR protocol for the MHC genotyping.

PCR1 were carried out in a 10 μ L reaction volume using 5 μ L of Multiplex PCR Kit (Qiagen) and 0.5 mM of each primer, with 1.5 μ L of sample DNA. This PCR consists of an initial denaturation at 95°C for 15min, followed by 30 cycles including denaturation at 94°C for 30s, annealing at 50°C for 45s and extension at 72°C for 2min, with a final extension phase at 72°C for 10min. We mixed the 96 index i5 and 96 index i7 to get 1001 unique combinations in the same MiSeq run. PCR2 were carried out in a 10 μ L reaction volume using 5 μ L of Multiplex PCR Kit (Qiagen), 4 μ L of a unique index combination and 1 μ L of PCR1 product. PCR2 consists of an initial denaturation at 95°C for 15min, followed by 8 cycles including denaturation at 95°C for 40s, annealing at 55°C for 45s and extension at 72°C for 2min, with a final extension phase at 72°C for 10min. We pooled PCR products by volume and a 2×250 bp paired-end MiSeq (Illumina) run was conducted at the ECOGENO laboratory.



Appendix S3. Identification of stocked individuals with population admixture analyses.

Results of the analyses run in STRUCTURE for quantifying the introgression into the sampled populations. Each bar represents one individual, and each color one genetic cluster. PISSou is the Soueich hatchery used for stocking in Haute-Garonne and Ariège, and PISCau the Cauterets hatchery used for stocking in the Hautes-Pyrénées. STRUCTURE was run 3 times with a burn-in period of 5000 replications and a run length of 50000 Markov Chain Monte Carlo iterations, for K=1 to K=4 clusters. We kept the results for K=2 and K=3 (ARGFoi, BOUSer) according to the homogeneity of the hatchery cluster. The individuals with a proportion > 0.75 (arbitrary threshold) were considered as stocked individuals, that we removed from the analyses (31 individuals). GARVil and GERPoi were especially concerned by these individuals.

Chapter 4. A multifaceted index of population health to detect risk-prone populations and underlying stressors in wildlife

This chapter is published as a scientific article in *Biological Conservation*.

Eloïse Duval^{1,2}, Erwan Quéméré², Géraldine Loot^{3,4}, Lisa Jacquin^{3,4}, Charlotte Veyssière³ & Simon Blanchet^{1,3}

¹ UAR 2029, Station d'Ecologie Théorique et Expérimentale, CNRS, 2 route du CNRS, 09200 Moulis, France

² UMR DECOD (Ecosystem Dynamics and Sustainability), INRAE, Institut Agro, IFREMER, 65 rue de Saint Brieuc, 35000 Rennes, France

³ UMR-5174, EDB (Laboratoire Evolution & Diversité Biologique), CNRS, IRD, Université Toulouse III Paul Sabatier, 118 route de Narbonne, 31077 Toulouse, France

⁴ Institut Universitaire de France, Paris, France

Graphical abstract



Abstract

Local declines of wild populations represent the most visible part of biodiversity loss, and their detection often relies on long-term surveys. An alternative to identify risk-prone populations is to use indicators informing on their general health (i.e., their general fitness and ability to cope with changing environment) based on simple and complementary parameters estimated from snapshot sampling. However, most studies on wildlife population health focus on one or only a few parameters, yielding potentially biased conclusions for conservation. Here, we developed a multifaceted index of population health by combining 3 complementary indicators, namely pathological, ecological, and genetic indicators, based on an integrative approach traditionally used to assess ecosystem multifunctionality. We investigated their complementarity and relevance for detecting brown trout (Salmo trutta) risk-prone populations at a large spatial scale, and the underlying environmental stressors. The multifaceted health index properly represented the individual indicators' complementary information. It identified a cluster of moderately risk-prone populations and raised the alarm for one population. Each indicator was individually associated with distinct environmental stressors relevant for brown trout requirements. The multifaceted health index highlighted surrounding agricultural land and oxygen concentration as the most impacting environmental factors for the general health and sustainability of brown trout populations. The implementation of such integrative index can be transferred to a wide range of species and contexts. This index therefore provides to environmental managers and conservationists a snapshot and easily operated tool to identify risk-prone populations and areas to restore or conserve.

Keywords: population demography, sustainability, biological indicator, parasitology, genetics, environmental variability

Abbreviations: MFI: multifaceted indicator of population health PI: pathological indicator EI: ecological indicator GI: genetic indicator

Introduction

By altering environmental conditions and biotic interactions, global change is threatening many plant and animal species, thereby increasing their risk of extinction (Radchuk et al., 2019; Spooner et al., 2018; Thomas et al., 2004). Species extinction is systematically preceded by the decline in abundance of local populations, which actually represents the most important and visible part of the global biodiversity loss (Collen et al., 2011; Hughes et al., 1997). Local declines of populations may therefore serve as early warning signals (EWS) for managers to mitigate the aftermaths of global change (Drake & Griffen, 2010). However, characterizing these declines requires long-term surveys of populations' abundance that are labor-intensive and still scarce in wild populations (Atkinson et al., 2006).

Monitoring indicators informing the general health of populations may be a relevant alternative to long-term surveys to infer their ability to cope with new environmental conditions (Stephen, 2014), and to identify risk-prone populations (Clements & Ozgul, 2016; Goff et al., 2020). The general health of populations is here defined as their general status (i.e., fitness) and their demo-genetic sustainability over short- to long-term periods (Hoban et al., 2013; Kophamel et al., 2021). Population health is therefore intrinsically multifaceted, and it can be informed by multiple indicators (generally genetic, phenotypic, or demographic information), measured during snapshot sampling events. For example, a decrease in the mean body size of whales appeared as a good EWS of local populations' declines (Clements et al., 2017). Similarly, intraspecific genetic diversity (or related indices such as effective population sizes) informs on the adaptive potential of populations in changing environments (Hoban et al., 2013; Lande & Shannon, 1996). Likewise, pathogenic infection can represent a silent threat to population fitness by provoking sudden mortality under new environmental conditions (Valenzuela-Sánchez et al., 2017). Hence, a thorough monitoring of wildlife populations' characteristics informing their general health may help noticing EWS and setting pro-active management decisions.

Wildlife population health is multifaceted and encompasses several intrinsic parameters such as ecological (e.g. population abundance, body condition), genetic (e.g. genetic diversity) or pathological (e.g. pathogen infection) parameters, which are complementary and influenced by multiple environmental factors (Stephen, 2014). Most studies on wildlife population health focus on one or only a few parameters. However, when interpreted independently, these indicators can yield opposite conclusions, and therefore potentially biased outcomes for wildlife population management. For instance, a population can show high local abundance, making it resilient to stochastic demographic fluctuations but a low level of genetic diversity, leaving it vulnerable under fluctuating environmental conditions (Maebe et al., 2019). Similarly, pathogen infection may differently affect a genetically diversified or an inbred host population (King & Lively, 2012). In addition, different indicators might be differently modulated by environmental factors. For example, an increase in temperature may benefit

individual growth, while favoring pathogen development detrimental to individual fitness and population health, so that the use of multiple indicators may better capture the global impact of environmental stressors. Besides, the combination of several indicators into a single index of population general health could buffer potential uncertainties related to the measurement of each individual indicator. Therefore, more integrative studies incorporating several indicators are needed to grasp the multiple dimensions of wild population health.

This query is timely because recent advances in field and laboratory techniques enable the development of such integrative indicators. For instance, remote sensing mapping landscape types (Skidmore, 2003) or molecular tools assessing genetic diversity and environmental DNA quantifying the abundance of species (Bohmann et al., 2014; Hoban et al., 2013) offer new non-invasive monitoring tools to address this challenge while minimizing the impact on sampled populations. Building on such new field and molecular techniques, this study aims at developing a multifaceted index of population health (MFI) combining individual indicators related to the pathological, ecological, and genetic status of populations. The construction of this MFI is inspired from studies measuring ecosystem multifunctionality by synthetizing multiple ecosystem functions into a single score (e.g. Manning et al., 2018). We first tested the covariation between each component indicator and their covariation with the MFI to investigate their complementarity or redundancy for informing population general health. We then tested the link between each of these indicators and potential natural and anthropogenic environmental stressors to identify the most impacting for population health.

We focused on populations of a common freshwater fish, the brown trout *Salmo trutta*, that has important economic, cultural and recreational values (Unfer & Pinter, 2018). This species is highly sensitive to environmental stressors such as water warming and chemical alterations (Eklöv et al., 1999; Elliott & Elliott, 2010). We sampled populations along a large spatial gradient associated with water physicochemical variations. For each population, we quantified 3 component indicators informing on their pathological, ecological and genetic status and combined them into a MFI. Our final aims were to identify low-health populations that are the most risk-prone and likely to decline in the sampled area, and the underlying environmental factors. We expected the component indicators to provide complementary information, revealed by a weak covariation among them within populations. Consequently, combining these indicators into a MFI should provide a better assessment of population health and its underlying environmental factors. We thus predicted a tighter association between environmental factors and the MFI relative to each individual component indicator.

Methods

Brown trout sampling

We developed the MFI in brown trout populations because the ecology of this salmonid fish is well known. This species is particularly sensitive to water temperature and quality (Elliott, 1994), so that water characteristics may be tightly associated with population health. We sampled 46 wild populations experiencing a wide range of environmental conditions in southern France, at the foothills of the Pyrenean mountains (Fig. 1, Table A1). During summer 2019, we electro-fished up to 20 individuals per site (when local abundances allowed it), representing 865 sampled brown trout in total (Table 1, Table A1). We targeted juveniles (mean size \pm SD 75 \pm 14mm), corresponding mainly to young-of-the-year (0+) fish because it is the most sensitive stage to environmental stressors and pathogen infection, and the most abundant cohort. After manipulations (see hereafter), we released the fish alive into their sampling sites. Authorizations to sample brown trout were provided by the Directions Départementales des Territoires of Ariège, Haute-Garonne and Hautes-Pyrénées respectively.

Type of parameter	Method	Measure	Range	Mean	SD
	Count	Number of brown trout sampled (N)	10-20	19	3
Environmental factors		% Agricultural land in a 2km buffer	0-89	49	24
	GIS software	Altitude (m)	232-844	447	136
		Distance from source (km)	3-105	28	21
	HOBO logger	Mean summer temperature (°C)	12.3-19.4	16.0	1.9
	Ponctual dosage	PO ₄ ³⁻ (µg/L)	0-205	58	55
		NNO3 ⁻ NNO2 ⁻ (µg/L)	0-2440	747	661
	Ponctual measure (InSitu)	рH	7.7-8.7	8.3	0.2
		Conductivity (µs/cm)	24-460	216	117
		O ₂ concentration (mg/L)	8.5-11.4	9.8	0.5
Pathological indicator	Parasite DNA detection in fish excretion	Infection prevalence (%)	0-100	30	40
		Parasite DNA excretion (copies/µL/g/min)	0-17	2	3.6
Ecological indicator	Measures on the field	Condition factor	1.05-1.43	1.24	0.08
		Brown trout density (N/min)	0.12-2.40	0.89	0.52
Genetic indicator	CND ₂	MLH ^a	0.13-0.21	0.17	0.02
	ONES	Ne ^b	6-681	138	174

Table 1. Summary of the measures in the 46 sampled brown trout populations, see Table A1 for details on each population.

^a Multilocus heterozygosity

^b Effective population size

Pathological indicator (PI)

The PI focused on the most impacting pathogen for brown trout in our study area: the myxozoa *Tetracapsuloides bryosalmonae*. This pathogen is the causative agent of the proliferative kidney disease (PKD), an emerging disease that has been increasingly reported during the last two decades, in Europe and North America. PKD can cause up to 100% mortality during severe outbreaks, especially in young-of-the-year cohorts, representing a threat for infected populations (Okamura et al., 2011).

We assessed the infection by *T. bryosalmonae* and its load in sampled fish using a non-lethal method developed in Duval et al. (2021) quantifying the DNA of pathogen excreted in the fish urine, considered as a proxy for the level of kidney infection. After capture, the fish recovered for 30min, and were placed individually into plastic bottles with 1.2L of commercial mineral water for 1h to let time for the infected fish to excrete pathogen spores or DNA through urine. Then, we used a Vampire sampler® to filter 1L of the water and extracted DNA from 1.2µm filters. Lastly, we used specific droplet digital PCRs (ddPCRs) to quantify *T. bryosalmonae* DNA (see Duval et al. (2021) for the complete procedure). Population infection prevalence is the number of positive individuals for *T. bryosalmonae* DNA detection divided by the number of sampled individuals. The fish pathogen load corresponds to the concentration of *T. bryosalmonae* DNA divided by the time of excretion and the fish body mass to correct for variation in urine excretion (Hunn, 1982).

We scaled the infection prevalence and the mean pathogen load in each population into scores ranging between 0 (the population with the highest prevalence or highest mean pathogen load) and 1 (the population with the lowest prevalence or lowest mean pathogen load), with the R *scales* package (Wickham & Seidel, 2020). We averaged these two scores and rescaled the mean between 0 and 1 to build the PI. Populations with the lowest pathogen prevalence and load were considered the healthiest from a pathological point of view.

Ecological indicator (EI)

The EI is based on the population density and the fish mean body condition. We estimated fish density at each site as the number of juveniles caught per minute through single-pass electrofishing surveys ("catch-per-unit-effort", CPUE, Kruse et al., 1998). After the urine excretion step (2.1.1.), we anaesthetized each fish with benzocaine, measured and weighed them to the nearest mm and 0.1g respectively. We calculated fish body condition (K) according to equation (1) (Fulton, 1904), with W as fish mass in g, and L as fish length in cm.

(1)
$$K = \frac{W}{L^3} \times 100$$

As for the PI, we scaled the mean body condition and fish density into scores between 0 and 1, averaged them and rescaled the mean to build the EI. Populations with the highest density of juveniles and the highest mean body condition were considered the healthiest from an ecological point of view (Lobón-Cerviá, 2005; Robinson et al., 2008).

Genetic indicator (GI)

We fin-clipped each fish for genetic analyses and stored the fins in 70% ethanol. The LGC® Group (UK) used the KASPar allele-specific fluorescent genotyping system to genotype each individual at 175 SNPs, among which 158 were confidently genotyped (Table A2). These included 141 SNPs evenly spread on the brown trout linkage genetic map from Saint-Pé et al. (2019), as well as 17 SNPs from Ahmad et al. (2018), identified as candidate loci in the resistance/tolerance to the infection by *T. bryosalmonae*. To ensure the reliability of the genotyping, we duplicated 17 individuals that indicated a 0.2% error in scoring (5 mismatches in 2584 comparisons). To identify potential genotyping errors, we ran Hardy-Weinberg equilibrium tests with the *genepop* package (Rousset, 2008). We removed from the analyses 6 loci that departed from the equilibrium in 5 to 22 populations (Table A2) and 7 individuals that did not amplify properly so that 152 loci and 858 individuals were genotyped.

The GI is based on the effective population size (N_e), a measure of population sustainability (Waples & Do, 2010) and the mean multilocus heterozygosity (*MLH*), a genomic inbreeding estimator often positively correlated with individual fitness in wild populations (Hansson & Westerberg, 2002). We computed the *MLH* with the package *inbreedR* (Stoffel et al., 2016). We computed populations' N_e with the linkage disequilibrium method (Waples & Do, 2007) in NeEstimator 2.1 (Do et al., 2014) with a lowest allele frequency of 0.05. These values were logged to homogenise variance.

As for the other indicators, we scaled *MLH* and N_e into scores between 0 and 1, averaged them and rescaled the mean to build the GI. Populations with the highest N_e and *MLH* harbor higher genetic variation, enhancing their response to environmental changes and reducing their sensitivity to genetic stochasticity, so that they were considered the healthiest from a genetic point of view (Evans & Sheldon, 2008; Palstra & Ruzzante, 2008).

Multifaceted health index (MFI)

We then calculated the MFI of each population by combining these 3 component indicators. We averaged the PI, EI and GI, before rescaling the mean between 0 and 1 to obtain the final MFI.

Environmental data

To investigate the impact of natural and anthropogenic factors on these different indicators, we measured key environmental factors (Table 1). Brown trout is highly sensitive to temperature, oxygen concentration, pH and conductivity (Elliott, 1994). Therefore, we recorded water temperature in each site every 4h between July and August 2019 with a HOBO® logger and we acquired O₂ concentration, pH, and specific conductivity through snapshot measures with the In-Situ® Aqua TROLL 500 Multiparameter Sonde in August 2020. In addition, we quantified PO_4^{3-} , NO_2^{-} and NO_3^{-} concentrations in the water reflecting water eutrophication, based on 50mL samples collected in May 2020 at the Laboratoire Ecologie Fonctionnelle et Environnement according to the ISO 15681-2 and ISO 13395 norms, respectively.

We estimated the percentage of agricultural land in a 2km buffer around each site with the CORINE Land Cover 2018 dataset (European Environment Agency), as it represents the main anthropogenic pressure in the sampled area. We also used the QGIS software (2022) to estimate the distance from the source and the altitude of the sampled sites. We expected that the general health of brown trout populations would be lower in the warmest waters with the lowest oxygen concentration, the highest values of eutrophication and the highest surfaces of agricultural land in their surroundings (Jonsson et al., 2011; Molony, 2001).

Statistical analyses

We conducted the statistical analyses in the R environment (R 4.0.3, R Core Team 2020).

Covariance between the indicators

We quantified the covariance between the PI, EI and GI and with the MFI respectively using pairwise Spearman correlation tests with Holm's correction for multiple inference (n=6 pairs of indicators) from the *RcmdrMisc* package (Fox, 2020). A positive and significant correlation between two indicators would indicate that they co-vary and support redundant information.

Impacts of the environmental factors on the indicators and multifaceted health index

To investigate the impacts of the environmental factors on the component indicators and the MFI, we used a redundancy analysis (RDA) with the *vegan* package (Oksanen et al., 2020). This analysis is a constrained form of principal component analysis which explains a set of response variables (here the indicators) by a set of explanatory variables (here the environmental factors). We assessed the validity of the RDA through an analysis of variance (ANOVA, 999 permutations).

Additionally, we ran path analyses to outline the statistically significant relationships between the environmental factors and the indicators while taking into account the inter-dependence of the environmental factors, using the lavaan (Rosseel, 2012) and semPlot (Epskamp et al., 2019) packages. We added and removed incrementally the links between the variables, only keeping significant relationships until reaching the lowest Akaike's Information Criterion (AIC), together with a nonsignificant Chi² p-value (>0.05), a Comparative Fit Index (CFI) >0.95 and a Root Mean Square Error of Approximation (RMSEA) <0.05 (Hu & Bentler, 1999; Rigdon, 1996). Because the MFI was highly correlated with the component indicators, we ran two path analyses separately: one including the 3 component indicators and one including only the MFI. However, as the covariations among environmental factors remained the same, we joined both analyses for graphical representation.

Results

Covariance between the component indicators and with the multifaceted index

The PI, EI and GI did not significantly covary (Spearman correlation tests and adjusted Holm's p-values; PI vs. EI, r=0.14, 95% CI -0.16 to 0.41, P=1; PI vs. GI, r=0.06, 95% CI -0.23 to 0.34, P=1; GI vs. EI r=0.03, 95% CI -0.26 to 0.32, P=0.1; Fig. A2). In addition, each of the 3 component indicators were significantly and positively correlated to the MFI (Spearman correlation tests; MFI vs. PI r=0.69, 95% CI 0.50-0.82, P<0.001; MFI vs. EI, r=0.52, 95% CI 0.27-0.70, P=0.001; MFI vs. GI, r=0.52, 95% CI 0.27-0.70, P=0.001; Fig. A2). These correlation coefficients did not differ significantly among them (95% CI overlap among the coefficients, Fig. A2), hence indicating that the 3 component indicators equally contributed to the MFI.

Effect of environmental factors on the indicators

The highest MFI scores were mostly located upstream and closer to the Pyrenean mountains, whereas the lowest scores were found further downstream, with a specific cluster in the western part of the studied area (Fig. 1). This spatial pattern was also detectable with the PI but not with the EI and GI (Fig. A1). The population with the lowest MFI score was BAUSou, which had a low score for each component indicator (Fig. 1, Fig. A1).



Figure 1. Map of the 46 sampled populations and their multifaceted health index. Dark red represents the populations with a low multifaceted health index score, and dark green the populations with a high score. The inset map shows the location of the studied area, in Southern France at the foothill of the Pyrenees. Codes for the sampled sites (in bold) and departments (standard police) are indicated.

The ANOVA confirmed the validity of the RDA (df=9, F=3.11, P=0.001). The global variance of the indicators' scores (component and MFI) constrained by the environmental factors was of 43.71%, including 39.93% explained by the two first axes (Fig. 2). The PI was negatively associated with the mean summer water temperature and the NNO2-NNO3 concentration (Fig. 2), suggesting that fish in colder and less eutrophicated water were less frequently and severely infected by *T. bryosalmonae*. The EI was poorly defined by both axes, and thus poorly explained by the measured environmental factors (Fig. 2). The GI was positively associated with the distance from the source, and negatively with the percentage of agricultural land, suggesting that fish further downstream and in less agricultural areas had a better genetic status. The MFI was strongly and negatively associated with the percentage of agricultural land, and positively associated with the O2 concentration. Overall, the first axis was positively related to high scores for all the indicators, therefore the populations on the left part of the plot were identified as the less healthy and the most likely to decline (Fig. 2).



Figure 2. Biplot of the redundancy analysis showing the relation between the indicators (response variables, in red) and the environmental factors (explanatory variables, in blue). Dots represent the sampled populations together with their code.

The path analyses for the component indicators (χ^2 =20.68, df=19, P=0.36; CFI=0.99; RMSEA=0.04) and the MFI (χ^2 =6.070, df=7, P=0.53; CFI=1; RMSEA=0) both met the validation requirements. Consistently with the RDA, they revealed statistically significant relationships between some environmental factors and the indicators, while evaluating the covariances between environmental factors, i.e., considering their non-independence (Fig. 3). For instance, the concentration of NNO2-NNO3, the percentage of agricultural land and the mean summer water temperature were positively correlated: agricultural sites were thus warmer and more eutrophicated. The mean summer temperature and the percentage of agricultural land were negatively correlated with the altitude, implying that warm agricultural sites were mostly located at low altitudes. After considering the environmental factors covariations, the PI was negatively associated with the nutrient concentration and the mean water temperature (Fig. 3, see Fig. A3 for a visual representation). The EI was negatively associated with the percentage of agricultural land, as was the GI which was additionally positively associated with the distance from the river source (Fig. 3, see Fig. A3 for a visual representation). The set of measured environmental factors explained a consistent part of the variance of the PI and the GI (R²=0.45 and $R^2=0.37$, respectively), but barely explained the variance in the EI ($R^2=0.09$, Fig. 3). The path analysis run separately for the MFI identified a positive relationship with the O2 concentration and a strong

negative relationship with the percentage of agricultural land (Fig. 3, see Fig. A4 for a visual representation). The MFI had the highest percentage of variance explained by the model ($R^2=0.55$, Fig. 3).



Figure 3. Plot of the best path analyses explaining the 3 indicators and the multifaceted health index. Plain arrows represent the impact of the environmental factors on the indicators, and double arrows represent the covariations between the environmental factors. Dotted arrows towards the multifaceted health index and the dotted rectangle indicate that this variable was part of a different analysis but represented on the same figure for the sake of clarity (see 2.3.2.). Red arrows represent negative relationships and green arrows represent positive relationships, and the estimates are indicated. All the relationships represented on the graphic are statistically significant (see Table A3 for details). T°C: temperature.

Discussion

We developed an easy-to-build operational multifaceted index informing on the general health of wild populations, as a first management step to identify risk-prone populations and guide conservation priorities. To that end, we combined indicators of population pathological, ecological, and genetic status into a multifaceted health index (MFI) by adapting a framework traditionally used to assess ecosystem multifunctionality. We showed that the 3 component indicators measured in brown trout populations did not covary, suggesting that they carry non-redundant and complementary information on population
health. The MFI equally synthetized their information and enhanced the detection of risk-prone populations. The component indicators were associated with different environmental stressors, and the MFI highlighted the most important stressors regarding the populations' general health, which can help prioritizing conservation actions.

Identifying risk-prone populations

The pathological, ecological, and genetic indicators (PI, EI and GI, respectively) did not covary, indicating that they carry complementary information regarding population health. For instance, the population ARRGou showed a good GI suggesting a high adaptive potential, but its PI and EI were weak. The discrepancy between indicators illustrates the need for a more integrative index to get reliable insights of populations' general health. The combination of the indicators into a MFI therefore enhanced the accuracy of health assessment and ultimately classified ARRGou as a moderately risk-prone population.

This is especially true because the 3 component indicators equally contributed to the MFI, indicating that each indicator was accurately represented by the MFI. The MFI identified a cluster of populations with low scores in the north-western part of the studied area, in which especially one population (BAUSou) showed a high risk of decline. Congruently, we observed that its density has decreased markedly in 2020 (data not shown). This particular case illustrates the potential of the MFI to detect early warning signals of populations' declines (Clements & Ozgul, 2016; Drake & Griffen, 2010), even though further temporal surveys would be needed to validate this hypothesis.

Identifying underlying environmental stressors

We further showed that the component indicators were influenced by different environmental factors. We found associations between the indicators and the environmental factors tested that were mutually coherent and consistent with the biological requirements of brown trout, hence comforting the robustness of the multifaceted index.

For instance, the PI decreased with increasing nutrient concentration and water temperature. This result is consistent with *T. bryosalmonae* life cycle: increased water temperature favors its multiplication and transmission as well as the growth of its main host, a bryozoan, that is also favored by increasing concentration in nutrients, which in turn increases the available niche for the pathogen (Okamura et al., 2011).

Likewise, the GI increased with increasing distance from the source, following the prediction that genetic diversity usually increases downstream in riverine fish (Paz-Vinas et al., 2015), and decreased with increasing percentage of agricultural land. In addition, we found that our agricultural

sites were warmer and more eutrophicated (3.2.). Since high concentration in nutrients, high temperature and low oxygen concentration are unfavorable conditions for brown trout survival and reproduction (Burkhardt-Holm & Scheurer, 2007; Elliott & Elliott, 2010), large agricultural surroundings may thus be associated with a rapid decline in the population demographic performance, ultimately decreasing its genetic diversity, as observed in other aquatic species (Blum et al., 2012; Nicol et al., 2017).

The EI also decreased with the percentage of surrounding agricultural land, but this indicator was globally poorly explained by the measured environmental factors.

The MFI was better explained by the measured environmental stressors than the 3 individual indicators, showing its relevance in indicating the most impacting factors for brown trout populations' health, i.e., the percentage of agricultural land and the oxygen concentration in the water. Consistently, the populations identified as the most likely to decline had the highest percentages of agricultural land in their surroundings. This corroborates the negative impact of agricultural land on the density and production found in other wild salmonid populations (Jonsson et al., 2011; Vondracek et al., 2005).

Lastly, an important advantage of the MFI is that it revealed the most impacting environmental stressors among those measured, including both the direct and indirect impacts of other environmental factors as revealed by the path analysis. Indeed, this integrative MFI approach showed that the presence of agricultural land was the main driver of low population health, combined with low oxygen concentration. Investigating the impact of environmental factors on the different facets of population health could enhance existing knowledge on the species' ecological niche and tolerance ranges (Sax et al., 2013) and help refining management practices.

Implementation and implications for wildlife conservation

This study shows how integrating different facets of health at the intraspecific level can improve risk assessment in wild populations, which is a major target for managers and stakeholders. Biological indicators combining multiple parameters at different scales of the ecosystem (communities, species, populations, genes) generally outperform single-parameter indicators (Alric et al., 2021; Friberg, 2014). Our MFI likewise demonstrated that the combination of several indicators appears wiser to limit the individual indicators' imperfections. Nonetheless, some individual parameters must be interpreted cautiously. For instance, a low genetic diversity could merely reveal past demographic history rather than the consequence of a recent contemporary stress (Matocq & Villablanca, 2001), and the body condition may misleadingly reflect individual health as it can be density-dependent or associated with some diseases (Bruneaux et al., 2017). This implies that the use of an integrative index should not impede complementary analyses of the individual parameters to ensure that some important information

is not lost during the process, blurring special patterns (e.g., especially alarming pathological or genetic status).

Importantly, the implementation of such integrative health indexes is highly flexible depending on management objectives as they can be built from a wide variety of parameters depending on the particularities of the studied systems. In this study, the parameters chosen were relevant for the brown trout, and easily implemented in the field, which improves the operationality of this index by managers and stakeholders. In other studies or species, additional indicators could be integrated such as physiological parameters (e.g. haematocrit, oxygen consumption, Bruneaux et al., 2017; cortisol, Sadoul & Geffroy, 2019), telomere length informing on the exposure to environmental stressors (e.g. in Dupoué et al., 2017), or demographic parameters (e.g. population age structure, Hixon et al., 2014; sex ratio, Le Galliard et al., 2005). Furthermore, our PI focused on the most threatening pathogen for our populations, but it could be accommodated to other pathogens and even to a wider community of pathogens so as to more realistically reveal the stresses imposed by pathogens. For instance, high sequencing throughput methods can be developed to screen the entire community of parasites infecting individuals, as it has been done for Chinook salmon (Oncorynchus tshawytscha) (Bass et al., 2017). The PI could be applied to mammal or avian species, based on fecal pathogen eggs counts (Kumar et al., 2019) or pathogen identification in the blood (Anjos et al., 2021). The MFI tested in our study could thus be applied to a wide range of species and ecosystems, and therefore help wildlife managers in prioritizing conservation measures into a wide variety of ecological contexts.

The implications of this new index are multiple. First, to understand how this MFI could guide species and habitat conservation actions, a parallel can be drawn with the community indicators of river quality (López-López & Sedeño-Díaz, 2015). For instance, the Fish Index, developed at the European scale, uses information from fish assemblages to assess the water quality of rivers (Pont et al., 2007). These indicators are very useful to assess the environmental quality of rivers at large spatial scales, but may be too coarse to compare sites with similar species composition. For instance, brown trout is the main species contributing to fish biomass in many mountainous areas in Europe, so that a community indicator based on fish assemblage does not allow the assessment of habitat quality. In these cases, gaining information at the *intraspecific level* is more appropriate to estimate the variation in habitat quality and identify potential key environmental stressors. Our indicator is therefore extremely valuable for indicating population and habitat qualities of ecosystems with dominant species, which is actually the norm rather than an exception in many areas in the World (Avolio et al., 2019). Focusing on the dominant species conservation may therefore benefit the whole ecosystem through conservation measures enhancing the habitat quality, corresponding to the concept of umbrella species (Roberge & Angelstam, 2004). Second, since it is based on the assessment of a single species, our indicator is also

extremely useful for managers focusing on emblematic (or economically important) species for which specific actions are often needed.

Conclusions

The development of multifaceted health indexes brings more integrative insights on wildlife population health and potential risk of decline in changing environments. The development of such indexes is timely as cutting-edge field and molecular technologies enable digging into complex processes at the intraspecific level along fine gradients of anthropogenic perturbations. Moreover, non-invasive methods now enable to sample at wider geographic scales and a high number of populations, while minimizing the time spent on the field and the impact on the sampled populations. The framework used in this study could thus be transferred to a wide range of species and different component indicators, as a tool to anticipate wildlife populations' declines and to guide future management decisions. An important perspective would be to normalize this index according to reference populations (as in Pont et al., 2007), which would undoubtedly ease its interpretation and implementation. We hope that our work will motivate researchers to improve this tool and make it operational for managers and stakeholders.

Acknowledgements

We warmly thank Perrine Huet-Conan, Pierre Girard, Keoni Saint-Pé, Orlane Scelsi, Héloïse Bossuat, Jérôme Prunier and Camille Poésy who contributed to the fieldwork. We thank the two anonymous reviewers for their highly relevant comments on the first version of the manuscript. This work was funded by the French Office for Biodiversity (OFB). ED was supported by a PhD grant from the French Ministry of Higher Education, Research and Innovation (MESRI). This work was supported by the "Laboratoires d'Excellences" (LABEX) TULIP (ANR-10-LABX-41).

Author contributions

SB and ED coordinated the study. ED, SB, CV, GL, LJ and EQ contributed to the fieldwork. GL and CV performed laboratory work at EDB. ED ran the statistical analyses. ED, SB, EQ, GL and LJ interpreted the data and wrote the first draft of the manuscript. CV read the initial draft, and all authors gave final approval for publication. All the authors agreed on the revised version of the manuscript.

Data availability

Raw data are available on Figshare at the following link: https://figshare.com/articles/dataset/Multifaceted_health_index/20732092.

Literature cited

- Ahmad, F., Debes, P. V., Palomar, G., & Vasemägi, A. (2018). Association mapping reveals candidate loci for resistance and anaemic response to an emerging temperature-driven parasitic disease in a wild salmonid fish. *Molecular Ecology*, 27(6), 1385–1401. https://doi.org/10.1111/mec.14509
- Alric, B., Dézerald, O., Meyer, A., Billoir, E., Coulaud, R., Larras, F., Mondy, C. P., & Usseglio-Polatera, P. (2021). How diatom-, invertebrate- and fish-based diagnostic tools can support the ecological assessment of rivers in a multi-pressure context: Temporal trends over the past two decades in France. *Science of The Total Environment*, 762, 143915. https://doi.org/10.1016/j.scitotenv.2020.143915
- Anjos, C. C., Chagas, C. R. F., Fecchio, A., Schunck, F., Costa-Nascimento, M. J., Monteiro, E. F., Mathias, B. S., Bell, J. A., Guimarães, L. O., Comiche, K. J. M., Valkiūnas, G., & Kirchgatter, K. (2021). Avian Malaria and Related Parasites from Resident and Migratory Birds in the Brazilian Atlantic Forest, with Description of a New Haemoproteus Species. *Pathogens*, 10(2), 103. https://doi.org/10.3390/pathogens10020103
- Atkinson, P. W., Austin, G. E., Rehfisch, M. M., Baker, H., Cranswick, P., Kershaw, M., Robinson, J., Langston, R. H. W., Stroud, D. A., Turnhout, C. V., & Maclean, I. M. D. (2006). Identifying declines in waterbirds: The effects of missing data, population variability and count period on the interpretation of long-term survey data. *Biological Conservation*, 130(4), 549–559. https://doi.org/10.1016/j.biocon.2006.01.018
- Avolio, M. L., Forrestel, E. J., Chang, C. C., La Pierre, K. J., Burghardt, K. T., & Smith, M. D. (2019). Demystifying dominant species. *New Phytologist*, 223(3), 1106–1126. https://doi.org/10.1111/nph.15789
- Bass, A. L., Hinch, S. G., Teffer, A. K., Patterson, D. A., & Miller, K. M. (2017). A survey of microparasites present in adult migrating Chinook salmon (*Oncorhynchus tshawytscha*) in south-western British Columbia determined by high-throughput quantitative polymerase chain reaction. *Journal of Fish Diseases*, 40(4), 453–477. https://doi.org/10.1111/jfd.12607
- Blum, M. J., Bagley, M. J., Walters, D. M., Jackson, S. A., Daniel, F. B., Chaloud, D. J., & Cade, B. S. (2012). Genetic diversity and species diversity of stream fishes covary across a land-use gradient. *Oecologia*, 168(1), 83–95. https://doi.org/10.1007/s00442-011-2078-x
- Bohmann, K., Evans, A., Gilbert, M. T. P., Carvalho, G. R., Creer, S., Knapp, M., Yu, D. W., & de Bruyn, M. (2014). Environmental DNA for wildlife biology and biodiversity monitoring. *Trends in Ecology & Evolution*, 29(6), 358–367. https://doi.org/10.1016/j.tree.2014.04.003
- Bruneaux, M., Visse, M., Gross, R., Pukk, L., Saks, L., & Vasemägi, A. (2017). Parasite infection and decreased thermal tolerance: Impact of proliferative kidney disease on a wild salmonid fish in the context of climate change. *Functional Ecology*, 31(1), 216–226. https://doi.org/10.1111/1365-2435.12701
- Burkhardt-Holm, P., & Scheurer, K. (2007). Application of the weight-of-evidence approach to assess the decline of brown trout (*Salmo trutta*) in Swiss rivers. *Aquatic Sciences*, 69(1), 51–70. https://doi.org/10.1007/s00027-006-0841-6
- Clements, C. F., Blanchard, J. L., Nash, K. L., Hindell, M. A., & Ozgul, A. (2017). Body size shifts and early warning signals precede the historic collapse of whale stocks. *Nature Ecology & Evolution*, 1(7), 0188. https://doi.org/10.1038/s41559-017-0188
- Clements, C. F., & Ozgul, A. (2016). Including trait-based early warning signals helps predict population collapse. *Nature Communications*, 7(1), 10984. https://doi.org/10.1038/ncomms10984
- Collen, B., McRae, L., Deinet, S., De Palma, A., Carranza, T., Cooper, N., Loh, J., & Baillie, J. E. M. (2011). Predicting how populations decline to extinction. *Philosophical Transactions of the Royal Society B: Biological Sciences*, 366(1577), 2577–2586. https://doi.org/10.1098/rstb.2011.0015
- Do, C., Waples, R. S., Peel, D., Macbeth, G. M., Tillett, B. J., & Ovenden, J. R. (2014). NEESTIMATOR v2: Re-implementation of software for the estimation of contemporary effective population size (*N* _e) from genetic data. *Molecular Ecology Resources*, 14(1), 209–214. https://doi.org/10.1111/1755-0998.12157
- Drake, J. M., & Griffen, B. D. (2010). Early warning signals of extinction in deteriorating environments. *Nature*, 467(7314), 456–459. https://doi.org/10.1038/nature09389

- Dupoué, A., Rutschmann, A., Le Galliard, J. F., Clobert, J., Angelier, F., Marciau, C., Ruault, S., Miles, D., & Meylan, S. (2017). Shorter telomeres precede population extinction in wild lizards. *Scientific Reports*, 7(1), 16976. https://doi.org/10.1038/s41598-017-17323-z
- Duval, E., Blanchet, S., Quéméré, E., Jacquin, L., Veyssière, C., Lautraite, A., Garmendia, L., Yotte, A., Parthuisot, N., Côte, J., & Loot, G. (2021). Urine DNA (uDNA) as a non-lethal method for endoparasite biomonitoring: Development and validation. *Environmental DNA*, edn3.228. https://doi.org/10.1002/edn3.228
- Eklöv, A. G., Greenberg, L. A., Bronmark, C., Larsson, P., & Berglund, O. (1999). Influence of water quality, habitat and species richness on brown trout populations. *Journal of Fish Biology*, 54(1), 33–43. https://doi.org/10.1111/j.1095-8649.1999.tb00610.x
- Elliott, J. M. (1994). Quantitative Ecology and the Brown Trout (Oxford University Press).
- Elliott, J. M., & Elliott, J. A. (2010). Temperature requirements of Atlantic salmon *Salmo salar*, brown trout *Salmo trutta* and Arctic charr *Salvelinus alpinus*: Predicting the effects of climate change. *Journal of Fish Biology*, 25.
- Epskamp, S., Stuber, S., Nak, J., Veenman, M., & Jordgensen, T. D. (2019). *semPlot: Path Diagrams* and Visual Analysis of Various SEM Packages' Output. https://CRAN.Rproject.org/package=semPlot
- Evans, S. R., & Sheldon, B. C. (2008). Interspecific Patterns of Genetic Diversity in Birds: Correlations with Extinction Risk. *Conservation Biology*, 22(4), 10.
- Fox, J. (2020). *RcmdrMisc: R Commander Miscellaneous Functions. R package version 2.7-1.* https://CRAN.R-project.org/package=RcmdrMisc
- Friberg, N. (2014). Impacts and indicators of change in lotic ecosystems: Impacts and indicators of change in lotic ecosystems. Wiley Interdisciplinary Reviews: Water, 1(6), 513–531. https://doi.org/10.1002/wat2.1040
- Fulton, T. W. (1904). The rate of growth of fishes. Twenty-Second Annual Report, pp 141-241.
- Goff, C. B., Walls, S. C., Rodriguez, D., & Gabor, C. R. (2020). Changes in physiology and microbial diversity in larval ornate chorus frogs are associated with habitat quality. *Conservation Physiology*, 8(1), coaa047. https://doi.org/10.1093/conphys/coaa047
- Hansson, B., & Westerberg, L. (2002). On the correlation between heterozygosity and fitness in natural populations. *Molecular Ecology*, *11*(12), 2467–2474. https://doi.org/10.1046/j.1365-294X.2002.01644.x
- Hixon, M. A., Johnson, D. W., & Sogard, S. M. (2014). BOFFFFs: On the importance of conserving old-growth age structure in fishery populations. *ICES Journal of Marine Science*, 71(8), 2171–2185. https://doi.org/10.1093/icesjms/fst200
- Hoban, S., Hauffe, H. C., Pérez-Espona, S., Arntzen, J. W., Bertorelle, G., Bryja, J., Frith, K., Gaggiotti, O. E., Galbusera, P., Godoy, J. A., Hoelzel, A. R., Nichols, R. A., Primmer, C. R., Russo, I.-R., Segelbacher, G., Siegismund, H. R., Sihvonen, M., Vernesi, C., Vilà, C., & Bruford, M. W. (2013). Bringing genetic diversity to the forefront of conservation policy and management. *Conservation Genetics Resources*, 5(2), 593–598. https://doi.org/10.1007/s12686-013-9859-y
- Hu, L., & Bentler, P. M. (1999). Cutoff criteria for fit indexes in covariance structure analysis: Conventional criteria versus new alternatives. *Structural Equation Modeling: A Multidisciplinary Journal*, 6(1), 1–55. https://doi.org/10.1080/10705519909540118
- Hughes, J. B., Daily, G. C., & Ehrlich, P. R. (1997). Population Diversity: Its Extent and Extinction. *Science*, 278(5338), 689–692. https://doi.org/10.1126/science.278.5338.689
- Hunn, J. B. (1982). Urine Flow Rate in Freshwater Salmonids: A Review. *The Progressive Fish-Culturist*, 44(3), 119–125. https://doi.org/10.1577/1548-8659(1982)44[119:UFRIFS]2.0.CO;2
- Jonsson, B., Jonsson, N., & Ugedal, O. (2011). Production of juvenile salmonids in small Norwegian streams is affected by agricultural land use: Salmonid production in streams. *Freshwater Biology*, *56*(12), 2529–2542. https://doi.org/10.1111/j.1365-2427.2011.02678.x
- King, K. C., & Lively, C. M. (2012). Does genetic diversity limit disease spread in natural host populations? *Heredity*, 109(4), 199–203. https://doi.org/10.1038/hdy.2012.33
- Kophamel, S., Illing, B., Ariel, E., Difalco, M., Skerratt, L. F., Hamann, M., Ward, L. C., Méndez, D., & Munns, S. L. (2021). Importance of health assessments for conservation in noncaptive wildlife. *Conservation Biology*, cobi.13724. https://doi.org/10.1111/cobi.13724

