Anxiété et manipulation parasitaire chez un invertébré aquatique : approches évolutive et mécanistique

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ANXIETE ET MANIPULATION PARASITAIRE CHEZ UN INVERTEBRE AQUATIQUE : APPROCHES EVOLUTIVE ET MECANISTIQUE

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Mots clés : acanthocéphale, amphipode, comportement, état émotionnel, manipulation parasitaire, prédation

Les parasites à transmission trophique sont connus pour les changements phénotypiques qu'ils induisent chez leurs hôtes. Ces changements sont supposés favoriser la transmission des parasites vers un hôte définitif à travers la prédation de l'hôte intermédiaire. Ce phénomène de « manipulation parasitaire » a longtemps été vu comme un trait adaptatif des parasites. La manipulation reposant sur des interactions proie-prédateur, il est nécessaire de comprendre comment les comportements antiprédateur sont modulés par des facteurs exogènes (pression de prédation) et endogènes (infection parasitaire, état émotionnel, …) aux individus. Au cours de cette thèse, nous avons tenté d'approfondir la compréhension de ce phénomène, chez les crustacés amphipodes, en répondant à plusieurs questions : (1) quelle est l'étendue de la multidimensionnalité de la manipulation parasitaire par les Acanthocéphales, quantifiée au travers d'une méta-analyse ; (2) l'amplitude des changements de comportements antiprédateur varie-t-elle selon le contexte local de prédation ? ; (3) les comportements antiprédateur présentent-ils une flexibilité en lien avec le contexte local de prédation ? ; (4) le parasite "exploite" t-il la flexibilité comportementale des gammares sains, notamment en lien avec leur régulation émotionnelle de type anxiété ?. Au niveau interspécifique à l'échelle du phylum des Acanthocéphales, nous avons mis en évidence une altération marquée des comportements en lien avec la défense antiprédateur des hôtes (changement de microhabitat, protection et réponse à des stimuli). Au niveau du couple hôte-parasite *Gammarus fossarum, Pomphorhynchus tereticollis*, nous avons montré que l'intensité des changements de comportements antiprédateur induit par l'infection (phototaxie et utilisation du refuge) présentait une variabilité interpopulationnelle, en lien avec le risque de prédation : la manipulation semble d'autant plus forte que la pression de prédation locale, i.e. les opportunités de transmission, est faible. Chez les individus sains, nous avons mis en évidence, par une approche corrélationnelle, une variabilité interpopulationnelle de l'intensité des comportements antiprédateur en lien avec le risque local de prédation, et la densité de gammares conspécifiques. Nous avons également montré une flexibilité de la réponse au stimulus de prédation chez des individus provenant de populations où le risque de prédation était élevé, la réponse augmentant avec l'intensité du stimulus. En revanche, les individus provenant de populations à faible risque de prédation semblent montrer une réponse relativement forte indépendamment de l'intensité du stimulus, ce qui suggère une hypersensibilité. Ces études corrélationnelles, portant sur l'analyse de la variabilité interpopulationnelle selon les pressions de prédation locales, nous ont amenés à supposer que ces différentes stratégies seraient intimement liées à l'expérience d'un stress chronique de la prédation. Nous suggérons alors l'existence d'un état de long-terme de type anxiété qui pourrait être la résultante de la répétition d'épisodes de court-terme de peur. Nous avons effectué un premier pas en montrant expérimentalement l'existence de comportements de peur et de type anxiété chez *G. fossarum*. L'ensemble de ces travaux a démontré la place centrale des interactions proie-prédateur dans l'étude de la manipulation parasitaire. Plus précisément, nous avons mis en évidence une variabilité et une modulation complexe des comportements antiprédateur des hôtes en lien avec le contexte local de prédation, et qui pourraient trouver racine dans un état émotionnel lié au stress chronique de la prédation. Ces travaux ouvrent alors quelques pistes à investiguer telles que les mécanismes sous-jacents de cet état et l'éventuel rôle des parasites dans la modulation de cet état de type anxiété qui viendrait modifier l'expression des comportements antiprédateur.

Title : Anxiety and parasite manipulation in an aquatic invertebrate : evolutive and mechanistic approaches

Keywords : acanthocephala, amphipoda, behaviour, emotional state, parasite manipulation, predation

Trophically transmitted parasites induce changes in their host's phenotype. These changes are supposed to increase transmission probability to definitive hosts through the predation of intermediate hosts. This phenomenon is known as 'parasite manipulation' has been hypothesized to be an adaptive trait of parasites for a long time. As manipulation involves predator-prey interactions, it is therefore necessary to understand how antipredatory behaviours are modulated by exogenous (predation pressure) and endogenous (infection, emotional state) factors. We tried to go into this phenomenon in depth, in amphipods, by responding to several questions : (1) what is the extent of the multidimensionality in parasite manipulation by Acanthocephalan, quantified through a meta-analysis ; (2) is there variation in the magnitude of antipredatory behaviours according to local predation risk? (3) are antipredatory behaviours flexible with respect to local predation risk? (4) Do parasites exploit behavioural flexibility in uninfected individuals, in relation to an emotional state such as anxiety-like state? Within the Acanthocephala phylum, we evidenced notable changes, more particularly of host antipredatory behaviours (microhabitat changes, protection and responses to stimuli). Considering *Gammarus fossarum, Pomphorhynchus tereticollis* host-parasite couple, we showed that there was variation in the magnitude of antipredatory behavioural changes induced by infection (phototaxy and refuge use), with respect to local predation risk : host manipulation seemed as strong as predation risk, i.e. transmission opportunities, is low. With a correlational approach, we also evidenced variation in the magnitude of antipredatory behaviours according to local predation risk and conspecifics density, in uninfected individuals. In addition, we emphasized flexibility of behavioural responses to predator cues: individuals from populations experiencing high predation risk exhibited increased responses as predator cues concentrations increased. In contrast, individuals from populations experiencing low predation risk appeared to exhibit strong responses independent of predator cues concentrations, suggesting hypersensitivity. We supposed that these strategies would be closely related to the exposure to chronic predation risk. We therefore suggest the existence of an anxiety-like state that could result from the repetition of acute stress, i.e. fear, episodes. We made a first step through an experimental approach, evidencing the existence of both anxiety-like and fear behaviours in *G. fossarum*. Overall, these works pointed out the importance of predator-prey interactions in the study of parasite manipulation. More particularly, we evidenced variation and complex modulation of host antipredatory behaviours in accordance with local predation risk, and which may be closely related to an emotional state stemming from chronic predation stress. We therefore suggest some future directions to investigate, such as the underlying mechanisms of anxiety-like state, and the role of parasite in the modulation of this state that would modify the expression of antipredatory behaviours.

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TABLE DES MATIERES

INTRODUCTION GENERALE

Contexte de la manipulation parasitaire

Et si on vous disait que les parasites représentent environ la moitié des espèces présents sur Terre (Poulin & Morand, 2000) et que chaque organisme vivant, plante ou animal, héberge un ou plusieurs parasites (May & Anderson, 1990) ? Les parasites sont bien plus nombreux, en densité, sur Terre que les organismes non parasites (Windsor, 1998), mais qu'entend-on par « parasites » ? Les parasites renvoient généralement une image sanitaire relative chez l'homme à la pathologie et la mort (Dobson & Carper, 1996 ; Thornhill, Fincher, & Aran, 2009). Cependant, le terme « parasite » ne fait en réalité pas seulement référence aux bien connus vers ou tiques, dont tout le monde connaît les conséquences néfastes sur l'animal porteur, humain ou non. Malgré les attentes, le parasite, au sens large du terme, représente tout organisme qui se sert d'un autre organisme vivant (pour se nourrir par exemple) (Price, 1980). Ainsi, il ne serait pas étonnant de qualifier de parasites les petits de la femelle coucou, *Cuculus canorus*, que cette dernière pond dans les nids d'autres oiseaux, afin qu'ils soient à la charge de la mère « adoptive » (Davies, Kilner, & Noble, 1998). Les spécialistes de la parasitologie adoptent cependant une définition plus stricte selon laquelle un parasite est un organisme vivant dans (endoparasite) ou sur (ectoparasite) un autre organisme qualifié d'hôte, cette interaction ayant des conséquences négatives sur le phénotype de ce dernier (Poulin, 2011). Tout aussi important que l'aspect préjudiciable de cette interaction sur l'hôte, c'est son caractère à long terme (Combes, 2001), certains parasites pouvant vivre à l'intérieur de leur hôte pendant plusieurs années (Chauvin *et al.*, 2009). Plus surprenant encore, les parasites recouvrent une diversité de taxons remarquable, des virus et bactéries aux invertébrés comme les helminthes (vers), mollusques (bivalves et gastéropodes) ou encore arthropodes (arachnides, crustacés et insectes) (May & Anderson, 1990 ; Poulin & Morand, 2000). Même si cette nouvelle définition tend à diminuer le nombre estimé de parasites, *sensu stricto*, existant, celui-ci reste néanmoins sans le

moindre doute impressionnant étant donné que nous ne pourrons jamais répertorier tous les organismes vivant sur Terre.

Les parasites ont un rôle écologique à ne pas négliger. En effet, le phénotype d'hôtes altéré par l'infection est susceptible d'avoir un impact sur la dynamique et l'écologie de la population d'hôtes et par conséquent, sur le réseau trophique du milieu (Lefèvre *et al.*, 2009). Par exemple, l'infection par un parasite impacte généralement, avec très peu d'exceptions, la survie et/ou la reproduction des individus d'une population (Tompkins, Greenman, & Hudson, 2001 ; Thomas *et al.*, 1995). Les parasites peuvent également fortement moduler la compétition interspécifique (Tompkins *et al.,* 2001). Par exemple, *Gammarus pulex* (espèce native) et *G.roeseli* (invasive) sont deux espèces de gammares (Crustacés : Amphipodes) vivant en sympatrie, avec un avantage compétitif pour *G.roeseli*. Bauer *et al.*, (2000) a montré que seule l'espèce native était impactée par le parasite *P.laevis*, avec une photophobie (comportement antiprédateur naturel) inversée pour les individus infectés. En comparant ces deux mêmes espèces en milieu naturel, Lagrue *et al.*, (2007) a montré une mortalité liée à la prédation augmentée pour les individus natifs infectés comparés aux individus sains de la même espèce et aux individus invasifs quel que soit leur statut infectieux. Les parasites sont alors capables d'influencer la structure locale des communautés à travers leur impact sur les interactions biotiques, comme les relations proies-prédateurs (Lafferty *et al.*, 2008). C'est principalement pour cette place dans les relations proies-prédateurs et les éventuelles conséquences qui y sont associées sur le plan écologique, que les parasites à transmission trophique par exemple, sont les plus étudiés (Lafferty & Kuris, 2012).

Au cours de l'évolution, le cycle de vie des parasites subit des changements dans sa complexité (Poulin, 2011). Tous les parasites n'ont pas un mode de vie similaire et présentent

une grande diversité de cycles (Poulin, 2011). Même si la transmission est primordiale, les étapes qui y contribuent et ce qu'elles impliquent peuvent varier. Certaines variations dans la complexité des cycles (ajout ou suppression d'un hôte par exemple), qu'elles arrivent accidentellement ou par pressions de sélection, vont permettre l'augmentation de l'efficacité de transmission des parasites (Poulin, 2011). D'une façon générale, on observe une augmentation de la complexité des cycles avec l'ajout d'un second hôte dans des cycles initialement simples (Parker *et al.*, 2003 ; Poulin, 2011). Le cycle de vie est maintenant qualifié de complexe. Pour qu'un cycle de vie simple évolue en un cycle complexe, deux voies sont possibles. La première fait appel à l'incorporation d'un hôte qui se trouve être une proie régulière de l'hôte de base du parasite et qui ingèrera les œufs du parasite dans le milieu (Choisy *et al.*, 2003 ; Parker *et al.*, 2003 ; Poulin, 2011). Dans un système proie-prédateur, l'abondance de proies étant supérieure à celle du prédateur, le parasite à son stade larvaire libre aura plus de chance de rencontrer une proie dans laquelle se développer avant d'atteindre l'hôte définitif (Lafferty & Kuris, 2012). La seconde voie intègre un hôte qui est alors prédateur de l'hôte déjà présent dans le cycle et qui devient intermédiaire (Parker *et al.*, 2003 ; Poulin, 2011). Si l'hôte intermédiaire est régulièrement prédaté par le nouvel hôte, les parasites qui réussiront à se reproduire dans ce dernier, qualifié de définitif, seront alors avantagés. Lors d'un tel cycle de vie, la transmission du parasite de l'hôte intermédiaire vers le définitif repose alors sur la relation proie-prédateur entre ces deux hôtes (Médoc & Beisel, 2011). Ainsi ces parasites à cycle complexe sont plus communément appelés parasites à transmission trophique (Fig.1).