- Kruse, C. G., Hubert, W. A., & Rahel, F. J. (1998). Single-Pass Electrofishing Predicts Trout Abundance in Mountain Streams with Sparse Habitat. North American Journal of Fisheries Management, 18, 940–946.
- Kumar, S., Kumara, H. N., Santhosh, K., & Sundararaj, P. (2019). Prevalence of gastrointestinal parasites in lion-tailed macaque *Macaca silenus* in central Western Ghats, India. *Primates*, 60(6), 537–546. https://doi.org/10.1007/s10329-019-00751-y
- Lande, R., & Shannon, S. (1996). The Role of Genetic Variation in Adaptation and Population Persistence in a Changing Environment. *Evolution*, 50(1), 434–437.
- Le Galliard, J.-F., Fitze, P. S., Ferrière, R., & Clobert, J. (2005). Sex ratio bias, male aggression, and population collapse in lizards. *Proceedings of the National Academy of Sciences*, 102(50), 18231–18236.
- Lobón-Cerviá, J. (2005). The importance of recruitment for the production dynamics of stream-dwelling brown trout (*Salmo trutta*). *Canadian Journal of Fisheries and Aquatic Sciences*, 62, 10.
- López-López, E., & Sedeño-Díaz, J. E. (2015). Biological Indicators of Water Quality: The Role of Fish and Macroinvertebrates as Indicators of Water Quality. In R. H. Armon & O. Hänninen (Eds.), *Environmental Indicators* (pp. 643–661). Springer Netherlands. https://doi.org/10.1007/978-94-017-9499-2_37
- Maebe, K., Karise, R., Meeus, I., Mänd, M., & Smagghe, G. (2019). Level of Genetic Diversity in European Bumblebees is Not Determined by Local Species Abundance. *Frontiers in Genetics*, *10*, 1262. https://doi.org/10.3389/fgene.2019.01262
- Manning, P., van der Plas, F., Soliveres, S., Allan, E., Maestre, F. T., Mace, G., Whittingham, M. J., & Fischer, M. (2018). Redefining ecosystem multifunctionality. *Nature Ecology & Evolution*, 2(3), 427–436. https://doi.org/10.1038/s41559-017-0461-7
- Matocq, M. D., & Villablanca, F. X. (2001). Low genetic diversity in an endangered species: Recent or historic pattern? *Biological Conservation*, 98(1), 61–68. https://doi.org/10.1016/S0006-3207(00)00142-7
- Molony, B. (2001). Environmental requirements and tolerances of Rainbow trout (*Oncorhynchus mykiss*) and Brown trout (*Salmo trutta*) with special reference to Western Australia: A review. *Fisheries Research Reports*, 130, 32.
- Nicol, E., Stevens, J. R., & Jobling, S. (2017). Riverine fish diversity varies according to geographical isolation and land use modification. *Ecology and Evolution*, 7(19), 7872–7883. https://doi.org/10.1002/ece3.3237
- Okamura, B., Hartikainen, H., Schmidt-Posthaus, H., & Wahli, T. (2011). Life cycle complexity, environmental change and the emerging status of salmonid proliferative kidney disease. *Freshwater Biology*, *56*(4), 735–753. https://doi.org/10.1111/j.1365-2427.2010.02465.x
- Oksanen, J., Blanchet, F. G., Friendly, M., Kindt, R., McGlinn, D., O'Hara, R. B., Simpson, G. L., Solymos, P., Stevens, M. H. M., Szoecs, E., & Wagner, H. (2020). *Vegan: Community Ecology Package. R package version 2.5-7.* https://CRAN.R-project.org/package=vegan
- Palstra, F. P., & Ruzzante, D. E. (2008). Genetic estimates of contemporary effective population size: What can they tell us about the importance of genetic stochasticity for wild population persistence? *Molecular Ecology*, 17(15), 3428–3447. https://doi.org/10.1111/j.1365-294X.2008.03842.x
- Paz-Vinas, I., Loot, G., Stevens, V. M., & Blanchet, S. (2015). Evolutionary processes driving spatial patterns of intraspecific genetic diversity in river ecosystems. *Molecular Ecology*, 24(18), 4586–4604. https://doi.org/10.1111/mec.13345
- Pont, D., Hugueny, B., & Rogers, C. (2007). Development of a fish-based index for the assessment of river health in Europe: The European Fish Index. *Fisheries Management and Ecology*, 14, 427– 439.
- QGIS Development Team. (2022). QGIS Geographic Information System. https://www.qgis.org
- R Core Team. (2020). R: A language and environment for statistical computing. *R Foundation for Statistical Computing, Vienna, Austria.* https://www.R-project.org/
- Radchuk, V., Reed, T., Borràs, A., Senar, J. C., & Kramer-Schadt, S. (2019). Adaptive responses of animals to climate change are most likely insufficient.

- Rigdon, E. E. (1996). CFI versus RMSEA: A comparison of two fit indexes for structural equation modeling. *Structural Equation Modeling: A Multidisciplinary Journal*, *3*(4), 369–379. https://doi.org/10.1080/10705519609540052
- Roberge, J.-M., & Angelstam, P. (2004). Usefulness of the Umbrella Species Concept as a Conservation Tool. *Conservation Biology*, *18*(1), 76–85. https://doi.org/10.1111/j.1523-1739.2004.00450.x
- Robinson, M. L., Gomez-Raya, L., Rauw, W. M., & Peacock, M. M. (2008). Fulton's body condition factor K correlates with survival time in a thermal challenge experiment in juvenile Lahontan cutthroat trout (*Oncorhynchus clarki henshawi*). *Journal of Thermal Biology*, 33(6), 363–368. https://doi.org/10.1016/j.jtherbio.2008.05.004
- Rosseel, Y. (2012). lavaan: An R Package for Structural Equation Modeling. *Journal of Statistical Software*, 48(2), 1–36.
- Rousset, F. (2008). genepop'007: A complete re-implementation of the genepop software for Windows and Linux. *Molecular Ecology Resources*, 8(1), 103–106. https://doi.org/10.1111/j.1471-8286.2007.01931.x
- Sadoul, B., & Geffroy, B. (2019). Measuring cortisol, the major stress hormone in fishes. *Journal of Fish Biology*, 94(4), 540–555. https://doi.org/10.1111/jfb.13904
- Saint-Pé, K., Leitwein, M., Tissot, L., Poulet, N., Guinand, B., Berrebi, P., Marselli, G., Lascaux, J.-M., Gagnaire, P.-A., & Blanchet, S. (2019). Development of a large SNPs resource and a lowdensity SNP array for brown trout (*Salmo trutta*) population genetics. *BMC Genomics*, 13.
- Sax, D. F., Early, R., & Bellemare, J. (2013). Niche syndromes, species extinction risks, and management under climate change. *Trends in Ecology & Evolution*, 28(9), 517–523. https://doi.org/10.1016/j.tree.2013.05.010
- Skidmore, A. (2003). *Environmental Modelling with GIS and Remote Sensing*. https://www.taylorfrancis.com/books/e/9780203302217
- Spooner, F. E. B., Pearson, R. G., & Freeman, R. (2018). Rapid warming is associated with population decline among terrestrial birds and mammals globally. *Global Change Biology*, 24(10), 4521– 4531. https://doi.org/10.1111/gcb.14361
- Stephen, C. (2014). Toward a modernized definition of wildlife health. *Journal of Wildlife Diseases*, 50(3), 427–430. https://doi.org/10.7589/2013-11-305
- Stoffel, M. A., Esser, M., Hoffman, J., & Kardos, M. (2016). *InbreedR: Analysing Ibreeding Based on Genetic Markers*. https://CRAN.R-project.org/package=inbreedR
- Thomas, C. D., Cameron, A., Green, R. E., Bakkenes, M., Beaumont, L. J., Collingham, Y. C., Erasmus,
 B. F. N., de Siqueira, M. F., Grainger, A., Hannah, L., Hughes, L., Huntley, B., van Jaarsveld,
 A. S., Midgley, G. F., Miles, L., Ortega-Huerta, M. A., Peterson, A. T., Phillips, O. L., &
 Williams, S. E. (2004). *Extinction risk from climate change*. 427, 4.
- Unfer, G., & Pinter, K. (2018). Fisheries Management of Stream-Resident Brown Trout Populations— Possibilities and Restrictions. In J. Lobón-Cervía & N. Sanz (Eds.), *Brown trout: Biology,* ecology and management (pp. 649–665). Wiley.
- Valenzuela-Sánchez, A., Schmidt, B. R., Uribe-Rivera, D. E., Costas, F., Cunningham, A. A., & Soto-Azat, C. (2017). Cryptic disease-induced mortality may cause host extinction in an apparently stable host-parasite system. *Proceedings of the Royal Society B: Biological Sciences*, 284(1863), 20171176. https://doi.org/10.1098/rspb.2017.1176
- Vondracek, B., Blann, K. L., Cox, C. B., Nerbonne, J. F., Mumford, K. G., Nerbonne, B. A., Sovell, L. A., & Zimmerman, J. K. H. (2005). Land Use, Spatial Scale, and Stream Systems: Lessons from an Agricultural Region. *Environmental Management*, 36(6), 775–791. https://doi.org/10.1007/s00267-005-0039-z
- Waples, R. S., & Do, C. (2007). *LDNE: a program for estimating effective population size from data on linkage disequilibrium*. 5.
- Waples, R. S., & Do, C. (2010). Linkage disequilibrium estimates of contemporary N_e using highly variable genetic markers: A largely untapped resource for applied conservation and evolution: Precision and bias of LD estimates. *Evolutionary Applications*, 3(3), 244–262. https://doi.org/10.1111/j.1752-4571.2009.00104.x
- Wickham, H., & Seidel, D. (2020). Scales: Scale Functions for Visualization. R package version 1.1.1. https://CRAN.R-project.org/package=scales

L
\cap
5
e a
D.
5
¥.
Ň
+

Supporting information

Genetic indicator

N.

84.6 113

107.8

11.6

76.6 13.5

12.3

350

281.5 92.1

59.2

15.3

145.6

17

40.4

78.3

305

97.3

28.1

6.7

630

52.7

48.4

8.7

21.2

411.8

32.5

89.6

35.7

30.9

63.4

40.6

11.3

123.1

98.5

176.1

438

489.3

238.2

490.4

28.5

60.3

35.4

52.8

680.5

6

Site	Date of sampling N		% Agricultural land	d Altitude (m) source (km)	temperature (°C)	PO4 ³⁻ (µg/L)	(µg/L)	pН	(µS/cm)	(mg/L)		prevalence	(copies/µL/g/min)	factor	c	lensity (N/min)	MLH	
ADOAur	22/07/2019	20	6	9 2	59	60	18.98	40.9	703.8	8. 8.	24 23	2.54	9.48	0.55		1.94	1.28	0.7	7 0.1	6
ALACas	12/08/2019	20	6	1 2	79	21	17.77	27.7	646.0	8.	39 16	4.42	9.70	0.90		2.43	1.18	1.2	8 0.1	15
ARABas	15/07/2019	20	3	4 4	59	27	16.90	42.1	550.7	8.	28 7	9.51	10.02	0.00		0.00	1.24	0.5	3 0.1	16
ARBBar	25/07/2019	20	4	4 3	90	3	15.26	1.6	653.9	8.	35 39	5.78	9.79	0.05		0.10	1.27	1.4	1 0.1	7
ARBMan	16/07/2019	19	7	0 3	10	18	18.37	32.6	1390.0	8.	70 38	6.69	10.30	0.05		0.16	1.28	0.3	3 0.1	18
ARGFoi	18/07/2019	19	3	1 3	83	20	17.22	42.3	958.0	8.	19 8	4.42	9.88	0.00		0.00	1.28	2.1	0 0.1	ί7
ARGPon	24/07/2019	19	6	9 4	67	13	15.89	117.3	669.4	7.	92 7	6.17	10.05	0.00		0.00	1.18	0.5	3 0.1	18
ARIVar	25/09/2019	20	6	2 3	26	87	17.15	8.6	92.4	8.	50 9	0.59	10.64	0.75		11.69	1.13	0.7	7 0.1	9
ARRGou	08/08/2019	20	4	6 2	99	27	16.95	110.2	1489.0	8.	54 45	9.86	9.55	0.90		5.58	1.11	0.6	7 0.1	ι5
ARRMou	17/07/2019	11	8	9 2	32	43	19.43	149.9	1198.0	8.	58 43	9.83	9.61	0.91		6.81	1.29	0.1	2 0.1	4
ARZDur	24/07/2019	20	6	i0 3	50	27	17.29	75.6	1776.0) 8.	43 32	9.29	9.74	0.60		5.58	1.24	0.5	3 0.1	ι5
ARZPon	15/07/2019	20	8	0 3	83	17	16.91	14.8	577.9	8.	02 21	5.46	9.13	0.00		0.00	1.24	0.4	5 0.1	4
BAUSou	30/07/2019	20	8	9 4	40	9	17.40	37.2	560.0	8.	22 23	3.26	9.21	1.00		6.17	1.19	0.3	3 0.1	4
BOUAuc	19/07/2019	19	4	5 5	50	17	15.30	193.3	894.0	8.	45 27	3.66	10.24	0.00		0.00	1.24	1.3	3 0.1	16
BOUSer	17/07/2019	20	8	0 3	70	18	18.03	15.8	2310.6	8.	09 10	9.36	9.09	0.95		3.09	1.28	1.1	0 0.2	21
DOUPer	14/08/2019	20	3	7 4	58	15	15.72	20.1	336.5	8.	59 38	6.44	9.24	0.05		0.02	1.23	0.8	7 0.1	ί4
ECHJui	08/08/2019	20	6	3 3	40	7	19.24	38.0	2208.0	8.	39 27	7.55	9.38	1.00		2.72	1.16	0.5	9 0.1	16
GARFos	23/07/2019	19		7 5	45	51	15.28	88.8	0.0	8.	44 14	2.52	10.03	0.15		0.04	1.21	1.2	5 0.1	19
GARVil	16/07/2019	20	7	8 3	B5	105	16.97	197.1	638.0	8.	51 17	8.87	10.06	0.25		0.36	1.24	1.1	0 0.1	17
GAVLau	26/07/2019	20	5	3 4	21	47	14.33	121.2	284.8	8. 8.	32 17	7.74	9.85	0.00		0.00	1.31	0.6	7 0.1	18
GAVRie	26/07/2019	13	6	51 3	40	71	15.96	46.7	175.0	8.	37 18	5.84	9.81	0.05		0.05	1.31	1.0	0 0.1	18
GERAsp	25/07/2019	19	3	1 4	49	16	14.61	44.3	470.6	8.	40 29	8.74	9.91	0.00		0.00	1.19	1.3	3 0.1	15
GERPoi	07/08/2019	19	6	i1 3	30	32	17.89	0.0	1312.6	8.	39 37	2.57	10.07	0.00		0.00	1.20	0.4	3 0.1	8
GERSou	16/07/2019	20	7	0 3	78	25	16.48	125.5	1060.0) 8.	41 30	8.77	9.94	0.00		0.00	1.20	0.8	2 0.1	18
GRAMon	17/07/2019	20	6	57 3	25	17	17.25	31.4	1976.0	8.	12 11	0.01	9.33	0.80		2.45	1.30	0.7	9 0.1	17
GRSLas	09/08/2019	10	7	78 3	51	14	16.33	28.6	1877.0	8.	10 10	6.54	9.49	1.00		6.58	1.24	0.2	5 0.1	17
HERCam	14/08/2019	15	4	9 3	45	57	17.81	15.7	550.0	8.	37 34	0.72	9.99	0.80		16.98	1.29	0.4	0 0.1	18
JOBEnc	07/08/2019	20	4	7 3	70	17	16.47	97.4	1029.0	8.	21 44	4.52	9.42	0.95		6.37	1.24	2.4	0 0.1	17
JOBLab	25/07/2019	18	3	5 4	40	9	13.29	16.0	1024.0	8.	51 39	8.92	9.97	0.00		0.00	1.25	1.4	3 0.1	13
LEZArr	19/07/2019	20	5	5 4	17	32	16.52	11.5	219.0	8.	39 21	5.23	10.01	0.00		0.00	1.32	0.5	5 0.1	17
LEZAub	23/09/2019	20	3	7 4	90	26	16.63	104.6	0.0	8.	37 23	9.94	9.83	0.00		0.00	1.10	0.3	3 0.1	17
NESArr	06/08/2019	18	2	2 7	31	34	13.88	68.7	0.0	8.	26 12	1.29	9.91	0.00		0.00	1.21	0.8	4 0.1	18
NESSar	06/08/2019	19	4	2 6	30	44	13.07	3.8	208.0	8.	31 11	7.14	10.49	0.05		0.11	1.30	0.6	1 0.1	19
NISLom	12/08/2019	18	5	2 4	77	15	14.80	14.6	450.0	8.	42 20	5.60	10.24	0.00		0.00	1.21	0.7	1 0.1	ί4
ONELuc	13/08/2019	20		7 6	21	21	13.16	23.2	537.0	8.	42 11	3.56	10.31	0.00		0.00	1.43	2.0	0 0.1	16
ORIOrg	05/09/2019	20	1	.5 8	29	21	12.48	0.0	0.0) 7.	94 2	4.45	11.40	0.00		0.00	1.17	1.5	0 0.1	18
OURSar	07/08/2019	20	6	51 4	71	21	15.61	23.3	576.4	8.	30 26	2.41	9.81	0.00		0.00	1.10	0.9	2 0.1	16
PIQCig	23/07/2019	20	2	4 4	80	39	14.34	37.9	359.0	8.	13 14	0.22	10.26	0.00		0.00	1.31	1.4	4 0.1	18
PIQCI	23/07/2019	20	1	.7 5	89	29	13.73	26.8	347.2	8.	05 13	1.83	9.97	0.00		0.00	1.28	0.3	0 0.1	18
PIQRav	29/07/2019	12		0 8	44	12	12.30	204.5	0.0	8.	23 9	7.00	10.05	0.00		0.00	1.30	0.1	3 0.1	15
SALTau	23/09/2019	20	6	8 3	50	50	18.01	37.0	158.0	8.	46 22	1.26	9.55	0.25		1.80	1.16	1.2	0 0.1	ί7
SAVVil	09/08/2019	18	7	6 4	54	10	16.60	8.6	931.0	8.	04 10	3.15	9.31	1.00		3.59	1.40	0.9	1 0.1	.8
SIOSta	18/07/2019	20	2	8 4	50	4	15.88	105.7	604.0	8.	33 27	2.84	9.95	0.00		0.00	1.27	0.5	0 0.1	9
TORTuz	09/08/2019	13	6	5 5	30	10	17.50	61.3	2440.4	7.	70 14	1.94	8.48	0.77		8.17	1.17	0.8	0 0.1	19

Ponctual dosage

NNO3 NNO2

Ponctual measure (InSitu)

Conductivity O2 concentration

Pathological indicator

Parasite DNA excretion

Infection

Ecological indicator

Brown trout

Condition

143

Table A1. Sampled sites and their characteristics.

GIS software

Distance from

HOBO logger

Mean summer

TOUFre

VICNia

14/08/2019

11/10/2019

20

20

18

12

626

546

12

32

14.59

12.31

24.5

133.1

123.0

0.0

8.23

8.09

164.45

80.34

9.38

10.36

0.00

0.00

0.00

0.00

1.33

1.05

1.00

1.75

0.18

0.16

Table A2. List of the 175 SNPs genotyped in the study.

144

			Repualler
SNP ID Sequence	Provenance	genotyping	HWE check
St23I1_23468 AACTGCCTCAGGTCAGTAAGGGAATGACTGGGGGGCTGAGTGTGTTTCTGAATGAA	Ahmad et al. 2018	х	х
St23l1_9367 AGGCCGCATCGCAGTAAATGCTATACAGCCAATGCCAATGCCAGATGCCAGATGCAATGCTTTAGCTTTAGCTTTGCTTTACCTCTGCTATCCACTC[T/C]TCTTTAgaaaaaagtgttcaataaacccacagccctcccattgatgttgg	Ahmad et al. 2018	х	х
St23I2 20870 GGCAAGAGAGAGAGAGAGAGAGAGGGCGTTAAGTCATAAAACCGGCCCAAATCCATCC	Ahmad et al. 2018	х	х
St23I2 49157 GATTATTTTTGGCTGTAGCAAATTGGCTCAAATTAAGATTCTACATCTGTACATACTTT[G/T]TTTGCTCTGTTGACATCTCAGACACCCtggacccattccaattcacatac	Ahmad et al. 2018	х	х
St23I2 634 GTCCTGGGGTCAAGCAGGAGAGGGTCTGAAGGAGAGGAG	Ahmad et al. 2018	х	х
St411 23300 gcctctgagtctgctcctgtcggctctggcttgaccctgcagAAAAAAAT[G/T]ATCTGTTAAACTAACTATTTCATAAAACTATAATTGTGGGCAATAATATTTAATAAAAGCCATTATTTC1	Ahmad et al. 2018		
St4I2 93 CAGTCGTGTTCGAATATGTATGGGCCCTTCCGGACAAGTCCGTTAAAATTACAACTACCCAAAACCCGTGA[T/C]AATCAGATCAGATCTGacccagaacttatggaattatggatcc	Ahmad et al. 2018	х	х
St4I3 18317 CTGCAGTGACAATAAAGACTGCTTCCGGGTGAGAGGAATTCTCGCTGGGTA[T/C]GGGGGGAAAGGAAAGGAAAGGGAAAGGAACAGGGGAAAGATTGTCAGCACTCCGatacagc	Ahmad et al. 2018	x	х
St4I3 33646 acggagcaatgtttttaggcataggctacttatagaaCTGCAGAACATCT[C/A]TTTCATGTATGCATATICCAAACCTGTGCTTCAAAGATTCTATCCAATTAGCCTACTGCGAAAACTTCCAAATATTCA	Ahmad et al. 2018	х	х
St56/1 14011 AGTAACTAGTCCCCAACTGATGCCACCTCATCCCTCACCCCACCGCCTGACTCA[G/A]AAGAATTATCATTTACTATTACCATAGTGAATTATtgtaatgtttgttigts	Ahmad et al. 2018	х	х
St56/1 18065 tatccaacagagaactttcaactgagaactgcaggctatgtctgcagTG/C/T/AAAGCTCTGACCTTGAGTTTTTGGTGAACTGCTCTATTTAATTCACCTTTAGTTGACTCTAAACATCACCTTACACAGATATGTG	Ahmad et al. 2018	х	х
St56/2 21030 #ttt#ccTGCAGGGGACACCACCTCCACCTTCGACCAGCTGGGTGGACCTGTJA/CICATTCGGCTGGCTAGCCGGCGACGACGTCCAGAGCGGCGGGC	Ahmad et al. 2018	x	x
515612 26142 ctetegagatgagagaaccttcctgaaggacaaccatctCIGCAGCACTCIC/TIACCAATAAGGCATTITAIGGTAGAGTGGCCAGATGGAAGCCACTCCCAGTAAAAAGGCACATGACAGCCCTCTTGGAGTTIG	C Ahmad et al. 2018		
5156/2 347 CAGACTICTTCCCCCCTTCTCCCCGCACTCTCCTCTGCCCAGTACCTGACCCACACTGTACCACAGGA[A/C]TTCAAAAtaggaccaacaattttttacattcatetetatagggttctccc	Ahmad et al. 2018	х	х
556/2 8321 gctgtgcagatggtagggctctgtactggctcatagtacacactgcagGGG/TITTTTAATTICCTATTTTTTTACTTGAGGAGTICTATTAAAATGTCTTATCCGCTCTTTGTGGGGTTAGTACGAGGAACTGTCTG	Ahmad et al. 2018	x	x
St56i3 11053 CAGAAGCAACTGGCCACTGAACCAGAAGCAGCTGACCGCCATGCCCCCAGCAAGTGTGGGTATCAAGGT/CITAAAACATTCCAGCAGCaaaatttgectgccatggcacaggg	Ahmad et al. 2018	x	x
St56i3 15605 etcatercrtecaeteecaeteecaeteccetecaeTGGGAGAAGAGCAGC/C/TIGTGGGTCAGGCCACACCTGCGGCAAGTCAAGGGGACACTCAGGCCATCCCACCCCCAGGGGAGA	Ahmad et al. 2018	x	x
515613 21686 AGAGGCTGGACAAAATAGATAGATAGATGAAGAAGAAGATGCACCAATGCATTTTGGGTGAAATGAGCAAAAACACTAACTA	Ahmad et al. 2018	x	x
St56I3 7170 CIGCAGCIGCCAGICACAACATAICAACCIGIGATAATAACCGTAIGGGCITCCIT/GICIGCCACCCACACCIGAGAGAGGGGGAIGAAGGIGCIzagagagaga	Ahmad et al. 2018	x	x
170400 AATTCCCATGCTGGGGCACAGTGGGAAAGTTGACATCAAACACATAATAGGACTTGAAGCACA[C/TIATCCAGTGCATCTTGCTCCAATAATGACTTAAAACCTGTGAGCAGTCCACAAGAAC	Saint-Pe et al. 2019	x	x
223903 AATTCCTAGGGCAATGGCTTACATCTGCTAAGTAAATAGAAGTACACAGGTAGGT	Saint-Pe et al. 2019	x	x
66394 CGGTTGTTGATGTCCTCTCATTTTTGCCACAGGCTTTGAGCCGGCCTGTGACAGCACAAACTCAAAAACTCAAAAACTCGAGAAACTCCATGGAGAATAAGAGCACCTCCTCCCAGCT	Saint-Pe et al. 2019	x	x
68688 AATTCCCTGTGTGAGGGGGGGGGGGGGGGGGGGGGGGGG	Saint-Pe et al. 2019	x	x
68989 AATTCACTGTCTAAACCATGTGGTCGAACTAGTCCATGTGGTTCAATCTCAATCCCATGCCATGTGGGAGGATAATGAAATGGCAGCCTTGGCACCTCTCAAAAGCCTACCACCTATTGCTATC	Saint-Pe et al. 2019	x	x
70645 AATTCTTAACAGACTTCATGTCTATAACAGAGAGCACATATGTAGAGAAATTGTGCATAGIA/GICCTTTCGAATGTGTGACTGTTTGAAGAGGTCACAATGACATCTCTAAGAAGTGCAAGTTATT	Saint-Pe et al. 2019	x	x
72682 CGGGTAGIGCTGAGACCCGATACATAAGCTCCCCGCCACACTACTTCCACCTCTTCIG/CITTTCTCGGATCATTCCAGAGAGGGATAGACTTGGTAAGAGGCTCTATGTAGAGTGTATAAGGGCA	Saint-Pe et al. 2019	x	x
75940 AATTCTTCTGTCTCTTTTACGCAGCAGGCATATAACACAGACTGGCCAAGTAGGCATGIC/TJAATGGGCTATGGCCATTGCAGTTAATTACCACATTTTCTGCGCTAAACTATGTAGAATATT	Saint-Pe et al. 2019	x	x
76879 CGGCTGCATTGTCATCCTCCCCCTCTGGCCTGCAGGAGGGCCCTCAAACAGGTTAIC/TITGACTAGACTGCTCCTCTGCTCTTTTATAGGCAGAGAATGAGAATGAGAATGACAAATGACAAATAGGTGTTTT	Saint-Pe et al. 2019	x	x
76971 AATTCCCAGACTTCCTTAATGCCTGGTTGGCGGGCTCAGGCTGGCT	Saint-Pe et al. 2019	x	x
83411 AATTCTGGGTGGATGGATGGATGGACCGCCCTACTATTGGGCCCAGATCATATCTGCATG[C/G]CTGCTGCATCCCATCCCATCCCATACAGCCATACAGCCATACAGCTACGATGTATTCTACTC	Saint-Pe et al. 2019	x	x
84426 AATTCTCCTGCCTAAGTTAATATTTACCAGGGGAATGGATCCTCAGTGGGTAAT[A/T]GCAGGGCATGCTATGTAGCCAACGACGAGGTGTGCCTTGTAACTTCAGTCATGACACTTTA	Saint-Pe et al. 2019	x	x
88663 CGGTTATTCAACCATCGCCCCATACAGTATTTCATTTCA	Saint-Pe et al. 2019	x	x
89247 CGGACAGCTGATGAAATTGTAGGTTTGCACAACAGAGGAATTGTCAGAAACCGTCTCAGGGA/A/T/GCTCATTTGCGTGCTCGTCGTCCTCACCAGGGTTATGACCTGACGTGCAGTTCAGGGT	Saint-Pe et al. 2019	x	x
91305 CGGGGGCTCTGCAGGACCAGCCACGGGTGCAGGTGCAGGTGAGGACCTGTAGGATCACGCAGAGACAIT/GIATGATGGCATAGGGTTGGTAGAGGCGGCGGAGAACGTCCTTCAGGTGCAGGGTTGAGC	Saint-Pe et al. 2019	x	x
92989 CGGTAGAAATCTGTCCTTTGGTCTGATGAATCCAAATCTGAGATTGTTGATTCCAACCTC/C/T/GTGTCTTTGTGAAATACAGAATAGGTGAACGGATGATCTCCCGCATGTGTGGGTTCCCACC	Saint-Pe et al. 2019	x	x
95217 AATTCATGGAATGGAGTCAAACAGGTGGTTTCCATACAGTTTGATGTGATGGATG	Saint-Pe et al. 2019	x	x
97641 AATTCAGTGGTTTGGCCCTTAGTTIGACTAAAGAGAGCCAAACCTTGCACAGTC[C/A]TTGCAGTGAGTAATGTTTGTCCACCCAGGAGTCTTATTAAGGCTAATCAGAGACATCTTCATG1	Saint-Pe et al. 2019	x	x
99153 AATTCTATTGTTGACATGGTATTACAGGGTATTACATCACAACATCTTGTGTGC[G/TITACCACTGCATCATATTGTCTTGTACAATGTTGTACTGTATTGTCTTGACAATGTTGTACTGTATTGTCTTAAA]	Saint-Pe et al. 2019	x	x
99729 CGGCTICTTAAGAGAACCAGGCGAGGACAGTCCTGGCAACAACAAATAAAT	Saint-Pe et al. 2019	x	x
	Saint-Pe et al. 2019	x	x
103111 CGGGTTCCACTCCTATCAGCTAAAAATAACAAGAAGTGGGTCCAGTGGGCACATIA/GJATCACCAACACTGGACAATTGACGAGTGGAAAAACATTGCCTGGTCTGACGAATCCTGGTTCTTG	Saint-Pe et al. 2019	x	x
108574 CGGCTACTCACCATTTATTAATCATTACTCCCCACATTTGTAAATGTTAGTAAGCTAGT[6/T]ATTCACAGTTATTCACAGTATATACAGTATATTCCTCGAAACATGCTGTGTTGTGTGCTTG]	Saint-Pe et al. 2019	x	x
109230 AATTCCCTTTTCCCCCACCCTCTTCTCTCGCGTTGAACCCGTGAACATGGACATGGACATGGACTGCACATGTATT/CCATGATCACGTTCGACACATTT	Saint-Pe et al. 2019	x	x
111379 AATTCCTATGGCCCATAACACTGGTACACTATTTCATGACTCTTATCCACTCATATGGCTGAGAGAGA	Saint-Pe et al. 2019	x	x

114480	CGGTCCTGGGGGTGTGTGCGTGGTGGCCCACAGAAACATGCCTTGACAGTTGAAT[A/G]AGGCACASCAAGTTCTTCCATTTTTAAAGTATGATTCATTCATTCATT	Saint-Pe et al. 2019 X	×
115815	AATTCAGCCTTCTCCCTCTCTCCTCCTCTCTCTCCTCCTCTCTCT	Saint-Pe et al. 2019 X	×
125692	AATTCTAGGTCATTTAACAAAACAGGGCCAAAGGTCCATGGGGCCAGGCATTAAAGCAA[A/C]CAACCTACAATGTCTCTGGTTGCTTTTCTCTGTATATATCTCTCTC	Saint-Pe et al. 2019 X	×
133622	CGGATCCTCGCATTTATTCTCGGGGAAGGGGGGGGGGGG	Saint-Pe et al. 2019 X	×
134069	CGGAGGAATTGAGGACCGTGGCTAACGAATACGGCGCGCAGGTAAGTAA	Saint-Pe et al. 2019 X	
135108	AATTCAAATCAAATTAAATTACATTGCCATTGCCGGAATACAATAGTGT[A/C]GACATTACGGGGAAATGCTTACTGACAAGCCACTAACAATGCAGTTTAGGAAAAAA/	Saint-Pe et al. 2019 X	×
135228	AATTCATGAGCAATTTCGCCTCCGAATTTCTGCTCTTTGCGCAGCAAGAGGC(A/TJAATTTTCTCTTCGGGGGGGGGCGCCCTAGTTTCGGAAATATTAAGATTATTTAAGATTATTAAGACA	Saint-Pe et al. 2019 X	×
136951	AATTCTGTTGATTGACTGGGCTCGGGGTTCCAACGCATTGTCCCCTCCAGGCTC[G/A]TCATCAAGCTTAGGAACCTGGGGGCTGGGGACTGGGGTCGGGGTCGGGGCTTGCTGA	Saint-Pe et al. 2019 X	
144289	AATTCACAGCTTTTAGTGGTGAAGCCCAAGGTAGTTGTGGGTTGTGTAGCT[A/G]TGCCAGACAGTTCCACGTGCCTGCTCGCGCGAACAGTAGGTGCGTATCACGGGGATTTAGGCAT	Saint-Pe et al. 2019 X	×
144553	AATTCAGCTTTTGAACCTTGAATCATTCAATGATACAACGATGATTTTT[C/A]TACAAAAGTCAAGCCCAACTCTAAACTTGAGTTAGGATAGGAGGAGGAGGAGACTTTCTCATGG1	Saint-Pe et al. 2019 X	×
158613	AATTCAATAGTTTTTCTCCCTTATCAATCTACACAATACCACATAATGACAAGGCAAAA[G/A]CAGGTTTTTCAATGTCACAATTTCAATGTATTACATTAC	Saint-Pe et al. 2019 X	×
159298	CGGGTCTTTAAAAGCCCTTCCGTTGGGGGGGGTTTCCAGTGAGGAGGAGCGCACCACCAC(A/G)CCCCTCTGGGGGCTCTTAACCATTAACAAAGGCCTGTTGTTTGT	Saint-Pe et al. 2019 X	×
161205	CGGTTGCCAATGTCAACAAATCTCCTTAGTTACGTGTGTAGTCATAGCAACTGCGGATT[A/G]CTCCCCGTCTACAAAGCAGGATGGACTGCAGAATTAGCTAGAGATTGAATTAGTTGAATTAC	Saint-Pe et al. 2019 X	×
164126	AATTCTCTCTCTTGCTTCCTTCTCAAAAATCATTGGGGGGGG	Saint-Pe et al. 2019 X	×
166053	AATTCCTTAGGCAAACGCCAGTTGGAAAATTCAGTCAGAAATCTGCCAAAGT[C/1]TrGCCTCAATTAGAAATGAGCAAGCATGGCCGGGACCTGTTGTACTGACCTGTTGTCACATTG1	Saint-Pe et al. 2019 X	×
171900	CGGCCAAAGTAAACAATGGGGGGTTACTCGAGGTTTGGGATGGTAGCTAGATAGC(A/C)ATTTCCCTTGGAGATTAGAGTTCAATTGCCTTTTCTTGGCCTGGCATCGTTACCCAGAAGTAA	Saint-Pe et al. 2019 X	×
174130	AATTCTGCCTCTTTCATTTTCATCACTGGTTACCATGTTCAAAGGGGGCTACTGGGCGTTTGTGGGGG/CJCTATGAGGTTCTTCCATTGACAGGCTCTTAACGTCTATCATT	Saint-Pe et al. 2019 X	×
179991	CGGCTCCGCGCCTCTTGATCATGGCGCTCCCGAATAGAGAGCGAATGTAAT[A/T]AACGTCTAGTCGGCTAGACTACAATGCCTTCAAGCATATCATACCTTTTGA	Saint-Pe et al. 2019 X	×
181162	AATTCTCGCCCCCGCTACGAATTTTGCGCCTTTTCTCGAGATCAGTGGCGCTCATAGTACTT[C/T]GCCCAGTTGGAAGATAAAAGAGAACCTCCACGAGGGATGCCATTAAAATGCAAC	Saint-Pe et al. 2019 X	×
184886	AATTCTTGAAGATTTTGGAAAGCAGGAGAAAATGGATGCCACTGATATCCTTTCTAAAGG(C/T)GTCAATACTCTAGCGTCCAGCCACCCCGGGCTCCTGCCGGGAGAGTGTAATCTG	Saint-Pe et al. 2019 X	×
186215	CGGGGACAGGGACAGGGATCTGGATGTTGTCAGTGGGGGGCTCATTGTCT[G/A]TGGTCTCATTGATCCATTGTGACAGCCCTCTGGGGGGCCCTTTTAACAATGTTTT	Saint-Pe et al. 2019 X	×
192002	AATTC4CGTT4GCTG4C4CTC4ACCAAATGTAAATGTAAACGTTGAACTGAAGTCTGTGCCC4[G/A]TG6GATATGTGTCCTAAGTGATGGCGTCATAATATTTGTAGCTTAAAC	Saint-Pe et al. 2019 X	×
194339	AATTCAAAGTACCTTCAAGAACAGGGCCAAATAACAATATAAAGTTTCCTATGGAGTT[G/A]AGTTGAACAGGGGGGGGGGGGGGGGGGGGGGGGGGGGG	Saint-Pe et al. 2019 X	×
200738	AATTCGCAACAGATTTGTGTCACTTTGCAACATAGGACATTCTGCTTGATGATAGCAATA[6/A]GTGAGGCTGCAGAACGGATCACGGTTAGGGTTAGGCTAATGTAACTACAA	Saint-Pe et al. 2019 X	×
206307	AATTCATGAATAGTCAATAATATTTTCGGGCCTACGGTAAAACTGGTTGATGAACTGG[T/G]TCGATAAATAAAATCTCTTTTCTGCGATGTTCATATGGGTCCCACTATGTG	Saint-Pe et al. 2019	
211116	CG6TaGCCCGCCCCCCCCGCTGGTACTAGTAATAAAGCAGCTTGAGAAAGGAAC[1/C]GAAAAGAGTCAGTTAACAGTGCGTGCGTGCATGGGTTTCTTATT ^p	Saint-Pe et al. 2019 X	×
211842	CGGGGAGCTGATATGCGTTTTCTGGTAGGCATAAGCAAGC	Saint-Pe et al. 2019	
212565	CGGTTTAGCTATGATGGGGGGGGGTTACAAAAAAGGGCCACATTTTAAAAAAG[C/A]CCACAAACTAGTGTCTCTTGGCTCTTGCGGGAAATATAGTCACTCTCGCCCTACGGGAAGATCTC	Saint-Pe et al. 2019 X	×
216750	AATTCCTGACGGGGCGGGGGGGGGGGGGGGGGGGGGGGG	Saint-Pe et al. 2019 X	×
221677	AATTCATGATATAGCCATTGATGATGATGATGATGATGATGATGCCTCATGAGC(A/G)GAGTCAAATGTGAGAGTGGTCACATTTCCCGGGCCGCTCGGCTGTTTACCCGGAAAC	Saint-Pe et al. 2019 X	×
226019	ΔΑΤΤCTACAGGGGGGGTACAATATGGGGGGGGGTGTAAAATTCCATGG[G/C]ATTCATTTTAGTGAACTTGATTGCGGTGGGGATACCTTTTCAATAAGTAAG	Saint-Pe et al. 2019 X	×
226261	CGGTTGGGGGTCTGTCCCCTTAATAATTCCCATTAATGTCGGCAATCTTTTATATTTGTGCC(A/T)GAAGTGAGGCACATTTTGTAATTGAGGGGGAAATTATCTAATTAAGTGGGGG	Saint-Pe et al. 2019 X	×
227480	CGGTATGTTTAATCCTATAGCTTCCTCTTCAATGTAATGGCAAAATGTAGATTT[C/G]CACCTAAATAGACACCTCAAAAGTAACTGCTATTCATGTTTAATTACATGATACGACATGCA ^F	Saint-Pe et al. 2019 X	×
229445	AATTCTCCTTACAATAGACAAGACTCATTCTGTTCAGAACAACGCGGGGGGGG	Saint-Pe et al. 2019 X	×
231417	CG6TG6ATACTGTAGTGCGGCAGCAGCTTGGCAGCTTTGGAATCAATTTCCTCAGATA[G/C]ATTTGGCCACAGAATCAGATTAGGACAGTAGTAGTAGTAGTGGCACAATGGATA	Saint-Pe et al. 2019 X	×
232523	AATTCTCATC6G6CTCTAAGAATCCT6GTTC6GTTG6GT4G6GT4GCAATCAA[6/A]AGTAGCAGCAGAAAAAAAGTGCATGTTAACAGC4GT6GAGCCTGAAGCCTTGTTGACAGAG	Saint-Pe et al. 2019 X	×
236933	CGGCTTTGAGGGAGGGGGGGGGGGGGGGGTTGGGTCCAAAATGAAACGCGTATCCCGGCAG[J/C]AGTGCAGTATTTTGAACAGGGGGCTCTCTTTTAAAATTTGCACTGAGGGGAATAGATTGCTAT	Saint-Pe et al. 2019 X	×
240122	AATTCCCATGAGCCATGAGGGGTGGAGCCTAATTGACCTGTTGCATAAATGTATCTCTG[G/C]CTTACAATCTCCGGTGAACACTGAAAACATTTTCTCTTCATAACGGCGAGAGGGGGGG	Saint-Pe et al. 2019 X	×
244245	AATTCAGAATAAGGAAATGGTGCATAAATGGCCGGTCTGGGGCCAATAAAA[A/T]GCTAGTTGTGGCATCAGGCATTAGGGCATGGCCTGTGCATGGCCTGGCCTGGCCTGGTCAG	Saint-Pe et al. 2019 X	×
260589	CGGATTACTATGTTACATCTATCTATGAGACCAGGTTGCCTTGTAAAAAGTAA(A/TJ)ACTACGTGCTATGACCAAGGAGGAAAAGGATTAACGCTTTACAACGGGGGGGG	Saint-Pe et al. 2019 X	×
266817	AATTCAATGATGATCATCAGTGGTGGGGGGGGGGGGGGG	Saint-Pe et al. 2019	
269458	CGGATTC4CGATTC1GGCAGTAGAGCCACAGATTTTTTATTC7CTGCTTTAGTG1T[6/T]1TTCTTATGACTAACTAACTAACTAAGATATTTTG1CTTTTGCGTCC7CACTTTGCC	Saint-Pe et al. 2019 X	×
272760	CGGGCTAGTATTATTATATCAAATGTTTTACTTTCACTGACAGTATAAGCGACATATTTCTTTAG[6/A]AGCAGCAATATAGTAATGATTGAAAAGGATATCCAAAATGAAACTTT	Saint-Pe et al. 2019 X	×
277359	CGGGGGAATTGGTATACCAAGAAACTTTTGGGAACAACTTTTGGGAAACAAGCAGCGG[T/C]ACTTATTTGAAGATGGATAGATAGATGGATGGAAATGGCTGAAAGAGAGGGGGGGG	Saint-Pe et al. 2019 X	×
278625	AATTCTAGTGAGGGGGGGGGGGGGGGGGGGGGGGGGGGG	Saint-Pe et al. 2019 X	×
279383	CGGCATGTCAACTGAGTTGGGATGTAGGATAGGGTCAGAAAAGTGCTCCCAAGTGTTGGAGGGGGCGCCATCACTTGTGTGGGGGACACTTACTGTTATCTGTTAGAAACATG	Saint-Pe et al. 2019 X	×