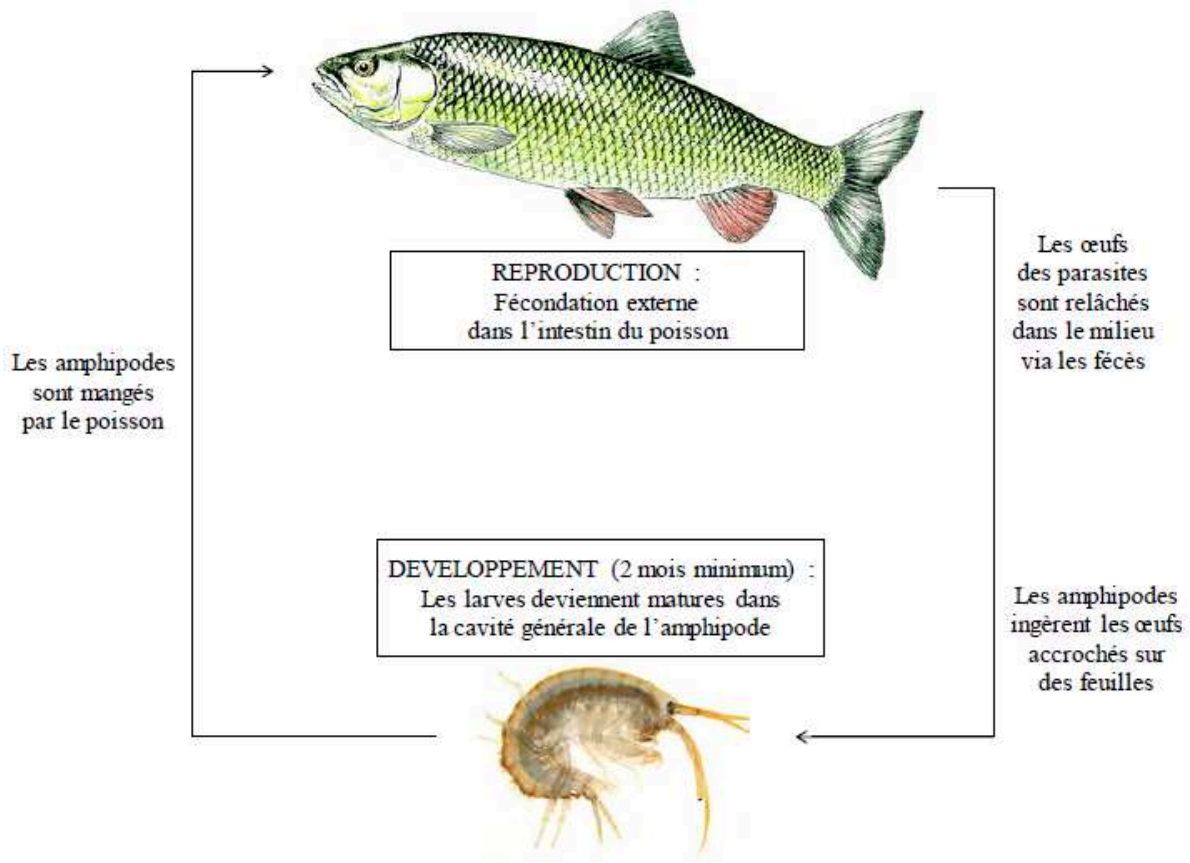

Fig.1 Description du cycle de vie d'un parasite à transmission trophique (complexe) incluant un hôte intermédiaire (amphipode : gammare) et un hôte définitif (poisson : chevesne) dans lesquels le parasite effectue les deux étapes de son cycle (développement et reproduction), respectivement.

S'ils sont beaucoup étudiés, c'est parce que les parasites à transmission trophique sont responsables de changements phénotypiques, notamment ciblés sur le comportement ou l'apparence, relativement bien visibles chez leur hôte intermédiaire (Lafferty, 1999). Ces changements phénotypiques sont censés être bénéfiques au parasite, le bénéfice résidant en la réussite de la transmission vers l'hôte définitif dans lequel le cycle pourra s'achever via la reproduction (Médoc & Beisel, 2011 ; Lafferty & Kuris, 2012). La manipulation parasitaire a longtemps été perçue comme un trait adaptatif chez ces parasites, permettant d'augmenter la probabilité de prédation de l'hôte intermédiaire par l'hôte définitif (Holmes & Bethel, 1972 ; Thomas, Adamo, & Moore, 2005). Cependant, de nombreuses critiques ont récemment mis en doute cette hypothèse finaliste (voir Cézilly *et al.*, 2010), certains changements phénotypiques

pouvant émerger en tant qu'effets secondaires de l'infection (Thomas *et al.,* 2005). De plus, le lien de causalité directe entre changement phénotypique et probabilité de prédation n'a encore jamais été établi (Cézilly *et al.*, 2010). Le caractère adaptatif de la manipulation est encore aujourd'hui un sujet de débat. Les mécanismes impliqués dans ces changements phénotypiques, bien que difficiles à comprendre, méritent que l'on y consacre encore plus de temps ne serait-ce que pour estimer les coûts associés à la manipulation pour le parasite (Thomas *et al.,* 2005). Cependant, les mécanismes mêmes élucidés ne permettraient sans doute pas de trancher entre caractère adaptatif ou secondaire de la manipulation.

Le comportement : dimension phénotypique la plus étudiée, aussi la plus impactée ?

La manipulation parasitaire est reconnue pour être multi-dimensionnelle (Thomas, Poulin, & Brodeur, 2010 ; Cézilly, Favrat, & Perrot-Minnot, 2013), pouvant toucher le comportement, la morphologie, la reproduction ou la physiologie de l'hôte. Cependant, la dimension comportementale est depuis longtemps, celle qui est la plus étudiée, loin devant les autres. Plusieurs raisons, qu'elles soient d'ordre écologique ou non, peuvent expliquer cette tendance. Premièrement, les interactions proies-prédateurs et plus particulièrement le risque de prédation est dépendant du comportement en général et davantage des comportements antiprédateur des proies (Lima & Dill, 1990 ; Brown *et al.*, 2006). Deuxièmement, la première réponse, et la plus rapide surtout, qu'exprime un individu face à un changement environnemental est de nature comportementale, via une modification ajustée du comportement à la situation (Nagelkerken & Munday, 2016). Ainsi, les changements de comportement sont supposés être plus facilement observables que tout autre changement phénotypique. Enfin, parmi toutes les dimensions phénotypiques, le comportement est aussi celle qui montre la plus grande plasticité contrairement à la morphologie (West-Eberhard, 1989 ; Price, Qvarnström, & Irwin, 2003), car c'est sur cette plasticité que l'intensité de sélection sera plus forte (Garland & Kelly, 2008). La

plasticité du comportement c'est la capacité qu'aura un génotype donné à produire différents comportements adaptés à diverses situations (Price, Qvarnström, & Irwin, 2003). Cette plasticité joue donc un rôle crucial dans la variation des réponses des individus face aux changements environnementaux (Sih, 2013). De plus, comme pour les traits physiologiques, le comportement est aussi la dimension phénotypique qui présente un haut degré de flexibilité, contrairement à la morphologie dont le changement en une forme peut parfois être irréversible (Pigliucci, 2006). La flexibilité correspond donc à la réversibilité d'un phénotype exprimé, sur un pas de temps relativement court. Autrement dit, la flexibilité comportementale est d'une utilité importante lorsqu'elle permet la modulation rapide d'un comportement de réponse (défenses augmentées ou diminuées) face à l'apparition de nouveaux changements dans un environnement donné (Sih, 2013). Avec une telle capacité de réversibilité, les traits comportementaux sont alors susceptibles d'être plus facilement et rapidement « manipulables » par les parasites. En faisant le lien entre flexibilité du comportement et interactions proie-prédateur, on peut alors supposer que la manipulation concernera davantage les comportements antiprédateur de l'hôte intermédiaire dans le cas de parasites à transmission trophique. Ainsi, le parasite modulerait les comportements de défense de son hôte, défenses elles-mêmes modulées par les conditions locales environnementales.

Récemment, à travers une synthèse quantitative, Fayard *et al.*, (2020) ont apporté la confirmation du constat selon lequel le comportement semble être la dimension la plus impactée par la manipulation. Pour aller encore plus loin, les comportements de réponse à un stimuli (olfactif, visuel, …) semblent être préférentiellement altérés par les parasites acanthocéphales (Moore, 2002 ; Fayard *et al.*, 2020), tout comme les comportements en lien avec le choix de microhabitat (Poulin, 1994 ; Fayard *et al.*, 2020). En effet, le changement de microhabitat

pourrait être une façon privilégiée par ces parasites à transmission trophique d'augmenter le taux de rencontre entre proies infectées et prédateurs (Lafferty & Shaw, 2013).

Modèles biologiques : la pertinence des Acanthocéphales et leurs hôtes intermédiaires, les gammares

Chez les parasites à transmission trophique, les acanthocéphales représentent un modèle d'exception pour l'étude de la manipulation parasitaire (Moore, 2002 ; Bakker, Frommen, & Thünken, 2017). Regroupés en un petit phylum d'environ 1300 espèces, ils sont répartis en quatre classes : les archiacanthocéphales (classe la plus ancienne), eoacanthocéphales, palaeacanthocéphales et polyacanthocéphales (dérivées) (Amin, 2013). Plus de la moitié des espèces décrites et identifiées appartiennent à la classe des palaeacanthocéphales (Kennedy, 2006 ; Amin, 2013), de ce fait largement sur-représentée et donc étudiée (Fayard *et al.*, 2020). Les acanthocéphales constituent un groupe assez homogène, ils présentent une certaine similarité morphologique (tête à crochets), écologique (même cycle de vie) et ont le même mode de reproduction (sexuée). Les archiacanthocéphales sont des parasites à hôte terrestre, principalement rongeurs (Table.1). Les palaeacanthocéphales utilisent des hôtes aquatiques, poissons et oiseaux (Table.1) (Fig.2). Les eocanthocéphales, quant à eux, se retrouvent aussi bien dans des hôtes aquatiques et terrestres (Ribas & Casanova, 2006). Tous endoparasites, ils vivent à l'intérieur du tractus digestif de leurs hôtes définitifs vertébrés (Kennedy, 2006). Tous les acanthocéphales requièrent un hôte intermédiaire invertébré arthropode (Near, 2002), et utilisent la cavité générale de ce dernier pour se développer et atteindre leur maturité sexuelle (Kennedy, 2006). Ils poursuivent leur cycle via la reproduction, dans l'hôte définitif vertébré (poissons, oiseaux, mammifères, amphibiens, …), en s'accrochant à la paroi intestinale de ce dernier (Near, 2002). Après fertilisation des œufs, ces derniers sont relâchés dans l'intestin de l'hôte pour enfin être relargués dans le milieu naturel où ces œufs pourront être ingérés et ainsi infecter de nouveaux hôtes intermédiaires.

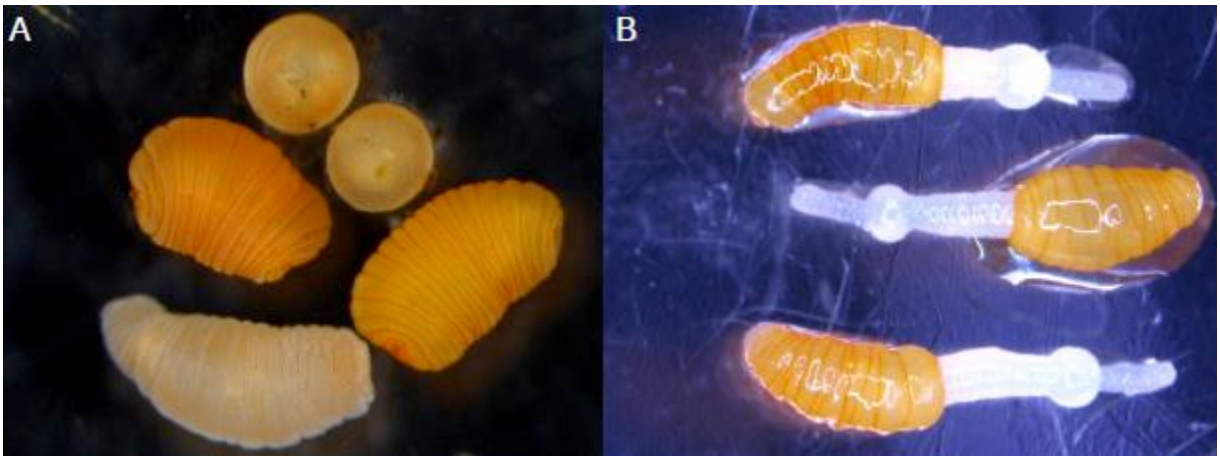

Fig.2 Parasites acanthocéphales de la classe des Palaeacanthocéphales (photos de Sophie Labaude). A) *Pomphorhynchus laevis* rond et lisse et *P. tereticollis* allongé et ridé et, B) *P. tereticollis* avec le proboscis dévaginé.

Table.1 Liste non-exhaustive d'espèces de parasites acanthocéphales et des changements qu'ils induisent dans le phénotype de leur(s) hôte(s) respectif(s).

Sp. acanthocéphale (classe)	Sp. hôte intermédiaire	Hôte définitif	Changement phénotypique	Source
Moniliformis moniliformis (archi-acanthocéphale)	*Periplaneta americana*	Rat	Phototaxie positive	Moore 1983
			Fuite altérée	Libersat & Moore 2000
Acanthocephalus anguillae (palae-acanthocéphale)	*Asellus aquaticus*	Poisson	Phototaxie positive	Lyndon 1996
Acanthocephalus dirus (palae-acanthocéphale)	*Caecidotea intermedius*	Poisson	Couleur altérée (plus claire)	Camp & Huizinga 1979 ; Park & Sparkes 2017
			Utilisation d'un refuge diminuée	Hechtel et al. 1993 ; Kopp et al. 2016
			Appariement pré-copulatoire diminué	Bierbower & Sparkes 2007 ; Caddigan et al. 2014 ; Sparkes et al. 2005 ; Kopp et al. 2016

			Concentrations en dopamine et sérotonine dans le cerveau diminuées	Kopp et al. 2016
			Concentrations en glycogène et lipides dans le corps entier augmentées	Korkofigas et al. 2016
Acanthocephalus lucii (palae-acanthocéphale)	*Asellus aquaticus*	Poisson	Utilisation d'un refuge diminuée	Benesh et al. 2008
			Couleur altérée (abdomen plus sombre)	Benesh et al. 2008
Leptorhynchus thecatus (palae-acanthocéphale)	*Hyalella azteca*	Poisson	Utilisation d'un refuge diminuée	Stone & Moore 2014
			Réaction face à un stimulus de type prédateur altérée	Stone & Moore 2014
Plagiorhynchus cylindraceus (palae-acanthocéphale)	*Armadillidium vulgare*	Oiseau	Utilisation d'un refuge diminuée	Moore 1983
			Faible gain de masse	Moore 1983
Echinorhynchus truttae (palae-acanthocéphale)	*Gammarus pulex*	Poisson	Taux d'approvisionnement augmenté	Laverty et al. 2017
			Phototaxie positive	MacNeil et al. 2003
			Utilisation d'un refuge diminuée	MacNeil et al. 2003
Polymorphus minutus (palae-acanthocéphale)	*Gammarus pulex*	Oiseau	Géotaxie (distribution verticale) inversée	Mariott et al. 1989 ; Bauer et al. 2005 : Tain et al. 2006
			Appariement pré-copulatoire diminué	Bollache et al. 2001, 2002
			Fécondité diminuée	Bollache et al. 2002
			Immunodépression	Rigaud & Moret 2003 ; Cornet et al. 2009
			Réaction face à un stimulus altérée	Mariott et al. 1989

	Gammarus lacustris	Oiseau	Phototaxie positive	Hindsbo 1972
	Echinogammarus berilloni	Oiseau	Géotaxie inversée	Jacquin et al. 2014
			Réaction face à un stimulus de type prédateur altérée	Jacquin et al. 2014
Pomphorhynchus laevis (palae-acanthocéphale)	*Gammarus pulex*	Poisson	Phototaxie positive	Kennedy et al. 1978 ; Perrot-Minnot 2003 ; Tain et al. 2006 ; Tain et al. 2007 ; Cornet et al. 2009 ; Dianne et al. 2010 ; Franceschi et al. 2010a ; Durieux et al. 2012 ; Perrot-Minnot et al. 2014
			Géotaxie inversée	Perrot-Minnot et al. 2014
			Utilisation d'un refuge diminuée	Perrot-Minnot et al. 2014
			Appariement pré-copulatoire diminué	Bollache et al. 2001, 2002
			Fécondité diminuée	Bollache et al. 2002
			Immunodépression	Rigaud & Moret 2003 ; Cornet et al. 2009 ; Cornet & Sorci 2010 ; Cornet 2011
			Agrégation avec des conspécifiques diminuée	Durieux et al. 2012
			Concentration en sérotonine dans le cerveau augmentée	Tain et al. 2007
	Gammarus fossarum	Poisson	Phototaxie positive	Perrot-Minnot et al. 2014
			Géotaxie inversée	Perrot-Minnot et al. 2014
			Utilisation d'un refuge diminuée	Perrot-Minnot et al. 2014
Pomphorhynchus tereticollis (palae-acanthocéphale)	*Gammarus pulex*	Poisson	Phototaxie positive	Tain et al. 2006 ; Cornet et al. 2009 ; Perrot-Minnot et al. 2012
			Immunodépression	Cornet et al. 2009

		Réaction face à un stimulus de type prédateur altérée	Perrot-Minnot et al. 2007
Gammarus fossarum	Poisson	Phototaxie positive	Fayard et al. 2019
		Utilisation d'un refuge diminuée	Fayard et al. 2019

Les acanthocéphales sont d'autant plus reconnus pour être responsables de fascinants et divers changements dans le phénotype de leur hôte intermédiaire (Table.1), tout particulièrement le comportement (Moore, 2002 ; Cézilly *et al.*, 2013 ; Bakker *et al.*, 2017). Ils sont supposés augmenter la probabilité de transmission vers l'hôte définitif. Ainsi, les acanthocéphales sont supposés être de réels "manipulateurs du comportement" jusqu'à modifier un type de comportement relatif au mode de prédation de l'hôte définitif, comme le choix du microhabitat (Lafferty & Shaw, 2013). Par exemple, *Pomphorhynchus laevis* et *tereticollis* qui utilisent certaines espèces de poissons comme hôtes définitifs sont reconnus pour changer la phototaxie de leur hôte intermédiaire *G. pulex* (Tain, Perrot-Minnot, & Cézilly, 2006 ; Perrot-Minnot *et al.*, 2012 ; Perrot-Minnot, Sanchez-Thirion, & Cézilly, 2014). En parallèle, *Polymorphus minutus*, qui nécessite un oiseau d'eau pour achever son cycle de vie, va plutôt altérer la géotaxie de ce même hôte intermédiaire (Bauer *et al.*, 2005 ; Tain *et al.*, 2006). Pour aller encore plus loin, certains cas de « protection » de l'hôte intermédiaire ont été mis en évidence quand les parasites, à un stade dit « non-infectieux » ou « immature », ne sont pas encore « prêts » à être transmis. Ces cas de protection de l'hôte intermédiaire s'expriment par une augmentation des comportements de défenses antiprédateur. Par exemple, des individus *G. pulex* infectés par un stade acanthelle (non-infectieux) du parasite *P.laevis* ont tendance à plus utiliser le refuge que des individus sains (Dianne *et al.*, 2011, 2014). En revanche, quand ils sont infectés par le stade cystacanthe (infectieux) du même parasite, leur utilisation du refuge est diminuée par rapport aux individus sains (Dianne *et al.*, 2011).

Tout ce qu'on observe peut alors laisser penser que la manipulation chez les acanthocéphales possède ce caractère adaptatif selon lequel elle aurait évolué pour permettre aux parasites de maximiser leur transmission. Plus précisément, les changements phénotypiques que l'on peut observer chez les hôtes infectés semblent être relativement bien conformes aux attentes quant aux fonctions supposées de ces changements (comme faciliter la prédation). Cependant, ce 'purposing design' (Poulin, 1995) caractérisant la manipulation parasitaire d'adaptative n'est peut-être qu'apparent. Même si l'idée de la sélection sur les gènes des parasites à cycle complexe conférant la capacité de modifier le phénotype de l'hôte pour la transmission est appréciable, une autre possibilité n'est pas à exclure. En effet, cette capacité d'altérer le phénotype aurait pu être présente chez les parasites avant même l'inclusion d'un deuxième hôte, auquel cas la manipulation ne représenterait plus un trait parasitaire sélectionné pour la transmission, qui est alors inexistante. Ainsi, l'autre scénario possible est donc l'inclusion d'un second hôte comme étant l'adaptation. Prenons le cas d'un cycle simple évoluant en cycle complexe par l'incorporation d'un hôte définitif, l'hôte intermédiaire est alors initialement le seul hôte présent dans le cycle parasitaire. En supposant que l'altération phénotypique vient de l'interaction entre le parasite avec le système neurologique et/ou immunitaire de l'hôte (Adamo, 2002), ce dernier pourrait alors exprimer phénotypiquement cette infection à travers un comportement ou une condition altérée. Pour citer Poulin (2010) personne ne s'attend à ce qu'un animal malade se comporte normalement. Cette condition altérée voire dégradée des individus infectés pourraient les amenés à être exposés à un risque de prédation plus élevé. Ceci aurait créer une pression de sélection sur le parasite pour lequel l'inclusion d'un prédateur régulièrement rencontré come hôte définitif reste la « meilleure solution » et donc la réelle adaptation. Cette discussion sur le caractère adaptatif de la manipulation par les acanthocéphales sera reprise et davantage approfondie dans la première partie du manuscrit.

Les acanthocéphales sont des parasites facilement accessibles sur le terrain. Il est aussi relativement aisé et rapide de procéder à des infestations expérimentales au laboratoire sans besoin de matériels spécifiques. De par l'homogénéité du phylum, leur capacité à "manipuler" leurs hôtes et l'absence de réelles difficultés à travailler avec eux, les acanthocéphales représentent un modèle de prédilection pour l'étude la manipulation parasitaire.

S'il existe un modèle phare chez les parasites, il en est de même pour les hôtes intermédiaires. Les arthropodes présentent une grande diversité d'espèces utilisées par les parasites, non seulement par les acanthocéphales, mais aussi par les cestodes, digènes, nématodes et nématomorphes (Hall, 1929). Les amphipodes (crustacés : amphipodes), représentent un modèle biologique très utilisé pour l'étude de la manipulation par les parasites acanthocéphales car ils sont les hôtes intermédiaires principaux de la classe palaeacanthocéphales (Kennedy, 2006). Ce sont des organismes qui occupent une large gamme de milieux aquatiques (eau douce, marine) (Väinölä *et al.*, 2008) et peuvent tolérer une certaine variation dans plusieurs paramètres physicochimiques. Les amphipodes ont des stratégies alimentaires aussi diverses, ils peuvent être herbivores, carnivores, omnivores ou détritivores et sont les proies de nombreux poissons (Väinölä *et al.*, 2008). En général, les acanthocéphales utilisent donc comme hôtes intermédiaires des espèces qui ont un rôle important dans les réseaux trophiques (Kennedy, 2006). Au sein de l'ordre amphipodes, les gammares (sous-ordre des gammaridés) sont prépondérants avec plus de 4000 espèces et recouvrant plus de 75% des amphipodes (MacNeil, Dick, & Elwood, 1997), sont parmi les organismes les plus étudiés pour la manipulation parasitaire (Fayard *et al.*, 2020). En milieu naturel, ils peuvent souvent représenter la plus grosse fraction de macro-invertébrés que l'on peut échantillonner (MacNeil, Dick, & Elwood, 1997). Les gammares sont la proie de diverses et nombreux prédateurs, tels que les poissons, oiseaux ou mêmes d'autres invertébrés. Même s'ils sont prédateurs d'autres

invertébrés, ils sont davantage reconnus pour décomposer la matière organique du milieu (MacNeil, Dick, & Elwood, 1997) et ainsi être des indicateurs relativement fiables de la bonne qualité de l'eau.

Cette place centrale dans les relations trophiques des milieux aquatiques fait des gammares des hôtes intermédiaires optimaux pour beaucoup d'espèces d'acanthocéphales. Comme pour ces derniers, l'accès à une population de gammares dans leur milieu naturel est relativement aisé, tout comme leur infestation et leur maintenance en laboratoire, ce qui en facilite l'étude.

Objectifs de la thèse

Les comportements de défense antiprédateur semblent avoir un rôle essentiel dans la transmission du parasite vers son hôte définitif. De par leur rôle dans la modulation des interactions proie-prédateur, ils représenteraient une "cible" dans l'évolution de la manipulation. Ainsi la sélection se ferait sur les gènes des parasites permettant la modification des comportements de l'hôte, ce qu'on appelle phénotype étendu. De plus, ces comportements exprimés suite à l'exposition à un stress présenteraient une grande flexibilité (Perrot-Minnot & Cézilly, 2013). Cette flexibilité comportementale pourrait prendre source dans la fluctuation des niveaux de certains neuromodulateurs (Adamo, 2002), tels que la sérotonine, et qui interviennent dans la régulation des états émotionnels. Ainsi les comportements liés à la prise de décision seraient modulés par l'état émotionnel des individus et par les différents stress perçus.

Les prédateurs représentent une source importante de stress (Clinchy *et al.*, 2004 ; Clinchy, Sheriff, & Zanette, 2013 ; Newman *et al.*, 2013). Ils sont perçus à travers des stimuli

(olfactifs, visuels, …) auxquels sont exposés les individus dans l'environnement, et qui vont provoquer chez ces derniers des réponses au niveau physiologique et / ou comportemental (Romero, 2004). Face à un stress relatif à la prédation, la perception, l'évaluation et la réponse sont des étapes cruciales dont dépendent la survie ou le succès reproducteur des individus (Brown, 2003). Ainsi, plus la perception, l'évaluation et la réponse sont affinées, plus la survie ou le succès reproducteur en sera élevé. On s'attend alors à ce que certains traits phénotypiques qui contribueront au mieux à la perception, l'évaluation et la réponse soient sélectionnés. En effet, les comportements de défenses comme la fuite ou l'affrontement (« *fight or flight* ») se révèlent être des réponses généralement exprimées par les individus exposés à un risque de prédation (Ford & Reeves, 2008 ; Sheriff & Thaler, 2014). Ces réponses comportementales sont couplées à des réponses physiologiques (Sheriff & Thaler, 2014). Par exemple, face à un stress l'organisme va subir une décharge de glucose conduisant à une hyperglycémie, possible coût du stress physiologique perçu (Jentoft *et al.*, 2005). Chez la crevette *Litopenaeus vannamei*, la réponse de fuite à un stimulus est associée à une concentration élevée de lactate dans l'hémolymphe (Robles-Romo, Zenteno-Savín, & Racotta, 2016). Ceci traduit une importante libération de glucose résultant de la glycolyse au niveau musculaire afin de soutenir une importante activité musculaire et d'éviter un épuisement de l'adénosine triphosphate (ATP). Ainsi, sur la base d'un rapport coût / bénéfice, la sélection naturelle aurait modelé certains traits phénotypiques maximisant la survie et / ou le succès reproducteur des individus exposés à différents stress. Dès lors qu'un individu est en mouvement dans son environnement pour la prospection de ressources, qu'elles soient alimentaires ou relatives à la reproduction, il est sujet à un certain risque de prédation. Il existe alors un compromis dans l'énergie que l'individu va allouer entre ces fonctions de survie et / ou de reproduction, et des comportements de défenses. Ainsi, les individus les plus efficaces dans leur interaction avec leur environnement, à travers

des comportements d'exploitation et de réponses adéquats, verront leurs chances de survie et donc leur succès reproducteur augmentés.

L'exposition aux prédateurs et/ou à des indices de la présence de prédateurs est susceptible d'induire chez les proies une forme de stress prolongé, en plus du stress topique du moment de rencontre (Clinchy *et al.*, 2013). Par exemple, un individu qui fait directement face à un prédateur sur un pas de temps très court va, pour la plupart du temps, fuir. Cette réponse comportementale est brève et traduit un état de peur (**Box**) chez l'individu. Cependant, un stress prolongé pourrait être accompagné de modifications sur le long terme. Qu'elles soient comportementales, physiologiques en lien avec l'approvisionnement ou la reproduction, ces modifications sont censées favoriser les stratégies d'évitement de futures rencontres (Clinchy *et al.*, 2013). Autrement dit, un autre individu qui fait face à des épisodes répétés d'exposition à des signaux de prédation, devrait adopter un comportement marqué d'appréhension de son environnement. C'est en réponse à cette perception du risque que la proie va sélectionner un niveau basal et optimal de vigilance qu'elle mettra en place en l'absence même d'indice du prédateur dans le milieu. Un niveau basal d'appréhension trop bas fera que la proie se fera prédater plus souvent tandis qu'un niveau trop élevé lui fera sans doute manquer des opportunités d'approvisionnement ou de reproduction (Brown, Laundré, & Gurung, 1999). Adopter un comportement efficace dépendra alors fortement de la perception du risque qui doit être la plus fine possible. Les procédés cognitifs à la base de la modulation du comportement des proies en réponse au risque de prédation s'inscrivent dans un nouveau concept appelé « écologie de la peur » (Brown *et al.*, 1999 ; Clinchy *et al.*, 2013). L'exposition au risque de prédation pourrait non seulement affecter la survie mais également induire des effets prolongés sur la physiologie et la cognition des individus.