286205	CGGACCATATATGGGGGTAGAAGACCCACACCTCTCCGTTTCTGGCTTGGGAG(G/A)CAGTGGTTGCAAGAATGTGTTCCAAGTGCCACCCGATTGGTGCAATAGTGCAATAC	Saint-Pe et al. 2019 X	×	
286531	AATTCTTTCCAAATAATAATAATGGAAAATTAACAAATGTACCAAAAGATCAGTGG[G/C]AAGGCCAAATTACAGGGGAGGAACTTTTTGAGGGCTATTAAATCCTTTGTCTGGGAAAAAGC	Saint-Pe et al. 2019 X	×	
288953	AATTCCAAAGAATGATAAGATGATGATGATGATGAATTAATAAATCAT[I/A]ATTTTTAAATCTTAGATGTGTGTGTGTGGTAAACTATCACAGTGGTCATCACAGAGTCAGGAGTCAGGGTCAGGTGGTGT	Saint-Pe et al. 2019 X	×	
293037	AATTCTCAGTTTTGTGCAGGAGGTGTGGAAAACATTTTGGATCCGAGGCTCTGGCAAACTCGGGGA[T/A]GCAAGTACTAAAACTACAAGGGGCTTTTTCGCAGTGGAGGGGGTCTGATGGCAGTC	Saint-Pe et al. 2019 X	×	
293559	AAITCAGCCGATAAAGCAATCAAGGACTIGCTGGTACAGGGCTGCGGTCCGTTCAGT[C/T]CTCAGCACTTTGGGCCTGCAAGTAATCACCAAGCGTGGCTGCTCCTCCAAACG1	Saint-Pe et al. 2019 X	×	
294902	CGGGGGCGCGCGGCCCAATTGCGCGCCCCTATGGGGCTCCCTTAAATCATA(ACTA(A/C)ATTTTTAATAACTTGTCGCTCCCTTATAATGAATAGTCCTTAAGTAATCATTCAAAAAAT7	Saint-Pe et al. 2019 X	×	
295415	CGGAGGGAAATTACAGGGAGAAGTGACGAGGCGCCAGGAGCAGGAGGGCCATCTACTGGT[A/T]GACGCATATAATAATAATAATGATGTGAGAGGGAGGGGGGGG	Saint-Pe et al. 2019 X		
299470	CGGCTCTGTGAGCGGGGGGGGGGGGGGGGGGGGGGGGGG	Saint-Pe et al. 2019 X	×	
302642	CGGTATAAATATTTCCTTTGGTCTGGTGGTGAAATTTGGGAAATTTGGTTCCAACCGCC[A/G]TGAAGCTTGGAGGGGGGGGGGGGGGGGGGGGGGGGGGG	Saint-Pe et al. 2019 X	×	
303106	AATTCTGGGAAATGTATCAGGAAGAGGACAGGACGAGAGAGT[G/A]GAGTGTTGAAATGTTGCCTGTAGAAGAAATCTTTCAACCAAGGGCAAGGTTTGTCTCACAAAAAAC	Saint-Pe et al. 2019 X	×	
308316	AATTCCCTATCTTAGAATATTGGAATCTTAACATCCCTGACCCATGCAGGAGTATGAGATGTG[G/C]ATGCGTGGGTGGGCGCTCCTTGTCTCCATATCCCAGACTGCAGTTGAAAGCGGACAC	Saint-Pe et al. 2019 X	×	
309901	CGGTGACGAACAGAAATGAGTCTAGCTTGTCCACCTTGTAGCAAAATGAGGGGG[A/G]AAAAACTTTGAAGTTTTGATCCAAGGTAGGTGGGGGGGGG	Saint-Pe et al. 2019 X		
310747	AATTCCACAATTATCGCCGCGTGCAAGACAACTCGGAAATGGAAAACGTTGATCGACC[A/G]ATAGGAAACTCAAAATGTCCGGAATTGTCCAATAAACAATCTGTC2	Saint-Pe et al. 2019 X	×	
316925	AATTCATTGACTTGTTAAATCCTTTGTTAAATAAACCTTTATTGACCCATAAT[A/G]TACTGGATTTTCCTTTCCACTGGGGTAGTGAGGGATTTTCCTTTCCCCCGCGGTCGGT	Saint-Pe et al. 2019 X	×	
317909	AATTCTCTGGGGGGTTCAAGCAATTTCTCCATCATCAATGAATAGACGACGTGACGGGGGTGACGGCGTTTGATGGGGGATTTTGGGGAAGGTGGGGGTGGGGGTAGGGGT	Saint-Pe et al. 2019 X	×	
318154	AATTCATTGGGGGGGGGGGGGGGGGGGTCTTTACCGGGGCTATGGGGCTATGGGGC[A/C]TCTGCGCTGCTGTGTCGCTGGCTGGTGGGGAGGGAGCCTTTTCCTGC	Saint-Pe et al. 2019 X	×	
320246	CGGGTAGCTATTTGGTTAACTATTTAGCAGTCCTATGGCTTGTGGCTGGTGT[A/6]TCAGAACCGCTTGTCATGCGGTAGCAAGAGAGAGCAGTCTATAACTTGGGTGGCTGATTCG	Saint-Pe et al. 2019 X	×	
321153	CGGTATCTAGCCAAACCGATGCTAAATAAACTAAATGAAAACATCGGTTGCAAAGTAA[G/C]TGGCTAATAGGCCTATATTCTGTCCAGGTTGACCACATGAGCACATTACATTA	Saint-Pe et al. 2019 X	×	
323835	AATTCTTTCTGAATGGGTGTGTTATATATCGTTAGAGATGTGTTAAAAATAACAACAAT[G/A]ITAGCGATAACCTTACGTTTTTAAAGAATGGGTTGATTAAATAAA	Saint-Pe et al. 2019 X	×	
325312	CGGGTGAGTCCAAAAATGTTGTATGCTGCCTGCATAATGATGATATGCCGGGGGGATATG[T/CJATACTGAGGAAGGTAATACTAACTATATTGTGTGTGGTGGTGGTGGT	Saint-Pe et al. 2019		
327761	CGGGCTGTGTGGGGGGGGAGGTGATGACAGTGGGGCTCCTATGAGTGTAAATGTCCCGC[T/G]GGCCATGGCCAGGGGGCGGCGGCGGCGGGGGGGGGGGG	Saint-Pe et al. 2019 X	×	
330754	CGGTCCGTCTGCTGGAGAGTTCATCGTCTGTCTCTCTCTC	Saint-Pe et al. 2019 X	×	
333806	CGGAACTGAGACCTGTTCTGGTTGAGGGGGCTTGGGGGACACTTTGGTCCTATGGT7AJCACATCACCAAAAACAAATTATGACCAATGGTGGTAACAAAAATAGTTCAGTTTATT	Saint-Pe et al. 2019 X	×	
337455	AATTCACACC6AGGGTGGGTATTTAGTGCTGATCACTGCCACTGGAAGAAAAA(A/T)AC6CATTCCTCACACCTTTCGGGGGTTTTCTTCGGTGGTTATTCTTGGTAA1	Saint-Pe et al. 2019 X	×	
339526	CGGTTGTCCTGGTTTCCAGTGGTTGCAGAGGGATGTCTTCCTTAATTGACCCCCCATAAAC(A/G)TAACGGGGCCATATACCAGGGGCTGTGCCCGGCCTGCTGCTGTTGACTCATCC	Saint-Pe et al. 2019		
341174	AATTCACAGTATCAGACATCCTCAGAGTGCCTTAAATAGAGAAATCTATTTTTC[A/G]ACAACAGAGAGAAATGGTTAGTCCCATCCAGCAGAAATTCCTTAGCTCTAACACTATTAGTGAAGC	Saint-Pe et al. 2019 X	×	
343612	CG6TAGGTGCATTGCACTGCATTGCTTGTGTGATGACTTGA[C/T]CTCTTAAATGCACTGTAGTGCTGCATGGAAGAATGGGGTCATGTTGTTCAATGTACTTCCTACCTA	Saint-Pe et al. 2019 X	×	
344809	AAITICTGFGGGGGGTGTTGTATTCCGCTGCTATGGGGGGCTAATGGGGGGGG	Saint-Pe et al. 2019 X	×	
348454	AATTCT6666TTT6666ACTTCT6T6CTCC66666CTTT666CATCT6C6T646[6/A]C6C6666AA6A66G66GTTAT6AA66AGGT6TCACC6T66ACAGATATTTF66C6A6666A66	Saint-Pe et al. 2019 X	×	
353264	AAITCTACCTCGTGTTGTTAGGGTTAGTGTATATTTCCCAAGCTGCGGGAGGACAGGGAAGATGTGTCTACCCTCGGGGTCGGGTTAACACATCCTCCCCATCTCCCATCC	Saint-Pe et al. 2019 X	×	
358809	CGGTCTCAGCTGGGTATCCTATAGACGTTTGAAATAATTGAAAGATTAGTGGAATCTG[C/T]GTTGGTAGCTGGACGGTAGTGAGTGAAGGTGAAGGTGAAGGTGACAGGGAGGAAGGA	Saint-Pe et al. 2019 X	×	
363982	CGGTGCCAACACTGGTTTTCACCACAGAGGGCTATGCCAGGACTCTTCATCCTCAGGGGGAAGT[A/G]TGTGGCACACACACACATTCTTGTTTTCAATTCCCAGGTATCTGTCA	Saint-Pe et al. 2019 X	×	
365638	AATTCAGGAAGCCATTTCCTGAAATAAACACACAAAACCCACAACACAGAAA[C/T]TGTGGAATGTAACAATCGGCCTCCACCATGATGGTGGGAGGGTCAGGGTGAAACAGGGTGGGT	Saint-Pe et al. 2019 X	×	
372957	AATTCAGATCCAAGGTTTAAGGTTGTTGCTGAAGTCACGGCAACAT[1/c]ACATGATTATGTGCCTTCTCATTTAGTGTTCCACCATCGTTGAAGAGGCATGGTTTCTCATTTAC1	Saint-Pe et al. 2019 X	×	
374057	CGGGGGACATATCCCGGTCGGTGATCAAAACATTCCTGAAGCATGGTTTCC[A/G]ATTGGTCGGTGGGTGGATGATGGTCCTTAGCATGGGTGGG	Saint-Pe et al. 2019 X	×	
375073	AATTCTTTCCCAACAGTGTGTGTGGGTGGTGGTGGAAGCTGTGAACCAACAGCGGGGGCGCCTT[1/G]AACATTTCCAACAGTGACGTTTCGGTGGGGGGGTTGTAACATTA	Saint-Pe et al. 2019		
375988	CGGTAGTTATGAGTGAGGTGGGGGGGGGGGGGGGGGGGG	Saint-Pe et al. 2019 X	×	
382931	AATTCATTTGCCTTGGTTAATCCATTCCAAGGGCGGGGGGGG	Saint-Pe et al. 2019 X	×	
384919	CGGTTCTGTATTCGCAGAGATCTCGTGCTAAAGATAGCGATCACTACCGAGCCAGCC	Saint-Pe et al. 2019 X	×	
386171	сб6Т66АААбСТСТТА6А6АСТТАСССА6ААА6АСТСАСАТСА6ТААТТ6СТ6ССААА66Т6[A/C]T1СТАСААА6ТПТА6АТАТПТСТ6ТАПТПТСАА6АСТПТ6САААААТ6Т₽	Saint-Pe et al. 2019 X	×	
389677	AATTCTTCAG666CAT66AAACGTT6CTCAATT66TATCAA666AACCTCAT6T6T6CCG666AA4[A/6]CATTCCCCATACCATTACCCCCCCCCCG6CCG6TA6ACTT6ACCCG66CG66CG66	Saint-Pe et al. 2019 X	×	
390732	AATTCACATATCCTGGCCAACAGCAGCAGCTAAAAATGTAATCAAGCCACTAGAAAT[G/T]TCAATGTATCATTCTGCAATGCAGGACAGGACAGGAGAGTATAGCGCTAAACAAAC	Saint-Pe et al. 2019 X	×	
394880	CGGAGTGAAGTGAGTGAGTAGTAGTGTTGTAAAGCCACGTTACACCCATTCTGTCTCT[1/G]TCCCCAGATAATATGCCTAACCTTCCAGAGTAGCTCTCTTAAACAACGAC	Saint-Pe et al. 2019 X		
398850	AATTCAGTAAGAAGGACCGCACATCGTTGCTCGCATGTTCTGTTAA[T/C]AAGAACTACCATATTCTAAAAGTGATTTCTGCCATTCTGAGCACCGTGGTGGCGCCCCTAATC	Saint-Pe et al. 2019 X	×	
402210	CGGAGTCGGTATATTTTCCCAACTTGCGGGGGCAATGGAGCTCATCCCCCGGATGCATCATGATTTCCT[G/a]CAACAGGGGGGGGGGGGGGGGCGCCCCGGGGGGCGTTCCCTAAATTCGTGCA	Saint-Pe et al. 2019 X	×	
406172	CGGGTTATGTGTTATAAGCTAACCACAATAAGGATTAGCCACAATAATGGAATTTGC/A/TTTCACCTTCATCTTTGAAAGTGATGGAGGAGGTTACAATTGGTGGAATCATGGCCATATT	Saint-Pe et al. 2019 X	×	

414128	AATTCAGAGAGATAGGGAAATAGCTGTACACATTATGCATCCAAGGCCAC(AYT)AAGCCTGGGGGCTCCCAGAGTACTGACGAGGAGGAGAATTACTGGGGCAACTGAAC	Saint-Pe et al. 2019 X	×
415925	CGGTTAATATTTCACACTGTGTTCTAGAACTCATSCAAGACATTGAAT[1/C]GATTTTATTACATCTATCTAACCTATTTATTGGTGGTGGTGGTGGTGGTGCTGTTCAGGG	Saint-Pe et al. 2019 X	×
418712	AATTCTTTGGGGGGATAATATGGTCGACATTTAATTGTGGGGGGATGGAT	Saint-Pe et al. 2019 X	×
420928	CGGATATACAGTCCCCGTGAATATAGCCAGCTGGGGTTCTCAGTAGTGGGATATAGGAATATAGAACTCTCCAGGAAGAAGGAGGAGGAGGAGGAGGAGTATTCATGATTAACTACTAT	Saint-Pe et al. 2019 X	×
422809	AAITTCTCATATAGCAGTGGGGACTTCGGGCCCTCTTTCTCGGCCCCCTCTTGAGGCAGATCA[A/C]GGGGGATCTGGGGGGGGGGGGGGGGGGGGGGGGGGGGG	Saint-Pe et al. 2019 X	×
424552	AATTCCAAGAGTGTGCAAAGGTGTCATCAAGGGCAAGGGTGGCTATTAGAAGAATCTCCAAATAAA[1/A]AAAAAAATATTTTGATTTAGATTACTACTATAGTGTGATTCCATATGTGT	Saint-Pe et al. 2019 X	×
428815	AATTCTGTATGCAGGGCTGAGTGGGGCGGGGGGGGGGGG	Saint-Pe et al. 2019 X	×
432538	AATTCTTCAAAGTAGGGAGGTAGTTACACGCCTCGGCATTTCAAGGGCTGTGTGTTCTAAAAT[6/T]AAAGGGCGGTAAAGGGGGAGTATCTCGGAAGTACATCTGAAACTCCA	Saint-Pe et al. 2019 X	×
433018	AATTCTATAGACAACCATATGTGGGGCAAAAAAATAAATTGGCAAAAAATGGCATC[T/C]TTCTAGTAGCTTTATAATCTAGAGGCATTATGGAGGAATAAGTTGGCCATTGACC1	Saint-Pe et al. 2019 X	×
435476	CGGTTCACTGTACTGTCTGTGTAAGGTCATACATCAAGGCACAATAAGGTAACTATACCAT[T/C]AAAATCATAAAATGCACCACTGCTTTCATATTAGTAGCTAGC	Saint-Pe et al. 2019 X	×
436125	CGGATGAATAGCACGGGTCGAAGGGCTGAAGGAGGGGGAAGGGGGAAGGGGGAAGGGGGCGCGGGTGTGGGTTTCCGGGGGGGG	Saint-Pe et al. 2019 X	×
438997	CGGGTAGAACGGAACAGCAATCGATTGCAGGCAGCGCGCGGAATATTGAGATGTGGATACTTGCCTTTGAGGCTTTGGGCTGGGCGGGGGGGG	Saint-Pe et al. 2019 X	×
440321	AATTCCGGTGGGATCAAATATTCTGGTCCGCGTATAGTTCCAACGCTTATCAATAGG[C/T]TAATAAATAAAATGGCCAAATTCGCCATAAAATCATTTCGCCATTGCAC	Saint-Pe et al. 2019	
442477	ΑΑΤΤCTGTGATGAAGAAGCTATCTAGCTAATTTAGCCTGCTAACAAGAAAG[6/T]CATTTCTCTIGAGAAATAATCCTCACTATTCTCTTTAGTTATGGGTCATGTGGTCGTCGATAA	Saint-Pe et al. 2019 X	×
454655	CGGGGGGCCTGAGCTGGCGCGGCTCCTGGTTCGCTGGTGGCGGGGAGGATAG[A/C]GGGGGGGGGGGGGCGCGCCTCCTGGGAGGGGGGGGGGG	Saint-Pe et al. 2019 X	×
458529	AATTCAAATGAGCAGATCAAATTCAAATTAGTCACATGCGCCGATGCTTACTTA	Saint-Pe et al. 2019 X	×
461330	AATTCAAAAGTATCAATATTCTCCAGGTCTGTGAAAAAAGTTAGTT	Saint-Pe et al. 2019 X	×
462768	AATTCATAACAAAAGTCCCTGCAAACAAAAATGTGTCTTTAGGACTTCCTCGGGAATGTCa[C/T)GAAATGGTCGCCTAAAGTGGTCGGGAAATTGTGGGTCCTGGGGGGTTTTGTCTAGTTGT	Saint-Pe et al. 2019 X	×
464800	CGGTATAGTCATTTGTGACCGTGTCCTCATCATCACCACTAGAATGACCAGCAGGAA[G/A]AATGTATTAAATACCTTTAGAAGGAGTATGCGTACAACACATTTCAATATCCATTTG	Saint-Pe et al. 2019 X	×
470946	AATTCTGGTTTTGAGCAACAGCTGGCAGCTGATTCACAGCTGTATTGAGTCTCTTT[G/T]TCAATTCAGGTTTTGGCTCACAGTGGTGGGGGAGGGATAGTGCTCGATGTGTTCTGC	Saint-Pe et al. 2019 X	×
471081	AATTCTGGCTGTAACACATGTGGGGATACTTTCTGGAGGCGCTGATAACTTCTATGGAATCTG[6/C]CGTCTATATACAACTTCTGATGACTTTACAAAACAGAAGTTGAATGAT	Saint-Pe et al. 2019	
472311	CGGTGACGCGTTGGCTGGGGGGGGGGGGGGGGGGGGGGG	Saint-Pe et al. 2019 X	×
472745	CGGCCaCTAGGGGCAACAGTAAGCGCTGTTACCTTCAATATGGTTTGGTGTT[6/C]CTATGGCGTTG1GGGGGGGGGGGGGGGGGGGGGGGGGGGG	Saint-Pe et al. 2019 X	×
474810	CGGAGAAAAGTCTTTATGAGAGTCTGAGTGATATATTAACCCCAAAATGACCCCA[C/A]TCGCTGCGTAGCGAAGCTCCTGTTATTGGAAGTTATCACATGCGCCAAGGGA	Saint-Pe et al. 2019 X	×
475988	CGGCTTCATCTTTAAGAGCTAATACCAGTAGCACCCCTTATCCAACTGGCTTGG(A/6)TCCAAACCTAGCCTGGCATCCTTCCCTCCTCCTGCTGCAGATGACATTGCCATACAC	Saint-Pe et al. 2019 X	×
139783	CGGTCAAAGGGTAGTTTTTCAGGTGGCACAAACAGACTCTGTTTGGCTGGGGGCGGGG(A/TJTATTCTGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGG	Saint-Pe et al. 2019 X	×
142311	AATTCCATTGTGGCTGGCTAGAAAATCTTCCCACATCTCATGGATCTCTCCCTCC	Saint-Pe et al. 2019 X	×
154148	AATTCTGTCGGTGTTTTAATGTCCTGGTTGATTATTTATT	Saint-Pe et al. 2019 X	
274678	CGGGATTGACAGTGTAAAACGTTGATGTTGGTAGGTAGGT	Saint-Pe et al. 2019 X	×
395955	CGGTTCAGACACTGCTGTGGGATTAAGCCATTCCCTCTACAATTGCACAACATCA(C/T)CAGGACAATCCAAGCCAAATTTCTGTCCTATAGACACTGCAAATGTATAGGAACGGTC	Saint-Pe et al. 2019 X	×
424516	ΑΑΤΤCCTTATATGAACTGTAAACTGTGTAAAAAATTGTAAATTGTTGTGGTTATTGTTTT[C/T]GTTCAGTGTGGGGTGGGCTGGGCTAAGCAATGAACCATAATCCCAACTC/	Saint-Pe et al. 2019	
432899	CGGTTTTACGTATCATCGTTGAGTCCATCCTCCTCCTCCATCACAGTCTGGGTTTGGGTCTG[G/C]GTCTGCCCACTGCAAGGGGCAAAGCTGCATGCATGTTCTGTCCGCCAGAAGGGTCAT	Saint-Pe et al. 2019 X	×
465017	AATTCATGAATTAAATGAAATTAAATGAAATGAATGAAGGATGGGTAGCCTATA(C/T)GCATAAATTATGTACACGAGCGTGCAATTGAGTCTGAGGTCTGACGTGCGGGCTTCCATGTGAAA	Saint-Pe et al. 2019 X	×

Table A3. Details of path analyses regressions and covariances.

Regressions:							
		Estimate	Std.Err	z-value	P(> z)	Std.lv	Std.all
	Pathological	~					
	NNO2-NNO3	-0.389	0.132	-2.946	0.003	-0.389	-0.401
	temp	-0.354	0.138	-2.562	0.010	-0.354	-0.349
	Ecological	~					
	% agricultural land	-0.307	0.14	-2.191	0.028	-0.307	-0.307
	Genetic	~					
	Distance from source	0.437	0.12	3.636	0.000	0.437	0.426
	% agricultural land	-0.443	0.12	-3.681	0.000	-0.443	-0.432
	Multifaceted health	~					
	% agricultural land	-0.524	0.116	-4.533	0.000	-0.524	-0.518
	02	0.324	0.112	2.891	0.004	0.324	0.331
Covariances:							
		Estimate	Std.Err	z-value	P(> z)	Std.lv	Std.all
	temp	\sim					
	Altitude	-0.793	0.186	-4.272	0.000	-0.793	-0.811
	% agricultural land	0.741	0.181	4.096	0.000	0.741	0.758
	NNO2-NNO3	~~					
	temp	0.607	0.164	3.702	0.000	0.607	0.594
	temp	~~~					
	02	-0.556	0.161	-3.447	0.001	-0.556	-0.551
	NNO2-NNO3	~~					
	Distance from source	-0.420	0.134	-3.147	0.002	-0.420	-0.411
	Distance from source	~~					
	02	0.360	0.133	2.697	0.007	0.360	0.357
	NNO2-NNO3	~~					
	Altitude	-0.554	0.160	-3.465	0.001	-0.554	-0.542
	Altitude	~~					
	02	0.455	0.154	2.952	0.003	0.455	0.452
	% agricultural land	~~~					
	Altitude	-0.700	0.177	-3.945	0.000	-0.700	-0.715
	NNO2-NNO3	\sim					
	% agricultural land	0.571	0.161	3.541	0.000	0.571	0.558
	% agricultural land	\sim					
	02	-0.505	0.158	-3.208	0.001	-0.505	-0.501
	NNO2-NNO3	~~					
	02	-0.599	0.164	-3.646	0.000	-0.599	-0.568







Figure A2. Pairwise Spearman correlation coefficients between the component indicators and the multifaceted health index. The dashed line indicates an absence of correlation (r = 0). The black bars represent the 95% CI of the correlation coefficients. The significance of the correlation coefficients is indicated by **: P=0.001, ***: P<0.001.



Figure A3. Linear regressions between the indicators and their most impacting environmental factors. Pathological indicator's score according to the NNO_2 - NNO_3 concentration in the water (a) and the mean summer water temperature (b). Genetic indicator's score according to the percentage of agricultural land (c) and the distance from the source (d). Ecological indicator's score according to the percentage of agricultural land (e).

Figure A4: Biplots of the relation between the multifaceted health index and the most impacting environmental factors. (a) Multifaceted health index score according to the percentage of agricultural land in the surroundings. (b) Multifaceted health index score according to the water concentration in oxygen. Adjusted R² for the linear regressions and their p-values are indicated.

Synthesis of the main results

Understanding the emergence and spread of parasites and their associated diseases, together with their outcomes in host populations requires a thorough study of the host-parasite-environment interactions at multiple levels, both at the spatial and temporal scales (Penczykowski et al., 2016). An integrative study framework is therefore needed to accurately grasp the mechanisms involved in the disease triangle (comprising the interactions between host, parasite and the environment). This thesis aimed at using such a framework to investigate the ecological and evolutionary outcomes of a host-parasite system in the wild. Across the different chapters, I developed and implemented new non-lethal biomonitoring techniques to detect the parasite and infected hosts at a large spatial scale from individuals to groups of populations across river networks. This has provided a unique opportunity to assess how host-parasite interactions vary across gradients of environmental conditions and to identify risk-prone populations to be prioritised for conservation. The conjoint study of all stages of the parasite life cycle also provided a better understanding of the factors favouring its development within and outside its hosts.

Chapter 1 introduced a new non-lethal molecular method for endoparasite detection (uDNA for urine DNA), relying on parasite excretion by the infected hosts. I demonstrated its ability to accurately assess individual infection status and population infection prevalence. This methodological development is at the core of this thesis as I further used uDNA in all the subsequent chapters (Fig. 11). It is also a very promising diagnostic tool for epidemiology as it could be applied to a wide range of parasites and hosts species to infer infection parameters of wildlife populations at large spatial scales.

In Chapter 2, the detection of the parasite and its hosts from water environmental DNA (eDNA) combined with sensor data, available databases related to the river network and land use from remote sensing for measuring a wide range of environmental parameters at a large geographical scale (83 sites spread over 6000km² and 54 different streams, including 46 sampled brown trout populations) allowed to identify the most important biotic and abiotic variables for parasite establishment and disease emergence in trout populations. I highlighted the importance of hosts' abundance for parasite distribution and showed that abiotic environmental factors acting directly on the host physiology were more important than the abundance of parasite propagules in the environment for determining the infection prevalence, hence illustrating the context-dependence of this host-parasite interaction outcomes. I investigated two different levels of the parasite life cycle, i.e., the parasite into its vertebrate host that was of interest for conservation purposes, and the parasite into its transmission stages. Studying a maximum of a parasite component populations allows for a finer understanding of the mechanisms shaping host-parasite interaction outcomes.

In Chapter 3, I worked at the intra-population level to investigate the responses of brown trout to environmental stressors involved into disease development (infection prevalence and water temperature) at the demographic, genetic and phenotypic levels with a before/after outbreak study design. I found that the infection prevalence in fish was linked to a decline in host populations density, but only in populations showing low to medium infection prevalence the year before, suggesting that initial great declines due to PKD might be followed by rapid population adaptation. The infection prevalence had no impact on the neutral genetic diversity but was linked to a loss of diversity at an immune gene, suggesting that directional selection might operate at this adaptive locus. At the phenotypic level, I found a negative effect of the infection prevalence and the water temperature on host body condition and colouration, suggesting that these stressors could have an impact on the future fitness of surviving individuals.

Finally, in Chapter 4, I worked at the inter-population level to identify populations that were the most at risk of decline, not only because of the parasitic context, but also in relation to other parameters related to population global health status. I therefore associated the pathological status of wild host populations to other relevant indicators for population conservation, informing on their genetic and ecological status, both important for their adaptive potential in a context of environmental change, to develop an integrative multifaceted index informing on wild populations global health. This multifaceted index properly represented its component indicators (pathological, genetic and ecological) and highlighted the most relevant abiotic drivers of the studied wild populations' health. The construction of this multifaceted index is highly flexible and should be transposable to a wide range of ecosystems and species.

In the following part, I discuss the methods and findings of this thesis, including for the hostparasite system studied, together with the adding of some extra-analyses not shown within the chapters. Altogether, this work provides a new methodological framework for studying the eco-evolutionary dynamics of host-parasite systems that further improves the understandings of the complex association between environmental factors and parasite infections in wild populations confronted with changing environments. More specifically, it identified some clues for the surveillance and management of wildlife infectious diseases and stressed the main environmental drivers to the studied case of *T*. *bryosalmonae* and wild brown trout populations.

155

Figure 11. Schematic representation of the different facets of the epidemiological triangle (host in blue, parasite in orange and environment in green) that were investigated throughout the thesis. D in the center describes the potential disease outcome. Only the environmental factors identified as the most relevant are shown. The methods used are indicated in italic. *uDNA* was developed in Chapter 1 to infer fish infection status, a *before/after* study design was used in Chapter 3, and a *multifaceted health index* of wild populations was developed in Chapter 4. Arrows width on the parasite part indicate the relative importance of the abiotic and biotic (hosts abundance and parasite abundance, estimated through *eDNA*) for parasite abundance and fish infection, as identified in Chapter 2.

The use of non-lethal methods for wildlife disease surveillance

Monitoring population infection status

The complexity and ubiquity of parasite-host-environment interactions stresses the need for considering parasitism when evaluating multiple stressors acting on wildlife, especially in the context of environmental change (Lafferty & Kuris, 1999). Parasite distribution and prevalence are most probably larger than disease distribution and prevalence (mostly reported by the detection of noticeable diseased individuals via passive surveillance), because of asymptomatic parasite carriers. Therefore, an active and efficient monitoring of wildlife population infection status is required to provide early warnings and forecast future disease outbreaks (Ryser-Degiorgis, 2013). Alternative methods for detecting individual and population infection status are needed to get a large-scale overview of the populations that are the most at risk of developing infectious disease. Wildlife population screening for endoparasite infections usually relies on lethal sampling and the examination of the affected organs (e.g. in McAllister et al., 2016). Yet, except for the parasites that are trophically transmitted (Kuris, 2003), most parasites have to be excreted from an infected host to find their way to their next host. In the present work, I showed how a non-lethal method taking advantage of the release of parasite spores and DNA along with host fluid excretion could inform the individual infection status and population infection prevalence (Chapter 1). I therefore expect that such non-lethal methods could be implemented in the study of most host-parasite interactions.

Moreover, infection diagnoses based on host excretion inform on the amount of infective spores released towards the other susceptible hosts, so that the comparison of DNA quantity excreted at different sites could inform on the ability of a parasite to complete its life cycle into different environmental conditions. Furthermore, it may help to identify diseases' super-spreaders. In wildlife infectious diseases, 20% of the individuals (super-spreaders) are considered responsible for 80% of the infection transmissions (White et al., 2017). The identification of super-spreader individuals or hotspots for parasite transmission (when heterogeneity in transmission occurs in space) may allow for better targeted control measures (Paull et al., 2012). This might also be beneficial for instance within farming structures, especially for parasites horizontally transmitted (i.e., with direct transmission and avoid too much spreading of the associated disease. For parasite release by the different hosts, contrary to traditional eDNA sampling in the river water, as eDNA cannot distinguish between spores infective to one host species or to the other (e.g., between spores released by bryozoans infective to the fish and spores released by fish infective to bryozoans; Fontes et al., 2017).

The use of non-lethal infection diagnoses may therefore be useful to the study of a lot of issues linked to wildlife infectious diseases. For instance, in this thesis, I applied the uDNA method to assess populations' infection prevalence at a large geographical scale, to decipher its environmental drivers, to identify hosts populations that were the most at risk of developing the disease and/or of declining in the context of environmental change (Chapters 2 and 4), and to study the combined impact of the infection and thermal stressor on host populations (Chapter 3). Overall, this represents a total of 1300 fish tested for parasite infection with uDNA, that would have otherwise been sacrificed for the infection assessment. This is substantial because it avoided the impoverishment of already declining wild populations.

Non-lethal sampling is therefore relevant for conservation purposes, but may also be used to avoid losses due to sampling in farming. For instance, da Cunha et al. (2020) developed a method based on blood sampling and qPCR to detect an intestinal endoparasite of *Colossoma macropomum* in farmed fish to avoid the economic loss due to lethal sampling. Likewise, Riepe et al. (2021) used mucus swabbing to detect kidney bacterial infection of the brook trout (*Salvelinus fontinalis*). However, fish infected in the kidney yielded less than 60% positive mucus swabbing, compared to the 87% found during the development of the uDNA method (Chapter 1). Moreover, 11 out of the 72 sampled fish (i.e., 15%) were found positive *via* mucus swabbing but not in the kidney. These false positives may illustrate the modulation of the compatibility filter through host resistance: the parasite went through the encounter filter to find its host but did not manage to enter its body and cause infecton (see Fig. 2 for an illustration of the encounter and compatibility filters). Detection methods based on hosts excretion might therefore be more accurate because they indicate parasite's ability to enter the host, translating effective infection.

After comparison to the traditional lethal method used for *T. bryosalmonae* detection in the fish host (e.g. in Bruneaux et al., 2017), uDNA revealed appropriate for inferring host infection status (qualitative information), but imperfect for parasite load quantification, probably due to the impaired excretion of severely diseased individuals (Chapter 1). Nonetheless, I found a better relationship between the parasite load measured in the kidney and the uDNA quantity of fish from a population showing no symptom of the disease. This suggests that uDNA used on older fish -that may be infected without developing the disease upon reinfection- could give a better estimation of the parasite load, as non-diseased and recovered fish still excrete parasite spores (Soliman et al., 2018).

The main drawback of the uDNA method compared to traditional lethal sampling and examination of the affected tissues is that it does not inform on the outcomes of the infection on the individual health. A perspective would be to further make use of the host excretions to monitor physiological parameters related to host health status. For instance, the detection of cortisol, a stress hormone, within fish excretion, is increasingly used to assess fish stress. Cortisol detection is therefore

useful to assess fish welfare in fish farms, or the adaptive capacities of fish in response to environmental change (Sadoul & Geffroy, 2019). Even though there remain some technical issues regarding the immediate impact of fish handling on the quantity of excreted cortisol, this method seems promising to get further information on chronic stress resulting from parasite infection, that we could not detect in this thesis with the single use of uDNA. In the case of the proliferative kidney disease caused by *T. bryosalmonae*, physiological measures such as haematocrit (% of blood cells in the blood), oxygen consumption or kidney hyperplasia are used to quantify the pathological effect of the infection (e.g. in Bruneaux et al., 2017). In severely diseased fish, external symptoms such as exophthalmia or swollen anterior body manifest and may inform, at least qualitatively, on PKD development. Accordingly, a quick supplementary investigation (based on only a few samples) showed that there might be a positive relationship between the measured kidney swollenness and the height of the fish body (Appendix 1). Even though a swollen anterior body can be the symptom of a wide range of fish diseases, the combined use of uDNA, cortisol measurement, haematocrit (with blood sampling done precautiously, Lawrence et al., 2020) and body height might represent a reasonable alternative to fish lethal sampling for assessing PKD development.

eDNA as a tool for studying the dynamics of host-parasite interactions

Environmental DNA is being increasingly used in parasitology to detect and quantify otherwise invisible microscopic parasite species that are highly diluted in the open environment (Huver et al., 2015; Bass et al., 2015). This is substantial to get an overview of parasite distribution, but eDNA has until now rarely been used to infer host-parasite interactions. The development of highly sensitive detection methods such as the droplet digital PCR, which can now target several species DNA in multiplex, represents a precious tool for wildlife monitoring. The conjoint detection of the different compartments of a parasite life cycle (hosts and parasite within and outside its hosts) is essential for identifying potential transmission sites to provide early warnings, evaluate the risks for disease outbreaks and understand the environmental conditions favouring the infection (Amarasiri et al., 2021). Such methodological framework allows to dig into finer details of host-parasite interactions, in terms of spatial distribution but also of timing, especially when vectors or reservoir hosts are involved. Here, "reservoir hosts" describes all the other potential host species in which a parasite can be permanently maintained and from which infection is transmitted to the defined target host (Haydon et al., 2002; e.g., in my study case, brown trout is the target host, and bryozoan represents the reservoir hosts).