Evaluer le risque de prédation passe par la perception de stimuli olfactifs, visuels ou sonores dans l'environnement. La nature des stimuli perçus semble déterminer un pattern de comportements défensifs spécifiques au risque de prédation (Kim & Jung, 2018). Ainsi, l'évaluation du risque, qui semble être une étape cruciale, serait fortement sous sélection. Par exemple, la perception de stimuli olfactifs laissés par les prédateurs se traduit par un comportement d'évitement à travers une diminution de la fréquence et du temps passé à exploiter l'environnement (Kim & Jung, 2018). Ceci aura pour effet de diminuer la probabilité de rencontre entre la proie et le prédateur. En revanche, la perception visuelle du prédateur se traduit souvent par deux stratégies distinctes, le « freezing » ou la fuite, qui dépendent de la distance entre la proie et le prédateur (Kim & Jung, 2018). En effet, détecter une proie à distance semble difficile si cette proie ne bouge pas, et fuir serait la meilleure option quand le prédateur a perçu sa proie et se déplace rapidement. Les stratégies antiprédateur pourraient aussi dépendre du mode de chasse du prédateur (à l'affût ou actif), de l'écologie de la proie (vie en groupe ou solitaire) ainsi que du microhabitat (Miller, Ament, & Schmitz, 2014). En réponse à la présence de prédateurs qui chassent à l'affût, la proie diminuerait drastiquement son activité dans ce même espace, tandis qu'en présence de prédateurs actifs, la proie augmenterait son activité pour sortir de cet espace.

La prédation constitue une forte pression de sélection. Le risque de prédation présente aussi de la variabilité sur plusieurs plans : intensité, distribution spatio-temporelle et imprévisibilité. Par conséquent, les comportements antiprédateur des individus doivent présenter une certaine flexibilité. La variabilité interindividuelle dans les comportements antiprédateur pourrait alors être innée (résultant de la sélection) ou trouver son origine dans l'expérience des individus. Face à des changements, il n'est pas non plus exclu que les composantes génétique et plastique des réponses puissent agir ensemble (Gienapp, Leimu, &

Merilä, 2007). Buskirk, Mulvihill, & Leberman (2012) ont suggéré une action combinée de la sélection et de la plasticité pour expliquer les changements dans les périodes de migration chez les oiseaux en réponse au changement climatique. En effet, s'il existe une part innée dans les stratégies de défenses antiprédateur, la flexibilité de ces comportements semble essentielle puisque le stress lié à la prédation n'est jamais constant dans toutes ses caractéristiques. Ainsi, les individus ayant une stratégie de défenses plastique seraient avantagés par rapport aux individus à stratégies fixes dans un environnement variable. Nous pouvons alors parler de plasticité adaptative (Henry, Roitberg, & Gillespie, 2006). Chez la daphnie *Daphnia sp.*, cette plasticité phénotypique se transmettrait de génération en génération, malgré les potentiels coûts mécanistiques, permettant une meilleure adaptation aux conditions environnementales changeantes (Weiss, 2019).

L'approche « écologie de la peur » pourrait permettre de mieux comprendre comment les amphipodes construisent et ajustent leurs comportements antiprédateur en fonction de la chronicité des épisodes de stress de prédation, et d'identifier un éventuel niveau basal d'appréhension de leur environnement. Chez les amphipodes, les comportements antiprédateur sont facilement modulables expérimentalement. Il est possible en laboratoire de modifier l'intensité de l'expression du comportement d'utilisation du refuge. Par exemple, *G. pulex* utilise plus le refuge après l'introduction d'odeur de prédateur (poisson) dans le milieu qu'avant (Perrot-Minnot, Kaldonski, & Cézilly, 2007 ; Dianne *et al.*, 2014). Un des autres avantages que présentent les amphipodes pour travailler sur la perception du risque de prédation et l'ajustement (ou la modulation) de comportements qui en résulte, c'est le milieu dans lequel ils vivent. En effet, les amphipodes perçoivent les informations dans l'environnement notamment à travers des indices olfactifs (Dicke & Grostal, 2001 ; Derby & Schmidt, 2017) dont le milieu aquatique permet la circulation de façon assez aisée. Le milieu aquatique comprend des facteurs abiotiques (écoulement, turbidité) et biotiques (communauté de prédateurs) modulant l'intensité

et la fréquence d'apparition des signaux de prédation. Ces facteurs sont variables à la fois dans l'espace et dans le temps. Selon l'hypothèse '*risk allocation*', cette variabilité dans la fréquence d'apparition du risque de prédation et son intensité va impacter l'expression des comportements de défenses/vigilance et d'activité des individus (Lima & Bednekoff, 1999 ; Sih & McCarthy, 2002). Cette hypothèse sera présentée, discutée et proposée pour l'étude des comportements antiprédateur des amphipodes dans les perspectives proposées en fin de manuscrit.

Si les conditions locales modulent les comportements antiprédateur des amphipodes à travers la mise en place d'un état de vigilance ou d'appréhension optimal, il existe alors une réelle piste à explorer sur les voies d'action du parasite pour manipuler son hôte. L'hypothèse la moins parcimonieuse serait de proposer que le parasite à travers son hôte, arrive à percevoir le risque de prédation via la communauté de prédateurs présent dans le milieu, pour ensuite moduler le comportement. En revanche, sans avoir de « connaissances » sur le milieu extérieur à l'hôte, le parasite pourrait interagir avec "l'état émotionnel" de son hôte, cet état modulant les comportements déclenchés par les facteurs externes tel que le risque de prédation. Dans cette optique, le parasite ne manipulerait aucun comportement, mais sa simple présence pourrait interagir avec le système neurophysiologique de son hôte, impliqué dans la modulation des états émotionnels. Il nous faut alors tenter d'identifier et comprendre les mécanismes et voies d'action modulant ces états émotionnels chez les hôtes, et que les parasites seraient susceptibles de modifier. La sérotonine est un neuromodulateur connu pour intervenir dans les états d'anxiété (Fossat *et al.*, 2014 ; Zangrossi & Graeff, 2014). Chez *G. pulex* son injection est responsable de modifications de comportements qui coïncident avec les comportements exprimés (défenses diminuées) par des individus infectés par *P.laevis* (Perrot-Minnot *et al.*, 2014). Cependant, nous ne savons pas si ce changement de comportements passe par une modification de l'état d'anxiété basal des individus après injection. En effet, l'injection topique de sérotonine n'inverse que la taxie des individus mais pas l'utilisation du refuge (Perrot-

Minnot *et al.*, 2014). Ainsi, l'injection topique de sérotonine ne semble pas modifier l'état émotionnel des individus mais seulement un certain type de comportements n'impliquant a priori pas de processus cognitifs spéciaux. Aussi serait-il davantage pertinent de tester l'administration chronique de sérotonine afin de voir si davantage de traits phénotypiques sont modifiés et si cela semble se traduire par un état émotionnel altéré.

Le but de cette thèse était d'essayer d'établir un lien entre la manipulation parasitaire et la notion d'anxiété, mise en évidence récemment dans la littérature, chez un modèle biologique d'invertébré aquatique, le gammare. Avant d'introduire la notion d'état émotionnel et plus précisément d'anxiété, les deux premières parties de ce manuscrit de thèse ont été axées (i) sur la manipulation parasitaire en faisant l'état des lieux via une analyse critique (méta-analyse) et (ii) sur la variation de l'expression des comportements antiprédateur en lien avec les facteurs externes du milieu (risque de prédation). La troisième et dernière partie axée sur les états émotionnels récapitulera ce qui est connu chez les invertébrés, l'expression et le lien entre peur et anxiété chez de tels animaux, ainsi les futures pistes à explorer.

PARTIE 1 –

BILAN CRITIQUE ET QUANTITATIF SUR LA MANIPULATION PARASITAIRE

Chapitre 1 -

Magnitude and direction of parasite-induced phenotypic alterations: a meta-analysis in Acanthocephalans

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RESUME

Certaines espèces de parasites sont capables de modifier le phénotype de leur hôte, favorisant probablement la transmission vers un hôte définitif. Ce phénomène connu sous le nom de manipulation parasitaire, s'interprète comme l'expression du phénotype étendu du parasite. Les parasites manipulateurs sont souvent responsables de multiples changements dans le phénotype de l'hôte, affectant plusieurs types de traits. Cependant, le caractère adaptatif de cette manipulation multidimensionnelle ne reste que peu étudiée. Nous avons réalisé une méta-analyse phylogénétiquement corrigée afin de clarifier cette multidimensionnalité, quantifier la manipulation parasitaire et tenter de comprendre les causes de la variation dans l'intensité des changements phénotypiques, en se focalisant sur un des modèles biologiques far de la manipulation, les parasites acanthocéphales. Les acanthocéphales représentent un phylum de parasites helminthes, qui utilisent des invertébrés et vertébrés comme hôtes intermédiaires et définitifs, respectivement. C'est aussi un taxon de parasites pour lequel la manipulation semble être un trait ancestral. A partir de 81 études regroupant 13 espèces de parasites acanthocéphales, nous avons analysé 279 estimateurs (tailles d'effet) de la valeur des changements phénotypiques. Pour chacun de ces estimateurs, nous avons affecté un signe (positif ou négatif) selon la conséquence du changement en termes de transmission, et classé chacun de ces changements dans des catégories phénotypiques (comportement, morphologie, physiologie ou trait d'histoire de vie). La variation dans les tailles d'effet n'est expliquée que dans une faible proportion par la phylogénie des parasites acanthocéphales. La moyenne méta-analytique représentant l'intensité générale des changements phénotypiques est modérée et positive, ce qui signifie que d'une manière globale, les changements phénotypiques induits par les acanthocéphales semblent favoriser leur transmission vers un hôte définitif. La variation observée dans l'intensité des changements semble être en partie expliquée par la catégorie de trait. En effet, les traits comportementaux, tels que la taxie-phobie ou la réponse à un stimulus, semblent plus

fortement affectés. Parmi les autres catégories de traits, la reproduction (traits d'histoire de vie) et l'immunité (physiologie) sont aussi très impactées. Ainsi, des changements dans le microhabitat de l'hôte et dans son comportement antiprédateur augmenteraient la probabilité de transmission des parasites acanthocéphales qui, en même temps, favoriseraient les stratégies d'économie d'énergie de l'hôte. Pour aller plus loin, les changements phénotypiques induits par le stade non-infectieux (acanthelle) des parasites semblent être relativement bien opposés en termes de transmission, mais si de même intensité, à ceux induits par le stade infectieux (cystacanthe). Cependant, ce résultat est à prendre avec précaution étant donné le faible nombre d'estimateurs concernant les acanthelles. Cette méta-analyse nous permet alors de soulever quelques pistes/problèmes qui doivent être pris en considération pour les futurs travaux qui s'intéresseront au caractère adaptatif de la manipulation parasitaire, pas seulement par les acanthocéphales, mais aussi par les autres taxons de parasites. Plus précisément, quantifier et comprendre la contribution des traits altérés à la transmission, à travers la clarification d'un éventuel lien de causalité par exemple, représente une piste qui nécessite toute notre attention. De plus, la relation entre comportement et immunité (hypothèse neuropsychoimmune) tout au long de l'ontogénèse du parasite et en tenant compte des divers systèmes hôte-parasite est toujours en attente de travaux expérimentaux. Toutes ces pistes devraient s'appliquer de façon plus étendue à tous les cas reportés de manipulation parasitaires chez d'autres taxons.

Magnitude and direction of parasite-induced phenotypic alterations: a meta-analysis in acanthocephalans

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ABSTRACT

Several parasite species have the ability to modify their host's phenotype to their own advantage thereby increasing the probability of transmission from one host to another. This phenomenon of host manipulation is interpreted as the expression of a parasite extended phenotype. Manipulative parasites generally affect multiple phenotypic traits in their hosts, although both the extent and adaptive significance of such multidimensionality in host manipulation is still poorly documented. To review the multidimensionality and magnitude of host manipulation, and to understand the causes of variation in trait value alteration, we performed a phylogenetically corrected meta-analysis, focusing on a model taxon: acanthocephalan parasites. Acanthocephala is a phylum of helminth parasites that use vertebrates as final hosts and invertebrates as intermediate hosts, and is one of the few parasite groups for which manipulation is predicted to be ancestral. We compiled 279 estimates of parasite-induced alterations in phenotypic trait value, from 81 studies and 13 acanthocephalan species, allocating a sign to effect size estimates according to the direction of alteration favouring parasite transmission, and grouped traits by category. Phylogenetic inertia accounted for a low proportion of variation in effect sizes. The overall average alteration of trait value was moderate and positive when considering the expected effect of alterations on trophic transmission success (signed effect sizes, after the onset of parasite infectivity to the final host). Variation in the alteration of trait value was affected by the category of phenotypic trait, with the largest alterations being reversed taxis/phobia and responses to stimuli, and increased vulnerability to predation, changes to reproductive traits (behavioural or physiological castration) and immunosuppression. Parasite transmission would thereby be facilitated mainly by changing mainly the choice of microhabitat and the antipredation behaviour of infected hosts, and by promoting energy-saving strategies in the host. In addition, infection with larval stages not yet infective to definitive hosts (acanthella) tends to induce opposite effects of comparable

magnitude to infection with the infective stage (cystacanth), although this result should be considered with caution due to the low number of estimates with acanthella. This analysis raises important issues that should be considered in future studies investigating the adaptive significance of host manipulation, not only in acanthocephalans but also in other taxa. Specifically, the contribution of phenotypic traits to parasite transmission and the range of taxonomic diversity covered deserve thorough attention. In addition, the relationship between behaviour and immunity across parasite developmental stages and host–parasite systems (the neuropsychoimmune hypothesis of host manipulation), still awaits experimental evidence. Most of these issues apply more broadly to reported cases of host manipulation by other groups of parasites.

Key words: behaviour, helminth; host–parasite interaction, manipulation, multidimensionality, phylogenetic meta-analysis, publication bias, ribosomal DNA, trophic transmission.

CONTENTS

(3) Recommendations for future research

V. Conclusions

VI. Acknowledgements

VII. References

VIII. Supporting information

I. INTRODUCTION

Several parasites bring about phenotypic alterations in their hosts that appear to increase their own fitness at the expense of that of their hosts (Poulin, 1995; Moore, 2002; Thomas, Adamo & Moore, 2005; Cézilly *et al.*, 2010). Such parasite-induced phenotypic alterations (PIPAs) can take different forms, through affecting, for instance, the physiology (Plaistow, Troussard & Cézilly, 2001; Tain, Perrot-Minnot & Cézilly, 2006; Perrot-Minnot & Cézilly, 2013; Guler *et al.*, 2015; Kopp *et al.*, 2016; Perrot-Minnot, Maddaleno & Cézilly, 2016), reproduction (Bollache, Gambade & Cézilly, 2001; Bollache, Rigaud & Cézilly, 2002; Rauque & Semenas, 2009; Bollache, 2016) or appearance (Lewis, 1977; Camp & Huizinga, 1979; Oetinger & Nickol, 1981; Amato *et al.*, 2003; Wesołowska & Wesołowski, 2014) of infected hosts. However, most studies of PIPA concern the altered behaviour of host species. For instance, several species of ectoparasitoid wasps are known to modify the web-building behaviour of their spider hosts (Eberhard, 2000; Matsumoto, 2008; Korenko *et al.*, 2014; Takasuka *et al.*, 2015; Kloss *et al.*, 2017). Just before the wasp enters its final stage of development, the spider host builds a modified web in the form of a 'cocoon' (Eberhard, 2000) that appears to enhance the survival of the parasitoid pupae. Both rodents and chimpanzees infected with *Toxoplasma gondii* famously lose their innate aversion to the urine of feline predators (Berdoy, Webster & Mcdonald, 2000; Dass & Vyas, 2014; Poirotte *et al.*, 2016), a phenomenon that presumably increases the transmission of the parasite to its final host. Similarly, several species of helminths with complex life cycles are known to alter the antipredation behaviour of their intermediate arthropod hosts in ways that appear to enhance trophic transmission to final hosts (Hechtel, Johnson & Juliano, 1993; Kaldonski, Perrot-Minnot & Cézilly, 2007; Sánchez, Georgiev & Green, 2007). For instance, whereas uninfected crustacean amphipods are significantly repulsed by the chemical cues originating from a fish predator, infected ones are significantly attracted to the odour (Baldauf *et al.*, 2007; Perrot-Minnot, Kaldonski & Cézilly, 2007). Most of the time,

such phenotypic alterations are interpreted as expressions of the extended phenotype (*sensu* Dawkins, 1982) of the parasite species, whose ability to 'manipulate' its host has evolved by natural selection (Moore, 2002; Thomas *et al*., 2005; Hughes, Brodeur & Thomas, 2012). Alternatively, they could correspond to simple pathological effects (Chow & Mackauer, 1999; Edelaar, Drent & De Goeij, 2003; Schutgens *et al.*, 2015) or to an adaptive host response (Smith Trail, 1980; Poulin, 1992; Poulin, Brodeur & Moore, 1994). Whether the magnitude of parasite phenotypic alterations varies in relation to its consequences for the parasite and its host is poorly documented.

Although adaptive host manipulation has become a sort of paradigm in evolutionary parasitology and behavioural ecology (Poulin, 2000; Moore, 2002; Thomas *et al.*, 2005; Bakker, Frommen & Thuenken, 2017), growing evidence suggests that the 'purposive design' (*sensu* Poulin, 1995) of phenotypic alterations induced by parasites should be examined with more caution. A crucial step in validating the manipulation hypothesis is to show convincingly that a direct *causal* relationship exists between altered host phenotype and enhanced completion of the life cycle (Cézilly *et al.*, 2010). Indeed, behavioural alterations observed in infected hosts that *seemingly* enhance the completion of the parasite's life cycle may not *actually* contribute to it. For instance, the behavioural alterations displayed by tenebrionid beetles infected with *Hymenolepis diminuta*, including reduced activity, concealment and photophobia (Hurd & Fogo, 1991; Robb & Reid, 1996), were initially interpreted as a case of manipulation. However, such phenotypic alterations do not necessarily result in a differential vulnerability of infected and uninfected beetles to predation by rodent final hosts (Webster *et al.*, 2000). Similar conclusions have been drawn from recent studies of two historical models of host manipulation. Crustacean amphipods serve as intermediate hosts for various acanthocephalan parasites that use different species of vertebrates as final hosts. Inside their intermediate hosts, larval acanthocephalans progressively develop into cystacanths, the infective stage for the definitive

host. Cystacanths of several acanthocephalan species have a carotenoid-based, bright orange colouration (Gaillard *et al.*, 2004) that can be seen through the translucid cuticle of their hosts, such that infected hosts are particularly conspicuous, at least to the human eye. In addition, gammarids infected with acanthocephalans show altered behaviour, including reduced photophobia. Bethel & Holmes (1973, 1977) were the first to provide evidence for a causal link between the altered behaviour of gammarids infected with larval acanthocephalans and their increased vulnerability to predation, and the phenomenon was quickly regarded as a compelling example of host manipulation (Dawkins, 1982). Bakker, Mazzi & Zala (1997) went further by arguing that both the modified appearance and the altered phototactic behaviour of *Pomphorhynchus laevis*-infected *Gammarus pulex* were responsible for their increased vulnerability to predation by three-spined sticklebacks, *Gasterosteus aculeatus*. However, more recent investigations using phenotypic engineering to manipulate one trait at a time (Kaldonski *et al.*, 2009; Perrot-Minnot *et al.*, 2012) demonstrated convincingly that neither parasite's colour nor the altered phototactic behaviour of infected hosts *alone* contribute to the increased vulnerability of *P. laevis*-infected gammarids to fish predation. Therefore, several phenotypic changes might act synergistically to enhance trophic transmission.

Similarly, Worth, Lymbery & Thompson (2013) questioned the adaptiveness of behavioural alterations induced by *T. gondii* in rodents, based on several lines of evidence. First, studies of mice and rats have resulted in conflicting results about what behaviours are or are not affected by infection. Second, behavioural alterations similar to those coincidental with *T. gondii* infection can also be induced by *Eimeria vermiformis*, a parasite that does not rely on trophic transmission to complete its life cycle [Kavaliers & Colwell, 1995; see also Cator *et al.* (2013) for a related result in a markedly different host–parasite association]. Third, there is, surprisingly enough, no direct evidence that rodents infected with *T. gondii* are more vulnerable to predation by cats. Fourth, even if such evidence was available, it appears that cats and sexual

reproduction might not be crucial for the survival, transmission, and maintenance of *T. gondii* in a population (Worth *et al*., 2013). The overall evidence thus suggests that the apparent 'purposive design' of parasite-induced phenotypic alterations does not guarantee a causal relationship between manipulation and enhanced trophic transmission. More to the point, it is still unclear to what extent the consequences of host manipulation, in terms of enhanced completion of the parasite's life cycle, depends on its magnitude.

In addition, although most studies have considered a single phenotypic alteration at a time, it is increasingly acknowledged that, most often, manipulative parasites affect more than one phenotypic dimension in their hosts (Gotelli & Moore, 1992; Cézilly & Perrot-Minnot, 2005; Cézilly, Favrat & Perrot-Minnot, 2013). Such multidimensionality might be adaptive if, for instance, it allows the parasite to enhance the completion of its life cycle under a large range of ecological circumstances (Thomas, Poulin & Brodeur, 2010). Under this scenario, multidimensionality may have arisen from the progressive addition of several phenotypic dimensions that are manipulated independently of each other through distinct physiological pathways. Alternatively, multidimensionality in manipulation may stem from the major disruption of some specific physiological mechanism, with cascading effects affecting various phenotypic dimensions (Cézilly & Perrot-Minnot, 2010). For instance, crustacean amphipods infected with fish acanthocephalans show a variety of modified phenotypic traits (Cézilly *et al*., 2013), including an increased serotonergic activity in the brain (Tain *et al*., 2006). Interestingly, multidimensionality in manipulation as observed in *G. pulex* infected with *P. laevis* can be partly mimicked in uninfected individuals by the injection of serotonin (Perrot-Minnot, Sanchez-Thirion & Cézilly, 2014), thus providing support for the second hypothesis. To what extent this finding applies to other cases of multidimensionality in manipulation remains an open question. In addition, whether the existence of a single mechanism would result in covariation among individuals in the magnitude of the various phenotypic alterations

simultaneously brought about by a parasite species remains unclear (see Bailly, Cézilly & Rigaud, 2018).

The interest in manipulative parasites is however not limited to their value as a supposedly perfect example of an extended phenotype. Growing attention has been given to the role that such parasites play in ecosystems through their influence on the behaviour and trophic niches of their hosts and, ultimately, on trophic cascades (Thomas *et al.*, 1997; Thomas *et al.*, 1998; Lefèvre *et al.*, 2009; Lafferty & Kuris, 2012; Sato *et al.*, 2012; Boze & Moore, 2014; Britton & Andreou, 2016; Reisinger & Lodge, 2016). Still, the precise impact of manipulative parasites on ecosystem dynamics remains unclear, partly because the relationship between the magnitude of phenotypic alterations and their ecological consequences is difficult to assess. More to the point, the ability of parasites to manipulate their hosts might be modulated by various environmental variables. For instance, temperature recently has been shown to affect the extent of manipulation of phototaxis in amphipods infected by acanthocephalans, but not that of geotaxis or refuge use (Labaude, Cézilly & Rigaud, 2017*a*). Environmental influences and infection with manipulative parasites may thus have interactive or additive effects on the phenotype of infected hosts (see Labaude, Rigaud & Cézilly, 2017*b*) and, therefore contribute directly to the observed variation in the magnitude of manipulation within and among host–parasite associations, with potential consequences at the level of ecosystems.

Whether host manipulation is studied from the point of view of its evolutionary routes (Thomas, Rigaud & Brodeur, 2012), its underlying mechanisms (Perrot-Minnot & Cézilly, 2013) or its ecological consequences (Lafferty & Kuris, 2012; Labaude, Rigaud & Cézilly, 2015*b*), an important question is what causes variation at different levels in the magnitude of phenotypic alterations coincidental with infection by manipulative parasites. Variation in the extent of such phenotypic alterations exists both within and among infected individuals in a single host population, as well as among host populations (Thomas *et al.*, 2011; Fayard, Cézilly

& Perrot-Minnot, 2019) or among host species infected with the same parasite (Gotelli & Moore, 1992; Bauer *et al.*, 2000; Tain, Perrot-Minnot & Cézilly, 2007). The relative importance of host and parasite phylogenies, the type of altered trait or the consequences in terms of enhanced completion of the parasite's life cycle remain however poorly documented, although a few attempts have been made to provide quantitative reviews of the existing literature on host manipulation (Moore & Gotelli, 1990; Poulin, 1994, 2000; McElroy & de Buron, 2014; Nakagawa *et al.* 2015). In the latter approach, meta-analysis constitutes a valuable tool (Poulin & Forbes, 2012), particularly to quantify the heterogeneity observed in the magnitude of host manipulation. So far, however, meta-analysis has seldom been used for that purpose. Using a meta-analytic framework, Poulin (1994, 2000) and Nakagawa *et al*. (2015) provided valuable insights on the influence of parasite taxa and behavioural traits on the magnitude of the effect of parasites on their hosts. Interestingly, based on 137 comparisons between the behaviour of infected and uninfected hosts, Poulin (2000) found that the reported values of effect size indicating host manipulation tended to decrease over time. He further suggested that this may be due to the fact that most of the earlier investigations of host manipulation concerned acanthocephalan parasites, in which the ability to manipulate host phenotype is regarded as an ancestral, well-established character (Moore, 1984), whereas, later on, evidence for manipulation was sought in a larger range of host–parasite associations (Poulin, 2000). The same, although non-significant, trend for effect size becoming smaller over time was reported in an updated analysis based on 202 effect sizes (Nakagawa *et al*., 2015). As acanthocephalans tend to have marked effects on their hosts (Bakker *et al.*, 2017), it might have been difficult to obtain similar results in other parasites with a relatively smaller ability to manipulate their hosts (Nakagawa *et al*., 2015). Another recent analysis, focusing on host performance (defined as a physical quantity that measures how well an organism can execute a given behaviour or task) and considering the literature published until 2013, failed to detect the same effect, but found

some evidence for an increase in the magnitude of the effect of parasites on their hosts with publication year (McElroy & de Buron, 2014). However, the final data set in that study was based on only 49 studies.

The use of meta-analysis to analyse both the direction and magnitude of parasite-induced phenotypic alterations introduces several difficulties. First, not all published articles provide enough statistical information to allow the computation of effect sizes, such that final data sets available for meta-analysis might be of reduced size, thus increasing the risk of type II error (Arnqvist & Wooster, 1995). Second, there exists an unequal representation of the various species of hosts and parasites in the scientific literature on host manipulation, and this taxonomic bias is likely to result in non-random data sets (Lajeunesse, 2010). This is why it is highly recommended to incorporate phylogenetic information in ecological meta-analyses (Chamberlain *et al.*, 2012).

Here, we provide a meta-analysis of the existing literature about the phenotypic alterations induced by acanthocephalan parasites in their intermediate hosts. Although around 1300 species of acanthocephalan parasites have been described, their phylogenetic relationships within Metazoans remain controversial (García-Varela & León, 2015). Based on morphological, ecological and molecular evidence, acanthocephalan parasites have been divided into four classes: Archiacanthocephala, corresponding to the most basal clade, and Palaeacanthocephala, Eoacanthocephala, and Polyacanthocephala, corresponding to three derived clades (Amin, 1987; Kennedy, 2006; García-Varela & León, 2015). Although the evolution of acanthocephalans is characterized by a multiplicity of host-switching events (García-Varela & León, 2015), they tend to have strong and diversified effects on the phenotype of their hosts (Cézilly *et al*., 2013; Bakker *et al*., 2017). Palaeacanthocephalans represent the most diverse and best-studied class of acanthocephalans, while published studies of host manipulation in other acanthocephalan groups are scarce (see Section II).