Following the work from Carraro et al. (2017; 2018), I used eDNA to conjointly detect and quantify the parasite and its bryozoan host, adding the quantification of brown trout abundance and expending it to a large geographical scale (Chapter 2). I investigated the main environmental drivers of the parasite distribution within the open environment (eDNA) and within the fish host (infection prevalence) through uDNA, therefore adding another compartment of the parasite life cycle, to

disentangle the relative importance of abiotic and biotic factors in shaping the parasite distribution and disease risk. While the occurrence and abundance of the free-living parasite propagules (infectious either to the fish or the bryozoan host) were mainly driven by the abundance of the two hosts (biotic factor); the infection within fish was driven by the abiotic environmental conditions experienced by the fish hosts, and only poorly linked to the propagule abundance in the water (biotic factor, see Fig. 11 for a visual representation). This suggests that the abiotic environment modulates host susceptibility to infection, probably through a negative effect of water temperature on this poikilotherm immune response (overexpression leading to immunopathology; Råberg et al., 1998; Bowden, 2008), and/or through the increase of the parasite proliferation within the host due directly to warmer temperature and/or through a decreased control of its development by the host (Macnab & Barber, 2012; Lauringson et al., 2021).

These results suggest that even though a parasite is present, it is most likely the direct impact of the environment on host physiology that will determine the infection prevalence and disease risk, probably through a decreased host tolerance under altered environmental conditions. Therefore, a change in environmental conditions may have a stronger direct impact on the host than indirect impact *via* the effect on the parasite transmission from reservoir hosts (illustrated by the arrow's width on Fig. 11), so that management actions might better focus on the abiotic environmental conditions than on the control of the parasite and its potential reservoir hosts' propagation (see below).

Because of the importance of the hosts abundance in shaping the parasite distribution, I additionally applied the analyses of Chapter 2 (i.e., model selection) to identify the abiotic environmental factors that were the most important for the brown trout and the bryozoan distributions. Bryozoan distribution is patchy (Okamura & Wood, 2002) and previously unknown in our study area, so that the study of its environmental drivers could have further revealed the indirect importance of abiotic factors for *T. bryosalmonae*'s distribution (Appendix 2). These analyses revealed that it is difficult to predict bryozoan distribution, as the model containing all the relevant abiotic variables (confirmed by model selection) had a low R² (0.14). However, it seems that the finer the sediments, the higher the chances are of finding *F. sultana*'s DNA in the environment (Appendix 2b). Brown trout eDNA was relatively more abundant in cooler water, and on sites with mild slope and mild urbanisation ($R^2 = 0.33$ for the model containing these variables and their quadratic terms, Appendices 2c-g). These results are coherent with what is known of brown trout ecology, especially for the water temperature (Elliott & Elliott, 2010), thus comforting on the accuracy of the eDNA quantifications.

The screening of the studied area through eDNA revealed the presence of the parasite and/or its bryozoan host at sites where infection of the fish host had not been detected yet, raising the alarm for potential future infection and/or disease development depending on the environmental abiotic conditions. This illustrates how eDNA may be used as an early warning signal of a biotic threat towards wildlife or farmed populations. For instance, eDNA detection of the protozoan parasite *Chilodonella*

hexasticha was positively correlated to mortality events of barramundi *Lates calcarifer* within a fish farm (Bastos Gomes et al., 2017). In this latter study, they also showed that abiotic factors such as water temperature and rainfall were important drivers of fish mortality, also illustrating the importance of environmental conditions in driving the outcomes of a parasite infection.

The temporal dynamics of a parasite life cycle may also be substantial to the infection outcomes. During my thesis, I also carried out a temporal eDNA sampling, surveying the concentrations of T. bryosalmonae, F. sultana and S. trutta DNA approximately every two months during a whole year, from June 2019 to June 2020. The aim was to investigate the temporal patterns of the parasite life cycle and to identify a potential synchronism between the activity of the parasite and that of its hosts. In the end, I did not have time to process the data during my thesis, but I had the opportunity to do preliminary analyses with Chiara Mercier, a MSc student that I co-supervised (see Appendix 3 for her report). The sampling consisted in stream water collection in sterile bags and vacuum filtration back in the laboratory (see Appendix 4 for an illustration of the different filtration methods used for eDNA and uDNA collection throughout the thesis). I sampled 11 sites known to house infected fish populations for 7 sampling sessions, collecting each time 6L of water per site. Chiara showed coordinated peaks of abundance for parasite and bryozoan in the early August and early September sessions (Fig. 2 of Appendix 3). We expected such peaks to occur in spring, when the conditions become favourable for bryozoan growth with an increase in temperature and food availability, coinciding with bryozoan overt infection and the onset of parasite spore release (see Fig. 8 and the general introduction). In that sense, there was a small increase in parasite DNA concentration in May, even though not significant (Fig. 2 of Appendix 3). The unexpected summer peak in parasite abundance could reflect brown trout spore excretion, but its synchronisation with the bryozoan peak suggests that the detected parasite DNA may mostly come from bryozoans, either through spore release, or perhaps from an early release of infected bryozoan statoblasts (see Fig. 8 for an illustration of bryozoan life cycle). These dormant stages are usually massively produced by bryozoan colonies prior to senescence when environmental conditions become unfavourable (usually in autumn), and bryozoan colonies spend the winter in these reduced forms (Hartikainen et al., 2009, 2013; Hartikainen & Okamura, 2015). Alternatively, bryozoans have also been reported to release infective parasite spores in autumn, witnessing a resurgence of overt infections, probably as a terminal effort of the parasite to escape future bryozoan senescence (Tops et al., 2009; Hartikainen & Okamura, 2015). Additionally, an extra-analysis on the onset of parasite release by infected fish also revealed that a stricter study of temperature regimes beginning in the winter might be important to understand the timing of T. bryosalmonae's life cycle and the onset of spore release in the wild, both by its bryozoan and by its fish hosts (Appendix 5, Rubin et al. 2022). To better understand these results, it would be beneficial to increase the eDNA sampling frequency during spring, autumn, but also in winter, coupled with fine temperature recording to get a finer overview of the temporal patterns at these critical periods for the continuity of the parasite life cycle.

A future perspective for the study of T. bryosalmonae's life cycle with eDNA tools would be to identify the whole potential host community present in the rivers, including all salmonid and bryozoan species known to be hosting the parasite. Our fish sampling showed that brown trout broadly dominated fish community, with only two sites housing another salmonid, the Atlantic salmon Salmo salar, that was identified through photograph inspection of morphological traits differentiating S. salar and S. *trutta*, and/or confirmed by a bad SNP genotyping (one site of the Garonne and one site of the Neste). However, it would be interesting to try to detect T. bryosalmonae DNA from the other most common sympatric fish species in the Pyrenees streams such as the stone loach (Barbatula barbatula), the European bullhead (Cottus gobio), the gudgeon (Gobio gobio) or the common minnow (Phonixus phonixus). Indeed, even though these species would most probably represent dead-end hosts for the parasite as only two species within the salmonids have been identified as permissive hosts releasing infective parasite spores towards bryozoans (Grabner & El-Matbouli, 2008), they could play a role of parasite population sink by decreasing the parasite pressure for brown trout and for the bryozoans (Doherty & Poulin, 2022). Moreover, the screening of other bryozoan species known to host T. bryosalmonae such as Plumatella rugosa, Cristatella mucedo or Pectinatella magnifica could be further interesting as the accurate distribution of these species is unknown in the Pyrenees, and they might rapidly colonise new environments, therefore increasing the presence of T. bryosalmonae's reservoir hosts (Vuorio et al., 2018).

Environmental DNA detection is until now especially used in the aquatic environment, but recent uses of the air eDNA (Clare et al., 2021; Lynggaard et al., 2022) or plant eDNA (Lyman et al., 2022) suggest that its use could be extrapolated to almost all the species and types of environment, to infer a wide range of species interactions (e.g., plant-animal interactions; Banerjee et al., 2022), therefore opening new avenues for wildlife species (communities) monitoring.

Reliability of eDNA quantification

Previous eDNA studies have shown that it is often more efficient to increase the number of field replicates than the volume filtered per sample to get accurate detection and quantification of the targeted species (e.g. in Capo et al., 2020). For eDNA detection in Chapter 2, we thus decided to filter up to 1.5L per filter, to use 8 filter per site (representing a maximum total volume of 12L) that we grouped by 2 into the same sample, to avoid too many extractions, leading to 4 field replicates per site. I found variation in eDNA concentration between the field replicates for the three targeted species, highlighting the importance of multiplying the field replicates to ensure an optimal detection of rare or patchy-distributed species such as *T. bryosalmonae*, but also to compute a more accurate mean concentration in the case of positive detection (Appendix 6). For *T. bryosalmonae*, each field sample was run twice with ddPCR, and the visual exploration of the results showed that the two PCRs yielded very similar *T. bryosalmonae* DNA concentrations, therefore confirming that field replication seems more important

than PCR replication (Appendix 6a). This repeatability found between PCR replicates further confirms the great precision of ddPCR quantification (Doi, Uchii, et al., 2015; Doi, Takahara, et al., 2015).

We found parasite DNA in the water for 21 of the 27 sites where we detected infection within fish (Chapter 2). Another study on the same parasite previously found parasite eDNA with qPCR at only half of the sites with infected fish (Sieber et al., in prep). The better performance of our sampling might be due to the higher sensitivity of ddPCR (Doi, Uchii, et al., 2015; Doi, Takahara, et al., 2015). However, the lack of detection of parasite DNA in the water was especially surprising for two sites (GRAMon and BOUSer) where we found a very high infection prevalence within fish (respectively 80 and 100%). This points at the limits of eDNA and some difficulties that still have to be overcome. This could reveal a timing mismatch between the sampling and the period of parasite release, but this is not supported by the temporal study that identified a peak of parasite detection in August (Appendix 3), i.e., at the very same period of the eDNA sampling of Chapter 2. Moreover, the fish infection prevalence at these sites was also measured in August via uDNA, thus confirming that parasite DNA is released in the milieu by infected fish. Alternatively, high water temperatures during the sampling could have precipitated DNA degradation (Strickler et al., 2015), but the detection of brown trout DNA at all sites seems to exclude this hypothesis. The explanation may come from the main difficulty encountered during the eDNA sampling, that is the difference in turbidity across the 54 sampled streams. For this eDNA sampling, we decided to constrain the number of filters used per site (8, that we grouped per 2 for the extraction), so that at the clearest sites we were able to filter 12L (we had set up a limit of 1.5L per filter), but at turbid sites, the amount of filtered water could be way less (for instance 2.4L for BOUSer, the most turbid site, and 4.9L for GRAMon, see Appendix 7 for a distribution). Although we were able to detect parasite DNA at some sites with only 5L filtered, this might represent the biggest limit of our sampling. For further sampling, I would therefore suggest increasing the number of filters used (thus increasing the number of extraction and field replicates) at the turbid sites to ensure the filtration of at least 7-8L per site. Alternatively, I would test the use of filtering cartridges allowing higher filtration volumes (Miya et al., 2016) with a pre-calibration at the most turbid sites to homogenise the maximum volume to be filtered per site, using at least 2 cartridges per site to have field replicates. The choice between these two sampling alternatives would be a trade-off between time, number of replicates, homogenisation between the sampled sites and budget (as with all the sampling methods), because increasing the number of filters at turbid sites takes time on the field and in the lab, while filtration cartridges are more expensive and restrict the number of field replicates. Additionally, I would try to identify different microhabitats apparently favourable to the parasite transmission (such as tree roots, sediment areas, rock shelters..., see below) and locate the sampling downstream of these habitats to diversify the field replicates and thus maximise the chances of finding the highly diluted parasite eDNA (found in low quantities at positive sites). However, this sampling design is easier to perform on small wadable streams than on large rivers such as the Ariège or the Garonne.

Additionally, I also tested the reliability of eDNA for estimating brown trout abundance by analysing the relationship between the quantity of brown trout eDNA detected in Chapter 2 and the brown trout biomass (including all-age trout) found at 26 sites that were common to another project led by the team. Even though both samplings (eDNA and biomass) were not performed at the very same time, I found a positive and significant relationship between the two measures ($R^2adj = 0.28$, P = 0.003, Appendix 8). This positive relationship highlights the ability of eDNA to inform on brown trout abundance, in agreement with most studies investigating the correlation between fish biomass and eDNA concentration in freshwater (Rourke et al., 2021), even though the relation was weaker than that found in experimental studies (e.g. for pike, *Esox lucius*, $R^2 = 0.74-0.87$ in Karlsson et al., 2022) due to the hazards encountered on the field that are avoided in controlled experiments.

In conclusion, non-lethal methods such as uDNA and eDNA should be increasingly used to study wildlife populations while avoiding the harm to the sampled organisms as much as possible, for ethics and conservation purposes (Parris et al., 2010; Waugh & Monamy, 2016). Although host sampling may not be 100% risk-free to the hosts, as the capture and handling generate stress, and eDNA for parasite detection in the open water may still require sampling refinements, the combination of uDNA and water eDNA methods in this thesis allowed to track population infection prevalence and to further understand the dynamics of an endoparasite life cycle without killing the hosts, while studying several parasite component populations (within the host and as transmission stages), therefore opening new perspectives for future studies of endoparasites. The development of such methods, together with the monitoring of environmental factors, allows wildlife studies to encompass large geographical scale, required for an efficient targeting of threatened populations into management plans (for instance, 83 sites were sampled for eDNA within two weeks by two persons on over 6000km² for Chapter 2). The French SAGIR network involves professional and citizen science for the recording of mortality or morbidity events affecting wild populations of birds and mammals through passive surveillance. This network, encompassing the whole country, reveals efficient in detecting early signal of wildlife disease outbreaks. When disease cases and/or suspicious death are reported, investigations are led to determine the causes (infectious agents, but also pollution; see Millot et al., 2017). Such a national network could be initiated within streams to routinely detect the presence of wildlife parasites via eDNA in the water and provide insights on infectious disease risks.

The impacts of multiple stressors on host populations

The second part of this thesis focused on the ecological and evolutionary impacts of multiple stressors, including parasite infection, on the host populations and their global health. Two studies were led at two different levels: at the intra-population level in Chapter 3, investigating the impact of multiple stressors before and after the favourable period for disease outbreak, and at the inter-population level in Chapter 4, by developing a multifaceted index of population health, compared between populations while investigating the driving environmental factors, therefore allowing to identify risk-prone populations.

Monitoring wildlife populations intraspecific variation in time

In Chapter 3, I took a first step towards the understanding of a parasite impact on its host species recruitment (i.e., the number of juveniles that incorporate into the population as a result of the annual spawning), that is substantial for the population size and sustainability (Lobón-Cerviá, 2005). Most studies about host-parasite interactions in the wild are led after the detection of a disease outbreak, or during a specific period that is favourable to the detection of infected individuals. However, such sampling might miss the less tolerant/resistant individuals that succumbed to the infection prior to the sampling, so that the parasite effect on its host populations might be underestimated (Rubin et al., 2022). Here, this before/after study investigated the combined impacts of multiple stressors, represented by parasite infection and water temperature, on host survival and intraspecific variation during the critical period for disease development (i.e., the summer). *T. bryosalmonae* was identified as the main threat to its host survival in the study area, causing a temperature-dependent disease, so that water temperature might act at two different levels: directly on the host physiology (brown trout, the studied host, is a poikilotherm and cool-water fish), and indirectly by triggering the disease development (either by an increased development of the parasite, an increased propagule pressure in the environment -but see Chapter 2-, or an impaired host immune response).

The results showed that the abundance of brown trout decreased further during the summer at highly infected sites, except at the sites that were already highly infected the year before. This is a very interesting result, suggesting that naive populations show a higher risk of decline, whereas the juvenile density of populations with an infection history and a high percentage of infected individuals during previous years could be maintained. This could illustrate transient dynamics where the initially high mortality caused by a pathogen could rapidly fade due to micro-evolutionary response of the host population (Hastings, 2004; Voyles et al., 2018). A recent experimental study also suggested that sudden environmental changes (such as a disease outbreak) may favour a more rapid adaptation of populations compared with slower environmental changes (Guzella et al., 2018). Accordingly, I found that high infection prevalence was linked to a decline in diversity at an immune gene, whereas no such decline was detected at the neutral loci. This decrease in immune-gene variation could result from the directional

selection of advantageous alleles and highlights the importance of standing genetic variation for a rapid adaptation (Eizaguirre et al., 2012; Phillips et al., 2018). This supposed ongoing adaptation to T. bryosalmonae infection could rapidly erase the initial disease-related declines, but the resulting decrease in standing genetic variation at the immune genes could also favour co-infection by other parasites, even though the impact of reduced MHC diversity in the wild remains unclear (Radwan et al., 2010; Pauls et al., 2013). At the phenotypic level, I found that water temperature and infection prevalence were negatively linked to body condition and had an interactive effect on carotenoid-based colouration, suggesting phenotypic plasticity and a reallocation of resources under the adverse environmental conditions experienced during the summer. These reallocations due to a poor early-life environment may have further effects on individual lifetime fitness including future reproduction and survival to other potential stressors (Lindström, 1999; Pigeon & Pelletier, 2018). Overall, these results suggest that brown trout populations of the studied area may show the adaptive potential (i.e., the ability to respond to a selection pressure by means of phenotypic and/or genetic changes; Eizaguirre & Baltazar-Soares, 2014) to counter an initial loss of density due PKD emergence, even though the negative impact of infection and water temperature on phenotypic traits related to fitness could have long-lasting negative effects on surviving individuals. Furthermore, the apparent tolerance linked to phenotypic plasticity could be efficient on the short-term, but may show some limits on the long-term, especially if the environmental conditions continue to alter (Nussey et al., 2007; Hansen et al., 2012).

Wildlife population monitoring before and after a critical period for the action of environmental stressors on several consecutive years may help to confirm possible transient dynamics, and evolution in genetic and phenotypic variation (Royle et al., 2018; Vredenburg et al., 2019). Moreover, as wildlife populations are not closed to dispersion (immigration and emigration of individuals), a study at a finer scale, allowing to quantify dispersion between sampled sites, could reveal important dynamics relative to a metapopulation system (Jiao et al., 2020). Indeed, it is possible that the initial mortality at a site could be buffered by the arrival of new individuals. This immigration could either provide genetic rescue to a host population lacking standing genetic diversity, or on the contrary weaken the effect of directional selection that might be required for rapid adaptation (Tallmon et al., 2004). A long-term individual-based study, involving the tagging of surviving individuals and the following of their subsequent reproduction could inform on the long-lasting effects of the early-life environment (Clutton-Brock & Sheldon, 2010). Such a study would require several years of sampling for the construction of an accurate pedigree and thus intensive sampling of whole populations at a few sites. Although very costly in sampling effort, it could give precious information on the potential evolution in resistance and tolerance in a host population after a disease emergence.

Alternatively, a common garden experiment in rivers categorised by different temperatures, infection prevalence at the year n-1 and infection histories (ideally more than one year ahead), with

165

caging of individuals originating from these different rivers would help to further understand the impacts of these two stressors on brown trout juveniles and the mechanisms involved (adaptation through directional selection or phenotypic plasticity; Jensen et al., 2008; Blanquart et al., 2013; Grimaudo et al., 2022). Such in situ experiment could be coupled to the test of different treatments within the same river, testing for instance the effect of different microhabitats (e.g., river substrate, as Chapter 2 suggests an effect of sediment size on both the infection prevalence and parasite distribution). The test of different microhabitats (related to river substrate but also close to tree roots, dead wood, shelters near large rocks, i.e., favourable habitats to bryozoan colonies establishment) seems substantial as a previous caging experiment in rivers where T. bryosalmonae eDNA was detected in the water failed to induce infection in the caged fish, also suggesting that microhabitat preferentially chosen by free ranging fish represents infection hotspots (Hutchins et al., 2021). Overall, such an experiment would enable a comparison of individual survival according to the infection and genetic background of their source population, to get further insights on the potential higher survival of the offspring of infected breeders, and on potential hotspots of infection (Mathieu-Bégné et al., 2021). Moreover, the infection could be surveyed through time with the uDNA method, the only mean for temporal individual infection survey (because nonlethal, Chapter 1), together with the evolution of phenotypic traits to investigate the trade-off hypothesis between immune, growth and reproduction functions. eDNA sampling upstream of the cages targeting T. bryosalmonae and F. sultana as in Chapter 2, combined with a fine recording of water temperature, would further inform on the timing of infection and the exposure to parasite propagules. This experiment would thus provide a great quantity of information on the parasite life cycle and the impact of the infection on brown trout hosts. However, it would require a lot of individuals to ensure enough statistical power, and it may raise some issues to introduce exogenous individuals into a river, together with their parasite cohorts (potentially T. bryosalmonae and others) that could cause sanitary issues in their translocation area.

Monitoring wildlife populations health in space

If parasite infection may threaten wildlife populations, it is not the only parameter that may inform on a population risk of decline. In Chapter 4, I developed an integrative multifaceted index informing on wildlife population health, by combining information on the pathological, the ecological and the genetic status of populations (component indicators). Here, the term health goes beyond a disease-centric approach, and rather describes the propensity of a population to respond to environmental changes (Stephen, 2014; Wittrock et al., 2019). The results revealed that the component indicators were not correlated and therefore carried complementary information, and that the multifaceted index properly represented its component indicators. The multifaceted index identified the populations that were the most risk-prone, together with the most important environmental factors among a wide range in shaping populations' health, a substantial information for the elaboration of management plans.

Moreover, the use of a multifaceted index buffers the imperfection and incompleteness of the single component indicators. For instance, although in our case study T. bryosalmonae likely represented the main pathological threat in the sampled area, the pathological indicator could have been complemented by information about the whole community of pathogens likely to affect the host of interest. For instance, a high-throughput qPCR assay has been developed by Bass et al. (2017) to detect a wide array of microparasites (45 different taxa including viruses) in the Chinook salmon (Oncorhynchus tshawytscha) by amplifying RNA. The combination of non-lethal methods for parasite detection such as uDNA and/or biopsy with molecular screening would represent a great opportunity to further improve the significance of the multifaceted index. Additionally, the genetic indicator informing on population genetic diversity could be positively biased by the introgression from stocked individuals. The study area has a history of stocking wild salmonid populations with hatchery-reared fish, even though this is especially true for elevated altitudes, and stocking has been reduced in the last decades (Chat et al., 2015; Galop, 2020). I run some additional analyses to potentially identify individuals that would come either directly from hatcheries, or that would be partly introgressed with these hatcheries' strains (Sanz et al., 2009). Population admixture analyses run with STRUCTURE (Pritchard et al., 2000) together with 125 SNPs data and 30 individuals from the two main hatcheries used for stocking in the region revealed that the sampled sites were only weakly concerned with introgression from farmed fish, as we found a mean introgression of 0.08 (with 0: wild cluster and 1: hatchery cluster), with a maximum of 0.47 for TOUFre which showed a weird introgression pattern (Appendix 9). If what we detect in this population is truly introgression, it means that almost all the individuals are the offspring of an interbreeding between purely "wild" and purely "farmed" fish, which seems very unlikely and may illustrate some limitation in the introgression quantification (that could be overcome with an analysis population per population, such as in Appendix S3 from Chapter 3). I could have added the introgression measure within the genetic indicator to balance a potential "positively biased" genetic diversity, but it is not straightforward whether the introgression should have a positive of negative effect on the population genetic health score. Indeed, if the resulting increase in standing genetic variation might be beneficial, the introgression could also lead to outbreeding hindering local adaptations. For instance, Currens et al. (1997) found that the introgression with hatchery rainbow trout reduced the ability of wild rainbow trout to survive the infection by the myxozoan Ceratomyxa shasta. I also run similar analyses to detect stocked fish with the individuals genotyped in the before/after study (Chapter 3), where I chose to remove the individuals identified as stocked (with introgression > 0.75, arbitrary threshold) to ensure that the before and after densities would not be influenced by these "external" fish that might respond differently to the environmental stressors, or that might have been stocked between the two sampling sessions. Interestingly, we found firm evidence of stocking at a site before the summer, but not after, suggesting that the stocked fish may not have survived the summer, or that they dispersed further downstream.

Another perspective to this work would be to investigate whether the parasite shows different strains over the study area, which could represent another level of variation in virulence and disease severity that I did not explore. A recent study used eDNA to identify the strains of a *Ranavirus* and found that the eDNA strains differed from that found within earlier infected amphibian hosts (Kaganer et al., 2021). These results therefore suggest that eDNA and/or uDNA methods could be used for parasite strains identification across a spatial range, but also to follow parasite evolution across time. The sequencing of *T. bryosalmonae*'s DNA could therefore add some more insights to the eco-evolutionary pathways of this host-parasite system.

Moreover, I focused on only one parasite species, because *T. bryosalmonae* has been identified as the main pathogen threat in the area, but co-infection with other parasites, that is the rule rather than the exception in natural systems, may add to the multiple stressors (as part of the biotic environment) shaping the host-parasite-environment interaction outcomes (Lively et al., 2014). The study of multiparasite infections is developing through high-throughput sequencing methods (Titcomb et al., 2019), and has already been used in chinook salmon (Bass et al., 2017). Such methods for detecting a whole host parasite community could be derived from parasite DNA detection in host excretion (uDNA method).

Relevance of this work for brown trout management in the Pyrenees

This thesis shed light on a parasite distribution in a previously unsampled area. With the use of uDNA and eDNA, I identified infected brown trout populations and areas where they could become infected or diseased in the near future. Moreover, I identified the most risk-prone populations in the area including other information than their sole infection status, which may have a different effect on populations with high densities, high genetic diversity and favourable phenotypic traits than in a small, inbred population with already weakened individuals.

Accordingly, I found in Chapter 3 that high infection prevalence in naive populations had a great impact on their post-summer density. This means that populations showing already low juvenile densities before the summer might risk a null-recruitment year. However, the surviving individuals, that are most likely protected from disease development upon reinfection (Feist & Longshaw, 2006), may have been selected for their favourable genetic background (as suggested by the reduced diversity at the MHC locus after the summer at infected sites), that they could pass on to the next generation (genetic heritability), even though this would require a strong selection. Moreover, the populations highly infected the year before the sampling showed a weak decline in density, that was comparable to non-infected populations. This may either illustrate a strong directional selection that already operated on the breeders, or a potential transfer of the immunity by infected mothers to their eggs that would facilitate the development of juveniles' immune response (maternal heritability, e.g. in Gasparini et al., 2002).

Overall, this means that after an initial decline in population recruitment, the surviving individuals may participate to a transient dynamic illustrating rapid micro-evolution (Ashley et al., 2003; Voyles et al., 2018). At the phenotypic level, I found that the surviving individuals had an altered body condition at the warmer and infected sites, and that their carotenoid ornamentation, which highly increased during the summer at cold non-infected sites, barely increased at warm sites and infected cold sites. The combined impact of the infection and the water temperature thus altered these fitness-related traits, probably because of a trade-off with immune functions (Rauw, 2012). The negative effects of high infection and/or high water temperature on growth during the first summer may also have long-term effect on brown trout over-winter survival, size at all age and future reproduction (Biro et al., 2004; Parra et al., 2009; Ros et al., 2022).

However, this before/after study focused on a single generation, and further investigation of these populations' reproduction and of the genetic background of the next generations would provide more accurate insights of the mechanisms involved. Moreover, a history of the population's dynamics, including the different age-cohorts, could help to identify major declines within juveniles' cohorts or potentially in older fish if the parasite recently emerged, and how it echoes on the populations' demographic structure.

The populations sampled both in 2019 and 2020 revealed that on the 6 populations that were uninfected in 2019, only one remained at a zero prevalence in 2020. In all the other populations, the infection prevalence increased, except for two populations in which it slightly decreased (see Fig. S1a from Chapter 3). These observations are going in the direction of the novel pathogen hypothesis (Rachowicz et al., 2005), in which the parasite infection represents a threat to the host population because it is newly arrived in the environment. However, the infection prevalence may also have been previously null because of the environmental conditions modulating the compatibility filter (Fig. 2), most probably through higher host resistance as I showed that it is the direct impact of the abiotic environment on brown trout that modulates the infection, therefore preventing the parasite from establishing into its brown trout host, so that the endemic pathogen hypothesis cannot be excluded.

The parasite distribution was mainly driven by the abundance of its two hosts. On the other hand, the infection prevalence within brown trout populations was mainly impacted by the abiotic factors, with the water temperature, the percentage of agriculture and the sediment size identified as the most important among the factors tested. Finer sediment size could better retain parasites' free-living spores and favour their contact with juvenile fish that on the other hand preferably use coarser substrate when available (Heggenes et al., 1999; Droppo et al., 2006). Greater fine-sediment patches on the riverbed may thus represent hotspots of infection, therefore associated to higher infection prevalence in fish (e.g. in Mathieu-Bégné et al., 2021). Additionally, I found that the bryozoan distribution, as inferred by eDNA quantification, was also driven by the sediment size, i.e., the finer the sediments, the more
bryozoan DNA detected (Appendix 2b). This is rather surprising and to take cautiously as bryozoan colonies are known to grow in sheltered locations protected from sedimentation, and our distribution model globally poorly explained the bryozoan distribution (Hartikainen & Okamura, 2015). In Chapter 4, the pathological status of brown trout populations was also negatively affected by both the water temperature and the concentration in nutrients in the water. Warmer and more eutrophicated sites corresponded to those showing the highest percentage of agricultural land in their surroundings, and the agriculture also had a negative impact on the ecological and the genetic status of populations and was also accordingly identified the main driver of the multifaceted health index. Since high concentration in nutrients, high temperature and low oxygen concentration are unfavourable conditions for brown trout survival and reproduction (Burkhardt-Holm & Scheurer, 2007; Elliott & Elliott, 2010), large agricultural surroundings may thus be associated with a rapid decline in the population demographic performance, ultimately decreasing its genetic diversity, as observed in other aquatic species (Blum et al., 2012; Nicol et al., 2017). Consistently, the populations identified as the most likely to decline with the multifaceted health index had the highest percentages of agricultural land in their surroundings. This corroborates the negative impact of agricultural land on the density and production found in other wild salmonid populations (Vondracek et al., 2005; Jonsson et al., 2011). However, the main drawback of studies in the wild is that most of the environmental variables are not independent and may also reflect the impact of other unmeasured variables. As mentioned above, the percentage of agricultural land is tightly linked to other environmental variables so that its effect might represent a combination of the water temperature, the concentration in nutrients and the distance from the source (agricultural lands are located further downstream in our study system), but also of decreased vegetation cover above the stream or water derivation for irrigation (Champagne et al., 2022). Nonetheless, I used statistical methods such as path analysis but also hierarchical partitioning (see Appendix 10) to take into account the interdependence of the environmental variables, and the agricultural land always appeared as a main driver. A laboratory experiment testing the combined effects of water temperature, sedimentation and nutrients for instance may help to disentangle between these related variables (e.g. in Piggott et al., 2012), but it would be difficult to include all the potential variables hidden under the agricultural land variable. Moreover, other studies on freshwater fish and amphibian species identified the agricultural landscape as a main threat to populations' health and adaptive potential (Vondracek et al., 2005; Jonsson et al., 2011; Blum et al., 2012; Nicol et al., 2017; Harfoot et al., 2021), therefore comforting our results and the most likely high importance of agriculture on the brown trout health in the Pyrenees.

The mapping of the parasite distribution drawn from eDNA detection in Chapter 2 shows a patchy distribution, with the upstream detection of the parasite not always linked to its presence at downstream sites, contrary to the hypothesis of a downstream accumulation of parasite spores due to the river flow (Carraro, Mari, et al., 2018). Accordingly, in 2019, JOBEnc was highly infected, but the downstream population of GERPoi had no infected individuals (Chapter 4, Fig. A1c). A stricter

-General discussion-

environmental characterisation inventorying for instance the percentage of different river substrates at a site, the composition of the riverside vegetation (indicating the type of root system available for the bryozoan establishment), together with the percentage of vegetation cover above the streams could give precious information on the local scale and the finer mechanisms involved in the parasite infection.

Overall, my results identified the abiotic environment as the most important driver of brown trout infection and disease risk. Among the tested environmental factors, the agriculture, water temperature, nutrient and oxygen concentration were identified as the most important drivers of brown trout infection and global health, whereas the intensity of parasite exposure had only a weak impact on brown trout infection. Consequently, mitigation measures acting broadly on brown trout habitat restoration would appear more efficient for disease control than measures that would seek for parasite or bryozoan control. This is a substantial result, not to mention that the restoration of a suitable habitat should be easier to achieve than the control of parasite and bryozoan spread, especially when considering the unlimited reservoir offered by bryozoan clonal reproduction (Abd-Elfattah et al., 2013). These mitigation measures may include a greater connectivity within the dendritic system, that could enhance brown trout movement towards cooler areas and favour infection (or at least disease) avoidance, but also demographic and genetic rescue (Bellard & Hugueny, 2020), while avoiding the warming effect caused by dams (even small ones; Zaidel et al., 2021). Furthermore, anthropogenic stream modification most of the time simplifies the hydro-geomorphology, decreases the availability of cooler thermal refuges, while the management of bankside vegetation often leads to the loss of riparian shade, also contributing to a decreased thermal buffering (O'Briain et al., 2019). Riparian vegetation represents a shield against nutrient runoff from agriculture, and it has been suggested that it could avoid ecological degradation due to erosion, sedimentation, decreased water quality and that it could maintain aquatic ecosystem functions even in a context of high whole-watershed agricultural intensity (Broadmeadow & Nisbet, 2004; Turunen et al., 2021; Champagne et al., 2022). Accordingly, Ros et al. (2022) suggested that restoration of the riverine system and riparian vegetation for keeping cool water temperature (also associated with higher oxygen concentrations) would be the most efficient management measures to avoid the negative effects of T. bryosalmonae infections.

In light of the present work, it seems substantial to use an integrative framework for the study of the host-parasite-environment interactions in wildlife and for the settlement of adequate management measures. Opportunities in bridging the gap between conservation biology and the accelerating environmental changes are growing with the emergence of novel management tools (Reid et al., 2019). A better understanding of the context-dependent diseases that may represent a threat to biodiversity and related ecosystem services appears substantial to try to mitigate their outcomes as holistic, One Health-like approaches to the management and mitigation of the risks of emerging infectious diseases have been suggested to show the greatest chances of success (Cunningham et al., 2017).

Literature cited

- Abd-Elfattah, A., Fontes, I., Kumar, G., Soliman, H., Hartikainen, H., Okamura, B., & El-Matbouli, M. (2013). Vertical transmission of *Tetracapsuloides bryosalmonae* (Myxozoa), the causative agent of salmonid proliferative kidney disease. *Parasitology*, 141(04), 482–490. https://doi.org/10.1017/S0031182013001650
- Amarasiri, M., Furukawa, T., Nakajima, F., & Sei, K. (2021). Pathogens and disease vectors/hosts monitoring in aquatic environments: Potential of using eDNA/eRNA based approach. *Science* of The Total Environment, 796, 148810. https://doi.org/10.1016/j.scitotenv.2021.148810
- Ashley, M. V., Willson, M. F., Pergams, O. R. W., O'Dowd, D. J., Gende, S. M., & Brown, J. S. (2003). Evolutionarily enlightened management. *Biological Conservation*, 111(2), 115–123. https://doi.org/10.1016/S0006-3207(02)00279-3
- Banerjee, P., Stewart, K. A., Antognazza, C. M., Bunholi, I. V., Deiner, K., Barnes, M. A., Saha, S., Verdier, H., Doi, H., Maity, J. P., Chan, M. W. Y., & Chen, C. Y. (2022). Plant–animal interactions in the era of environmental DNA (eDNA)—A review. *Environmental DNA*, edn3.308. https://doi.org/10.1002/edn3.308
- Bass, A. L., Hinch, S. G., Teffer, A. K., Patterson, D. A., & Miller, K. M. (2017). A survey of microparasites present in adult migrating Chinook salmon (*Oncorhynchus tshawytscha*) in south-western British Columbia determined by high-throughput quantitative polymerase chain reaction. *Journal of Fish Diseases*, 40(4), 453–477. https://doi.org/10.1111/jfd.12607
- Bass, D., Stentiford, G. D., Littlewood, D. T. J., & Hartikainen, H. (2015). Diverse Applications of Environmental DNA Methods in Parasitology. *Trends in Parasitology*, 31(10), 499–513. https://doi.org/10.1016/j.pt.2015.06.013
- Bastos Gomes, G., Hutson, K. S., Domingos, J. A., Chung, C., Hayward, S., Miller, T. L., & Jerry, D. R. (2017). Use of environmental DNA (eDNA) and water quality data to predict protozoan parasites outbreaks in fish farms. *Aquaculture*, 479, 467–473. https://doi.org/10.1016/j.aquaculture.2017.06.021
- Bellard, C., & Hugueny, B. (2020). Importance of metapopulation dynamics to explain fish persistence in a river system. *Freshwater Biology*, 65(11), 1858–1869. https://doi.org/10.1111/fwb.13571
- Biro, P. A., Morton, A. E., Post, J. R., & Parkinson, E. A. (2004). Over-winter lipid depletion and mortality of age-0 rainbow trout (*Oncorhynchus mykiss*). *Canadian Journal of Fisheries and Aquatic Sciences*, 61, 7.
- Blanquart, F., Kaltz, O., Nuismer, S. L., & Gandon, S. (2013). A practical guide to measuring local adaptation. *Ecology Letters*, 16(9), 1195–1205. https://doi.org/10.1111/ele.12150
- Blum, M. J., Bagley, M. J., Walters, D. M., Jackson, S. A., Daniel, F. B., Chaloud, D. J., & Cade, B. S. (2012). Genetic diversity and species diversity of stream fishes covary across a land-use gradient. *Oecologia*, 168(1), 83–95. https://doi.org/10.1007/s00442-011-2078-x
- Bowden, T. J. (2008). Modulation of the immune system of fish by their environment. Fish & Shellfish Immunology, 25(4), 373–383. https://doi.org/10.1016/j.fsi.2008.03.017
- Broadmeadow, S., & Nisbet, T. R. (2004). The effects of riparian forest management on the freshwater environment: A literature review of best management practice. *Hydrology and Earth System Sciences*, 8(3), 286–305. https://doi.org/10.5194/hess-8-286-2004
- Bruneaux, M., Visse, M., Gross, R., Pukk, L., Saks, L., & Vasemägi, A. (2017). Parasite infection and decreased thermal tolerance: Impact of proliferative kidney disease on a wild salmonid fish in the context of climate change. *Functional Ecology*, 31(1), 216–226. https://doi.org/10.1111/1365-2435.12701
- Burkhardt-Holm, P., & Scheurer, K. (2007). Application of the weight-of-evidence approach to assess the decline of brown trout (*Salmo trutta*) in Swiss rivers. *Aquatic Sciences*, 69(1), 51–70. https://doi.org/10.1007/s00027-006-0841-6
- Capo, E., Spong, G., Königsson, H., & Byström, P. (2020). Effects of filtration methods and water volume on the quantification of brown trout (*Salmo trutta*) and Arctic char (*Salvelinus alpinus*) eDNA concentrations via droplet digital PCR. *Environmental DNA*, 2(2), 152–160. https://doi.org/10.1002/edn3.52
- Carraro, L., Bertuzzo, E., Mari, L., Fontes, I., Hartikainen, H., Strepparava, N., Schmidt-Posthaus, H., Wahli, T., Jokela, J., Gatto, M., & Rinaldo, A. (2017). Integrated field, laboratory, and

theoretical study of PKD spread in a Swiss prealpine river. *Proceedings of the National Academy of Sciences*, *114*(45), 11992–11997. https://doi.org/10.1073/pnas.1713691114

- Carraro, L., Hartikainen, H., Jokela, J., Bertuzzo, E., & Rinaldo, A. (2018). Estimating species distribution and abundance in river networks using environmental DNA. *Proceedings of the National Academy of Sciences*, 201813843. https://doi.org/10.1073/pnas.1813843115
- Carraro, L., Mari, L., Gatto, M., Rinaldo, A., & Bertuzzo, E. (2018). Spread of proliferative kidney disease in fish along stream networks: A spatial metacommunity framework. *Freshwater Biology*, 63(1), 114–127. https://doi.org/10.1111/fwb.12939
- Champagne, E. J., Guzzo, M. M., Gutgesell, M. K., & McCann, K. S. (2022). Riparian buffers maintain aquatic trophic structure in agricultural landscapes. *Biology Letters*, 18(3), 20210598. https://doi.org/10.1098/rsbl.2021.0598
- Chat, J., Manicki, A., Guéraud, F., & Lepais, O. (2015). Continuité écologique et conservation de la diversité génétique et écotypique d'un grand migrateur (*Salmo trutta*). *Rapport INRA*, 55.
- Clare, E. L., Economou, C. K., Faulkes, C. G., Gilbert, J. D., Bennett, F., Drinkwater, R., & Littlefair, J. E. (2021). eDNAir: Proof of concept that animal DNA can be collected from air sampling. *PeerJ*, 9, e11030. https://doi.org/10.7717/peerj.11030
- Clutton-Brock, T., & Sheldon, B. C. (2010). Individuals and populations: The role of long-term, individual-based studies of animals in ecology and evolutionary biology. *Trends in Ecology & Evolution*, 25(10), 562–573. https://doi.org/10.1016/j.tree.2010.08.002
- Cunningham, A. A., Daszak, P., & Wood, J. L. N. (2017). One Health, emerging infectious diseases and wildlife: Two decades of progress? *Philosophical Transactions of the Royal Society B: Biological Sciences*, *372*(1725), 20160167. https://doi.org/10.1098/rstb.2016.0167
- Currens, K. P., Hemmingsen, A. R., Schreck, C. B., & Li, H. W. (1997). Introgression and Susceptibility to Disease in a Wild Population of Rainbow Trout. North American Journal of Fisheries Management, 17, 1065–1078.
- da Cunha, F. P., Cardoso, A. C. de S., Merlano, J. A. R., Nornberg, B. F. da S., Marins, L. F., Jerônimo, G. T., & Almeida, D. V. (2020). Non-lethal molecular diagnostic for acanthocephalosis in *Colossoma macropomum. Aquaculture*, 519, 734860. https://doi.org/10.1016/j.aquaculture.2019.734860
- Doherty, J.-F., & Poulin, R. (2022). Come with me if you want to live: Sympatric parasites follow different transmission routes through aquatic host communities. *International Journal for Parasitology*, 52(5), 293–303. https://doi.org/10.1016/j.ijpara.2021.11.009
- Doi, H., Takahara, T., Minamoto, T., Matsuhashi, S., Uchii, K., & Yamanaka, H. (2015). Droplet Digital Polymerase Chain Reaction (PCR) Outperforms Real-Time PCR in the Detection of Environmental DNA from an Invasive Fish Species. *Environmental Science & Technology*, 49(9), 5601–5608. https://doi.org/10.1021/acs.est.5b00253
- Doi, H., Uchii, K., Takahara, T., Matsuhashi, S., Yamanaka, H., & Minamoto, T. (2015). Use of Droplet Digital PCR for Estimation of Fish Abundance and Biomass in Environmental DNA Surveys. *PLOS ONE*, *10*(3), e0122763. https://doi.org/10.1371/journal.pone.0122763
- Droppo, I. G., Liss, S. N., Williams, D., & Leppard, G. G. (2006). River sediment/pathogen interactions: Importance for policy development on safe water practices. In J. S. Rowan, R. W. Duck, & A. Werritty, *Sediment Dynamics and the Hydromorphology of Fluvial Systems* (IAHS Publication 306, pp. 314–321).
- Eizaguirre, C., & Baltazar-Soares, M. (2014). Evolutionary conservation-evaluating the adaptive potential of species. *Evolutionary Applications*, 7(9), 963–967. https://doi.org/10.1111/eva.12227
- Eizaguirre, C., Lenz, T. L., Kalbe, M., & Milinski, M. (2012). Rapid and adaptive evolution of MHC genes under parasite selection in experimental vertebrate populations. *Nature Communications*, 3(1), 621. https://doi.org/10.1038/ncomms1632
- Elliott, J. M., & Elliott, J. A. (2010). Temperature requirements of Atlantic salmon *Salmo salar*, brown trout *Salmo trutta* and Arctic charr *Salvelinus alpinus*: Predicting the effects of climate change. *Journal of Fish Biology*, 25.
- Feist, S. W., & Longshaw, M. (2006). Phylum Myxozoa. In P. T. K. Woo, Fish diseases and disorders (CAB International, pp. 230–296).