We reviewed phenotypic alterations induced by acanthocephalan parasites by considering two features: (*i*) the alteration of mean trait value, measured as the increase or decrease in a host phenotypic trait value expected to increase parasitic transmission in infected hosts; (*ii*) the magnitude of alterations, quantifying the influence of the parasite on the host's phenotype irrespective of its consequence on parasite transmission. For the former, we used signed effect sizes measured at the last developmental stage infective to the final host (cystacanth). For the latter, we also included effect size estimates from larval stages not yet infective to final hosts (acanthella). We first examined the extent to which the alteration of mean trait value and the magnitude of alteration was affected by phylogeny. We then quantified the effects of acanthocephalans on their host phenotype to answer three questions: (1) how strong is the overall effect of infection? (2) Following the parasite manipulation hypothesis, are these alterations of host trait value likely to enhance parasite transmission on average? (3) How variable is alteration of the trait value according to several factors including trait categories (multidimensionality) and publication year?

II. METHODS

(1) Literature search

Studies on acanthocephalan-induced phenotypic alterations were searched in the the *Web of Science* and *Google Scholar* databases by using combinations of "acanthocephala*" and "behav*" or "physio*" or "morpho*" or "size" or "chang*" or "host" or "predat*" or "reproduct*" or "survival" or "mortality" key words. The search included studies published until January 2018. From 3531 studies, and after sequential removals because of lack of sufficient quantitative information, we obtained a database of 81 studies (PRISMA flow diagram, Fig. 1). All studies included in analyses are identified with an asterisk in the reference list.

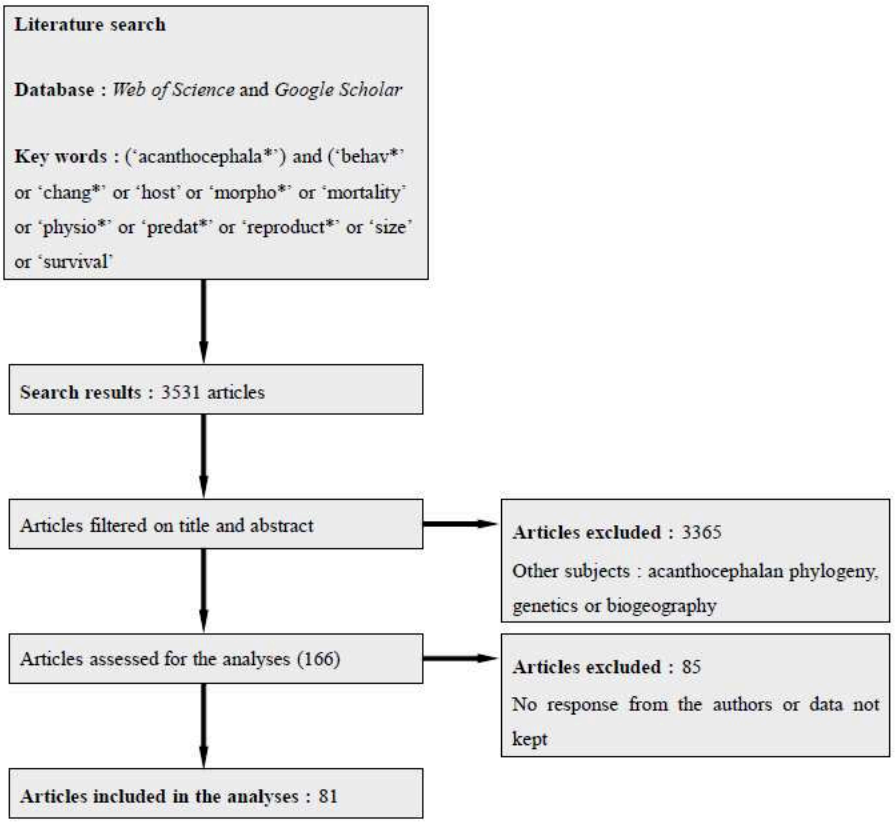

Fig. 1. Preferred reporting items for systematic reviews and meta-analyses (PRISMA) flow diagram (Liberati *et al*., 2009 ; Nakagawa *et al*., 2017) for this meta-analysis on variation in the intensity of host manipulation by acanthocephalans.

(2) Data collection

For each study, we recorded the year of publication, parasite taxonomy (from the class to the species) and stage (non-infective acanthella or infective cystacanth), intermediate host taxonomy (class and species), phenotypic traits measured and the magnitude of alteration associated with infection (effect size), sample size (infected and uninfected individuals) and infection type (natural or experimental). Following the recommendations of Noble *et al.* (2017), we sought for sources of non-independence stemming from within-study design, in addition to phylogeny-, species- and study-level non-independence. We identified two sources of within-study covariance: effect sizes estimated for different parasite species using the same controls

('shared treatment comparison' or 'shared controls'), and effect sizes measured on the same individual ('shared traits') (Noble *et al.*, 2017).

(3) Categorization of host phenotypic traits

We categorized host phenotypic traits into five groups: 'behaviour', 'life history', 'morphology' and 'physiology' according to Mousseau & Roff (1987), and vulnerability to 'predation' (Table 1). We further subdivided each category into trait subcategories: for behaviour, 'activity', 'protection', 'response to stimuli' and 'taxis/phobia'; for life history, 'body condition', 'foraging', 'intraspecific interaction', 'reproduction' and 'survival'; for morphology, 'growth' and 'colour'. We subdivided the category physiology into 'immunity', 'energy reserves/metabolism', and 'neurophysiology'. Finally, within the trait category predation, we differentiated two types of predators, 'non-host' and 'suitable host'.

(4) Calculation of effect sizes

Following the recommendation of Nakagawa *et al.* (2017) for comparisons between two treatments (here, parasite infection and control), we used standardized effect sizes based on means and standard deviations, mostly with Cohens *d* (Cohen, 1988). In some cases, we also extracted this information from figures with the 'digitize' R package (Poisot *et al.*, 2016). When means and standard deviations were not available in the publication, we attempted to contact the authors directly. When proportions of individuals were given, we used the Odds ratio (Borenstein *et al.*, 2009). As not all studies reported the same effect size metrics, their direct comparison was not possible. We used conversions from Borenstein *et al.* (2009) to obtain a common metric of effect size, the correlation coefficient *r*, allowing comparison between studies. To conduct the analyses, we then converted each *r* into a Fisher *Zr* using the Fisher *r* to *Z* transformation (Borenstein *et al.*, 2009), with $Zr = 0.5 \times \ln((1 + r) / (1 - r))$. After the analyses,

meta-analytic *Zr* means were back-transformed into meta-analytic *r* means. Values of 0.1, 0.3 and 0.5 were interpreted as low, moderate and strong effects, respectively (Cohen, 1988).

(5) Signed effect sizes according to parasite transmission

We assigned a sign to each effect size according to the direction of alteration, whether an increase or a decrease in mean trait value, that was expected to enhance trophic transmission. Positive values of effect sizes were associated with alterations in a host trait expected to enhance parasite transmission by increasing encounter rate between infected prey and predators (ERH: encounter rate hypothesis) (Table 1). When the effect of PIPA on parasite transmission did not directly affect encounter rate, positive values were assigned to alterations that would favour host survival independently of predation, for instance by decreasing host energetic expenditure (ESH: energy-saving hypothesis) (Table 1). The rationale is that parasite transmission relies on host survival until predation, in part modulated by energy reserves invested in host maintenance (traded-off against other functions) and parasite growth. Therefore, the fitness of trophically transmitted parasites relies partly on the survival of intermediate hosts until predation. In some cases, the direction of alteration for optimal parasite transmission was ambiguous, as either an increase or a decrease in the expression of one trait could contribute to increased parasite transmission (Table 1). We therefore ran alternative analyses without these ambiguous traits, following Cally, Stuart-Fox & Holman (2019), and present these additional results as online Supporting Information.

Table 1. Categories of host trait altered by acanthocephalan parasites that were incorporated in the meta-analysis. The predicted direction of alteration under the hypothesis of increased trophic transmission of the infective cystacanth stage to definitive hosts is provided as the main hypotheses. The direction of alteration is predicted from either increased encounter rate between infected prey and predators (ERH: encounter rate hypothesis), or decreased energetic expenditure by intermediate host (ESH: energy-saving hypothesis). In the former case, parasite-induced phenotypic alteration (PIPA) results in predation bias towards infected hosts. Non-exclusively in the latter case, energy saving/reallocation increases host and parasite survival and/or parasite growth rate.

Host trait category	**Host trait subcategory**	**Main hypothesis**	**Competing hypothesis (opposite signed effect)**
Behaviour	Activity	Higher activity increases conspicuousness; distance covered increases the probability of encounter (ERH)	Lower activity increases catchability (ERH) and saves energy (ESH)
	Protection	Decreased protective behaviour, increased exposure (ERH)	
	Response to stimuli	Decreased detection of stimulus or responsiveness to predator cues (ERH)	
	Taxis/phobia	Microhabitat overlaps with predators (decreased photophobia or negative geotaxis) (ERH)	
Life history	Body condition	Increased body condition (ERH and ESH)	
	Foraging	Higher exploration for resources (high food intake) increases prey exposure to predators (ERH)	
	Reproduction	Behavioural (male) and physiological (female) castration (ESH)	
	Intraspecific interaction (sociality)	Decreased agonistic behaviours (competition, cannibalism) (ESH)	
	Survival	Higher host survival increases the time frame for transmission (ERH and ESH)	
Morphology	Colour	Increased conspicuousness (ERH)	
	Growth	Higher growth/body size increases detection (ERH)	
Physiology	Immunity/resistance	Immunosuppression (ESH)	

	Energy reserves/metabolism	Higher energetic reserves (ERH and ESH) Lower metabolic rate (decreased oxidative stress: increased survival) (ERH and ESH)	Higher metabolic rate (sustains higher foraging and activity rate) (ERH and ESH)
	Neurophysiology	High serotonin level decreases anxiety (ERH) – associated with low dopamine level (5HT-DA opponency)	Low serotonin level impairs aversive learning, hence delays response to predation stimulus (ERH) – associated with high dopamine level (5HT-DA opponency)
Predation	Non-host	Decreased predation by non-hosts (ERH)	
	Suitable final host	Increased predation by suitable hosts (ERH)	

(6) Choice of moderators

Five factors were considered as fixed effects.

(*a*) *Category of traits*

As parasite transmission depends critically on prey–predator interactions, the behaviour of infected intermediate hosts is of prime importance relative to other phenotypic traits. In addition, behavioural traits are more plastic than, for instance, morphological traits (Price, Qvarnström & Irwin, 2003). One may therefore expect behavioural traits to be more easily altered by 'manipulative' parasites than morphological traits, resulting in differences in effect size between behavioural and morphological traits.

(*b*) *Infection type and environmental conditions*

One criticism of experimental studies on host manipulation by parasites is that laboratory conditions imperfectly reflect natural ones. Experimental infection procedure and maintenance conditions (light intensity, temperature, host density, stress of handling and maintenance) could impact the expression of phenotypic traits, and thus affect the estimates of effect size.

(*c*) *Parasite developmental stage*

Two different phenomena with opposite effects could alter the vulnerability to predation and survival probability of infected intermediate hosts (Parker *et al.*, 2009). 'Predation suppression' is used to refer to manipulations by immature parasites that decrease the vulnerability to predation of their intermediate host. Conversely, 'predation enhancement' is used to refer to manipulations that increase vulnerability to predation of the intermediate host at a developmental stage at which the parasite is infective to its final host. In acanthocephalans, acanthella are developmental stages at which the parasite is unable to establish in an appropriate final host, while the cystacanth is the last developmental stage in the intermediate host and is infective to final host. Dianne *et al.* (2011) found experimental evidence for both effects in *G. pulex* infected with *P. laevis*. Although opposite effects between acanthella and cystacanth infections have been reported several times, it is not clear whether they are of the same magnitude.

(*d*) *Publication year*

Host manipulation by parasites has been actively investigated in the field of host–parasite interactions since the study of Holmes (1972) pointed out the adaptive value of manipulation. Several criticisms of this hypothesis and alternative explanations emerged almost 20 years later from the review of Moore & Gotelli (1990). The approach used to study a phenomenon can change according to current paradigms, and this may lead to different conclusions (Poulin, 2000). As a consequence of growing interest in the topic, and methodological and technical progress, it is possible that trends in magnitude of acanthocephalan-induced alterations reported in the literature could appear through time.

(*e*) *Sample size*

Confidence intervals vary with sample size, being larger for small sample sizes. Therefore, we included the effect of sample size as a source of heterogeneity among effect sizes, estimated here using within-study sample size.

(7) Meta-analyses

We ran multi-level/hierarchical models with the MCMCglmm (Markov chain Monte-Carlo general linear mixed models) function in the MCMCglmm package (Hadfield, 2010), to investigate several types of non-independence. The first is widespread in evolutionary biology, and stems from phylogenetic relatedness among species (Harvey & Pagel, 1991; Nakagawa & Santos, 2012). To control for the potential non-independence of species data points, we implemented phylogenetic information as a variance–covariance matrix in the meta-analyses. As the most recent phylogenetic tree based on 18S rRNA gene sequences comprises only 36 acanthocephalan species (Verweyen *et al.*, 2011), we constructed a new tree based on 59 species (including three new species sequences) (Table S1). We retrieved the distances between species from an ultrametric tree derived from Bayesian inference (see Table S1). In addition to phylogenetic non-independence between effect sizes, we accounted for species- and study-level non-independence by including parasite species and study ID as random factors (Nakagawa & Santos, 2012). Finally, we explored the consequences of violating assumptions of independence among effect size estimates at the individual level ('shared-measures' and 'shared-controls') by running a sensitivity analysis, following the recommendations of several authors (Koricheva & Gurevitch, 2014; Noble *et al*., 2017). Shared measures are effect sizes estimated for different traits in the same individuals, shared controls are effect sizes estimated for at least two parasite species using the same control (uninfected) group. We assessed the robustness of the meta-analysis on signed effects of cystacanth infection after controlling for these sources of non-

independence, by running the same analysis on a subset of independent measures (Fig. S1B: (*i*) only one effect size was randomly chosen per individual whenever more than one trait was measured per individual within the same trait category or in two categories besides behaviour, (*ii*) when one or more behavioural traits were measured together with morphological, physiological or life-history traits on the same individual, we removed the behavioural trait(s) as this category was over-represented in the data set. This was a conservative approach, since behaviour was expected to be the trait category that was most impacted by infection.