- Fontes, I., Hartikainen, H., Holland, J., Secombes, C., & Okamura, B. (2017). Tetracapsuloides bryosalmonae abundance in river water. Diseases of Aquatic Organisms, 124(2), 145–157. https://doi.org/10.3354/dao03116
- Galop, D. (2020). Aux origines du peuplement piscicole des lacs d'altitude des Pyrénées. *Magazine truite & Cie*, 20.
- Gasparini, J., McCoy, K. D., Tveraa, T., & Boulinier, T. (2002). Related concentrations of specific immunoglobulins against the Lyme disease agent *Borrelia burgdorferi* sensu lato in eggs, young and adults of the kittiwake (*Rissa tridactyla*). *Ecology Letters*, 5(4), 519–524. https://doi.org/10.1046/j.1461-0248.2002.00345.x
- Grabner, D., & El-Matbouli, M. (2008). Transmission of *Tetracapsuloides bryosalmonae* (Myxozoa: Malacosporea) to *Fredericella sultana* (Bryozoa: Phylactolaemata) by various fish species. *Diseases of Aquatic Organisms*, 79, 133–139. https://doi.org/10.3354/dao01894
- Grimaudo, A. T., Hoyt, J. R., Yamada, S. A., Herzog, C. J., Bennett, A. B., & Langwig, K. E. (2022). Host traits and environment interact to determine persistence of bat populations impacted by white-nose syndrome. *Ecology Letters*, 25(2), 483–497. https://doi.org/10.1111/ele.13942
- Guzella, T. S., Dey, S., Chelo, I. M., Pino-Querido, A., Pereira, V. F., Proulx, S. R., & Teotónio, H. (2018). Slower environmental change hinders adaptation from standing genetic variation. *PLOS Genetics*, 14(11), e1007731. https://doi.org/10.1371/journal.pgen.1007731
- Hansen, M. M., Olivieri, I., Waller, D. M., Nielsen, E. E., & The GeM Working Group. (2012). Monitoring adaptive genetic responses to environmental change. *Molecular Ecology*, 21(6), 1311–1329. https://doi.org/10.1111/j.1365-294X.2011.05463.x
- Harfoot, M. B. J., Johnston, A., Balmford, A., Burgess, N. D., Butchart, S. H. M., Dias, M. P., Hazin, C., Hilton-Taylor, C., Hoffmann, M., Isaac, N. J. B., Iversen, L. L., Outhwaite, C. L., Visconti, P., & Geldmann, J. (2021). Using the IUCN Red List to map threats to terrestrial vertebrates at global scale. *Nature Ecology & Evolution*, 5(11), 1510–1519. https://doi.org/10.1038/s41559-021-01542-9
- Hartikainen, H., Fontes, I., & Okamura, B. (2013). Parasitism and phenotypic change in colonial hosts. *Parasitology*, 140(11), 1403–1412. https://doi.org/10.1017/S0031182013000899
- Hartikainen, H., Johnes, P., Moncrieff, C., & Okamura, B. (2009). Bryozoan populations reflect nutrient enrichment and productivity gradients in rivers. *Freshwater Biology*, 54(11), 2320–2334. https://doi.org/10.1111/j.1365-2427.2009.02262.x
- Hartikainen, H., & Okamura, B. (2015). Ecology and Evolution of Malacosporean-Bryozoan Interactions. In B. Okamura, A. Gruhl, & J. L. Bartholomew, *Myxozoan Evolution, Ecology and Development* (Springer International Publishing, pp. 201–216).
- Hastings, A. (2004). Transients: The key to long-term ecological understanding? *Trends in Ecology & Evolution*, 19(1), 39–45. https://doi.org/10.1016/j.tree.2003.09.007
- Haydon, D. T., Cleaveland, S., Taylor, L. H., & Laurenson, M. K. (2002). Identifying Reservoirs of Infection: A Conceptual and Practical Challenge. *Emerging Infectious Diseases*, 8(12), 1468– 1473. https://doi.org/10.3201/eid0812.010317
- Heggenes, J., Bagliniere, J. L., & Cunjak, R. A. (1999). Spatial niche variability for young Atlantic salmon (*Salmo salar*) and brown trout (*S. trutta*) in heterogeneous streams. *Ecology of Freshwater Fish*, 8(1), 1–21. https://doi.org/10.1111/j.1600-0633.1999.tb00048.x
- Hutchins, P. R., Sepulveda, A. J., Hartikainen, H., Staigmiller, K. D., Opitz, S. T., Yamamoto, R. M., Huttinger, A., Cordes, R. J., Weiss, T., Hopper, L. R., Purcell, M. K., & Okamura, B. (2021). Exploration of the 2016 Yellowstone River fish kill and proliferative kidney disease in wild fish populations. *Ecosphere*, 12(3). https://doi.org/10.1002/ecs2.3436
- Huver, J. R., Koprivnikar, J., Johnson, P. T. J., & Whyard, S. (2015). Development and application of an eDNA method to detect and quantify a pathogenic parasite in aquatic ecosystems. *Ecological Applications*, 25(4), 991–1002. https://doi.org/10.1890/14-1530.1
- Jensen, L. F., Hansen, M. M., Pertoldi, C., Holdensgaard, G., Mensberg, K.-L. D., & Loeschcke, V. (2008). Local adaptation in brown trout early life-history traits: Implications for climate change adaptability. *Proceedings of the Royal Society B: Biological Sciences*, 275(1653), 2859–2868. https://doi.org/10.1098/rspb.2008.0870

- Jiao, J., Gilchrist, M. A., & Fefferman, Nina. H. (2020). The impact of host metapopulation structure on short-term evolutionary rescue in the face of a novel pathogenic threat. *Global Ecology and Conservation*, 23, e01174. https://doi.org/10.1016/j.gecco.2020.e01174
- Jonsson, B., Jonsson, N., & Ugedal, O. (2011). Production of juvenile salmonids in small Norwegian streams is affected by agricultural land use: Salmonid production in streams. *Freshwater Biology*, 56(12), 2529–2542. https://doi.org/10.1111/j.1365-2427.2011.02678.x
- Kaganer, A. W., Nagel, L. D., Youker-Smith, T. E., Bunting, E. M., & Hare, M. P. (2021). Environmental DNA-derived pathogen gene sequences can expand surveillance when pathogen titers are decoupled in eDNA and hosts. *Environmental DNA*, *3*, 1192–1207.
- Karlsson, E., Ogonowski, M., Sundblad, G., Sundin, J., Svensson, O., Nousiainen, I., & Vasemägi, A. (2022). Strong positive relationships between eDNA concentrations and biomass in juvenile and adult pike (*Esox lucius*) under controlled conditions: Implications for monitoring. *Environmental DNA*, edn3.298. https://doi.org/10.1002/edn3.298
- Kuris, A. M. (2003). Evolutionary ecology of trophically transmitted parasites. *The Journal of Parasitology*, 89(suppl.), S96–S100.
- Lafferty, K. D., & Kuris, A. M. (1999). How environmental stress affects the impacts of parasites. *Limnology* and *Oceanography*, 44(3part2), 925–931. https://doi.org/10.4319/lo.1999.44.3_part_2.0925
- Lauringson, M., Nousiainen, I., Kahar, S., Burimski, O., Gross, R., Kaart, T., & Vasemägi, A. (2021). Climate change-driven disease in sympatric hosts: Temporal dynamics of parasite burden and proliferative kidney disease in wild brown trout and Atlantic salmon. *Journal of Fish Diseases*, 44(6), 689–699. https://doi.org/10.1111/jfd.13330
- Lawrence, M. J., Raby, G. D., Teffer, A. K., Jeffries, K. M., Danylchuk, A. J., Eliason, E. J., Hasler, C. T., Clark, T. D., & Cooke, S. J. (2020). Best practices for non-lethal blood sampling of fish *via* the caudal vasculature. *Fish Biology*, 97, 4–15.
- Lindström, J. (1999). Early development and fitness in birds and mammals. *Trends in Ecology & Evolution*, 14(9), 343–348. https://doi.org/10.1016/S0169-5347(99)01639-0
- Lively, C. M., de Roode, J. C., Duffy, M. A., Graham, A. L., & Koskella, B. (2014). Interesting Open Questions in Disease Ecology and Evolution. *The American Naturalist*, 184(S1), S1–S8. https://doi.org/10.1086/677032
- Lobón-Cerviá, J. (2005). The importance of recruitment for the production dynamics of stream-dwelling brown trout (*Salmo trutta*). *Canadian Journal of Fisheries and Aquatic Sciences*, 62, 10.
- Lyman, J. A., Sanchez, D. E., Hershauer, S. N., Sobek, C. J., Chambers, C. L., Zahratka, J., & Walker, F. M. (2022). Mammalian eDNA on herbaceous vegetation? Validating a qPCR assay for detection of an endangered rodent. *Environmental DNA*, edn3.331. https://doi.org/10.1002/edn3.331
- Lynggaard, C., Bertelsen, M. F., Jensen, C. V., Johnson, M. S., Frøslev, T. G., Olsen, M. T., & Bohmann, K. (2022). Airborne environmental DNA for terrestrial vertebrate community monitoring. *Current Biology*, 32(3), 701-707.e5. https://doi.org/10.1016/j.cub.2021.12.014
- Macnab, V., & Barber, I. (2012). Some (worms) like it hot: Fish parasites grow faster in warmer water, and alter host thermal preferences. *Global Change Biology*, *18*(5), 1540–1548. https://doi.org/10.1111/j.1365-2486.2011.02595.x
- Mathieu-Bégné, E., Blanchet, S., Rey, O., Scelsi, O., Poesy, C., Marselli, G., & Loot, G. (2021). A finescale analysis reveals microgeographic hotspots maximizing infection rate between a parasite and its fish host. *Functional Ecology*, *36*(2), 380–391. https://doi.org/10.1111/1365-2435.13967
- McAllister, C. T., Bursey, C. R., & Connior, M. B. (2016). New Host and Distributional Records for Helminth Parasites (Trematoda, Cestoda, Nematoda) from Amphibians (Caudata, Anura) and Reptiles (Testudines: Ophidia) of Oklahoma. *Proceedings of the Oklahoma Academy of Science*, 96, 76–82.
- Millot, F., Decors, A., Mastain, O., Quintaine, T., Berny, P., Vey, D., Lasseur, R., & Bro, E. (2017). Field evidence of bird poisonings by imidacloprid-treated seeds: A review of incidents reported by the French SAGIR network from 1995 to 2014. *Environmental Science and Pollution Research*, 24(6), 5469–5485. https://doi.org/10.1007/s11356-016-8272-y

- Miya, M., Minamoto, T., Yamanaka, H., Oka, S., Sato, K., Yamamoto, S., Sado, T., & Doi, H. (2016). Use of a Filter Cartridge for Filtration of Water Samples and Extraction of Environmental DNA. *Journal of Visualized Experiments*, 117, 54741. https://doi.org/10.3791/54741
- Nicol, E., Stevens, J. R., & Jobling, S. (2017). Riverine fish diversity varies according to geographical isolation and land use modification. *Ecology and Evolution*, 7(19), 7872–7883. https://doi.org/10.1002/ece3.3237
- Nussey, D. H., Wilson, A. J., & Brommer, J. E. (2007). The evolutionary ecology of individual phenotypic plasticity in wild populations. *Journal of Evolutionary Biology*, 20(3), 831–844. https://doi.org/10.1111/j.1420-9101.2007.01300.x
- O'Briain, R., Coghlan, B., Shephard, S., & Kelly, F. L. (2019). River modification reduces climate resilience of brown trout (*Salmo trutta*) populations in Ireland. *Fisheries Management and Ecology*, 26(6), 512–526. https://doi.org/10.1111/fme.12326
- Okamura, B., & Wood, T. S. (2002). Bryozoans as hosts for *Tetracapsula bryosalmonae*, the PKX organism. *Journal of Fish Diseases*, 25(8), 469–475. https://doi.org/10.1046/j.1365-2761.2002.00376.x
- Parra, I., Almodóvar, A., Nicola, G. G., & Elvira, B. (2009). Latitudinal and altitudinal growth patterns of brown trout *Salmo trutta* at different spatial scales. *Journal of Fish Biology*, 74(10), 2355– 2373. https://doi.org/10.1111/j.1095-8649.2009.02249.x
- Parris, K. M., McCall, S. C., McCarthy, M. A., Minteer, B. A., Steele, K., Bekessy, S., & Medvecky, F. (2010). Assessing ethical trade-offs in ecological field studies. *Journal of Applied Ecology*, 47, 227–234.
- Paull, S. H., Song, S., McClure, K. M., Sackett, L. C., Kilpatrick, A. M., & Johnson, P. T. (2012). From superspreaders to disease hotspots: Linking transmission across hosts and space. *Frontiers in Ecology and the Environment*, 10(2), 75–82. https://doi.org/10.1890/110111
- Pauls, S. U., Nowak, C., Bálint, M., & Pfenninger, M. (2013). The impact of global climate change on genetic diversity within populations and species. *Molecular Ecology*, 22(4), 925–946. https://doi.org/10.1111/mec.12152
- Penczykowski, R. M., Laine, A.-L., & Koskella, B. (2016). Understanding the ecology and evolution of host-parasite interactions across scales. *Evolutionary Applications*, 9(1), 37–52. https://doi.org/10.1111/eva.12294
- Phillips, K. P., Cable, J., Mohammed, R. S., Herdegen-Radwan, M., Raubic, J., Przesmycka, K. J., van Oosterhout, C., & Radwan, J. (2018). Immunogenetic novelty confers a selective advantage in host–pathogen coevolution. *Proceedings of the National Academy of Sciences*, 115(7), 1552– 1557. https://doi.org/10.1073/pnas.1708597115
- Pigeon, G., & Pelletier, F. (2018). Direct and indirect effects of early-life environment on lifetime fitness of bighorn ewes. *Proceedings of the Royal Society B: Biological Sciences*, 285(1870), 20171935. https://doi.org/10.1098/rspb.2017.1935
- Piggott, J. J., Lange, K., Townsend, C. R., & Matthaei, C. D. (2012). Multiple Stressors in Agricultural Streams: A Mesocosm Study of Interactions among Raised Water Temperature, Sediment Addition and Nutrient Enrichment. *PLoS ONE*, 7(11), e49873. https://doi.org/10.1371/journal.pone.0049873
- Pritchard, J. K., Stephens, M., & Donnelly, P. (2000). Inference of Population Structure Using Multilocus Genotype Data. *Genetics*, 155(2), 945–959. https://doi.org/10.1093/genetics/155.2.945
- Råberg, L., Grahn, M., Hasselquist, D., & Svensson, E. (1998). On the adaptive significance of stressinduced immunosuppression. *Proceedings of the Royal Society of London. Series B: Biological Sciences*, 6.
- Rachowicz, L. J., Hero, J.-M., Alford, R. A., Taylor, J. W., Morgan, J. A. T., Vredenburg, V. T., Collins, J. P., & Briggs, C. J. (2005). The Novel and Endemic Pathogen Hypotheses: Competing Explanations for the Origin of Emerging Infectious Diseases of Wildlife. *Conservation Biology*, 19(5), 1441–1448. https://doi.org/10.1111/j.1523-1739.2005.00255.x
- Radwan, J., Biedrzycka, A., & Babik, W. (2010). Does reduced MHC diversity decrease viability of vertebrate populations? *Biological Conservation*, 143(3), 537–544. https://doi.org/10.1016/j.biocon.2009.07.026

- Rauw, W. M. (2012). Immune response from a resource allocation perspective. *Frontiers in Genetics*, 3. https://doi.org/10.3389/fgene.2012.00267
- Reid, A. J., Carlson, A. K., Creed, I. F., Eliason, E. J., Gell, P. A., Johnson, P. T. J., Kidd, K. A., MacCormack, T. J., Olden, J. D., Ormerod, S. J., Smol, J. P., Taylor, W. W., Tockner, K., Vermaire, J. C., Dudgeon, D., & Cooke, S. J. (2019). Emerging threats and persistent conservation challenges for freshwater biodiversity. *Biological Reviews*, 94(3), 849–873. https://doi.org/10.1111/brv.12480
- Riepe, T. B., Vincent, V., Milano, V., Fetherman, E. R., & Winkelman, D. L. (2021). Evidence for the Use of Mucus Swabs to Detect *Renibacterium salmoninarum* in Brook Trout. *Pathogens*, 10(4), 460. https://doi.org/10.3390/pathogens10040460
- Ros, A., Schmidt-Posthaus, H., & Brinker, A. (2022). Mitigating human impacts including climate change on proliferative kidney disease in salmonids of running waters. *Journal of Fish Diseases*, 45(4), 497–521. https://doi.org/10.1111/jfd.13585
- Rourke, M. L., Fowler, A. M., Hughes, J. M., Broadhurst, M. K., DiBattista, J. D., Fielder, S., Wilkes Walburn, J., & Furlan, E. M. (2021). Environmental DNA (eDNA) as a tool for assessing fish biomass: A review of approaches and future considerations for resource surveys. *Environmental* DNA, 4(1), 9–33. https://doi.org/10.1002/edn3.185
- Royle, J. A., Fuller, A. K., & Sutherland, C. (2018). Unifying population and landscape ecology with spatial capture-recapture. *Ecography*, *41*(3), 444–456. https://doi.org/10.1111/ecog.03170
- Rubin, A., Bailey, C., Strepparava, N., Wahli, T., Segner, H., & Rubin, J.-F. (2022). Reliable Field Assessment of Proliferative Kidney Disease in Wild Brown Trout, *Salmo trutta*, Populations: When Is the Optimal Sampling Period? *Pathogens*, 11(6), 681. https://doi.org/10.3390/pathogens11060681
- Ryser-Degiorgis, M.-P. (2013). Wildlife health investigations: Needs, challenges and recommendations. BMC Veterinary Research, 9(1), 223. https://doi.org/10.1186/1746-6148-9-223
- Sadoul, B., & Geffroy, B. (2019). Measuring cortisol, the major stress hormone in fishes. *Journal of Fish Biology*, 94(4), 540–555. https://doi.org/10.1111/jfb.13904
- Sanz, N., Araguas, R. M., Fernández, R., Vera, M., & García-Marín, J.-L. (2009). Efficiency of markers and methods for detecting hybrids and introgression in stocked populations. *Conservation Genetics*, 10(1), 225–236. https://doi.org/10.1007/s10592-008-9550-0
- Sieber, N., King, A., Zenker, A., Vorburger, C., & Hartikainen, H. (in prep). A survey of multiple aquatic wildlife pathogens of concern in Switzerland using an eDNA-based method for detection in water. *Thesis Dissertation: Detection of Aquatic Wildlife Pathogens from EDNA in Water Samples Validation and Application in Switzerland, Chapter 3.*
- Soliman, H., Kumar, G., & El-Matbouli, M. (2018). *Tetracapsuloides bryosalmonae* persists in brown trout *Salmo trutta* for five years post exposure. *Diseases of Aquatic Organisms*, 127(2), 151– 156. https://doi.org/10.3354/dao03200
- Stephen, C. (2014). Toward a modernized definition of wildlife health. *Journal of Wildlife Diseases*, 50(3), 427–430. https://doi.org/10.7589/2013-11-305
- Strickler, K. M., Fremier, A. K., & Goldberg, C. S. (2015). Quantifying effects of UV-B, temperature, and pH on eDNA degradation in aquatic microcosms. *Biological Conservation*, 183, 85–92. https://doi.org/10.1016/j.biocon.2014.11.038
- Tallmon, D., Luikart, G., & Waples, R. (2004). The alluring simplicity and complex reality of geneticrescue.TrendsinEcology& Evolution,19(9),489–496.https://doi.org/10.1016/j.tree.2004.07.003
- Titcomb, G. C., Jerde, C. L., & Young, H. S. (2019). High-Throughput Sequencing for Understanding the Ecology of Emerging Infectious Diseases at the Wildlife-Human Interface. *Frontiers in Ecology and Evolution*, 7, 126. https://doi.org/10.3389/fevo.2019.00126
- Tops, S., Hartikainen, H.-L., & Okamura, B. (2009). The effects of infection by *Tetracapsuloides* bryosalmonae (Myxozoa) and temperature on *Fredericella sultana* (Bryozoa). International Journal for Parasitology, 39(9), 1003–1010. https://doi.org/10.1016/j.ijpara.2009.01.007
- Turunen, J., Elbrecht, V., Steinke, D., & Aroviita, J. (2021). Riparian forests can mitigate warming and ecological degradation of agricultural headwater streams. *Freshwater Biology*, 66(4), 785–798. https://doi.org/10.1111/fwb.13678

- Vondracek, B., Blann, K. L., Cox, C. B., Nerbonne, J. F., Mumford, K. G., Nerbonne, B. A., Sovell, L. A., & Zimmerman, J. K. H. (2005). Land Use, Spatial Scale, and Stream Systems: Lessons from an Agricultural Region. *Environmental Management*, 36(6), 775–791. https://doi.org/10.1007/s00267-005-0039-z
- Voyles, J., Woodhams, D. C., Saenz, V., Byrne, A. Q., Perez, R., Rios-Sotelo, G., Ryan, M. J., Bletz, M. C., Sobell, F. A., McLetchie, S., Reinert, L., Rosenblum, E. B., Rollins-Smith, L. A., Ibáñez, R., Ray, J. M., Griffith, E. J., Ross, H., & Richards-Zawacki, C. L. (2018). Shifts in disease dynamics in a tropical amphibian assemblage are not due to pathogen attenuation. *Science*, 359(6383), 1517–1519. https://doi.org/10.1126/science.aao4806
- Vredenburg, V. T., McNally, S. V. G., Sulaeman, H., Butler, H. M., Yap, T., Koo, M. S., Schmeller, D. S., Dodge, C., Cheng, T., Lau, G., & Briggs, C. J. (2019). Pathogen invasion history elucidates contemporary host pathogen dynamics. *PLOS ONE*, 14(9), e0219981. https://doi.org/10.1371/journal.pone.0219981
- Vuorio, K., Kanninen, A., Mitikka, S., Sarkkinen, M., & Hämäläinen, H. (2018). Invasion of Finnish inland waters by the alien moss animal *Pectinatella magnifica* Leidy, 1851 and associated potential risks. *Management of Biological Invasions*, 9(1), 1–10. https://doi.org/10.3391/mbi.2018.9.1.01
- Waugh, C. A., & Monamy, V. (2016). Opposing Lethal Wildlife Research When Nonlethal Methods Exist: Scientific Whaling as a Case Study. *Journal of Fish and Wildlife Management*, 7(1), 231– 236. https://doi.org/10.3996/072015-JFWM-061
- White, L. A., Forester, J. D., & Craft, M. E. (2017). Using contact networks to explore mechanisms of parasite transmission in wildlife. *Biological Reviews*, 92(1), 389–409. https://doi.org/10.1111/brv.12236
- Wittrock, J., Duncan, C., & Stephen, C. (2019). A determinants of health conceptual model for fish and wildlife health. *Journal of Wildlife Diseases*, 55(2), 285. https://doi.org/10.7589/2018-05-118
- Zaidel, P. A., Roy, A. H., Houle, K. M., Lambert, B., Letcher, B. H., Nislow, K. H., & Smith, C. (2021). Impacts of small dams on stream temperature. *Ecological Indicators*, *120*, 106878. https://doi.org/10.1016/j.ecolind.2020.106878

Appendices

Appendix 1.



A1. (a) Positive relationship between kidney swollenness (measured as the K/B ratio between kidney height (K) and body heigh (B), as shown on (b)) and standardised fish body height (body height measured as shown on (c) divided by fish fork length). This relationship was found among 23 individuals sampled in 2018 from 2 different sites with infection prevalence of 100% (ARIPam and ARIVar).



Appendix 2.



Appendix 3.

Synchronisme temporel entre un parasite et ses deux hôtes: une approche basée sur l'ADN environnemental

Stage réalisé dans le cadre du module Initiation à la Recherche, M1-Mention Écologie Université Toulouse III - Paul Sabatier

Chiara MERCIER

Encadrantes :

Géraldine LOOT¹, geraldine.loot@univ-tlse3.fr Eloïse DUVAL², eloise.duval@sete.cnrs.fr

¹ Laboratoire Évolution & Diversité Biologique EDB, UMR 5174, UPS ; CNRS ; ; IRD, Université Toulouse III Paul Sabatier, Toulouse, France

² Station d'Écologie Théorique et Expérimentale SETE, UMR 5321, UPS, CNRS, Moulis, France







AVANT-PROPOS

Les données utilisées pour ce stage étaient en partie préalablement disponibles. En effet, l'échantillonnage de terrain a eu lieu avant la période de stage et les données concernant la caractérisation de l'environnement étaient déjà disponibles. Concernant les méthodes moléculaires, la filtration et l'extraction ADN ont aussi été réalisées avant la période de stage par Eloïse Duval (doctorante) et Charlotte Veyssière (IE, CDD). Au début de mon stage, les échantillons ADN de chaque site étaient donc près à amplifier. En revanche, j'ai pu réaliser l'amplification de l'ADN par ddPCR et son interprétation informatique. Grâce à ces amplifications j'ai pu créer un jeu de données complet rassemblant les concentrations en ADN que nous avions trouvées et les données environnementales.

Le tableau formé était constitué de données se référant à l'échantillonnage d'une trentaine de sites situés dans les Pyrénées. Néanmoins, certains sites n'ayant pas été échantillonnés au même rythme que les autres et ne présentant pas une série temporelle complète, nous avons décidé de les enlever de l'analyse afin d'avoir des résultats plus cohérents et homogènes. Une partie du stage a donc été dédiée au nettoyage des données et à la formation de tableaux structurés.

Le script utilisé afin de répondre aux différentes questions a été créé directement pendant le stage. Le script que j'ai créé a permis la modification des données, la création de nouveaux tableaux mais aussi le traitement statistique. Concernant le raisonnement statistique, de nombreuses analyses et hypothèses ont été testées mais ne se sont pas montrées cohérentes avec les hypothèses de départ. Un tri a donc été fait afin de cibler seulement les hypothèses et les modèles qui nous intéressaient. Pour avoir les meilleures analyses possibles, j'ai eu recours à de l'aide extérieure et notamment de forums de discussions qui mettent en relation les utilisateurs de Rstudio.

REMERCIEMENTS

Je tiens tout d'abord à remercier profondément mes encadrantes de stage Géraldine Loot, enseignante chercheuse, et Eloïse Duval, doctorante, qui m'ont accompagnées et m'ont accordé leur confiance pour réaliser ce travail. Je remercie Géraldine Loot, pour tout ce qu'elle m'a appris pendant les séances de manips au labo, ses précieux conseils de rédaction ainsi que son accompagnement tout au long du stage. Par ailleurs, je remercie Eloïse Duval, qui s'est rendue disponible pour répondre à chacun de mes problèmes et questionnements. Je lui suis très reconnaissante pour son soutien sans faille et les innombrables conseils qu'elle a pu me donner durant ces 4 mois.

Je remercie bien évidemment Simon Blanchet, chercheur à la Station d'Écologie Théorique et Expérimentale du CNRS de Moulis, qui a su donner de son temps, de sa patience et m'aider à mettre en place le raisonnement statistique approprié mais aussi à réaliser un travail clair et structuré. Sa contribution à la direction de ce stage m'a beaucoup aidé et je le remercie infiniment pour ses nombreux conseils éclairants.

Je tiens également à remercier mes collègues du bureau 26 qui ont été très présents pour répondre à mes questions et qui ont su faire de chaque jour de ce stage des moments agréables. Ainsi je remercie les doctorants Laurine Gouthier, Sean Heighton et mon voisin de bureau, stagiaire M1, Olivier Brisset.

De plus, je remercie les nombreux doctorants et stagiaires de l'EDB avec qui j'ai passé du temps, pour leur accueil et la bonne ambiance qu'ils ont apporté pendant le stage. Chacun d'eux s'est montré très disponible et m'a soutenu lors de mes différents questionnements. Parmis eux je remercie notamment Paul Castagné, Amandine Vidal, Mathias Launay, Tristan Martin et les joueurs motivés de l'équipe de rugby du mardi.

Je remercie également Léonie Gratacap et Tanya Liao Rasamoelina, mes fidèles amies du Master qui m'ont soutenu et ont été très réconfortantes pendant ces 4 mois.

Enfin, je tiens à remercier Emilie Lecompte et Monique Gardes, responsables pédagogiques du module « *Stage d'initiation à la recherche* » pour leur accompagnement et suivi durant ce stage.

SOMMAIRE

RÉSUMÉ	1	
I. INTRODUCTION	1	
II. MATÉRIEL ET MÉTHODES	4	
A. Modèles d'études	4	
B. Échantillonnage	5	
C. Mesures de concentration ADN au sein des échantillons	7	
1. Filtration et extraction ADN	7	
2. Amplification et quantification ADN	7	
D. Analyses statistiques	8	
III. RÉSULTATS	9	
A. Variation saisonnière de la concentration	9	
B. Synchronisme entre sites pour chaque espèce	11	
C. Synchronisme entre les hôtes et le parasite	12	
IV. DISCUSSION	13	
RÉFÉRENCES BIBLIOGRAPHIQUES		

Synchronisme temporel entre un parasite et ses deux hôtes: une approche basée sur l'ADN environnemental

RÉSUMÉ

Le succès des cycles parasitaires dépend de nombreux facteurs dont le synchronisme temporel qui peut exister entre le parasite et ses hôtes. Néanmoins l'étude de ce synchronisme en milieu naturel s'avère complexe à mesurer en raison de limites méthodologiques. La présente étude a pour objectif de tester la dynamique temporelle de l'interaction entre le parasite *Tetracapsuloides bryosalmonae* et ses hôtes, un bryozoaire et un salmonidé, en utilisant une approche par ADN environnemental. Cette méthode et l'utilisation de la ddPCR ont permis une quantification absolue de chaque espèce à différents moments de l'année. Grâce à ces mesures, nous avons testé s'il existait un synchronisme dans les variations de concentration en ADN d'abord entre sites pour chaque espèce, puis entre le parasite et ses hôtes. Nos résultats ont montré qu'il existait des variations de concentration en ADN au cours du temps (pics d'abondance en été) et que ces variations étaient synchronisées entre le parasite et ses hôtes. Néanmoins, l'intensité du synchronisme était variable entre les différents sites échantillonnés, ce qui s'explique en partie par leur éloignement géographique. Ces résultats offrent de nouvelles perspectives sur l'étude du synchronisme temporel entre parasites et hôtes et suggèrent que l'étude approfondie de ces mécanismes serait nécessaire.

Mots-clés : Tetracapsuloides bryosalmonae, Salmo trutta, bryozoaire, milieu naturel, synchronie

ABSTRACT

The success of parasite cycles depends on many factors, including the temporal synchrony that may exist between the parasite and its hosts. However, the study of this synchronism is complex to measure in the natural environment because of methodological limitations. The present study aims to test the temporal dynamics of the interaction between the parasite *Tetracapsuloides bryosalmonae* and its hosts, a bryozoan and a salmonid, using an environmental DNA approach. This method and the use of ddPCR allowed an absolute quantification of each species at different times of the year. Using these measurements, we tested whether there was synchronism in the variations in DNA concentration first between sites for each species and then between the parasite and its hosts. Our results showed that there were variations in DNA concentration over time (peaks in abundance in summer) and that these variations were synchronised between the parasite and its hosts. Nevertheless, the intensity of synchronism was variable between the different sites sampled, which is partly explained by their geographical distance. These results offer new perspectives on the study of temporal synchrony between parasites and hosts and suggest that further study of these mechanisms is needed.

Keywords: Tetracapsuloides bryosalmonae, Salmo trutta, bryozoan, wildlife, synchronisation

I. INTRODUCTION

Le parasitisme peut se définir comme étant une interaction biotique entre un organisme parasite (protozoaire, virus, bactéries, métazoaire...) et un organisme hôte. Le parasite est dépendant de son hôte pour accomplir son cycle de vie, en effet, ce dernier représente à la fois une source de nourriture, un habitat et un outil de dissémination pour le parasite (Combes, 2001). Certains cycles parasitaires sont complexes car ils font intervenir plusieurs hôtes, jusqu'à 4 pour *Halipegus occidatus* (Krull, 1935), c'est ce qu'on appelle les cycles hétéroxènes. Le fait d'avoir un cycle de vie complexe composé de plusieurs hôtes en succession complique considérablement les chances de rencontre entre l'hôte et le parasite et donc le succès de reproduction du parasite. En effet, l'hôte et le parasite doivent être présents dans le même habitat au même moment pour que le cycle puisse s'accomplir, autrement dit il doit y avoir une synchronisation temporelle de leurs cycles biologiques. Cette synchronisation temporelle constitue la composante essentielle du filtre de rencontre, tel que décrit par Combes (2001). Ce filtre se répétant à chaque passage d'un hôte à l'autre, l'accomplissement du cycle parasitaire en devient très complexe.

Afin d'augmenter la probabilité de rencontre avec les hôtes, les parasites peuvent mettre en place toutes sortes de stratégies. Par exemple, de nombreuses études ont mis en évidence l'usage de la modification comportementale de l'hôte par le parasite (Hughes et al., 2012). C'est notamment le cas du parasite Toxoplasma gondii responsable de la toxoplasmose, qui infecte le chat et la souris. Ce parasite modifie l'aversion du rongeur pour les odeurs de chats et donc augmente la probabilité de rencontre entre la souris et le chat, autrement dit "la souris n'a plus peur du chat" (da Silva & Langoni, 2009). Il existe également des stratégies basées sur le synchronisme entre hôte et parasite. En effet, il est cohérent de penser que la disponibilité en hôtes dans l'environnement va conditionner la présence/abondance des parasites. D'un point de vue évolutif, un parasite a tout intérêt à ajuster sa dynamique temporelle à celles de ses hôtes. Cette adaptation lui permettra d'accomplir son cycle plus facilement en maximisant ses chances de rencontre dans l'environnement (Mathieu Bégné et al., 2022). Par exemple, il y a quelques années, il a été montré que l'abondance et la dynamique temporelle des espèces hôtes poissons et copépodes pouvaient influencer la rencontre avec le cestode parasite Ligula intestinalis (Loot et al., 2006). Néanmoins, l'étude de la dynamique temporelle des interactions hôte-parasite ne s'est que très peu développée, bien qu'une chronobiologie de la rencontre entre les émissions de métacercaires de Schistosoma et la présence de ses hôtes dans le milieu aie déjà été mise en avant par Combes et Théron il y a plus de 40 ans (Combes & Théron, 1977; Théron & Combes, 1988). C'est probablement en raison de la difficulté de détection des parasites dans leur environnement que ces mécanismes ne sont que très peu étudiés en milieu naturel.

La détection des parasites et de l'interaction en elle-même dans le milieu naturel se fait souvent à travers l'usage de méthodes invasives pour les animaux. Ce phénomène est largement amplifié lorsque les études concernent la détection d'endoparasites vivant à l'intérieur de leur hôte (Yusni et al., 2022; Xiao et al., 2004). En effet, les méthodes de détection pour ces organismes impliquent souvent la dissection et donc la mort des individus hôtes (McAllister et al., 2016; Salman et al., 2018). Ces pratiques soulèvent donc des questions éthiques en plus d'être peu adaptées aux situations dans lesquelles les populations hôtes sont déjà en péril. Afin de remédier à ce problème, de nouvelles méthodes non létales voient le jour depuis plusieurs années (Beja-Pereira et al., 2009). C'est en ce sens que récemment, de nouvelles méthodes non létales de détection de l'infection par des endoparasites ont été développées en utilisant la détection d'ADN environnemental (ADNe) à partir des excrétions de l'hôte chez l'anguille (Jousseaume et al., 2021) et chez la truite (Duval et al., 2021). L'ADNe peut se définir comme étant de l'ADN libre rejeté par les organismes dans le milieu (Taberlet et al., 2012). Ainsi, la collecte d'un échantillon de sol ou d'eau permet la détection et/ou la quantification d'une espèce cible dans son milieu grâce à l'amplification de son ADN (Rees et al., 2014). Grâce à cette méthode, il est alors possible de repérer la présence des espèces hôtes impliquées dans le cycle parasitaire mais aussi la présence du parasite. L'usage de l'ADNe semble donc particulièrement adapté pour répondre aux questions de synchronisme entre parasites et hôtes en milieu naturel (Hundermark, 2019).

Dans notre étude, nous avons utilisé cette approche par ADN environnemental afin d'étudier le synchronisme entre un parasite à cycle complexe et ses hôtes. Nous avons porté notre intérêt sur *Tetracapsuloides bryosalmonae*, un endoparasite myxozoaire responsable de la maladie rénale proliférative (PKD), une maladie émergente qui touche les populations de salmonidés sauvages et d'élevages (Okamura *et al.*, 2011). Initialement décrite en Allemagne en 1924 (Plehn, 1924), cette parasitose participe au déclin actuel des salmonidés en Europe et en Amérique du Nord (Sudhagar *et al.*, 2019). Au cours de son cycle, *T. bryosalmonae* alterne entre un hôte intermédiaire, un salmonidé, et un hôte définitif, un bryozoaire (Sudhagar *et al.*, 2019; Hartikainen & Okamura, 2015). Il a été démontré à plusieurs reprises que les différentes étapes de ce cycle sont sous le contrôle de la température (Gay *et al.*, 2001; Ros *et al.*, 2022; Tops *et al.*, 2006), notamment l'excrétion des spores par les bryozoaires et l'infection des poissons. L'activité même des bryozoaires, comme *Fredericella sultana*, pourrait être saisonnière et associée à la température (Raddum & Johnsen, 1983; Tops *et al.*, 2009).

La présente étude cherche à caractériser la dynamique temporelle de ce parasite et de ses hôtes dans plusieurs rivières pyrénéennes. L'objectif est de décrire les patrons temporels d'abondance des 3 espèces qui constituent l'interaction, à savoir : le parasite *Tetracapsuloides bryosalmonae*, le bryozoaire *F. sultana* et la truite commune *Salmo trutta fario*. L'étude a été menée sur une dizaine de

sites présentant un gradient de prévalence d'infection (Duval *et al.*, soumis). Ainsi, la première partie de ce projet s'intéresse à l'évolution de la concentration en ADN de chacune des espèces au cours du temps afin de déterminer s'il existe une variation saisonnière. Étant donné que les bryozoaires entrent en dormance durant l'hiver et que la prolifération de *T. bryosalmonae* dépend de leur activité (Tops *et al.*, 2009), nous nous attendons à ce que la concentration en ADN de bryozoaire et de parasite soit faible aux sessions d'hiver et plus forte au printemps et en été lorsque les températures remontent. À l'inverse, nous nous attendons à ce que la concentration en ADN de truite soit plus constante au cours du temps. Ensuite, nous avons testé l'existence d'une synchronie pour chaque espèce entre les différents sites d'échantillonnages. Nous avons également tenté d'identifier les facteurs environnementaux qui pouvaient influencer cette synchronie entre sites. Finalement, nous nous sommes intéressés à la présence de synchronie inter-spécifique temporelle entre les hôtes et le parasite. Ainsi nous supposions que les concentrations du parasite et ses hôtes seraient corrélées, ce qui pourrait augmenter la probabilité de rencontre entre le parasite et ses hôtes et donc favoriser le cycle parasitaire.

II. MATÉRIEL ET MÉTHODES

A. Modèles d'études

Afin de réaliser cette étude nous nous sommes concentrés sur l'interaction hôte-parasite impliquant le parasite agent de la PKD. Cette maladie est induite par le myxozoaire endoparasite Tetracapsuloides bryosalmonae phylogénétiquement décrit comme appartenant à la classe des Malacosporea (Canning et al., 1999; Canning et al., 2000). En Europe, Fredericella sultana et Plumatella emarginata sont les principaux bryozoaires pouvant devenir des hôtes pour le parasite (Okamura & Wood, 2002). F. sultana étant la plus répandue, l'étude que nous avons menée s'est focalisée seulement sur cette espèce de bryozoaire. Ces organismes se développent dans les cours d'eau en colonies de zooïdes ancrées dans le sol ou sur certains débris submergés. Si les conditions environnementales le permettent, les parasites présents dans les cellules des bryozoaires vont pouvoir se développer et former des sacs contenant jusqu'à 4000 spores (Okamura et al., 2011). Lorsque la température de l'eau atteint les 9°C, les spores sont excrétées par le bryozoaire et disséminées dans le milieu (Gay et al., 2001). Certains salmonidés, parmi lesquels la truite fario (Salmo trutta fario), dont il sera question dans cette étude, peuvent à leur tour être infectés. Cette infection se fait de manière aléatoire par insertion des spores libres dans les branchies ou le tégument du poisson. Ces spores rejoignent ensuite le système vasculaire puis le rein de l'animal (Feist et al., 2001). C'est généralement lorsque les températures environnantes dépassent les 15°C que la PKD se développe et

entraîne l'apparition de nombreux symptômes, parmi lesquels une hyperplasie rénale responsable d'une anémie chez les poissons les plus touchés (Ferguson & Ball, 1979; Bailey *et al.*, 2017; Bettge *et al.*, 2009; Okamura *et al.*, 2011). C'est à travers ces aggravations que la survie des poissons est mise en jeu : la maladie peut en effet entraîner jusqu'à 95% de mortalité dans les populations de truites d'élevage (Hedrick *et al.*, 1993). Certaines études localisées ont notamment montré que cette maladie était en partie responsable du déclin des populations sauvages aussi bien en Suisse qu'en Norvège (Burkhardt-Holm & Scheurer, 2007; Mo & Jørgensen, 2017). Pour finir, les spores de parasites sont excrétées par l'urine des poissons infectés. Cette excrétion de parasites peut persister 5 ans après l'infection (Soliman *et al.*, 2018). Elle va permettre aux parasites d'infecter de nouveau des bryozoaires.

B. Échantillonnage

La zone d'étude est située dans le piémont Pyrénéen dans les départements français d'Ariège, de Haute-Garonne et de Hautes-Pyrénées (Fig. 1). La présence de la maladie dans cette zone a été confirmée en 2016 par l'observation d'un fort taux de mortalité des juvéniles de truites par la Fédération Départementale de Pêche de l'Ariège (Lautraite, 2017).



Fig. 1. Carte de la zone étudiée faisant figurer les sites d'échantillonnages

Des prélèvements d'eau ont été réalisés sur un total de 11 sites (Table 1) et sur une période totale de 1 an allant de juin 2019 à juin 2020. Afin de tester l'existence d'une évolution des concentrations d'ADN des trois espèces (parasite, truite et bryozoaire) dans le temps, des prélèvements ont été effectués tous les 2 mois environ, chacun correspondant respectivement à une session : juin 2019, août 2019, septembre 2019, novembre 2019, février 2020, mai 2020, juin 2020. Pour chaque site et à chaque session, 6 prélèvements d'eau de 1L ont été réalisés à l'aide de sachets stériles Whirl-pak®. Une sonde multiparamètres In-Situ® mesurant les paramètres physico-chimiques de l'eau a également été utilisée lors de chaque échantillonnage afin de mesurer le pH, la conductivité, la concentration en oxygène dissous (RDO), la turbidité ainsi que la température de l'eau.

Table 1. Tableau récapitulatif de la position géographique des sites d'échantillonnages (degrés décimaux), de leur prévalence (% de truites parasitées) ainsi que de leur température moyenne sur la période d'étude.

Site	Rivière	Commune	Position latitudinale (°)	Position longitudinale (°)	Taux de prévalence parasitaire (%)	Température moyenne (°C)
ARBMan	L'Arbas	Mane	43.0725	0.9506	5	13.60
ARGFoi	L'Arget	Foix	42.9654	1.6030	0	11.21
ARIVar	L'Ariège	Varilhes	43.1064	1.2061	75	11.66
ARRMou	L'Arros	Moulédous	43.2299	0.2317	91	12.99
ARZDur	L'Arize	Durban-sur- Arize	43.0210	1.3461	60	12.43
GARVil	La Garonne	Villeneuve-de- Rivière	43.1057	0.6431	25	13.13
GERPoi	Le Ger	Pointis-Inard	43.0845	0.8138	0	13.93
HERCam	L'Hers	Camon	43.0086	1.9667	80	12.73
NESAne	La Neste	Anères	43.0664	0.4643	30	10.68
NESSar	La Neste	Sarrancolin	42.9635	0.3809	5	9.80
SALTau	Le Salat	Taurignan	43.0276	1.1124	25	10.80

C. Mesures de concentration ADN au sein des échantillons

1. Filtration et extraction ADN

La filtration de l'eau a été effectuée en laboratoire à l'aide d'un système de filtration Nalgène®. Chaque échantillon d'eau a été filtré à l'aide de filtres Sartorius® en nitrocellulose de diamètre 47 mm et d'une taille de pores de 0,45µm ou 0,8µm selon la turbidité de l'eau. Les 6 filtres (1 filtre par prélèvement de 1L) ont ensuite été regroupés par 2 pour constituer trois réplicats par session et par site. L'extraction d'ADN à partir des filtres a ensuite été réalisée à l'aide du kit DNeasy Power Soil Pro (Qiagen®) en suivant le protocole du fournisseur. Ces étapes ont été pratiquées par Éloïse Duval (Doctorante) et Charlotte Veyssière (IE, CDD) avant la période de stage.

2. Amplification et quantification ADN

L'ADN de chaque espèce (parasite, bryozoaire et truite fario) a ensuite été quantifié par ddPCR, soit digital droplet Polymerase Chain Reaction en anglais. La ddPCR est une méthode d'amplification récente et très précise (Baker, 2012; Quan *et al.*, 2018). Son utilisation sur des échantillons d'ADNe aquatiques a notamment montré une meilleure détection et quantification en ADN cible par rapport à la qPCR (Brys *et al.*, 2021, Mauvisseau *et al.*, 2019, Wood *et al.*, 2019).

Afin d'amplifier l'ADN de nos 3 espèces dans les échantillons d'eau nous avons utilisé des régions d'ADN propres à chaque espèce. Les régions cibles choisies pour le parasite et le bryozoaire sont celles développées par Carraro et al (2018) : respectivement l'ARN 16S mitochondrial et le gène COI mitochondrial. Concernant la truite fario, la zone amplifiée correspond à celle du cytochrome B mitochondrial (Carim *et al.*, 2016). Le mélange de réactifs pour la PCR a été effectué dans un volume total de 22µL composé de 2.2µL d'ADN extrait, 1.9µL d'amorce par espèce, 0.5µL de la sonde et 11µL de digital PCR Supermix (BioRad®). Cette préparation PCR est ensuite mélangée à une huile, puis une mise en émulsion permet de diviser la préparation en environ 20 000 gouttes à l'aide du QX200 Droplet Generator (BioRad®). Au sein de chacune de ces gouttes, une PCR a lieu de façon indépendante.

L'étape de dénaturation initiale de l'ADN s'est faite en chauffant les mélanges de réactifs à 95°C pendant 10 minutes. Ensuite, 40 cycles composés de 30s à 94°C et 1 minute à 60°C ont permis la dénaturation, l'hybridation des amorces puis l'élongation. Pour finir l'amplification, les mélanges ont été placés à 98°C pendant 10 minutes puis 30 minutes à 4°C. Ensuite, le QX200 Droplet Reader (BioRad®) a permis d'estimer la concentration absolue de chacun de nos échantillons.

Chaque plaque de 96 puits incluait 3 témoins négatifs ainsi qu'un témoin positif. La lecture des résultats de l'amplification s'est faite grâce au logiciel Quanta soft (Quanta Soft Software 1.7,

Regulatory Edition, BioRad®). Si de l'ADN cible est présent dans la goutte, l'amplification a lieu et la sonde permet la fluorescence. Le seuil de séparation des gouttes négatives et positives a été admis de façon arbitraire pour chaque plaque de 96 puits selon la distribution des gouttes des contrôles positifs et négatifs. Enfin, le ratio des gouttes positives sur le nombre total de gouttes formées a permis de quantifier la concentration d'ADN dans chaque échantillon en nombre de copies/µl.

D. Analyses statistiques

Toutes les analyses statistiques ont été réalisées avec R (R Core Team, RStudio 2021).

Tout d'abord, nous voulions tester l'existence d'une variation saisonnière (différence entre les sessions) pour chacune des trois espèces. Les données correspondant aux concentrations en ADN étant dépendantes dans le temps et dans l'espace, un modèle linéaire généralisé mixte (GLMM) a été réalisé pour chaque espèce grâce au package *glmmTMB* (Magnusson *et al.*, 2017; Bolker, 2016). Chaque modèle a été construit en considérant la session d'échantillonnage comme effet fixe et le site de prélèvement comme effet aléatoire selon le modèle suivant : concentration espèce cible ~ session + (1 | site). Le parasite et son hôte bryozoaire étant répartis de façon sporadique dans les rivières, la distribution de leurs concentrations est caractérisée par un excès de zéros. Ainsi, nos modèles ont été construits selon une distribution Binomiale négative de type I qui est adaptée aux données surdispersées. Une analyse de variance (ANOVA) de type II a été réalisée sur chacun d'eux.

Le second objectif était d'étudier s'il existait de la synchronie temporelle entre les différents sites pour chacune des trois espèces. Nous avons donc voulu tester si les concentrations en ADN de chaque espèce étaient corrélées entre les différents sites par des tests de corrélations de Spearman. Ces tests ont été réalisés par paires de sites permettant ainsi d'obtenir une matrice de coefficients de corrélation pour chaque espèce (le parasite, le bryozoaire et la truite). Les coefficients de corrélation obtenus ont été interprétés comme une mesure de synchronie entre sites. Ensuite, afin de mettre en évidence les facteurs qui pouvaient influencer la force de cette synchronie entre sites, nous avons construit des modèles de Régression Multiple sur des Matrices de distance (MRM) pour chaque espèce. La fonction MRM est disponible au sein du package *ecodist* (Goslee *et al.*, 2020) et permet d'implanter des matrices de distances entre paires de sites comme variables explicatives dans un modèle de régression. Dans notre cas, nous avons choisi de prendre comme variables explicatives : une matrice des différences de températures moyennes sur l'année, une matrice des différences environnementales moyennes (pH, conductivité, turbidité, concentration en oxygène dissous) et une matrice des distances entre coordonnées géographiques (Latitude et Longitude) qui permettent une mesure de similitude de climat. Nous avons préféré séparer la température des données

environnementales car nous nous attendions à ce que ce paramètre ait une influence importante dans la distribution de nos espèces. Ces MRM ont été réalisés sur la base de 1000 permutations.

Enfin, nous avons testé la synchronie temporelle entre espèces. Au sein de chaque site, des tests de corrélations de Pearson ont donc été réalisés. Encore une fois, les coefficients de corrélation ont été interprétés comme des mesures de synchronie entre chaque espèce. Seulement les combinaisons impliquant le parasite et les hôtes ont été testées, c'est-à-dire : parasite/bryozoaire, parasite/truite. Le calcul de l'intervalle de confiance autour de la valeur moyenne a permis de conclure sur la significativité de cette synchronie.

III. RÉSULTATS

A. Variation saisonnière de la concentration

Les modèles ont montré que quelle que soit l'espèce, c'est-à-dire *T. bryosalmonae, F. sultana et S. trutta*, la concentration en ADN variait significativement entre les sessions (respectivement $\chi^2 = 17.076$, df = 6, P < 0.01 ; $\chi^2 = 13.274$, df = 6, P < 0.05 ; $\chi^2 = 23.241$, df = 6, P < 0.001). Cependant, les valeurs prédites par les modèles ne semblent pas présenter les mêmes variations pour toutes les espèces (Fig. 2). Concernant les concentrations en ADN de parasite, on observe un pic au mois d'août 2019 (Fig. 2a). Les variations saisonnières de bryozoaires semblent avoir la même allure que celles du parasite avec des pics de concentration au mois d'août et septembre 2019 (Fig. 2b). En ce qui concerne la truite, un pic de concentration apparaît aux mois de septembre et novembre (Fig. 2c).



Fig. 2. Graphique représentant la prédiction du modèle pour la concentration en ADN (en nombre de copies/L) de (a) *Tetracapsuloides bryosalmonae*, (b) *Fredericella sultana* et (c) de *Salmo trutta* aux différentes dates de sessions d'échantillonnage. Le point représente la valeur moyenne et les barres d'erreurs correspondent à l'erreur standard (intervalle de confiance à 70%).

B. Synchronisme entre sites pour chaque espèce

La concentration moyenne en ADN des 3 espèces étudiées est corrélée positivement entre les sites (Fig. 3). Le bryozoaire et le parasite présentent une synchronie entre sites significative mais très faible (respectivement moyenne \pm IC95%; 0.24 \pm 0.10; 0.20 \pm 0.10). Néanmoins, la distribution des corrélations pour le parasite illustre à la fois une synchronie et asynchronie forte selon les sites comparés (Fig. 3a). L'étendue des mesures de synchronisme montre une variance forte entre les différents sites. Concernant la truite, les valeurs de corrélations entre sites sont significativement élevées (moyenne \pm IC95%, 0.43 \pm 0.08). Les abondances de truites des différents sites sont donc significativement synchronisées entre elles.



Fig. 3. Courbes de distribution des mesures de synchronie entre paire de sites pour (a) le parasite, (b) le bryozoaire et (c) la truite. La valeur de corrélation moyenne figure en ligne pleine et l'intervalle de confiance à 95% en pointillé.

Lors de l'identification des facteurs qui pouvaient influencer la variance de synchronisme entre site par MRM, seule la synchronie parasitaire a montré une réponse dépendante des facteurs étudiés. La distance géographique entre sites influence significativement le synchronisme entre les sites pour le parasite ($R^2 = -1.24$, P < 0.01). En effet, on observe que plus les sites d'échantillonnages sont proches géographiquement, plus l'évolution des concentrations en ADN de parasite ont tendance à être synchrones dans le temps (Fig. 4). En revanche, aucun des facteurs étudiés (température, variations environnementales, distance géographique) n'a montré une influence significative sur la synchronie des truites ou des bryozoaires.



Fig. 4. Mesure de la synchronie entre les sites (représentée par la corrélation entre les concentrations trouvées pour les différentes sessions d'échantillonnage) en fonction de leur distance géographique. La droite de régression figure en rouge (y = 0.41-0.49x)

C. Synchronisme entre les hôtes et le parasite

Les tests de corrélation ont montré l'existence d'un synchronisme significatif entre espèces au sein des sites. La mesure de synchronie moyenne entre la concentration en ADN de parasite et de bryozoaire est modérée et très variable selon les sites étudiés (moyenne \pm IC95%, 0.31 \pm 0.265). Comme précédemment, certains sites sont très synchrones et d'autres non, s'illustrant par deux pics, l'un supérieur à 0, l'autre inférieur (Fig. 5a). La synchronie entre le parasite et la truite est aussi modérée (moyenne \pm IC95%, 0.37 \pm 0.235) mais moins variable (Fig. 5b).



Fig. 5. Courbes de distribution des mesures de synchronie indépendamment au sein des sites entre (a) le parasite et le bryozoaire, (b) le parasite et la truite. La valeur de corrélation moyenne figure en ligne pleine et l'intervalle de confiance à 95% en pointillé.

IV. DISCUSSION

L'étude que nous avons menée avait d'abord pour objectif de décrire les patrons de variations temporelles de la concentration en ADN de *T. bryosalmonae* et de ses hôtes en milieu naturel. Les méthodes utilisées, notamment le couplage ADNe et ddPCR, se sont auparavant révélées être des bons estimateurs de l'abondance (Doi *et al.*, 2015; Carraro *et al.*, 2018). Nos résultats suggèrent qu'il existe des variations saisonnières dans l'abondance de chaque espèce et qu'elles peuvent différer en fonction de l'espèce étudiée. Comme nous l'attendions, nous avons mis en évidence un pic d'abondance de parasite et de bryozoaire en été. Cette forte concentration en ADN de bryozoaire dans l'eau en été est en accord avec de précédentes études (Tops *et al.*, 2009). En effet, ces organismes se multiplient de manière importante lorsque les températures de l'eau augmentent, c'est-à-dire plutôt en été ou au printemps. Le pic d'abondance parasitaire à la session du mois d'août pourrait correspondre à la période de libération des spores parasitaires par les bryozoaire et de parasite au cours de l'année semblait très similaire, nous permettant d'avoir un premier aperçu du possible synchronisme entre ces espèces (Fig. 2). Cependant, ces espèces étant très petites et se dispersant facilement dans

l'eau, il semble qu'en dehors des périodes de multiplication, elles ne laissent que très peu de traces ADN dans le milieu. Les concentrations aux sessions qui n'arborent pas de pic sont donc presque nulles voire égales à 0. Nos résultats montrent par ailleurs l'existence de pics de concentration aux sessions de septembre et novembre pour la truite fario (Fig. 2c) et semblent cohérent avec les études s'intéressant à son cycle biologique et notamment à la période de fraie (Baglinière *et al.*, 1979; Kocabas & Aksu, 2011). En effet, la reproduction des truites a lieu en hiver et commence habituellement au mois de novembre. L'excrétion massive de gamètes dans le milieu à cette période permet donc la présence de beaucoup de matériel génétique et favorise leur détection par ADN environnemental. L'étude de l'évolution des concentrations en ADN dans le temps a donc montré l'existence de patrons temporels similaires entre le parasite et le bryozoaire puis une variation de celle de la truite en accord avec la littérature.

Ensuite, les analyses visant à déceler le synchronisme entre les sites se sont avérées être significatives pour toutes les espèces étudiées. En ce qui concerne la truite fario, le synchronisme entre sites était fort et moins variable que chez les autres espèces (Fig. 3c). En comparaison, l'évolution des abondances de bryozoaire était assez faiblement synchrone entre sites (Fig. 3b). Pour la truite et le bryozoaire, nos résultats n'ont pas mis en évidence de paramètres expliquant le synchronisme entre sites. En effet, que ce soit la température, la distance géographique ou les paramètres environnementaux, aucun n'a eu d'effet sur la force du synchronisme spatial entre sites. Cependant, d'après les études au sujet du cycle de vie du bryozoaire, nous nous attendions à trouver un effet significatif de la température sur son abondance (Tops et al., 2006). Étant donnés ces attendus, il est possible qu'une mesure ponctuelle de température tous les 2 mois n'ait pas été suffisante pour mettre en évidence un effet significatif. La mesure des températures de l'eau de façon continue aurait pu s'avérer plus précise dans cette analyse. Ensuite, il semblerait que le synchronisme entre truites des différents sites ne soit pas non plus influencé par les paramètres étudiés. En accord avec nos résultats, plusieurs études s'intéressant à la dynamique des truites fario et à leur synchronie spatiale n'ont pas montré d'effet de l'environnement ni de la température au stade de vie adulte (Bret et al., 2016; Cattanéo et al., 2003). Selon ces recherches, seul le degré de synchronisation des truites juvéniles était influencé soit par la synchronie environnementale, soit par la synchronie hydrologique, en d'autres termes le débit, entre sites. Il est donc possible de supposer que la dynamique de cette espèce est régie par des phénomènes internes propres à son cycle de vie particulier.

Concernant le synchronisme du parasite entre sites, il était modéré et variait fortement selon les paires de sites considérées. En effet, certains sites étaient très synchrones et d'autres étaient au contraire complètement asynchrones. Cette forte hétérogénéité entre les différents sites échantillonnés s'expliquait par leur distance géographique. Nous avons ainsi montré que plus les sites d'échantillonnage étaient proches géographiquement, plus l'évolution de l'abondance du parasite

était synchrone. Ce synchronisme observé entre les sites proches géographiquement pourrait être dû à de la dispersion (Ranta et al., 1998; Cattadori et al., 2000). Les truites juvéniles présentent des capacités de dispersion (Solomon et al., 1976) qui pourrait mener à la dispersion des spores de T. bryosalmonae entre des sites proches lorsqu'ils sont excrétés dans l'environnement. De plus, des études antérieures qui cherchaient à tester les effets du climat sur les populations animales ont montré que la position géographique pouvait être un bon proxy du climat (Rieman et al., 2007). Des sites plus proches géographiquement ont tendance à avoir des climats plus similaires que des sites très éloignés. Notre mesure de distance géographique pourrait donc s'apparenter à une mesure de différence climatique. Ainsi, la synchronie entre sites pourrait également être régie par d'autres facteurs climatiques que ceux étudiés ici. La mesure de ces paramètres pourrait conduire à la détection d'un effet Moran. Moran a en effet démontré que le synchronisme temporel de 2 populations de lynx était égal à la corrélation calculée entre les facteurs climatiques des 2 habitats (Moran, 1953). En accord avec cette hypothèse, une étude plus récente a montré l'existence de l'effet Moran entre 4 populations de poissons dans l'Ouest de l'Afrique (Tedesco et al., 2004). Il est donc possible, qu'une caractérisation plus précise du climat prenant par exemple en compte la pluviométrie, le pourcentage d'ensoleillement ou la température extérieure puisse mettre en évidence un effet significatif du climat sur cette synchronie parasitaire entre les sites.

Finalement, nous voulions tester le synchronisme temporel du parasite et de ses hôtes. Comme nous l'attendions, nos résultats ont montré que l'évolution de l'abondance de T. bryosalmonae était synchronisée avec celle de ses 2 hôtes. Ce synchronisme pourrait faciliter la rencontre du parasite et de ses hôtes et ainsi maximiser le succès du cycle parasitaire. Il existerait donc une stratégie parasitaire afin de favoriser la rencontre et assurer la transmission et la dispersion de ce parasite. Cependant nous avons observé une forte variabilité entre les sites étudiés (Fig. 5), ce qui mériterait d'être plus précisément étudié. Le changement climatique en cours pourrait avoir un effet important sur la dynamique des interactions biotiques, en particulier les interactions hôtes-pathogènes, présentes dans les milieux aquatiques (Marcogliese, 2008). Par exemple, il a été démontré que la synchronie phénologique, c'est-à-dire du développement saisonnier des organismes, entre les Amphibiens et le trématode parasite Ribeiroia ondatrae était induite en partie par les changements climatiques à l'Ouest des États-Unis (McDevitt-Galles et al., 2020). Ces derniers ont montré que la prévalence parasitaire ainsi que les symptômes de la parasitose augmentaient de manière accrue avec la synchronie. En considérant le fait que Tetracapsuloides bryosalmonae est dépendant de la température pour accomplir son cycle (Tops et al., 2006), il serait possible que le changement climatique ait un impact d'autant plus fort sur les populations de truites sauvages et d'élevages. Cette hypothèse se révèle être cohérente avec la littérature. Certains articles ont notamment montré que l'augmentation des températures avait un effet direct sur les truites fario et leur tolérance thermique

et que la PKD s'avère être un coût supplémentaire pour ces populations déjà menacées (Bruneaux *et al.*, 2017).

Globalement, la synchronie que nous avons observée entre les espèces du système T. bryosalmonae/bryozoaire/truite fario reste faible, mais les patrons d'évolution de l'abondance semblent en tous points très similaires entre le bryozoaire et le parasite. L'approche par ADN environnemental s'est avérée être une approche pratique et efficace même si la complexité de détection du parasite subsiste. En effet l'ADN étant très dilué dans le milieu, la quantification précise de l'espèce quand elle est présente en très faible quantité n'est pas certaine. Malgré l'efficacité qu'a montré cette méthode à plusieurs reprises avec les parasites aquatiques (Sieber et al., 2020; Sengupta et al., 2019), il semble que certaines espèces, notamment les invertébrés, aient plus de mal à être détectées (Roussel et al., 2015). D'après cette analyse et celle de Troth et al (2021), ces effets sont accentués dans les études menées sur les cours d'eau, le transport et la forte dilution des échantillons étant responsable d'une plus forte hétérogénéité des résultats. En accord avec ces travaux, nos résultats montrent que quand le parasite et le bryozoaire ne sont pas dans leur période de développement respective, c'est-à-dire les sessions de novembre et février, il est très difficile de les détecter (Fig. 2). Afin de maximiser les chances de détection de T. bryosalmonae, il faudrait tester plusieurs protocoles d'échantillonnages et d'extraction ADN avant de débuter l'étude. En effet, il n'existe pas de méthode universelle utilisant l'ADNe. Outre la méthode d'amplification utilisée, le volume à prélever et la méthode de filtration font partie des paramètres pouvant influencer la précision de détection. Bien qu'une étude menée sur les salmonidés ait recommandé les mêmes conditions et méthodes que nous avons utilisés, à savoir des prélèvements de 1L et une filtration de 0.45µm (Capo et al. 2019), il se pourrait que le temps entre notre échantillonnage et l'extraction ADN ait suffi à la dégradation d'une partie de l'ADN.

En conclusion, cette étude révèle de nouvelles connaissances quant à l'étude de la dynamique du parasite émergent *Tetracapsuloides bryosalmonae*. Nous avons pu mettre en avant des périodes propices au développement de la PKD qui sont, d'après nos résultats, les mois d'août et septembre, ainsi qu'un synchronisme entre sites et entre espèces. Cependant, des prélèvements plus fréquents autour de ces pics de concentrations permettraient d'affiner les résultats et de définir avec précision quand ont lieu ces pics d'abondance. Il serait très intéressant de réaliser des mesures hebdomadaires pour avoir une idée plus juste du synchronisme entre espèces et en apprendre davantage sur le fonctionnement de cette interaction. Par ailleurs, cette étude offre de nouvelles perspectives d'études concernant le synchronisme dans les interactions hôtes parasites, à travers l'utilisation de méthodes précises et non invasives.

RÉFÉRENCES BIBLIOGRAPHIQUES

Baglinière, J. L., Champigneulle, A., & Nihouarn, A. (1979). La fraie du saumon atlantique (Salmo salar L.) et de la truite commune (Salmo trutta L.) sur le bassin du Scorff. *Cybium*, 7, 75-96.

Bailey, C., Segner, H., Casanova-Nakayama, A., & Wahli, T. (2017). Who needs the hotspot? The effect of temperature on the fish host immune response to Tetracapsuloides bryosalmonae the causative agent of proliferative kidney disease. *Fish & Shellfish Immunology*, 63, 424-437.

Baker, M. (2012). Digital PCR hits its stride. *Nature Methods*, *9*(6), 541-544.

Beja-Pereira, A., Oliveira, R., Alves, P. C., Schwartz,
M. K., & Luikart, G. (2009). Advancing ecological understandings through technological transformations in noninvasive genetics. *Molecular Ecology Resources*, 9(5), 1279-1301.

Bettge, K., Wahli, T., Segner, H., & Schmidt-Posthaus, H. (2009). Proliferative kidney disease in rainbow trout : Time- and temperature-related renal pathology and parasite distribution. *Diseases of Aquatic Organisms*, 83(1), 67-76.

Bolker, B. (2016). *Getting started with the glmmTMB package*. 8.

- Bret, V., Bergerot, B., Capra, H., Gouraud, V., & Lamouroux, N. (2016). Influence of discharge, hydraulics, water temperature, and dispersal on density synchrony in brown trout populations (Salmo trutta). *Canadian Journal of Fisheries and Aquatic Sciences*, 73(3), 319-329.
- Bruneaux, M., Visse, M., Gross, R., Pukk, L., Saks, L., & Vasemägi, A. (2017). Parasite infection and decreased thermal tolerance: impact of proliferative kidney disease on a wild salmonid fish in the context of climate change. *Functional Ecology*, 31(1), 216-226.

Brys, R., Halfmaerten, D., Neyrinck, S., Mauvisseau, Q., Auwerx, J., Sweet, M., & Mergeay, J. (2021). Reliable eDNA detection and quantification of the European weather loach (Misgurnus fossilis). *Journal of Fish Biology*, *98*(2), 399-414.

Burkhardt-Holm, P., & Scheurer, K. (2007). Application of the weight-of-evidence approach to assess the decline of brown trout (Salmo trutta) in Swiss rivers. *Aquatic Sciences*, 69(1), 51-70.

Canning, E.U., Curry, A., Feist S.W., Longshaw, M. & Okamura, B. (1999) Tetracapsula bryosalmonae n. sp. for PKX organism, the cause of PKD in salmonid fish. Bulletin of the European Association of Fish Pathologists 19, 203–206. Canning, E. U., Curry, A., Feist, S. W., Longshaw, M., & Okamura, B. (2000). A New Class and Order of Myxozoans to Accommodate Parasites of Bryozoans with Ultrastructural Observations on Tetracapsula bryosalmonae (PKX Organism). *The Journal of Eukaryotic Microbiology*, 47(5), 456-468.

Capo, E., Spong, G., Konigsson, H., & Bystrom, P. (2019). Effects of filtration methods and water volume on the quantification of brown trout (*Salmo trutta*) and Arctic char (*Salvelinus alpinus*) eDNA concentrations via droplet digital PCR. *Environmental DNA*, 2, 152-160.

Carim, K. J., Wilcox, T. M., Anderson, M., Lawrence,
D. J., Young, M. K., McKelvey, K. S., & Schwartz,
M. K. (2016). An environmental DNA marker for detecting nonnative brown trout (Salmo trutta). *Conservation Genetics Resources*, 8(3), 259-261.

Carraro, L., Hartikainen, H., Jokela, J., Bertuzzo, E., & Rinaldo, A. (2018). Estimating species distribution and abundance in river networks using environmental DNA. *Proceedings of the National Academy of Sciences*, 115(46), 11724-11729.

Cattadori, I. M., Merler, S., & Hudson, P. J. (2000). Searching for mechanisms of synchrony in spatially structured gamebird populations. *Journal of Animal Ecology*, 69(4), 620-638.

Cattanéo, F., Hugueny, B., & Lamouroux, N. (2003). Synchrony in brown trout, Salmo trutta, population dynamics: A 'Moran effect'on early-life stages. *Oikos*, 100(1), 43-54.

Combes, C. (2001). Parasitism: The ecology and evolution of intimate interactions. *University of Chicago Press*.

Combes, C., & Théron, A. (1977). Rythmes d'émergence des cercaires de Trématodes et leur intérêt dans l'infestation de l'homme et des animaux. *Inst Biol Publ*, *4*, 141-150.

da Silva, R. C., & Langoni, H. (2009). Toxoplasma gondii: Host-parasite interaction and behavior manipulation. *Parasitology Research*, *105*(4), 893-898.

Doi, H., Uchii, K., Takahara, T., Matsuhashi, S., Yamanaka, H., & Minamoto, T. (2015). Use of droplet digital PCR for estimation of fish abundance and biomass in environmental DNA surveys. *PloS* one, 10(3), e0122763.

Duval, E., Blanchet, S., Quéméré, E., Jacquin, L., Veyssière, C., Lautraite, A., Garmendia, L., Yotte, A., Parthuisot, N., Côte, J., & Loot, G. (2021). Urine DNA (uDNA) as a non-lethal method for endoparasite biomonitoring: Development and validation. *Environmental DNA*, *3*(5), 1035-1045.

Duval E., Quéméré E., Loot G., Jacquin L., Veyssière C. & Blanchet S. A multifaceted index of population health to detect risk-prone populations and underlying stressors in wildlife.

Feist, S. W., Longshaw, M., Canning, E. U., & Okamura, B. (2001). Induction of proliferative kidney disease (PKD) in rainbow trout Oncorhynchus mykiss via the bryozoan Fredericella sultana infected with Tetracapsula bryosalmonae. *Diseases of Aquatic Organisms*, *45*(1), 61-68.

Ferguson, H. W., & Ball, H. J. (1979). Epidemiological aspects of proliferative kidney disease amongst rainbow trout Salmo gairdneri Richardson in Northern Ireland. *Journal of Fish Diseases*, 2(3), 219-225.

Gay, M., Okamura, B., & Kinkelin, P. de. (2001). Evidence that infectious stages of Tetracapsula bryosalmonae for rainbow trout Oncorhynchus mykiss are present throughout the year. *Diseases of Aquatic Organisms*, 46(1), 31-40.

Goslee, S., Urban, D., & Goslee, M. S. (2020). Package 'ecodist'.

Hartikainen, H., & Okamura, B. (2015). Ecology and Evolution of Malacosporean-Bryozoan Interactions. In B. Okamura, A. Gruhl, & J. L. Bartholomew (Éds.), *Myxozoan Evolution, Ecology and Development* (p. 201-216).

Hedrick, R. P., MacConnell, E., & de Kinkelin, P. (1993). Proliferative kidney disease of salmonid fish. *Annual Review of Fish Diseases*, *3*, 277-290.

Hughes, D. P., Brodeur, J., & Thomas, F. (2012). Host manipulation by parasites. *Oxford University Press.*

Hundermark, E. (2019). An Environmental DNA based Analysis of the Life History of Eastern Hellbender Populations in the Susquehanna River Basin. Master's Theses. 217.

Jousseaume, T., Roussel, J. M., Beaulaton, L., Bardonnet, A., Faliex, E., Amilhat, E., ... & Launey, S. (2021). Molecular detection of the swim bladder parasite Anguillicola crassus (Nematoda) in fecal samples of the endangered European eel Anguilla anguilla. *Parasitology Research*, 120(5), 1897-1902.

Kocabas, M., & Aksu, O. (2011). The Reproduction Traits of Native Brown Trout. *Journal of Animal and Veterinary Advances*, 10(13), 1632-1637.

Krull, W. H. (1935). Studies on the life history of Halipegus occidualis Stafford, 1905. *American Midland Naturalist*, 129-143.

Lautraite, A. (2017). Rapport d'étude de la tétracapsuloïdose (« PKD ») infectant les truites fario

dans le réseau hydrographique de la région d'Ax-Les-Thermes (Ariège, Oriège, Lauze).

Loot, G., Park, Y.-S., Lek, S., & Brosse, S. (2006). Encounter rate between local populations shapes host selection in complex parasite life cycle. *Biological Journal of the Linnean Society of London*, *89*(1), 99-106.

Magnusson, A., Skaug, H., Nielsen, A., Berg, C., Kristensen, K., Maechler, M., & Brooks, M. M. (2017). *Package 'glmmtmb'*.

Marcogliese, D. J. (2008). The impact of climate change on the parasites and infectious diseases of aquatic animals. *Rev Sci Tech*, *27*(2), 467-484.

Mathieu-Bégné, E., Blanchet, S., Rey, O., Scelsi, O., Poesy, C., Marselli, G., & Loot, G. (2021). A finescale analysis reveals microgeographic hotspots maximizing infection rate between a parasite and its fish host. *Functional Ecology*. 36: 380-391.

Mauvisseau, Q., Davy-Bowker, J., Bulling, M., Brys, R., Neyrinck, S., Troth, C., & Sweet, M. (2019). Combining ddPCR and environmental DNA to improve detection capabilities of a critically endangered freshwater invertebrate. *Scientific Reports*, 9(1), 14064.

McAllister, C. T., Bursey, C. R., & Connior, M. B. (2016). New Host and Distributional Records for Helminth Parasites (Trematoda, Cestoda, Nematoda) from Amphibians (Caudata, Anura) and Reptiles (Testudines : Ophidia) of Oklahoma. *Proceedings of the Oklahoma Academy of Science*, 96.

McDevitt-Galles, T., Moss, W. E., Calhoun, D. M., & Johnson, P. T. (2020). Phenological synchrony shapes pathology in host–parasite systems. *Proceedings of the Royal Society B*, 287(1919), 20192597.

Mo, T. A., & Jørgensen, A. (2017). A survey of the distribution of the PKD-parasite Tetracapsuloides bryosalmonae (Cnidaria : Myxozoa: Malacosporea) in salmonids in Norwegian rivers – additional information gleaned from formerly collected fish. *Journal of Fish Diseases*, 40(5), 621-627.

Moran, P. A. (1953). The statistical analysis of the Canadian lynx cycle. *Australian Journal of Zoology*, 1(3), 291-298.

Okamura, B., Hartikainen, H., Schmidt-Posthaus, H., & Wahli, T. (2011). Life cycle complexity, environmental change and the emerging status of salmonid proliferative kidney disease : PKD as an emerging disease of salmonid fish. *Freshwater Biology*, *56*(4), 735-753.

Okamura, B., & Wood, T. S. (2002). Bryozoans as hosts for Tetracapsula bryosalmonae, the PKX organism. *Journal of Fish Diseases*, *25*(8), 469-475.

Plehn, M. (1924). Praktikum der Fischkrankheiten; Schweizerbart: Stuttgart, Germany.