Effect sizes (*Zr*) were used as the dependent variables and their variance was calculated using the formula: $1 / (n - 3)$ (Borenstein *et al.*, 2009), where n is the sample size associated with each effect size. The analyses were based on Bayesian hierarchical models which impose definitions of priors (Gelman, 2006). A prior is the strength of belief in the parameter value associated with the variable affecting the observed data. It is represented by the distribution of the parameter based on previous experience. In the absence of information on prior distribution, we used non-informative priors ($nu = 0.002$ and $V = 1$). To assess the influence of these priors on the results, we repeated the analyses with expanded priors ($nu = 1$, $V = 1$, $alpha.mu = 0$, $alpha.V = 1000$), with no detectable effect on our results. For each model, we ran 500,000 iterations which was large enough to minimize the level of autocorrelation (non-independence) between successive iterations: we checked that the autocorrelation coefficient was below 0.10, as suggested by J.D. Hadfield (personal communication). Model convergence was verified according to Gelman & Rubin (1992). To evaluate the reliability of the meta-analytic mean, we also assessed consistency among studies by calculating I^2, which quantifies heterogeneity between effect sizes for each random factor (Nakagawa & Santos, 2012). I^2 represents the variance accounted for by each random factor relative to the total variance. Heterogeneity was considered as low, moderate and high when $I^2 = 0.25$, 0.50 and 0.75, respectively (Higgins *et al.*, 2003).

First, we performed a meta-analysis using signed effect sizes of cystacanth infection, to quantify overall alteration in mean trait value. We also estimated the average magnitude of alterations by estimating the meta-analytic mean of absolute effect sizes on the complete data set, including effect sizes of infection with acanthella. We could not run the meta-analysis directly on absolute values of effect sizes, as the distribution of absolute effect sizes is a folded normal distribution (Morrissey, 2016*a*). Therefore, we used the procedure recommended by Morrissey (2016*a*, *b*), specifically the 'analyze-then-transform' approach. We first estimated the meta-analytic mean of all signed effect sizes (all infections with acanthella and cystacanth), and then derived the mean absolute value, we applied the formulae provided by Morrissey *et al*. (2016*a*) to convert both the posterior mean and confidence interval. The different analysis and their purposes are summarized in Fig. S2.

(8) Meta-regressions

We ran a meta-regression to assess the contribution of fixed effects to variation in signed effect sizes of cystacanth infection. The category of trait, infection type (natural or experimental), sample size and year of publication were entered as fixed factors, and parasite species, study and parasite phylogeny as random factors within the model. In the analysis on the entire data set including both acanthella and cystacanth infection to derive the average magnitude of alterations (Fig. S2), parasite developmental stage was added as an additional fixed factor. Since parasite species was already taken into account as a random factor, and was associated with host species (Fig. S1A), neither the host nor the parasite species were considered as fixed factors. We chose to keep these as random factors to control for non-independence between effect sizes. Starting with a global model (including all fixed effects), we performed model selection with the MuMIn package (Bartoń, 2016) using the deviance information criterion

(DIC) (Spiegelhalter *et al.*, 2002; Grueber *et al.*, 2011). For each factor level, the meta-analytic mean was estimated from the meta-regression.

(9) Analysis of parasite maturity

The aim of this analysis was to test whether the average magnitude and direction of parasite-induced phenotypic alterations varies according to whether parasite developmental stage is, or is not yet, infective to the final host (Fig. S2). First, we assessed to what extent parasite maturity could affect the overall meta-analytic mean of signed effect sizes, by comparing the output of two analyses, the main analysis based the data set restricted to the cystacanth stage, and the additional analysis based on the entire data set (acanthella and cystacanth stages) (Fig. S2). We expected the meta-analytic mean of signed effect sizes to be positive and larger when considering cystacanth infection only compared to both developmental stages. In addition, as for the overall mean absolute value, we derived the mean absolute values of effect sizes and their confidence intervals for each factor level, including parasite developmental stage. We used the 'analyze-then-transform' approach on the meta-analytic mean effect size of each factor level estimated from the meta-regression on signed effect sizes (both acanthella and cystacanth included).

(10) Publication bias

Statistically significant results are much more likely to be published than non-significant ones (Rosenthal, 1979). In addition, when published, studies reporting non-significant results tend to be based on large sample sizes which is expected to increase their leverage on the meta-analytic mean. We identified potential publication biases using funnel plot (Sterne & Egger, 2001). We quantified the magnitude of these publication biases using both Egger's regression (Egger *et al.*, 1997) and trim-and-fill (Duval & Tweedie, 2000) methods. We estimated the

number of missing studies using both L0 and R0 estimators (Duval & Tweedie, 2000). In order to remain conservative, we reported the estimator giving the largest number of missing studies. The associated correction was then applied to the first meta-analytic mean to see if the missing studies would have affected it significantly (Møller & Jennions, 2001; Rothstein, Sutton & Borenstein, 2005).

All analyses were run using the R software (version 3.4.3, R Core Team, 2018).

III. RESULTS

The full data set comprises 279 effect sizes obtained from 81 studies (Fig. 1), conducted on 13 species of acanthocephalan parasites (Table 2A), and 20 host species belonging to three orders of Crustacea and one order of Insecta (Fig. S1A). Our data set was composed of two phylogenetically different acanthocephalan classes: Archiacanthocephala and Palaeacanthocephala. Although these two classes were not equally represented (11% and 89%, respectively), we retained both in order to maximize statistical power given the variability in effect size estimates. The fish parasite *Pomphorhynchus laevis* accounted for 33% of the total number of effect size estimates, whereas estimates obtained for *Acanthocephalus anguillae*, *Hexaglandula corynosoma* and *Pseudocorynosoma constrictum* accounted for only 2.5% in total (Table 2A, Fig. S1A). Among the different trait categories, behaviour was the most frequently recorded trait, accounting for about 49% of all effect size estimates, whereas morphological traits represented only 9.7% (Table 2B, Fig. S1B). Effect size estimates of vulnerability to predation represented only 5.4% of the data set (Table 2B; Fig. S1B). Most effect sizes were estimated on intermediate hosts infected with the cystacanth stage (261 out of 279) as compared to acanthella (18).

Table 2. Number of studies and number of effect size estimates including both cystacanth and acanthella infection stages (with number of effect sizes for acanthella infections alone shown in parentheses) included in our data set for (A) acanthocephalan parasite species and (B) categories and subcategories of host phenotypic traits.

A

Parasite class	Parasite species	Number of studies	Number of effect sizes (acanthella only)
Archiacanthocephala	*Moniliformis moniliformis*	8	26 (0)
	Oncicola venezuelensis	2	5 (0)
Palaeacanthocephala	*Acanthocephalus anguillae*	1	2 (0)
	Acanthocephalus dirus	18	29 (2)
	Acanthocephalus lucii	12	25 (1)
	Echinorhynchus truttae	6	9 (0)
	Hexaglandula corynosoma	1	2 (0)
	Leptorhynchoides thecatus	1	8 (0)
	Plagiorhynchus cylindraceus	3	7 (0)
	Polymorphus minutus	27	56 (4)
	Pomphorhynchus laevis	39	92 (10)
	Pomphorhynchus tereticollis	7	15 (0)
	Pseudocorynosoma constrictum	2	3 (1)
TOTAL			**279 (18)**

B

Trait category	Trait subcategory	Number of studies	Number of effect sizes (acanthella only)
Behaviour	Activity	17	19 (1)
	Protection	15	23 (2)
	Response to stimuli	22	43 (4)
	Taxis/phobia	26	53 (2)
Life history	Body condition	4	4 (0)
	Foraging	4	8 (1)
	Reproduction	10	25 (3)
	Intraspecific interaction	1	1 (0)
	Survival	5	6 (1)
Morphology	Colour	5	7 (0)
	Growth	14	20 (3)
Physiology	Immunity/resistance	8	27 (0)
	Energy reserves/metabolism	12	19 (0)

	Neurophysiology	5	9 (0)
Predation	Suitable final host	10	13 (0)
	Non-host	2	2 (0)
TOTAL			**279 (18)**

Most effect size values were retrieved from studies reporting more than one estimate (93.5% of the overall data set), justifying the incorporation of study as a random factor in the model. Additionally, more than half the data set (58.8% of effect size values) comprised shared measures (55.9% of effect size values), with very few cases of shared controls (2.9%) (Fig. S1B). Shared measures were found in all trait categories except predation.

(1) Meta-analytic means and phylogenetic inertia

To incorporate phylogenetic information in the meta-analysis, we first estimated phylogenetic relationships among 59 acanthocephalan species using Bayesian inference of nuclear 18S rDNA sequences. The phylogeny was well resolved (Fig. 2), and our tree topology matches those published previously [see Verweyen *et al*. (2011), and references therein]. We confirmed paraphyly of the orders Echinorhynchida and Polymorphida within the most diversified class Palaeacanthocephala, in agreement with Verweyen *et al*. (2011) but with a larger data set (59 species instead of 29 species) (Fig. 2).

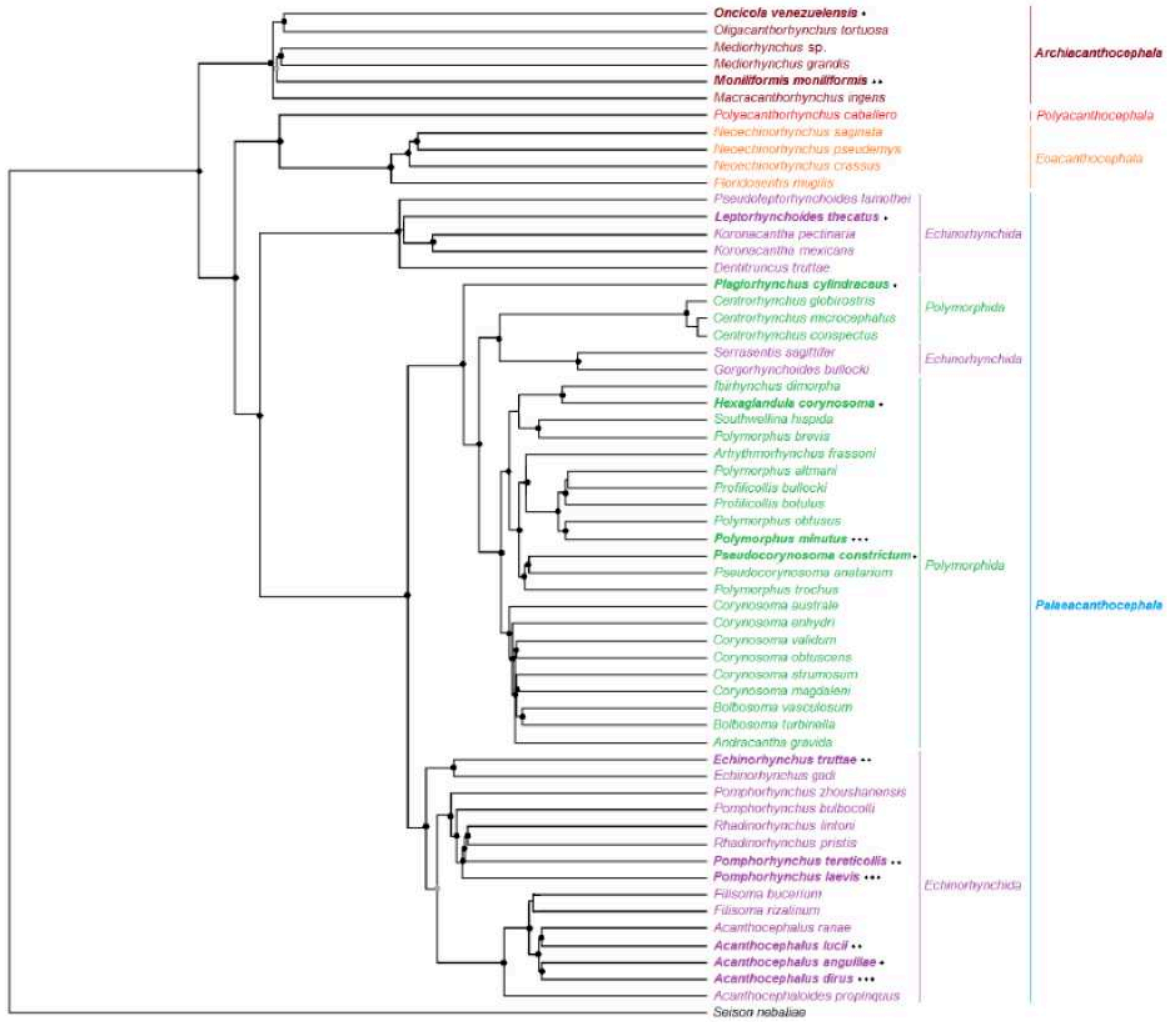

Fig. 2. Phylogenetic reconstruction of the phylum Acanthocephala based on 18S rRNA sequences from 59 species, and one outgroup species of Rotifera (in black), using Bayesian MCMC algorithms with MrBayes. Species included in the meta-analyses belong to the classes Archiacanthocephala (in brown, two species) and Palaeacanthocephala (in blue, 11 species). Species identified with one, two or three asterisks are represented in the data set by less than 5, between 5 and 15, or more than 15 estimates, respectively. Black and grey dots represent values of posterior probabilities higher than 0.90 and 0.80, respectively.