Quan, P.-L., Sauzade, M., & Brouzes, E. (2018). dPCR : A Technology Review. *Sensors*, *18*(4), 1271. Raddum, G. G., & Johnsen, T. M. (1983). Growth and feeding of Fredericella sultana (bryozoa) in the outlet of a humic acid lake. *Hydrobiologia*, *101*(1-2).

Ranta, E., Kaitala, V., & Lundberg, P. (1998). Population variability in space and time: the dynamics of synchronous population fluctuations. *Oikos*, 376-382.

Rees, H. C., Maddison, B. C., Middleditch, D. J., Patmore, J. R., & Gough, K. C. (2014). The detection of aquatic animal species using environmental DNA– a review of eDNA as a survey tool in ecology. *Journal of applied ecology*, 51(5), 1450-1459.

Rieman, B. E., Isaak, D., Adams, S., Horan, D., Nagel, D., Luce, C., & Myers, D. (2007). Anticipated climate warming effects on bull trout habitats and populations across the interior Columbia River basin. *Transactions of the American Fisheries Society*, 136(6), 1552-1565.

Ros, A., Schmidt-Posthaus, H., & Brinker, A. (2022). Mitigating human impacts including climate change on proliferative kidney disease in salmonids of running waters. *Journal of Fish Diseases*, 45(4), 497-521.

Roussel, J. M., Paillisson, J. M., Treguier, A., & Petit, E. (2015). The downside of eDNA as a survey tool in water bodies. *Journal of Applied Ecology*, 823-826.

Salman, H., Lahlah, M., & Shaaban, Q. (2018). Study of Endo-Parasitic Nematoda in Sargocentron Rubrum Fish at the Syrian Coastal Water. *International Journal of Agriculture & Environmental Science* (SSRG–IJAES), 5, 96-100.

Sengupta, M. E., Hellström, M., Kariuki, H. C., Olsen, A., Thomsen, P. F., Mejer, H., ... & Vennervald, B. J. (2019). Environmental DNA for improved detection and environmental surveillance of schistosomiasis. *Proceedings of the National Academy of Sciences*, *116*(18), 8931-8940.

Sieber, N., Hartikainen, H., & Vorburger, C. (2020). Validation of an eDNA-based method for the detection of wildlife pathogens in water. *Diseases of Aquatic Organisms*, *141*, 171-184.

Soliman, H., Kumar, G., & El-Matbouli, M. (2018). Tetracapsuloides bryosalmonae persists in brown trout Salmo trutta for five years post exposure. *Diseases of Aquatic Organisms*, *127*(2), 151-156. Solomon, D. J., & Templeton, R. G. (1976). Movements of brown trout Salmo trutta L. in a chalk stream. *Journal of Fish Biology*, 9(5), 411-423.

Sudhagar, A., Kumar, G., & El-Matbouli, M. (2019). The malacosporean myxozoan parasite Tetracapsuloides bryosalmonae: A threat to wild salmonids. *Pathogens*, 9(1), 16.

Taberlet, P., Coissac, E., Hajibabaei, M., & Rieseberg, L. H. (2012). Environmental dna. *Molecular ecology*, *21*(8), 1789-1793.

Tedesco, P. A., Hugueny, B., Paugy, D., & Fermon, Y. (2004). Spatial synchrony in population dynamics of West African fishes: a demonstration of an intraspecific and interspecific Moran effect. *Journal of Animal Ecology*, *73*(4), 693-705.

Théron, A., & Combes, C. (1988). Genetic analysis of cercarial emergence rhythms of Schistosoma mansoni. *Behavior genetics*, *18*(2), 201-209.

Tops, S., Hartikainen, H.-L., & Okamura, B. (2009). The effects of infection by Tetracapsuloides bryosalmonae (Myxozoa) and temperature on Fredericella sultana (Bryozoa). *International Journal for Parasitology*, *39*(9), 1003-1010.

Tops, S., Lockwood, W., & Okamura, B. (2006). Temperature-driven proliferation of Tetracapsuloides bryosalmonae in bryozoan hosts portends salmonid declines. *Diseases of Aquatic Organisms*, 70(3), 227-236.

Troth, C. R., Sweet, M. J., Nightingale, J., & Burian, A. (2021). Seasonality, DNA degradation and spatial heterogeneity as drivers of eDNA detection dynamics. *Science of the Total Environment*, 768, 144466.

Wood, S. A., Pochon, X., Laroche, O., Ammon, U., Adamson, J., & Zaiko, A. (2019). A comparison of droplet digital polymerase chain reaction (PCR), quantitative PCR and metabarcoding for speciesspecific detection in environmental DNA. *Molecular Ecology Resources*, *19*(6), 1407-1419.

Xiao, L., Ryan, U. M., Graczyk, T. K., Limor, J., Li, L., Kombert, M., & Lal, A. A. (2004). Genetic diversity of Cryptosporidium spp. in captive reptiles. *Applied and Environmental Microbiology*, 70(2), 891-899.

Yusni, E., Batubara, A., & Frantika, C. (2022). Detection of endoparasites in mackerel tuna (Euthynnus affinis) in north Sumatra province, Indonesia. *Iraqi Journal of Veterinary Sciences*, *36*(2), 519-524.

Appendix 4.



A4. The evolution of the filtration devices used to sample eDNA and uDNA during the PhD project. (a) Peristaltic pump used for uDNA in Chapter 1. (b) Vacuum filtration used for the eDNA temporal sampling in Appendix 3. (c) Vampire sampler used for uDNA in Chapters 3 and 4, and for eDNA detection in the river in Chapter 2.

Appendix 5.

Extra analysis on the onset of *Tetracapsuloides bryosalmonae* spore shedding by wild brown trout

The onset of parasite spore release by fish hosts may inform on the timing of the host-parasiteenvironment interaction. Strepparava et al. (2018, 2020) found experimentally that the onset of spore release occurred at 45 days post exposure to infective spores (dpe) for brown trout held at 15°C, while at 80dpe for brown trout held at 12°C. In the wild, parasite exposure starts when bryozoans release infective spores, i.e. when water temperature reaches 9°C (Gay et al., 2001). According to the water temperature records, all the sites sampled in the before/after study (Chapter 3) had a daily average temperature exceeding 9°C in April, so that parasite exposure may have started at the latest in April, or earlier in March at the warmest sites. The temperature of 12°C was reached in a stable way (with the daily average temperature > 12° C for most days) in May at all sites (i.e., at about 60 days before the first sampling). Therefore, at the first sampling of the before/after study, in late June (potentially more than 80 dpe), I would have expected to detect DNA in fish excretion at least at the moderately-highly infected sites (i.e. sites with infection prevalence > 50%). During this sampling, 10 fish individuals were tested for T. bryosalmonae's infection at most sites. This revealed that some individuals were indeed already shedding parasite spores (or at least DNA) in 6 out of 8 infected sites, with 10 to 80% of the individuals shedding. A brand-new study on wild brown trout populations estimated more precisely that parasite detection through histological examination of the kidney could occur after 1500 degree-days from the 1st of March (approximate date of brown trout hatching in Switzerland), or after cumulating 30 days with a mean temperature > 15°C (Rubin et al., 2022). Analysing my temperature records, I found that I could detect fish infection after 1413-1619 degree-days from the 1st of March (mean 1550), and with between 12-33 days (mean 19) with a mean temperature > $15^{\circ}C$ (A5a). This suggests that the detection of T. bryosalmonae infection in brown trout may occur earlier than after 30 days > 15°C, especially when using histological detection, which logically occurs before the onset of spore release by infected fish. This could be due to an earlier hatching and milder winter temperatures in the French rivers. As stressed by Rubin et al. (2022), there might be important between years variation in thermal regime, and extreme events such as the very early June canicule experienced in France in 2022 may disturb the hostparasite interactions and impact the timing and severity of fish infections (see Román-Palacios & Wiens, 2020). A stricter analysis of temperature regimes beginning in the winter might therefore be important to understand the timing of *T. bryosalmonae*'s life cycle and the onset of spore release in the wild, both by bryozoan and by fish. Additionally, the mean summer temperature used in Chapter 3, the corresponding degree-days, and number of days > 15° C were highly correlated, suggesting that the mean water temperature properly represented the temperature regime relative to the measurements used in this recent study (A5b).
-Appendices-

A5. (a) Infection prevalence (%) at the first sampling session and the degree days and number of days with water temperature > 15° C since the 1^{st} of March, for a comparison with Rubin et al. 2022. The sites are ranked by their mean summer temperature, and we can see that this ranking does not correspond to that of the number of days > 15° C, nor of that of the degree days. At TORTuz, the infection prevalence was already high at the first sampling session. This site is rather warm (mean summer temperature of 17.4° C), and already 33 days were registered with a mean temperature of > 15° C at the sampling date. Contrastingly, at ARZDur, a site with the same mean summer temperature, we detected no infection at the first sampling, most probably because only 8 days > 15° C were registered at that time.

Site	Date S1	Prevalence S1	Prevalence S2	Degree days S1	N days > 15°C S1	Mean summer temperature
GERAsp	24/06/2020	0	0	1259	0	14.51
ARBBar	29/06/2020	NA	22	NA	NA	15.29
ARGPon	03/07/2020	NA	5	1405	9	15.79
JOBEnc	26/06/2020	10	90	NA	NA	16.49
ARRGou	01/07/2020	22	100	1570	19	16.63
ARZPon	03/07/2020	0	5	1506	12	16.76
ARGFoi	22/06/2020	0	20	1303	2	17.00
GARVil	29/06/2020	0	78	1397	7	17.21
ARIVar	03/07/2020	13	67	1619	12	17.33
ARZDur	22/06/2020	0	85	1400	8	17.41
TORTuz	01/07/2020	80	89	1619	33	17.44
SALTau	02/07/2020	10	53	1413	12	17.62
BOUSer	01/07/2020	NA	100	1668	42	17.75
GERPoi	25/06/2020	0	42	1431	12	17.84
ARBMan	29/06/2020	30	50	1529	19	18.23

However, a short verification indicated that the mean summer temperature and the degree days and number of days >15°C were strongly and positively linked during the study period $(3^{rd} July - 14^{th} September, see the relationships in A5b).$



A5. (b) Mean summer temperature according to the summer degree days and to the number of days with a temperature exceeding 15°C.

Literature cited in Appendix 5

- Gay, M., Okamura, B., & de Kinkelin, P. (2001). Evidence that infectious stages of *Tetracapsula* bryosalmonae for rainbow trout *Oncorhynchus mykiss* are present throughout the year. *Diseases* of Aquatic Organisms, 46, 31–40. https://doi.org/10.3354/dao046031
- Román-Palacios, C., & Wiens, J. J. (2020). Recent responses to climate change reveal the drivers of species extinction and survival. *Proceedings of the National Academy of Sciences*, 117(8), 4211–4217. https://doi.org/10.1073/pnas.1913007117
- Rubin, A., Bailey, C., Strepparava, N., Wahli, T., Segner, H., & Rubin, J.-F. (2022). Reliable Field Assessment of Proliferative Kidney Disease in Wild Brown Trout, *Salmo trutta*, Populations: When Is the Optimal Sampling Period? *Pathogens*, 11(6), 681. https://doi.org/10.3390/pathogens11060681
- Strepparava, N., Ros, A., Hartikainen, H., Schmidt-Posthaus, H., Wahli, T., Segner, H., & Bailey, C. (2020). Effects of parasite concentrations on infection dynamics and proliferative kidney disease pathogenesis in brown trout (*Salmo trutta*). *Transboundary and Emerging Diseases*, tbed.13615. https://doi.org/10.1111/tbed.13615
- Strepparava, N., Segner, H., Ros, A., Hartikainen, H., Schmidt-Posthaus, H., & Wahli, T. (2018). Temperature-related parasite infection dynamics: The case of proliferative kidney disease of brown trout. *Parasitology*, 145(3), 281–291. https://doi.org/10.1017/S0031182017001482







A6. (a) eDNA concentration found for *T. bryosalmonae* at the positive sites (4 field replicates) for two PCR runs (laboratory replicates). Field replicates (i.e., each box) show variance in the estimated concentration for a site. However, consistency between the PCR replicates for a site are illustrated by the overall overlapping between the red and blue boxes for almost all the sampled sites.

208



A6. (b) eDNA concentration found with 4 field replicates for *Fredericella sultana* at the positive sites. Field replicates (i.e., each box) show variance in the estimated concentration for a site. Two sites with very high concentration (ADOAur and TORTuz) were removed from the representation for visual purposes.



A6. (c) eDNA concentration found for brown trout at the different sites with 4 field replicates. Field replicates (i.e., each box) show variance in the estimated concentration for a site.

210



A7. Total volume of water filtered for the eDNA sampling of Chapter 2. This histogram illustrates the great variance existing in the sampled sites turbidity.

Appendix 8.



A8. Relationship between brown trout eDNA concentration and brown trout biomass found at 26 sites sampled by our team by electrofishing for the iBEF project. The fish sampling occurred between July and August 2020, not at the exact same time than the eDNA sampling (early August).



A9. (a) Results of the analyses run in STRUCTURE for quantifying the introgression into the sampled populations of Chapter 3. Each bar represents one individual, and each color one genetic cluster. The analyses were run for the Hautes-Pyrénées on one side, and for Haute-Garonne and Ariège on the other side, because the populations of these two geographical regions are stocked with different hatcheries. STRUCTURE was run 3 times with a burn-in period of 5000 replications and a run length of 50000 Markov Chain Monte Carlo iterations, for K=1 to K=5 clusters. I represented K=5 for both analyses because this number of clusters discriminated the most both hatcheries. For the Hautes-Pyrénées we can see that a few individuals of the Neste (in NESArr and NESSar) are identified as stocked, but that otherwise the hatchery cluster (light yellow) is only patchily represented. In the Haute-Garonne (left part of the second analysis), more individuals are concerned with introgression (dark grey cluster), and a few individuals might be directly coming from a hatchery (e.g., in SAVVil, PIQCil and ARBMan). BOUSer was removed from the analyses because this population showed a strange pattern, resembling to that of TOUFre with all the individuals being identified as half wild, half introgressed. This population represented an admixture between two river basins and that it might disturb STRUCTURE analyses. This pattern observed for TOUFre could not be explained with the available elements

Appendix 9.



214

A9. (b) Map summarising the results of the STRUCTURE analysis in A8. (a). The mean introgression was computed by averaging the proportion of the hatchery cluster of all the individuals in a population. Most of the populations are barely concerned with introgression from stocked fish, especially in Ariège except for one population, TOUFre, for which the analysis found almost half hatchery's genes. Populations in Haute-Garonne show a moderate level of introgression, and it is less for those in Hautes-Pyrénées. The two catchment areas are represented, and populations from Hautes-Pyrénées are located on either side. The canal linking the two catchment areas is indicated in red. BOUSer had a particular profile, probably due to its location in between two separated geographical areas, so that this population was not included in the introgression analysis.

-Appendices-



Appendix 10.

A10. Independent effects of the environmental factors on the different indicators' scores, according to a hierarchical partition run with the hier.part 1.0-6 package (Mac Nally & Walsh, 2004).

Literature cited

Mac Nally, R., & Walsh, C. J. (2004). Hierarchical partitioning public-domain software. *Biodiversity* and Conservation, 13, 659–660.

Duval et al. BMC Ecol Evo (2021) 21:143 https://doi.org/10.1186/s12862-021-01876-9

RESEARCH

BMC Ecology and Evolution

Open Access

Long-term monitoring of a brown trout (*Salmo trutta*) population reveals kin-associated migration patterns and contributions by resident trout to the anadromous run

Eloïse Duval^{1,4*}, Øystein Skaala^{2*}, María Quintela², Geir Dahle², Aurélien Delaval^{2,5}, Vidar Wennevik², Kevin A. Glover^{2,3} and Michael M. Hansen^{1,2*}

Abstract

Background: In species showing partial migration, as is the case for many salmonid f shes, it is important to assess how anthropogenic pressure experienced by migrating individuals af ects the total population. We focused on brown trout (Salmo trutta) from the Guddal River in the Norwegian Hardanger Fjord system, which encompasses both resident and anadromous individuals. Aquaculture has led to increased anthropogenic pressure on brown trout during the marine phase in this region. Fish traps in the Guddal River allow for sampling all ascending anadromous spawners and descending smolts. We analyzed microsatellite DNA markers from all individuals ascending in 2006–2016, along with all emigrating smolts in 2017. We investigated (1) if there was evidence for declines in census numbers and effective population size during that period, (2) if there was association between kinship and migration timing in smolts and anadromous adults, and (3) to what extent resident trout were parents of outmigrating smolts.

Results: Census counts of anadromous spawners showed no evidence for a decline from 2006 to 2016, but were lower than in 2000–2005. Estimates of effective population size also showed no trends of declines during the study period. Sibship reconstruction of the 2017 smolt run showed signif cant association between kinship and migration timing, and a similar association was indicated in anadromous spawners. Parentage assignment of 2017 smolts with ascending anadromous trout as candidate parents, and assuming that unknown parents represented resident trout, showed that 70% of smolts had at least one resident parent and 24% had two resident parents.

Conclusions: The results bear evidence of a population that after an initial decline has stabilized at a lower number of anadromous spawners. The signif cant association between kinship and migration timing in smolts suggests that specif c episodes of elevated mortality in the sea could disproportionally af ect some families and reduce overall ef ective population size. Finally, the results based on parentage assignment demonstrate a strong buf ering ef ect of resident trout in case of elevated marine mortality af ecting anadromous trout, but also highlight that increased mortality of anadromous trout, most of which are females, may lower overall production in the system.

*Correspondence: eloise.duval@yahoo.fr; oystein.skaala@hi.no;

8000 Aarhus C, Denmark

² Department of Aquaculture, Institute of Marine Research, Nordnes, P.O.

Box 1870, 5817 Bergen, Norway

Full list of author information is available at the end of the article



©The Author(s) 2021. **Open Access** This article is licensed under a Creative Commons Attribution 4.0 International License, which permits use, sharing, adaptation, distribution and reproduction in any medium or format, as long as you give appropriate credit to the original author(s) and the source, provide a link to the Creative Commons licence, and indicate if changes were made. The images or other third party material in this article are included in the article's Creative Commons licence, unless indicated otherwise in a credit line to the material. If material is not included in the article's Creative Commons licence and your intended use is not permitted by statutory regulation or exceeds the permitted use, you will need to obtain permission directly from the copyright holder. To view a copy of this licence, visit http://creativecommons.org/joubliccomain/zero/1.0/) applies to the data made available in this article, unless otherwise stated in a credit line to the data.

mmh@bio.au.dk

¹ Department of Biology, Aarhus University, Ny Munkegade 114,

Keywords: Partial migration, *Salmo trutta*, Life-history types, Parentage assignment, Sibship reconstruction, Migration timing, Effective population size

Introduction

Individuals within species can exhibit different life history strategies which are often associated with important phenotypic variation, can differ between sexes and overall have pervasive ecological implications [1]. Hence, individuals representing different life history types may differentially allocate their amount of available energy between maintenance and reproduction functions to maximise their fitness. This polymorphism in life history strategies is maintained within species because their costs and benefits vary according to the environmental contexts [1]. As an example, within many species known to undertake migrations, some individuals migrate while others from the same population remain on the same site across their lifespan, referred to as partial or facultative migration [2, 3].

Among fishes, many salmonid species show anadromous life history forms, which means that juveniles hatch in freshwater and undertake feeding migrations at sea before returning to freshwater for spawning [4]. Their populations often include both sea-migratory and resident individuals that remain in freshwater, therefore showing facultative anadromy [5]. Coexistence between resident and migratory life-history strategies involves a fine balance between their respective costs and benefits [6]. Increased food availability in marine environments may lead to better growth and higher fecundity of anadromous individuals [7, 8]. On the other hand, residency can be advantageous when costs of migration become higher than benefits, due to factors such as predation risk, additional exposure to pathogens and parasites, or energetic costs for the migration process itself [6, 9].

Facultative anadromy is usually considered a quantitative trait, controlled by the action of multiple genes and their interaction with environmental factors [10, 11]. However, recent studies have shown that traits related to migration and life history in some salmonid species can be under control of single genes [12–14], whereas other studies point towards important elements of epigenetic regulation [15]. It is furthermore noteworthy that proportions of migrants and residents within a population may vary across years according to environmental factors or anthropogenically induced disturbances [16–18].

Migratory species, and not least salmonids, may be particularly susceptible to anthropogenic impact due to their dependence on several different habitats and connectivity between them [19]. For instance, fishing pressure in the sea and decreased access to marine environments due to dams represent important issues [20, 21]. Moreover, emerging threats related to climate change altering marine temperature regimes and adverse effects of marine aquaculture, such as accummulation of parasites that subsequently infect wild populations have become increasingly important [22–25]. In addition to general population declines, increased mortality at sea could also disproportionally affect the resident or migratory components of populations showing facultative anadromy. Also, in the case of sex-ratio differences between lifehistory types [26], increased mortality of one sex could reduce the total effective population size, resulting in a lower ratio between effective and census population size and leading to increased inbreeding and loss of genetic variation [27]. Finally, some studies have suggested that related fish tend to group together during migration [28, 29]. In addition to active association of kin [29], this could also reflect the mere fact that closely related individuals, especially smolts, may be "physiologically timed" to migrate at the same time [30]. If related individuals migrate together and are subjected to specific incidences of e.g., exposure to parasites, this could lead to high variance of survival among families and ultimately increase the variance of their reproductive success, a factor also leading to decreased effective population size [27].

The brown trout (Salmo trutta) is a species often showing facultative anadromy within populations [26, 31]. Recent studies have shown that environmental factors such as water temperature and food availability for brown trout juveniles can have contrasting effects on their migration tendency, with food limitation generally favoring anadromy and increasing temperature favoring residency [32-34]. These environmental factors interact with inherited genetic factors to shape life history strategies [33, 35, 36]. Brown trout shows a sex ratio typically skewed towards females among anadromous individuals and towards males among resident individuals, the latter including precocious mature male parr that can successfully fertilize eggs by adopting a sneaking behaviour [5, 26, 37-39]. Fjords in Norway have experienced an increased establishment of Atlantic salmon (Salmo salar) farms since the 1990s [40]. The aquaculture industry poses major problems for wild salmon and brown trout during marine migration [41]. Hence, the high concentration of farmed fish attracts and accummulates parasites such as sea lice Lepeophtheirus salmonis that subsequently infect wild salmon and trout passing by, often in lethal doses [23, 42-46]. Anadromous brown

trout in Norway typically do not migrate beyond a ca. 80 km range from their native river before returning to spawn [47]. Compared to Atlantic salmon, their home range is therefore more restricted to the fjords, where sea lice are concentrated. In some regions, low marine survival of brown trout has indeed been recorded during the last decades and ascribed to increasing exposure to salmon lice [43]. There is also evidence for genetic variation in susceptibility of brown trout populations to salmon lice infestation, further underpinning salmon lice as an important factor in marine mortality [48].

The present study focuses on the brown trout population of the River Guddal, located in the central region of the Norwegian Hardanger Fjord (Fig. 1). A trap facility encompassing two types of traps was installed in the early 2000's after a reported decrease in the number of sea trout in the fjord [49]. It allows for a full monitoring of ascending adults and descending smolts, from which phenotypic and genetic data have been secured annually. In turn, this provides a unique long-term data set on the down- and up-stream migratory patterns of brown trout in this river, located in a major farming region where marine survival is known to be low [49].

We analyzed microsatellite DNA variation in all anadromous trout ascending the river from 2006 to 2016 and in all smolts emigrating from the river in 2017. We assessed temporal trends in the number of ascending spawners across years and used genetic data to estimate effective population sizes. We tested the hypothesis that census and effective population sizes had declined over the time span studied. Second, as migratory behaviour in general may involve genetic components [33, 35, 36]



and specifically as some heritability has been shown in the timing of migration in other salmonid species [28, 50], we used genetically based parentage assignment and sibship reconstruction to test whether related individuals are more likely to migrate together than non-related individuals. Third, using parentage assignment of smolts from 2017 we assessed the degree to which anadromous and resident trout contributed to the smolt run, thus providing information on the possibility that resident trout can compensate for increased mortality in the marine environment.

Material and methods

Study site

The river Guddal (59° 58′ N, 6° 00′ E) is 13.5 km long and located in the central region of the Hardanger Fjord (Fig. 1), the second longest fjord in Norway and showing the highest concentration of Atlantic salmon farms [51]. Survival of wild anadromous trout in the system has been found to be low [43], presumably as a consequence of exposure to sea lice derived from salmon farms, as has recently been determined with genetic studies on lice [46, 52]. The river Guddal is partly fed by melting water running from the Folgefonna glacier, with relatively cold mean summer temperatures ranging from 8.5 to 11.9 °C between 2007 and 2016.

Sampling

Each year, all smolts descending the river for their first seaward migration are caught in a Wolf trap [53] that

covers the whole river transect, located at about 100 m from the tidal zone. The trap is operated annually from March or early April, depending on the water discharge, until the end of the smolt run. In principle, the trap should sample all downstream migrating smolts, but in some years there are shorter periods ranging from a few hours to a couple of days, where water discharge prevents full sampling, so that the trap is estimated to catch 90% of the migrating smolts. Smolts caught in the trap are anaesthetised with benzocaine, measured, weighed, adipose fin-clipped, and tagged with a passive integrated transponder (PIT tags 12×2 mm) since 2007 [49, 54] before being released downstream. A specific test showed that among 600 smolts retained in a prolonged recovery, only 4 individuals (0.7%) died $(\emptyset$. Skaala, unpublished results). Hence, we do not expect that handling has severely affected the smolts, and any effects should be randomly distributed among individuals and families. In addition, an upstream trap is operated each year during the upstream migration to capture all anadromous trout ascending the river. Each fish is anaesthetised, fin-clipped, checked for PIT tag, measured and weighed before being released upstream to continue its migration. Tissue samples for both smolts and ascending fish were stored in 95% ethanol. For this study, we used data on 711 ascending fish collected between 2006 and 2016 (Table 1), representing all the adults entering the system in this period. Census numbers (but not genetic data) of ascending spawners between 2000 and 2005 were also available [43].

Table 1Summary of brown trout trap sampling and genotyping, both for sea trout ascending the Guddal River in 2006–2016 and forthe smolts emigrating to sea in 2017

	Sample	Catches in the trap	Fish with no tissue sample*	Fish identified as salmon	Trout individuals genotyped
Ascending trout	2006	86	0	1	85
	2007	37	3	0	34
	2008	89	7	1	79
	2009	82	1	0	81
	2010	28	1	2	25
	2011	73	3	4	63
	2012	56	0	1	53
	2013	41	2	0	39
	2014	60	1	0	58
	2015	99	0	2	96
	2016	89	2	0	87
Smolt	2017	965	20	94	851

Catches in the trap: total number of ascending fish caught in the trap. * including individuals with too many loci missing after genotyping. Trout individuals genotyped: total number of individuals minus fish with no samples, fish identified as Atlantic salmon and sea trout individuals caught several times within the same year in the ascending trap for adults. Two individuals were caught twice in 2008, two twice in 2011, one individual three times in 2012, one twice in 2014, one twice in 2015 and one twice in 2016

Furthermore, we analysed the entire smolt run of 2017, representing 965 fish (Table 1).

Genotyping

DNA was isolated and extracted using Qiagen's DNeasy[®] Blood & Tissue kit (Qiagen Inc.), following the manufacturer's recommendations. DNA concentration was measured with a NanoDrop 1000 Spectrophotometer (Thermo Fisher Scientific Inc.). Dilutions were subsequently conducted using a Freedom EVOware[®] robot (Tecan Inc.), yielding ca. 16.6 ng/µL of DNA per sample.

All samples were genotyped at 21 microsatellite loci (Additional file 1: Appendix 1), divided into three multiplexes for the Polymerase Chain Reaction (PCR). The third multiplex included the sex-specific markers, Exon 2 and Exon 4 [55] from the sdY sequence, a male-specific-Y-chromosome gene that is highly conserved in salmonids [56]. Although not always 100% accurate, due to the occasional occurrence of autosomal pseudocopies of *sdY*, at least in brown trout's closest relative, Atlantic salmon [57], this provides more accurate sexing than phenotypic sex as morphological dimorphism can be difficult to ascertain in the field [58]. PCR was conducted using a Verity 96 well thermal cycler and GeneAmp PCR system 9700 (Applied Biosystems). Conditions for the cycling reactions and primer mixes used for the 3 multiplexes are detailed in Additional file 1: Appendix 2. PCR products were then diluted at 1:15 and separated by capillary electrophoresis using a 3730 DNA Analyzer (Applied Biosystems). Alleles were scored using GeneMapper 5 with GeneScan 500 LIZ size standards (Applied Biosystems). A total of 30 adults and 94 smolts were identified as Atlantic salmon as they showed several allele sizes that do not correspond to alleles known from brown trout, which left a total of 1560 trout sampled (711 adult ascenders and 851 smolt, see Table 1).

Two microsatellite loci (Ssa85 and Ssa197) were represented twice in separate multiplexes in order to provide a control of the allele scoring (Additional file 1: Appendix 1). PCR was repeated for each sample until data were obtained for most loci. In the whole dataset, 3 individuals had missing data at 3 loci, 4 at 2 loci and 56 at 1 locus, out of the 21 loci genotyped. When several PCRs had been conducted for the same individuals, they were compared and the one with the most clear-cut signal was considered right in case of conflict. The locus BG935488 was excluded from the dataset due to difficulties with reliable scoring of alleles. As loci MHC-I and Sasa-TAP2A are closely linked to loci associated with immune responses and have previously been suggested to be under diversifying selection among trout populations from the Hardanger Fjord [59]; they were omitted from analyses of effective population size but were used for sibship reconstruction (see below).

Tissue samples were accidentally missing for 20 of the ascending trout (Table 1). However, the PIT tag number from a missing fish of 2016 had a match with one captured in 2015, allowing to find its genotype based on the previous sampling.

Individual identification

The "matches" algorithm implemented in GenAlEx 6.5 [60] was used to identify identical genotypes (allowing for one mismatching locus), corresponding to individuals that had ascended the river Guddal several times among or within years. This procedure was implemented as PIT tags can be lost between captures [54], particularly in females during spawning, thus compromising individual identification.

Genetic diversity and effective population size

Total number of alleles and allelic richness (A_r) per locus were computed with the R package diveRsity [61], using R 3.6.1 [62]. Observed (H_O) and unbiased expected heterozygosity (H_E), as well as inbreeding coefficient (F_{IS}) were computed with GenAlEx 6.5. Tests for Hardy– Weinberg equilibrium were performed with Genepop 4.7 [63] using the following Markov chain parameters: 10,000 dememorisation steps, 1000 batches and 10,000 iterations per batch. False discovery rate (FDR) correction was applied to account for multiple testing [64], using the method by Y Benjamini and Y Hochberg [65] implemented in Myriads 1.1 [66].

Effective population sizes (N_e) for the ascending sea trout and the smolts were estimated with the linkage disequilibrium method implemented in NeEstimator 2.1 [67], using allele frequencies higher than 0.05. As several cohorts are represented among spawning individuals, we assume that the estimates measure N_e rather than N_b (the effective number of breeders in a single breeding event [68–70]). A smaller number of cohorts were expected to be represented in the sample of smolts and hence estimates could be shifted more towards estimating N_b .

Sibship and parentage assignment

COLONY 2.0.5.1 [71] was used to infer full and half sibships both in the 2006–2016 ascending trout and the smolt 2017 datasets. This software infers all possible relationships (siblings and parentage) of all individuals (all offspring and all candidate parents) simultaneously in a full likelihood framework. Analyses of the ascending trout dataset were conducted with no information on parental genotypes, assuming both male and female polygamy as well as possible inbreeding. The full-likelihood method was used at very high precision for

the full likelihood calculation and medium run length together with the options sibship scaling, no updated allele frequencies and no sibship priors. In the analysis of the smolt dataset, the trout ascending in or after 2010 were used as candidate parents. We assumed that when fish were last caught before 2010, they were unlikely to be candidate parents, as smolts at these latitudes leave the river between age 2 and 7 years [72]. Similarly, it is unlikely that reproduction in 2016 would result in smolts sampled in 2017, but several trout ascending in 2016 had also ascended in previous years (see Additional file 1: Appendix 2) and could consequently be parents of smolts as a result of previous spawning events. We made use of this information for providing an empirical assessment of the quality of parentage assignment. We thus predicted that anadromous individuals that had ascended the river in 2016 and were identified as parents of smolts from 2017 should also have ascended the river in previous years.

Three COLONY runs with different random number seeds were used to check the reliability of the results. Only the relationships found in the 3 runs were kept in the inferred pedigree. Allelic dropout rate in the input file was estimated with the PopGenReport R package [73]. Genotyping error rate was set to 0.01 for all loci. Each time, full and half-sibships were inferred from the "best configuration" COLONY output files, which is more accurate than the results found by the pairwise analysis in the files full and halfsib dyads [74].

More than 50% of male parr in a population can be precocious [75-77]. As it would be infeasible to sample all resident trout and not least precocious male parr from the system, we inferred the parentage of resident trout indirectly. Hence, when none or only one of the two parents of a smolt was identified among the anadromous candidate parents, it was assumed that missing parents were part of the resident proportion of the population. This was a realistic assumption as all ascending anadromous spawners are assumed to have been caught in the trap and genotyped (only 8 adults caught in the trap were accidentally not sampled for genotyping between 2010 and 2016, Table 1). Moreover, given sex ratio differences between anadromous and resident trout, we further corroborated this assumption by expecting a higher number of supposedly resident males than resident females.

Sibship and timing of migration events

To test whether related individuals tend to migrate at the same time (both for ascending adults and descending smolts), Mantel tests were conducted between a distance matrix comprising the sibship previously found between pairs of individuals, coded as 1 for unrelated, 0.75 for halfsibs and 0.5 for fullsibs, and a matrix with the distance in days between their date of capture in the traps. The R package ecodist [78] was used for these analyses, and significance was assessed using 10,000 permutations together with 1000 bootstraps. The test was conducted separately for each year in the ascending spawners. Fish that did not have any siblings within the samples of the year or in the smolt sample were removed from the analyses, in order not to bias the results.

Results

Numbers of ascending anadromous spawners

The number of anadromous trout ascending the river Guddal showed pronounced variation among years between 2006 and 2016, with a mean of 64.9 ± 22.9 individuals (\pm SD), ranging from 25 in 2010 to 96 in 2015, but with no general tendency for a decline over this time period (linear regression: $F_{(1,9)}=0.29$, $R^2adj=-0.07$, P=0.61; see also Table 1). However, compared to the mean number of anadromous trout recorded in the trap in in 2000–2005 in the same river (100 ± 43 (mean \pm SD), [43]) there were indications of a decline over a longer time span, although for the entire period of 2000–2016 the result remained non-significant (linear regression: $F_{(1,15)}=2.90$, $R^2adj=0.11$, P=0.11).

After genotyping, we identified 591 individuals among the 711 tissue samples from returning adult sea trout collected between 2006 and 2016. The matching genotypes showed that 82 individuals ascended the river more than once in the study period. Eight individuals were found returning to the river twice within the same year, and one returned three times in the same year (Table 1). Assignment tests using the simulation option in GeneClass2 [79] provided no evidence that the latter individuals were strayers from different populations, except for a single individual whose multilocus genotype was unlikely to occur based on the allele frequencies observed in the Guddal population (data not shown).

Individuals returned to the river on average 1.19 times, ranging from 1 to 6 times (recaptures are illustrated in Additional file 1: Appendix 3). Individuals ascending multiple times returned within a period of 2.7 ± 1.0 (\pm SD) years on average (Additional file 1: Appendix 3). During the study period, most of the sea trout ascended the river between mid-July and early September, but the spawning run had its earliest start in 2016 on the 14th of May and the latest end in 2010 on the 25th of November (Additional file 1: Appendix 4). The spawning run could be roughly divided into two peaks of migration for most of the years, one in summer and one in early autumn, but the pattern was not clear-cut (Additional file 1: Appendix 4).

A χ^2 goodness of fit test revealed that the number of females exceeded number of males among anadromous

trout across all years, with sex ratios (females:males) ranging from 1.04 in 2013 to 2.13 in 2009 (Table 2, $\chi^2 = 28.2$, df = 10, P = 0.002).

Genetic variation and effective population size estimated from ascending spawners

A total of three deviations from Hardy–Weinberg equilibrium were found in the microsatellite DNA dataset for annually ascending trout after Benjamini and Hochberg [80] False Discovery Rate correction (Table 2). As they concerned three different loci that were not deviating in other annual samples, they were all retained in the analyses. Mean unbiased expected heterozygosity (H_E) was high and stable across years, ranging from 0.74 to 0.76, and F_{IS} was not significantly different from 0, except in 2012 and 2013 where it nevertheless remained low (Table 2).

Estimates of mean effective population size (N_e) showed considerable variation, ranging between 83 in 2014 and 3646 in 2010 (Table 2). The highest N_e estimate coincided with the lowest sample size (N=25) and showed a very wide 95% confidence interval (94– ∞), indicating that the point estimate is not informative. Considering only the years with sample sizes > 50 (a total of 8 years), the N_e point estimates ranged from 83 to 548, with a mean of 223.1. Similar to the number of spawners per year, there was no evidence for a temporal decline of N_e.

Genetic variation and effective population size estimated from 2017 smolts

Mean unbiased expected heterozygosity (H_E) was similar to that observed in anadromous spawners (Table 2). In contrast, however, 17 deviations from Hardy–Weinberg equilibrium were found among the 18 loci analysed (Table 2), likely reflecting the large number of full- and half-sibs (see below) and essentially violating the criterion for Hardy–Weinberg equilibrium of infinite population size, combined with very high statistical power due to the sample size of N=851. Sex-ratio in the total sample of downstream migrating smolts was more biased towards females than for the anadromous spawners (2.15 against a mean of 1.48 in the spawners, Table 2). The point estimate of effective population size (N_e) was 51, much lower than estimates based on anadromous spawners (Table 2).

Relatedness and timing of migration events

Among the 851 smolts migrating to the sea in 2017, the consensus pedigree achieved after three different COL-ONY runs identified 3198 full and 11,065 halfsib dyads. This represents 317 different fullsib families, with a mean of 2.7 offspring per family (ranging between 1 and 42). The Mantel test between distance matrices composed of distance in Julian date of seaward migration and distance in inferred sibship relation yielded a significant positive correlation for the 815 smolts which had siblings in the run (r=0.027, P=0.0002, Table 3).

For the samples of anadromous spawners, the numbers of individuals with half- or full-sibs in the spawning

Table 2 Summary of the population genetic statistics, both for anadromous trout ascending the river Guddal in 2006–2016 and for smolts sampled in 2017

	Sample	Sample size	Sex-ratio (Nf:Nm)	Number of alleles	A _r	H _o	H _E	HWE deviations	F _{IS}	N _e (Cl _{95%})
Ascending trout	2006	85	1.43	193	8.14	0.75 ± 0.07	0.75 ± 0.07	1	-0.012 ± 0.024	99 (60–207)
	2007	34	1.83	169	7.98	0.76 ± 0.08	0.76 ± 0.07	0	0.000 ± 0.040	121 (51−∞)
	2008	79	1.55	196	8.15	0.72 ± 0.07	0.74 ± 0.07	0	0.019 ± 0.040	568 (202 −∞)
	2009	81	2.12	209	8.28	0.73 ± 0.07	0.74 ± 0.07	0	0.005 ± 0.027	280 (137–2854)
	2010	25	1.78	159	7.64	0.75 ± 0.09	0.76 ± 0.08	0	-0.007 ± 0.044	3646 (94-∞)
	2011	63	1.25	187	8.16	0.74 ± 0.07	0.75 ± 0.07	0	0.002 ± 0.030	196 (110–639)
	2012	53	1.21	199	8.46	0.71 ± 0.08	0.76 ± 0.08	2	0.049 ± 0.039	275 (96 −∞)
	2013	39	1.05	169	7.84	0.72 ± 0.08	0.76 ± 0.07	0	0.032 ± 0.039	108 (55–598)
	2014	58	1.42	191	8.17	0.73 ± 0.08	0.74 ± 0.08	0	-0.005 ± 0.040	83 (45–252)
	2015	96	1.18	192	8.02	0.74 ± 0.07	0.74 ± 0.07	0	-0.010 ± 0.025	135 (78–332)
	2016	87	1.42	203	8.23	0.73 ± 0.07	0.75 ± 0.07	0	0.010 ± 0.024	149 (93–313)
Smolt	2017	851	2.15	287	7.32	0.74 ± 0.04	0.75 ± 0.04	17	0.020 ± 0.006	51 (46–56)

N: number of individuals sampled, Nf:Nm: number of females divided by number of males, Ar: allelic richness, H_0 : mean observed heterozygosity, H_E : mean unbiased expected heterozygosity, F_{15} : mean individual inbreeding coefficient. H_0 , H_E and F_{15} are followed by their 95% confidence interval. HWE deviations: number of deviations from Hardy–Weinberg equilibrium out of the 18 loci tested, after false discovery rate correction. N_e : effective population size estimated by the linkage disequilibrium method, lowest frequency used 0.05, CI95%: Jack knife confidence interval, ∞ : infinite value

Sample	N	% Fullsib	% Halfsib	% Nonrelated	Mantel's r	Р
2006	47	0.65	5.92	93.43	0.112	0.0040
2007	12	1.52	10.61	87.88	0.225	0.0425
2008	27	0.28	4.84	94.87	0.092	0.0400
2009	43	0.00	3.32	96.68	0.023	0.2229
2010	-	-	-	-	_	-
2011	19	0.58	5.85	93.57	-0.007	0.5484
2012	7	4.76	19.05	76.19	0.182	0.1864
2013	17	1.47	5.88	92.65	-0.004	0.4909
2014	27	2.85	4.56	92.59	-0.025	0.6676
2015	56	1.36	4.29	94.35	0.055	0.0405
2016	44	1.90	3.07	95.03	-0.010	0.5978
smolt 2017	815	0.96	3.33	95.7	0.027	0.0002

Table 3 Mantel test results between sibship and distance in week of ascendance for ascending trouts between 2006–2016 and between Julian date of seaward migration for 2017 smolt run

Only individuals that were found to have kin within the samples were kept

N: number of individuals that had kins within the sample, %...: percentage of the total matrix that were full, halfsib or non related pair of individuals, Mantel's r: Pearson correlation between the two matrices, P: p-value for the test. In 2010, no ascenders were found to be related so the Mantel test could not be conducted

run of the same year ranged from 0 in 2010 to 56 in 2015 (Table 3). Mantel tests for association of sibship and migration timing conducted for ascending anadromous trout year by year used the week of ascendance in the matrix, as the time window for the ascending migration is larger than for descending migration of smolts. The results were significant for the adults ascending in 4 years (2006: r=0.112, P=0.0040; 2007: r=0.225, P=0.0425; 2008: r=0.092, P=0.0400, and 2015: r=0.055, P=0.0405; Table 3), but not for individuals ascending in the other years.

Resident trout contribution to anadromous trout run

Parents of the 2017 smolt run were found among the anadromous trout ascending the river Guddal between 2011 and 2016. Among 140 candidate anadromous fathers and 175 candidate anadromous mothers, 33 males and 52 females contributed to the total smolt sample. Among these individuals, four males and 12 females ascended the river in 2016, but as stated previously, the 2017 smolt run would be unlikely to include offspring from the previous year. In accordance with this, all but one of these individuals had in fact been recorded as ascending the river and presumably spawning in previous years, thus lending indirect empirical support to the robustness of parentage assignment. The putative parent recorded in 2016 but not found to ascend the river in previous years was a male. In addition to the possibility of incorrect parentage assignment, it is possible that the male reproduced as mature male parr and smolted and undertook migration to the sea afterwards. It is also possible that this male represents some of the individuals that were accidentally not genotyped (see Table 1).

Based on the assumption that a parent not represented by anadromous spawners caught in the trap must have been a resident individual, the freshwater resident part of the population was inferred to contribute to 70% of the 2017 smolt run (Fig. 2A). Identified anadromous females contributed to 72% of the smolt sample. The opposite pattern was observed for males, with putatively resident males (including precocious male parr) contributing to 66% of the smolt sample, which was significantly more than anadromous males (binomial tests, N=851, P < 0.001, Fig. 2B). These patterns are in accordance with the skewed sex ratio observed among anadromous spawners (Table 2). A total of 46% of the smolts had one putatively resident parent while the other was a migrant, 24% had two resident parents and 30% had two anadromous parents (Fig. 2A). Among these matings involving two different life history strategy types, 91% took place between a migrant female and a putatively resident male (Fig. 2A).

Discussion

Our study represents a unique long term monitoring effort of a salmonid population in an environment experiencing significant anthropogenic pressure. In addition to providing insights into demographic trends, the results raised the interesting possibility of concordance between migration timing and kinship in smolts and adult spawners. Finally, and most importantly, parentage assignment of an entire cohort of migrating smolts showed that resident parents contribute substantially to the anadromous **A** 100

80

60

40

20

0

B 80

Percentage of samples



anadromous

Percentage of 2017 smolt run 07 09 09 0 female male Parent Fig. 2 The parental origin of smolts in the 2017 run based on parentage assignment using COLONY 2.0.5.1 [71]. A Percentage of smolts according to their parental origin. **B** Contribution of sexes of anadromous and resident trout to the smolt run. Error bars represent the 95% confidence interval of the percentage. Significance of the binomial tests to compare contribution of anadromous and resident individuals of the same sex, ***: P < 0.001

run. We discuss these findings and their conservation implications in more detail below.

Temporal trends of census and effective population size

The number of anadromous trout ascending the Guddal River varied considerably among years, but did not show a general tendency for decline over the study period, although numbers of spawners were lower in 2006-2016 than in 2000-2006. Salmon farming in the Hardangerfjord accellerated during the 1990s, so it is expected that the most severe population declines occurred during that period and have subsequently stabilized, as indicated by our results.

Similar to census sizes of anadromous spawning run, effective population size estimates also showed no general trends towards declines during the study period. This comes with the caveat that estimates of N_e based on linkage disequilibrium are sensitive to low sample sizes [81], which for some years/samples resulted in low precision of estimates. It may seem surprising that the Ne estimate based on smolts (51) was much lower than any of the estimates based on anadromous spawners. However, as noted previously this estimate is not completely comparable to estimates from adult anadromous spawners that represent different numbers of sea-winters before they return to the river; the smolt run in a given year is expected to represent fewer cohorts compared to adult spawners and would therefore represent something in between N_e and N_b (the effective number of breeders in a single breeding event [68, 69]).

The N_e estimates of a few hundreds are comparable to most other estimates found in anadromous brown trout populations using temporal or LD-based methods [59, 82-84], but higher than most estimates from strictly resident populations [85-88]. Whereas Ne in the Guddal population is lower than the 500 or even 1000 assumed to be required for maintaining evolutionary potential [89-92], it should be noted that it is part of a larger system in the Hardanger Fjord where gene flow occurs among populations [59]. In general, anadromous brown trout populations have been found to exhibit a hierarchical genetic structure shaped by both geographical distance between populations and environmental parameters, with low genetic differentiation among local populations resulting from gene flow [93]. Evolutionary potential should thus be considered across several neighbouring populations, where total N_{e} is expected to be higher [94].

Concordance of migration timing of related individuals

Association of kin along with the possibility of kin selection has been studied intensively in salmonids [29, 95-99]. As sibs during the earliest life stages are situated in the same spawning redds, spatial association of kin would be expected to occur immediately after hatching, whereas subsequent drift and dispersal would lead to decreased kin association over time, unless active kin recognition and association takes place [99]. The significant association betwen migration timing and relatedness as observed in smolts in the present study can hardly be ascribed to reminiscent patterns of association of kin several years back in time in their spawning redds, but could reflect: (1) active aggregation of kin, (2) genetic components in the timing of smolt migration, and/or (3) similarity in size of sibs and thereby propensity for migrating at the same time, simply because sibs hatched and emerged from the same redds at the same time. Whereas the study does not allow for distinguishing between these possibilities, we note that (2) and (3) are the most parsimonious explanations and also indirectly supported

by empirical results [47, 100, 101], including data from Atlantic salmon demonstrating clear genetic components in migration timing [96, 102].

Interestingly, our results are at odds with those from a different study on migration timing and kinship in Atlantic salmon, which found no significant association between kinship and schooling and migration timing in smolts [103]. Part of the reason for the discrepancy of results could lie in different experimental set-ups. The study by Fernandes et al. [103] was based on experimental full-sib families stocked into a natural environment at the same point in time, whereas our study encompassed the total smolt run composed of families naturally spawned and hatched over an extended period of time. This would leave more statistical power in our study for detecting association between kinship and migration timing resulting from similar hatching time and size of sibs, without necessarily involving active kin aggregation or genetic components in migration timing.

We also found some support for association between kin and timing of upstream migration among anadromous trout, although significant associations were observed in only 4 out of 11 years. Few studies of possible kin-biased aggregation of adult salmonid fishes have been conducted, undoubtedly due to challenges with sampling. However, one study found kin associations to occur at the adult stage outside the spawning period in brook char (Salvelinus fontinalis) inhabiting a large freshwater lake [29]. Nevertheless, kinship analysis of spawners in a tributary to the same lake provided no evidence for association of kin [104]. As noted by the authors, this could be an effect of accummulated mortality over time, leaving few surviving related individuals at the time of spawning. This could also be the case in our present study, where numbers of anadromous spawners per year were overall low.

In total, there was evidence for association between kinship and migration time in smolts, and also evidence, albeit less consistent, for such an association in spawners returning to the river. The association found in smolts raises the possibility that episodes of increased marine mortality, e.g., due to salmon lice exposure or fluctuating aggregations of predators, could potentially increase variance in mortality among families, which could again lead to higher variance in reproductive success among families and lower effective population size. Sibs were found among anadromous spawners in all years except for 2010 (with only 25 ascending anadromous spawners), but whether this reflects a disproportionally high variance in mortality among families compared to undisturbed conditions cannot be assessed. This would require comparable data from the system before major environmental disturbance of the Hardangerfjord system took place.

To what extent does the resident stock of Guddal brown trout population contribute to the sea run?

In systems like the Hardanger Fjord with increased marine mortality [43–45, 49], it is important to assess to which extent the resident part of the population can compensate for recruitment in the case of a reduced number of anadromous spawners. Moreover, it is important to consider to what extent this will drive changes in anadromy.

We did not genotype candidate parents among the resident trout and made the assumption that non-genotyped parents corresponded to resident individuals. This is an important limitation of the study and for instance precludes distinguishing between parents that are mature male parr and adult resident trout. However, the trap in which ascending spawners were sampled is of a construction that makes it unlikely that individuals can escape further upstream without being registered. In very few instances tissue samples were by mistake not taken, but this is unlikely to account for all the parental genotypes not represented among the ascending anadromous spawners. Moreover, our findings of parentage are in accordance with expectations given the skewed sex-ratio observed among anadromous and resident spawners [5, 26, 37, 39], providing further confidence in our results. Hence, only 4% of all smolts in 2017 had an anadromous father and putatively resident mother, whereas 42% had a putatively resident father and anadromous mother. In total, 70% of the smolts had either one or two inferred resident parents, with the latter category accounting for 24% of all smolts.

In the case of resident males, it is likely that many of them are in fact precocious male parr, as studies of both brown trout and other salmonids have shown that they can be both numerous and have significant reproductive success [6, 38, 39, 105]. On the other hand, the contribution to the 2017 smolt run was higher for anadromous than putatively resident females (72% versus 28%), which could reflect a higher number of anadromous relative to resident females and/or the fact that it is more advantageous as a female to migrate to sea in order to maximize body weight and egg production and thereby reproductive success [31, 106]. In the context of elevated mortality rates at sea, these results demonstrate that resident trout may indeed have some buffering effects towards a decline of anadromous spawners and that a sizeable proportion of smolts in fact have two resident parents. Given the high contribution of anadromous females to the smolt run it is nevertheless also evident that strong declines of the anadromous portion of the population would likely have significant negative demographic consequences for the total population. It should also be stressed that high marine mortality would lead to reduced gene flow among

populations, hence reducing overall effective metapopulation size (as in for instance the entire Hardanger Fjord system) and potentially leading to inbreeding and loss of variation in individual populations [94].

Could long-term elevated marine mortality select against anadromy and ultimately remove it from the population? If we assume that anadromy is a quantitative trait with an environmentally-cued threshold [10], then in this case high levels of genetic variation could be preserved even under directional selection acting against it [107]. This way, even if migration costs are increasing, the propensity to migrate within a population may persist. This finds empirical support in studies of brown trout [84] and other salmonid species that have been landlocked for centuries (e.g., by dams) such as brook charr [108], rainbow trout Oncorhynchus mykiss [109] and bull trout Salvelinus confluentus [110], but where migratory behaviour is retained. Nevertheless, recent studies have demonstrated major quantitative trait loci for important life history and migratory traits in e.g., Chinook Oncorhynchus tshawytscha [12] and Atlantic salmon [13]. If such genetically based variation also exists in brown trout, then increased marine mortality could exert strong selection and lead to genetic and phenotypic changes in populations, even if anadromy per se is retained.

Conclusions

The unique long-term monitoring of ascending anadromous spawners in the Guddal River allowed us to track both census and effective population size over an extended time period coinciding with adverse anthropogenic conditions in the sea. We did not observe general trends of declines during this period, suggesting that the population had stabilized after initial declines prior to the study. We found a significant association between kinship of smolts and their timing of emigration from the river, which raises the possibility that periodically adverse conditions in the sea could disproportionally affect some families and potentially lead to decreased effective population size. A similar association between kinship and migration timing was also indicated in ascending anadromous spawners but was not consistent across years. It is possible that accummulated mortality until this life stage would decrease the number of surviving sibs and thereby weaken signals of an otherwise genuine association. Finally, using parentage assignment of the total smolt run within a year, we estimated that 70% of smolts had at least one resident parent, and in 24% of cases both parents were inferred to be resident. Hence, the resident proportion of the population played a major role in recruitment of anadromous trout and would be expected to provide some buffer against elevated marine mortality.

Nevertheless, as the majority of smolts had an anadromous mother, it is also envisaged that elevated marine mortality would have important negative consequences for production of the population as a whole and could also lead to altered selection pressure for important life history traits. In total, the study thus provides important new information about recruitment and dynamics of populations showing partial migration, and how this may interact with anthropogenic environmental disturbance.

Abbreviations

A,: Allelic richness; H₀: Observed heterozygosity; H_E: Expected heterozygosity; F₁₅: Fixation index in subpopulations ("inbreeding coefficient"); FDR: False discovery rate; N_a: Effective population size.

Supplementary Information

The online version contains supplementary material available at https://doi. org/10.1186/s12862-021-01876-9.

Additional file 1. Supplementary material on microsatellite loci used (Appendix 1), PCR conditions (Appendix 2), overview of anadromous trout returning to Guddal River several times during the study period (Appendix 3), and proportion of anadromous trout ascending the river in different weeks in specific years (Appendix 4).

Acknowledgements

We acknowledge Britt Iren Østebø for extensive field work and data punching, Bjørghild Breistein Seliussen for her help with the genotyping process, and Dr. Baocheng Guo and two anonymous reviewers for many insightful comments and suggestions that significantly improved the paper.

Authors' contributions

ØS, ED, KAG and MMH conceived and planned the study. ED, MQ, GD, AD and VW genotyped the samples and conducted the statistical analyses. ED, MMH, ØS, and KAG wrote the manuscript with input from the other authors. All authors read and approved the final version of the manuscript.

Funding

The study was funded by the Norwegian Ministry of Trade, Industry and Fisheries.

Availability of data and materials

The data that support the findings of this study have been deposited at the Institute of Marine Research electronic archive at: https://hdl.handle.net/ 11250/2740709. It consists of three text files: a genepop-format file of all the individuals genotyped at 20 microsatellite loci, and two files containing the phenotypic information for adults and smolts, respectively.

Declarations

Ethics approval and consent to participate

All experimental protocols were in accordance with permits obtained by the Hordaland County Governor, and protocols approved by the Norwegian Environment Agency and Norwegian Food Safety Authority. Specifically, this involves permits to capture, tag, and release smolt and recapture adult fish. Permit numbers are 07/13020-1, 2011/88155-1 and 17/15858-1. All applications and protocols for animal handling have been approved by the Institute of Marine Research's local animal welfare unit before further processing by the Norwegian Food Safety Authority.

Consent for publication

Not applicable.

Competing interests

The authors declare no conflict of interest.

Author details

¹Department of Biology, Aarhus University, Ny Munkegade 114, 8000 Aarhus C, Denmark. ²Department of Aquaculture, Institute of Marine Research, Nordnes, P.O. Box 1870, 5817 Bergen, Norway. ³Institute of Biology, University of Bergen, Bergen, Norway. ⁴Present Address: Theoretical and Experimental Ecology Station, UMR-5321, CNRS, University of Toulouse III Paul Sabatier, 2 route du CNRS, 09200 Moulis, France. ⁵Present Address: Faculty of Biosciences and Aquaculture, Nord University, 8049 Bodø, Norway.

Received: 29 April 2021 Accepted: 2 July 2021 Published online: 13 July 2021

References

- 1. Gross MR. Alternative reproductive strategies and tactics: diversity within sexes. Trends Ecol Evol. 1996;11(2):92–8.
- 2. Dingle H. Migration: the biology of life on the move. Oxford: Oxford University Press; 1996.
- Chapman BB, Brönmark C, Nilsson JÅ, Hansson LA. The ecology and evolution of partial migration. Oikos. 2011;120(12):1764–75.
- 4. McDowall R. Diadromy: origins and definitions of terminology. Copeia. 1992;1992(1):248–51.
- Jonsson B, Jonsson N. Partial migration: niche shift versus sexual maturation in fishes. Rev Fish Biol Fisher. 1993;3(4):348–65.
- Hendry AP, Bohlin T, Jonsson B, Berg OK. To sea or not to sea? Anadromy versus non-anadromy in salmonids. In: Hendry AP, Stearns SC, editors. Evolution illuminated: salmon and their relatives. New York: Oxford University Press; 2004. p. 92–125.
- Gross MR, Coleman RM, McDowall RM. Aquatic productivity and the evolution of diadromous fish migration. Science. 1988;239(4845):1291–3.
- Fleming IA, Gross MR. Breeding competition in a Pacific salmon (coho: Oncorhynchus kisutch): measures of natural and sexual selection. Evolution. 1994;48(3):637–57.
- Halttunen E, Gjelland KØ, Hamel S, Serra-Llinares RM, Nilsen R, Arechavala-Lopez P, Skarðhamar J, Johnsen IA, Asplin L, Karlsen Ø. Sea trout adapt their migratory behaviour in response to high salmon lice concentrations. J Fish Dis. 2017;41:953–67.
- Ferguson A. Genetics of sea trout, with particular reference to Britain and Ireland. In: Harris G, Milner N, editors. Sea trout: biology, conservation and management. Oxford: Blackwell publishing; 2006. p. 157–82.
- Ferguson A, Reed TE, McGinnity P, Prodöhl PA. Anadromy in brown trout (*Salmo trutta*): a review of the relative roles of genes and environmental factors and the implications for management and conservation. In: Harris GS (Ed.). Sea Trout: from Science to Management. Self-published; 2016.
- Thompson TQ, Bellinger MR, O'Rourke SM, Prince DJ, Stevenson AE, Rodrigues AT, Sloat MR, Speller CF, Yang DY, Butler VL, et al. Anthropogenic habitat alteration leads to rapid loss of adaptive variation and restoration potential in wild salmon populations. P Natl Acad Sci USA. 2019;116(1):177–86.
- Barson NJ, Aykanat T, Hindar K, Baranski M, Bolstad GH, Fiske P, Jacq C, Jensen AJ, Johnston SE, Karlsson S, et al. Sex-dependent dominance at a single locus maintains variation in age at maturity in salmon. Nature. 2015;528:405–8.
- Czorlich Y, Aykanat T, Erkinaro J, Orell P, Primmer CR. Rapid sex-specific evolution of age at maturity is shaped by genetic architecture in Atlantic salmon. Nat Ecol Evol. 2018;2(11):1800–7.
- Baerwald MR, Meek MH, Stephens MR, Nagarajan RP, Goodbla AM, Tomalty KMH, Thorgaard GH, May B, Nichols KM. Migration-related phenotypic divergence is associated with epigenetic modifications in rainbow trout. Mol Ecol. 2016;25(8):1785–800.
- Wilcove DS, Wikelski M. Going, going, gone: is animal migration disappearing? PLoS Biol. 2008;6(7):1361–4.
- 17. Wysujack K, Greenberg L, Bergman E, Olsson I. The role of the environment in partial migration: food availability affects the adoption

of a migratory tactic in brown trout Salmo trutta. Ecol Freshw Fish. 2009;18(1):52–9.

- 18. Shaw AK. Drivers of animal migration and implications in changing environments. Evol Ecol. 2016;30(6):991–1007.
- Webster MS, Marra PP, Haig SM, Bensch S, Holmes RT. Links between worlds: unraveling migratory connectivity. Trends Ecol Evol. 2002;17(2):76–83.
- Rand PS, Goslin M, Gross MR, Irvine JR, Augerot X, McHugh PA, Bugaev VF. Global assessment of extinction risk to populations of sockeye salmon Oncorhynchus nerka. PLoS ONE. 2012;7(4):e34065.
- 21. Limburg KE, Waldman JR. Dramatic declines in North Atlantic diadromous fishes. Bioscience. 2009;59(11):955–65.
- 22. Shelton AO, Sullaway GH, Ward EJ, Feist BE, Somers KA, Tuttle VJ, Watson JT, Satterthwaite WH. Redistribution of salmon populations in the northeast Pacific ocean in response to climate. Fish and Fisheries 2021, in press.
- Krkošek M, Lewis MA, Volpe JP. Transmission dynamics of parasitic sea lice from farm to wild salmon. Proc Royal Soc London B: Biol Sci. 2005;272(1564):689–96.
- Torrissen O, Jones S, Asche F, Guttormsen A, Skilbrei OT, Nilsen F, Horsberg TE, Jackson D. Salmon lice—impact on wild salmonids and salmon aquaculture. J Fish Dis. 2013;36(3):171–94.
- 25. Taranger GL, Karlsen O, Bannister RJ, Glover KA, Husa V, Karlsbakk E, Kvamme BO, Boxaspen KK, Bjorn PA, Finstad B, et al. Risk assessment of the environmental impact of Norwegian Atlantic salmon farming. ICES J Mar Sci. 2015;72(3):997–1021.
- Jonsson B. Life history patterns of freshwater resident and sea-run migrant brown trout in Norway. T Am Fish Soc. 1985;114(2):182–94.
- Palstra FP, Ruzzante DE. Genetic estimates of contemporary effective population size: what can they tell us about the importance of genetic stochasticity for wild population persistence? Mol Ecol. 2008;17(15):3428–47.
- Bentzen P, Olsen J, McLean J, Seamons T, Quinn T. Kinship analysis of Pacific salmon: insights into mating, homing, and timing of reproduction. J Hered. 2001;92(2):127–36.
- Fraser DJ, Duchesne P, Bernatchez L. Migratory charr schools exhibit population and kin associations beyond juvenile stages. Mol Ecol. 2005;14(10):3133–46.
- Killen SS, Marras S, Nadler L, Domenici P. The role of physiological traits in assortment among and within fish shoals. Philos Trans R Soc B. 2017;372(1727):20160233.
- 31. Elliott JM. Quantitative ecology and the brown trout. Oxford: Oxford University Press; 1994.
- Archer LC, Hutton SA, Harman L, McCormick SD, O'Grady MN, Kerry JP, Poole WR, Gargan P, McGinnity P, Reed TE. Food and temperature stressors have opposing effects in determining flexible migration decisions in brown trout (Salmo trutta). Global Change Biol. 2020;26(5):2878–96.
- 33. Archer LC, Hutton SA, Harman L, O'Grady MN, Kerry JP, Poole WR, Gargans P, McGinnity P, Reed TE. The interplay between extrinsic and intrinsic factors in determining migration decisions in brown trout (*Salmo trutta*): an experimental study. Front Ecol Evol 2019; 7.
- Nevoux M, Finstad B, Davidsen JG, Finlay R, Josset Q, Poole R, Hojesjo J, Aarestrup K, Persson L, Tolyanen O, et al. Environmental influences of life history strategies in partial anadromous brown trout (*Salmo trutta*, Salmonidae). Fish Fish. 2019;20(6):1051–82.
- Lemopoulos A, Uusi-Heikkila S, Huusko A, Vasemagi A, Vainikka A. Comparison of Migratory and resident populations of brown trout reveals candidate genes for migration tendency. Genome Biol Evol. 2018;10(6):1493–503.
- Lemopoulos A, Uusi-Heikkila S, Hyvarinen P, Alioravainen N, Prokkola JM, Elvidge CK, Vasemagi A, Vainikka A. Association mapping based on a common-garden migration experiment reveals candidate genes for migration tendency in brown trout. G3-Genes Genom Genet. 2019;9(9):2887–96.
- Bekkevold D, Hansen MM, Mensberg KLD. Genetic detection of sex-specific dispersal in historical and contemporary populations of anadromous brown trout *Salmo trutta*. Mol Ecol. 2004;13(6):1707–12.
- Klemetsen A, Amundsen PA, Dempson J, Jonsson B, Jonsson N, O'connell M, Mortensen E. Atlantic salmon Salmo salar L., brown

trout Salmo trutta L. and Arctic charr Salvelinus alpinus (L.): a review of aspects of their life histories. Ecol Freshw Fish. 2003;12(1):1–59.

- Cucherousset J, Ombredane D, Charles K, Marchand F, Baglinière J-L. A continuum of life history tactics in a brown trout (*Salmo trutta*) population. Can J Fish Aquat Sci. 2005;62(7):1600–10.
- Glover KA, Solberg MF, McGinnity P, Hindar K, Verspoor E, Coulson MW, Hansen MM, Araki H, Skaala O, Svasand T. Half a century of genetic interaction between farmed and wild Atlantic salmon: status of knowledge and unanswered questions. Fish Fish. 2017;18(5):890–927.
- Forseth T, Barlaup BT, Finstad B, Fiske P, Gjoaester H, Falkegard M, Hindar A, Mo TA, Rikardsen AH, Thorstad EB, et al. The major threats to Atlantic salmon in Norway. ICES J Mar Sci. 2017;74(6):1496–513.
- Thorstad EB, Finstad B. Impacts of salmon lice emanating from salmon farms on wild Atlantic salmon and sea trout. NINA Report. 2018;1449:1–22.
- Skaala Ø, Kålås S, Borgstrøm R. Evidence of salmon lice-induced mortality of anadromous brown trout (*Salmo trutta*) in the Hardangerfjord, Norway. Marine Biol Res. 2014;10(3):279–88.
- Johnsen IA, Stien LH, Sandvik AD, Asplin L, Oppedal F. Optimal estimation of lice release from aquaculture based on ambient temperatures. Aquacult Env Interac. 2020;12:179–91.
- Harvey AC, Quintela M, Glover KA, Karlsen O, Nilsen R, Skaala O, Saegrov H, Kalas S, Knutar S, Wennevik V. Inferring Atlantic salmon post-smolt migration patterns using genetic assignment. Roy Soc Open Sci. 2019;6(10):190426.
- 46. Fjørtoft HB, Nilsen F, Besnier F, Stene A, Bjorn PA, Tveten AK, Aspehaug VT, Finstad B, Glover KA. Salmon lice sampled from wild Atlantic salmon and sea trout throughout Norway display high frequencies of the genotype associated with pyrethroid resistance. Aquacult Env Interac. 2019;11:459–68.
- Thorstad EB, Todd CD, Uglem I, Bjørn PA, Gargan PG, Vollset KW, Halttunen E, Kålås S, Berg M, Finstad B. Marine life of the sea trout. Mar Biol. 2016;163(3):47.
- Glover KA, Skaala O, Nilsen F, Olsen R, Teale AJ, Taggart JB. Differing susceptibility of anadromous brown trout (*Salmo trutta* L.) populations to salmon louse (Lepeophtheirus salmonis (Kroyer, 1837)) infection. ICES J Mar Sci. 2003;60(5):1139–48.
- 49. Skaala Ø. A summary of 20 years (1998–2017) of scientific work on genetics and survival in anadromous brown trout (*Salmo trutta* L) and Atlantic salmon (*S. salar* L) in the river Guddalselva western Norway. Fisken Og Havet. 2017;5:46.
- Dodson JJ, Aubin-Horth N, Thériault V, Páez DJ. The evolutionary ecology of alternative migratory tactics in salmonid fishes. Biol Rev. 2013;88(3):602–25.
- Halttunen E, Gjelland KØ, Hamel S, Serra-Llinares RM, Nilsen R, Arechavala-Lopez P, Skarðhamar J, Johnsen IA, Asplin L, Karlsen Ø. Sea trout adapt their migratory behaviour in response to high salmon lice concentrations. J Fish Dis. 2017;00:1–15.
- Fjørtoft HB, Besnier F, Stene A, Nilsen F, Bjørn PA, Tveten A-K, Finstad B, Aspehaug V, Glover KA. The Phe362Tyr mutation conveying resistance to organophosphates occurs in high frequencies in salmon lice collected from wild salmon and trout. Sci Rep-Uk. 2017;7(1):14258.
- 53. Wolf P. A trap for the capture of fish and other organisms moving downstream. T Am Fish Soc. 1951;80(1):41–5.
- Gibbons JW, Andrews KM. PIT tagging: simple technology at its best. Bioscience. 2004;54(5):447–54.
- Eisbrenner WD, Botwright N, Cook M, Davidson EA, Dominik S, Elliott NG, Henshall J, Jones SL, Kube PD, Lubieniecki KP. Evidence for multiple sex-determining loci in Tasmanian Atlantic salmon (*Salmo salar*). Heredity. 2014;113(1):86.
- Yano A, Nicol B, Jouanno E, Quillet E, Fostier A, Guyomard R, Guiguen Y. The sexually dimorphic on the Y-chromosome gene (sdY) is a conserved male-specific Y-chromosome sequence in many salmonids. Evol Appl. 2013;6(3):486–96.
- Ayllon F, Solberg MF, Besnier F, Fjelldal PG, Hansen TJ, Wargelius A, Edvardsen RB, Glover KA. Autosomal sdY pseudogenes explain discordances between phenotypic sex and DNA marker for sex identification in Atlantic salmon. Front Genet. 2020;11:544207.
- Quéméré E, Perrier C, Besnard A-L, Evanno G, Baglinière J-L, Guiguen Y, Launey S. An improved PCR-based method for faster sex determination

in brown trout (*Salmo trutta*) and Atlantic salmon (*Salmo salar*). Conserv Genet Resour. 2014;6(4):825–7.

- Hansen MM, Skaala O, Jensen LF, Bekkevold D, Mensberg KLD. Gene flow, effective population size and selection at major histocompatibility complex genes: brown trout in the Hardanger Fjord, Norway. Mol Ecol. 2007;16(7):1413–25.
- Peakall R, Smouse P. GenAlEx 6.5: genetic analysis in Excel. Population genetic software for teaching and researchd-an update. Bioinformatics. 2012;28:2537–9.
- Keenan K, McGinnity P, Cross TF, Crozier WW, Prodöhl PA. diveRsity: an R package for the estimation and exploration of population genetics parameters and their associated errors. Methods Ecol Evol. 2013;4(8):782–8.
- 62. RCoreTeam: R: A language and environment for statistical computing. R foundation for Statistical Computing, Vienna. 2018.
- Rousset F. GENEPOP'007: a complete re-implementation of the genepop software for Windows and Linux. Mol Ecol Resour. 2008;8(1):103–6.
- 64. Waples RS. Testing for Hardy-Weinberg proportions: have we lost the plot? J Hered. 2014;106(1):1–19.
- Benjamini Y, Hochberg Y. Controlling the false discovery rate: a practical and powerful approach to multiple testing. J Roy Stat Soc: Ser B (Methodol). 1995;57:289–300.
- Carvajal-Rodriguez A. Myriads: P-value-based multiple testing correction. Bioinformatics. 2017;1:3.
- Do C, Waples RS, Peel D, Macbeth G, Tillett BJ, Ovenden JR. NeEstimator v2: re-implementation of software for the estimation of contemporary effective population size (Ne) from genetic data. Mol Ecol Resour. 2014;14(1):209–14.
- Ferchaud AL, Perrier C, April J, Hernandez C, Dionne M, Bernatchez L. Making sense of the relationships between Ne, Nb and Nc towards defining conservation thresholds in Atlantic salmon (*Salmo salar*). Heredity (Edinb). 2016;117(4):268–78.
- Waples RS. A generalized approach for estimating effective population size from temporal changes in allele frequency. Genetics. 1989;121(2):379–91.
- Waples RS. Genetic estimates of contemporary effective population size: to what time periods do the estimates apply? Mol Ecol. 2005;14(11):3335–52.
- Jones OR, Wang J. COLONY: a program for parentage and sibship inference from multilocus genotype data. Mol Ecol Resour. 2010;10(3):551–5.
- Økland F, Jonsson B, Jensen A, Hansen L. Is there a threshold size regulating seaward migration of brown trout and Atlantic salmon? J Fish Biol. 1993;42(4):541–50.
- 73. Adamack AT, Gruber B. PopGenReport: simplifying basic population genetic analyses in R. Methods Ecol Evol. 2014;5(4):384–7.
- 74. Wang JL. Estimating genotyping errors from genotype and reconstructed pedigree data. Methods Ecol Evol. 2018;9(1):109–20.
- L'Abee-Lund JH, Jensen AJ, Johnsen BO. Interpopulation variation in male parr maturation of anadromous brown trout (*Salmo trutta*) in Norway. Can J Zool. 1990;68(9):1983–7.
- Dellefors C, Faremo U. Early sexual maturation in males of wild sea trout, Salmo trutta L, inhibits smoltification. J Fish Biol. 1988;33(5):741–9.
- Birnie-Gauvin K, Thorstad EB, Aarestrup K. Overlooked aspects of the Salmo salar and Salmo trutta lifecycles. Rev Fish Biol Fisher. 2019;29(4):749–66.
- 78. Dray S, Dufour A-B. The ade4 package: implementing the duality diagram for ecologists. J Stat Softw. 2007;22(4):1–20.
- 79. Piry S, Alapetite A, Cornuet J-M, Paetkau D, Baudouin L, Estoup A. GENE-CLASS2: a software for genetic assignment and first-generation migrant detection. J Hered. 2004;95(6):536–9.
- Benjamini Y, Hochberg Y. Controlling the false discovery rate: a practical and powerful approach to multiple testing. J Royal Stat Soc Series B (Methodological). 1995;57:289–300.
- Wang J. Estimation of effective population sizes from data on genetic markers. Phil Trans Royal Soc B: Biol Sci. 2005;360(1459):1395–409.
- Hansen MM, Ruzzante DE, Nielsen EE, Bekkevold D, Mensberg KLD. Long-term effective population sizes, temporal stability of genetic composition and potential for local adaptation in anadromous brown trout (*Salmo trutta*) populations. Mol Ecol. 2002;11(12):2523–35.

- Hansen MM, Fraser DJ, Meier K, Mensberg KLD. Sixty years of anthropogenic pressure: a spatio-temporal genetic analysis of brown trout populations subject to stocking and population declines. Mol Ecol. 2009;18(12):2549–62.
- Hansen MM, Limborg MT, Ferchaud AL, Pujolar JM. The effects of Medieval dams on genetic divergence and demographic history in brown trout populations. BMC Evol Biol. 2014;14:122.
- Charlier J, Palme A, Laikre L, Andersson J, Ryman N. Census (NC) and genetically effective (Ne) population size in a lake-resident population of brown trout Salmo trutta. J Fish Biol. 2011;79(7):2074–82.
- Serbezov D, Jorde PE, Bernatchez L, Olsen EM, Vollestad LA. Short-term genetic changes: evaluating effective population size estimates in a comprehensively described brown trout (*Salmo trutta*) population. Genetics. 2012;191(2):579–92.
- 87. Jorde PE, Ryman N. Demographic genetics of brown trout (*Salmo trutta*) and estimation of effective population size from temporal change of allele frequencies. Genetics. 1996;143(3):1369–81.
- Charlier J, Laikre L, Ryman N. Genetic monitoring reveals temporal stability over 30 years in a small, lake-resident brown trout population. Heredity. 2012;109(4):246–53.
- Franklin IR. Evolutionary change in small populations. In: Soulé ME, Wilcox BA, editors. Conservation biology: an evolutionary-ecological perspective. Sunderland: Sinauer; 1980. p. 135–49.
- Frankham R, Bradshaw CJ, Brook BW. Genetics in conservation management: revised recommendations for the 50/500 rules, Red List criteria and population viability analyses. Biol Conserv. 2014;170:56–63.
- 91. Franklin IR, Frankham R. How large must populations be to retain evolutionary potential? Anim Conserv. 1998;1(1):69–70.
- 92. Reed DH, Briscoe DA, Frankham R. Inbreeding and extinction: the effect of environmental stress and lineage. Conserv Genet. 2002;3(3):301–7.
- Bekkevold D, Hojesjo J, Nielsen EE, Aldven D, Als TD, Sodeland M, Kent MP, Lien S, Hansen MM. Northern European Salmo trutta (L.) populations are genetically divergent across geographical regions and environmental gradients. Evol Appl. 2020;13(2):400–16.
- Fraser DJ, Hansen MM, Østergaard S, Tessier N, Legault M, Bernatchez L. Comparative estimation of effective population sizes and temporal gene flow in two contrasting population systems. Mol Ecol. 2007;16(18):3866–89.
- Griffiths SW, Armstrong JD. Kin-biased territory overlap and food sharing among Atlantic salmon juveniles. J Anim Ecol. 2002;71(3):480–6.
- Olsén KH, Petersson E, Ragnarsson B, Lundqvist H, Järvi T. Downstream migration in Atlantic salmon (*Salmo salar*) smolt sibling groups. Can J Fish Aquat Sci. 2004;61(3):328–31.
- 97. Hoare D, Krause J, Peuhkuri N, Godin JG. Body size and shoaling in fish. J Fish Biol. 2000;57(6):1351–66.
- Killen SS, Marras S, Nadler L, Domenici P. The role of physiological traits in assortment among and within fish shoals. Phil Trans R Soc B. 2017;372(1727):20160233.

- Carlsson J, Carlsson JEL, Olsen KH, Hansen MM, Eriksson T, Nilsson J. Kin-biased distribution in brown trout: an effect of redd location or kin recognition? Heredity. 2004;92(2):53–60.
- Bohlin T, Dellefors C, Faremo U. Date of smolt migration depends on body-size but not age in wild sea-run brown trout. J Fish Biol. 1996;49(1):157–64.
- Griffiths SW, Brockmark S, Höjesjö J, Johnsson J. Coping with divided attention: the advantage of familiarity. Proc Royal Soc B: Biol Sci. 2004;271(1540):695.
- 102. Skaala O, Besnier F, Borgstrom R, Barlaup B, Sorvik AG, Normann E, Ostebo BI, Hansen MM, Glover KA. An extensive common-garden study with domesticated and wild Atlantic salmon in the wild reveals impact on smolt production and shifts in fitness traits. Evol Appl. 2019;12(5):1001–16.
- Fernandes WPA, Ibbotson AT, Griffiths SW, Maxwell DL, Davison PI, Riley WD. Does relatedness influence migratory timing and behaviour in Atlantic salmon smolts? Anim Behav. 2015;106:191–9.
- Meli A, Fraser DJ. Kinship analysis of brook trout Salvelinus fontinalis during their breeding migration. J Fish Biol. 2013;82(5):1514–22.
- Martinez J, Moran P, Perez J, De Gaudemar B, Beall E, Garcia-Vazquez E. Multiple paternity increases effective size of southern Atlantic salmon populations. Mol Ecol. 2000;9(3):293–8.
- Goodwin JC, Andrew King R, Iwan Jones J, Ibbotson A, Stevens JR. A small number of anadromous females drive reproduction in a brown trout (*Salmo trutta*) population in an English chalk stream. Freshwater Biol. 2016;61(7):1075–89.
- Pulido F. Evolutionary genetics of partial migration-the threshold model of migration revis (it) ed. Oikos. 2011;120(12):1776–83.
- Curry RA, Bernatchez L, Whoriskey F, Audet C. The origins and persistence of anadromy in brook charr. Rev Fish Biol Fisher. 2010;20(4):557–70.
- Holecek DE, Scarnecchia DL, Miller SE. Smoltification in an impounded, adfluvial redband trout population upstream from an impassable dam: does it persist? T Am Fish Soc. 2012;141(1):68–75.
- 110. Quinn TP, Bond MH, Brenkman SJ, Paradis R, Peters RJ. Re-awakening dormant life history variation: stable isotopes indicate anadromy in bull trout following dam removal on the Elwha River, Washington. Environ Biol Fish. 2017;100(12):1659–71.

Publisher's Note

Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.

Ready to submit your research? Choose BMC and benefit from:

- fast, convenient online submission
- thorough peer review by experienced researchers in your field
- rapid publication on acceptance
- support for research data, including large and complex data types
- gold Open Access which fosters wider collaboration and increased citations
- maximum visibility for your research: over 100M website views per year

At BMC, research is always in progress.

Learn more biomedcentral.com/submissions