Overall, heterogeneity due to phylogenetic inertia (I^2) accounted for about 12–13% of the variation in signed effect sizes of infection with the cystacanth stage only (Table 3). Incorporating the signed effect sizes of infection with acanthella slightly increased this phylogenetic signal to 19% of overall variation (Table S2A). The meta-analytic mean effect size of infection with the cystacanth stage was significantly positive (0.28 [0.05–0.49], with phylogenetic correction) (Fig. 3). However, when incorporating the signed effect sizes of

infection with acanthella (entire data set including cystacanth and acanthella infection), the meta-analytic mean was no longer significant (0.23 [–0.19–0.57], with phylogenetic correction) (Fig. S3). Finally, the average magnitude of alteration induced by acanthocephalan infection independently of parasite transmission (absolute mean value) was moderate to large (0.40 [0.34–0.60]) (Fig. 4).

Table 3. Composition of the meta-analytic models run to explain variation in signed effect sizes of infection with cystacanths only. The corresponding deviance information criterion (DIC), and heterogeneity (I^2) arising from the random factors (study, parasite species and parasite phylogenetic distance) are provided. The best model, according to the lowest DIC, is shown in bold type.

			Heterogeneity I^2 (%) (random effects)		
Model	**Moderators (fixed effects)**	**DIC**	**Parasite species**	**Study**	**Parasite phylogenetic distance**
1	intercept only	215.36	6.55	31.18	11.84
2	category of traits	208.20	6.45	32.78	13.60
3	infection type	215.77	6.61	30.56	13.12
4	publication year	216	7.05	30.38	12.39
5	sample size	209.80	6.40	32.70	11.86
6	**category of traits + sample size**	**202.99**	**6.42**	**34.74**	**13.20**
7	category of traits + infection type + publication year + sample size	204.68	6.71	34.62	14.04

We ran another analysis on a subset of 230 signed effect size estimates, after removing ambiguous phenotypic traits with respect to whether an increase or a decrease would enhance parasite transmission in cystacanth-infected hosts. In this analysis, the meta-analytic mean remained significant (0.32 [0.05–0.52]) (Fig. S4).

Finally, to account for non-independence among effect size estimates caused by shared measures and shared controls, we ran a sensitivity analysis on a subset of 143 independent effect

size estimates: the meta-analytic mean of infection with cystacanths was still significant (0.31 [0.01–0.52]) (Fig. S5).

(2) Meta-regressions

To assess whether PIPAs enhance parasite transmission, we focused the meta-regression analysis on signed effect sizes including the cystacanth stage only (Fig. S2). The meta-regression revealed that trait category and sample size were the first factors driving variation in the magnitude of effect sizes, regardless of the incorporation of ambiguous traits (Fig. 3; Table 3), or not (Fig. S4; Table S2B). Specifically, two behavioural traits (response to stimuli and taxis/phobia), one life-history trait (reproduction), one morphological trait (colour), one physiological trait (immunity), and the vulnerability to predation of suitable final hosts, were significantly and positively affected by infection with cystacanths (Fig. 3). Here also, heterogeneity arising from random effects was consistent across models, regardless of the inclusion of fixed effects and their total number in the analyses (Table 3). Among the random effects, study ID accounted for about 32% of heterogeneity in signed effect sizes, whereas parasite species accounted for only 7% (Table 3).

The average magnitude of alteration, estimated for each factor as the mean absolute effect size, was comparable between developmental stages. In addition, behavioural and life-history traits seemed to be the most strongly affected ones (Fig. 4).

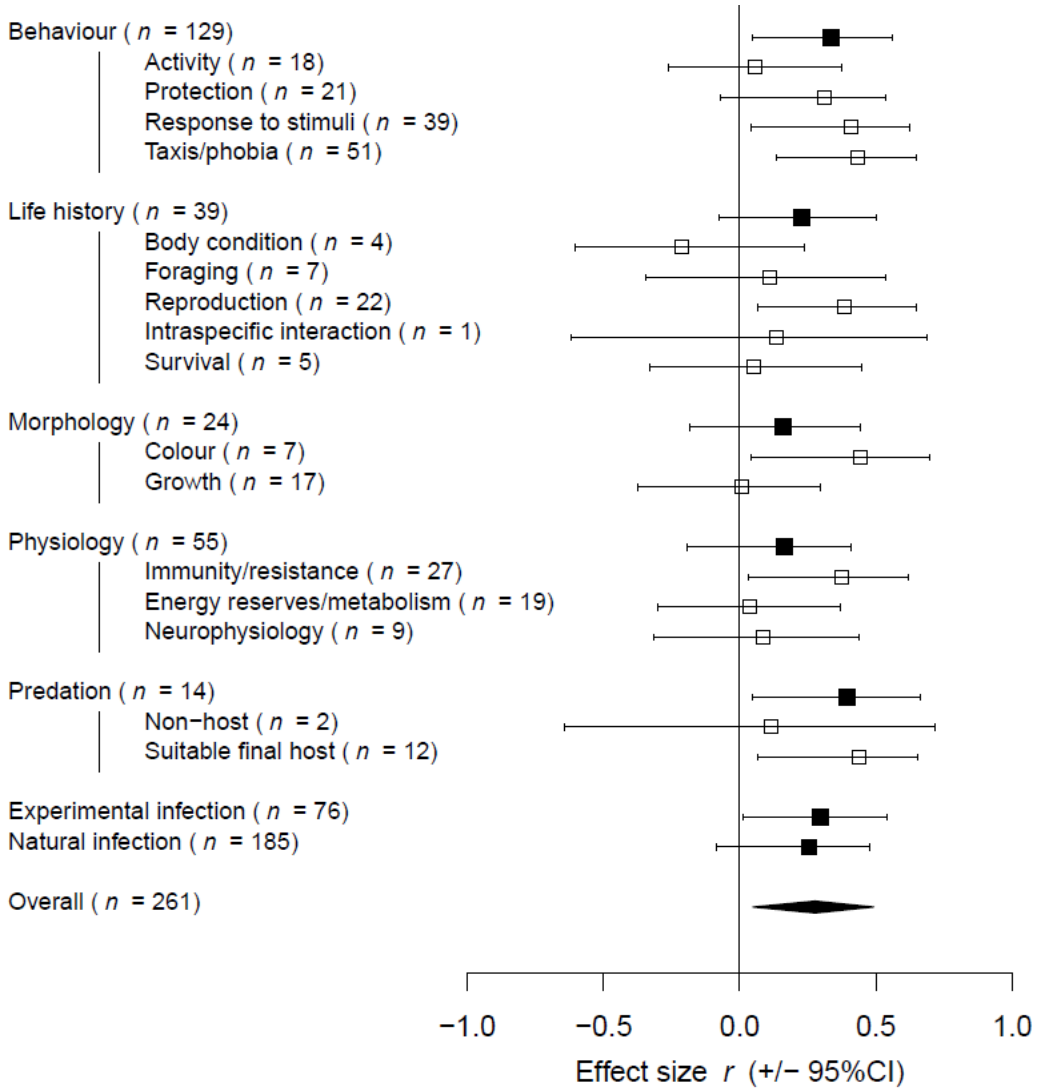

Fig. 3. Forest plots of the global meta-analytic mean of signed effect sizes (overall) based on cystacanth-induced alterations in host phenotype, and the meta-analytic mean for each moderator (categories and subcategories of traits and type of infection). Positive effect sizes represent infection-induced alterations of trait value expected to increase trophic transmission, whereas negative effect sizes represent infection-induced alterations expected to decrease trophic transmission. *n*, sample size.

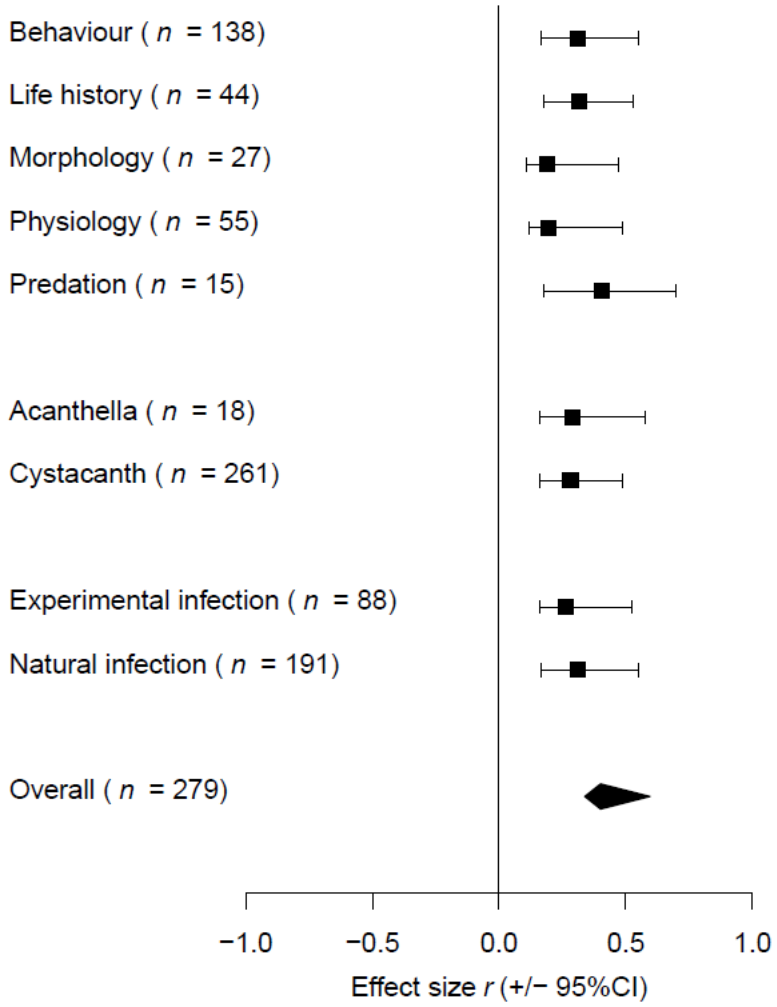

Fig. 4. Forest plots of the global meta-analytic mean of absolute effect sizes (overall), and the meta-analytic mean for each moderator (categories of traits, parasite maturity and type of infection) representing the magnitude of alterations induced by infection with acanthocephalans (both acanthella and cystacanth) on host phenotype. *n*, sample size.

(3) Publication bias

Based on Egger's regression, there was no significant evidence for a publication bias (intercept = 0.05, 95% CI = –0.02–0.11). This was further confirmed by the trim-and-fill analysis. Although 48 effect size estimates were likely to be missing on the left side of the funnel plot (Fig. 5), the funnel plot was almost symmetrical and the correction of –0.09 from the trim-and-fill did not alter the meta-analytic mean significantly (0.26, 95% CI = 0.03–0.45).

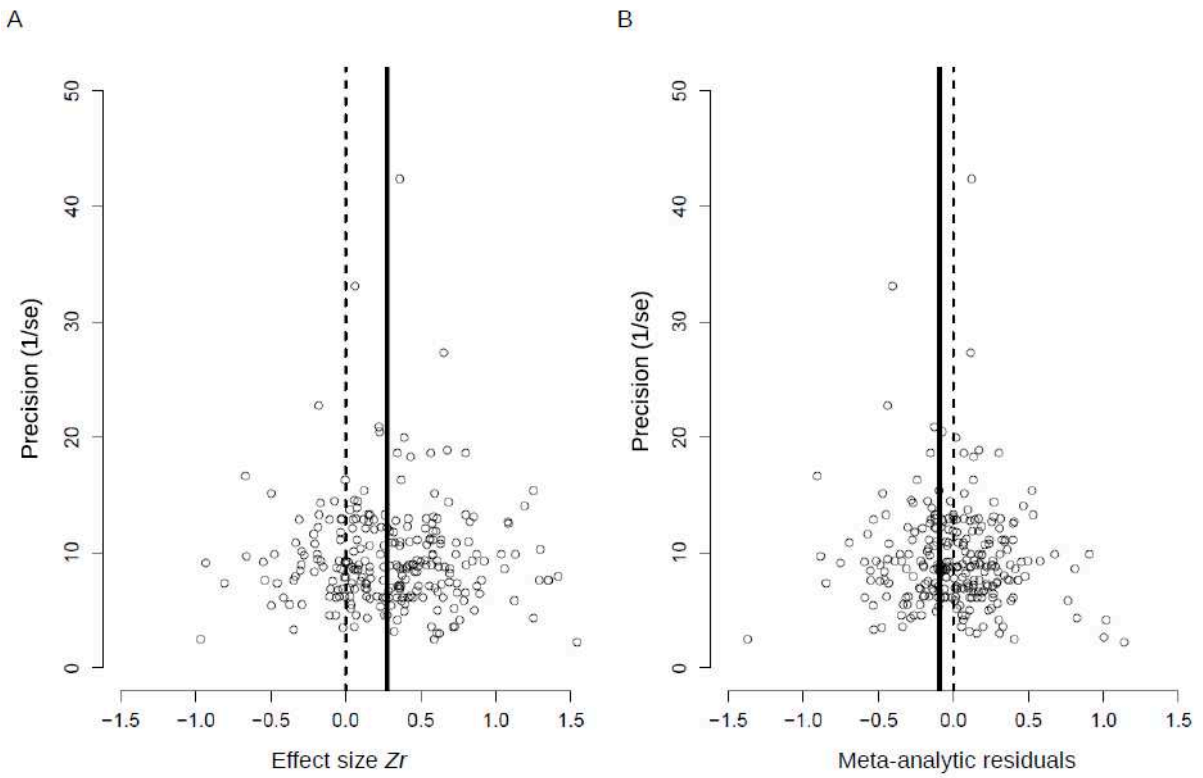

Fig. 5. Funnel plots of (A) original data points (effect size estimates) and (B) residuals from model 1 (Table 3), plotted against precision (the inverse of standard error). Bold lines represent the meta-analytic mean in (A) and the correction (calculated using the trim-and-fill method) in (B).

IV. DISCUSSION

The aim of our study was to undertake a critical review on host manipulation by acanthocephalan parasites, in the framework of phylogenetic meta-analysis. We believe the originality and strength of our analysis lies in several features. First, we ran a phylogenetically corrected meta-analysis using a more exhaustive and multidimensional data set (N = 279 estimates of effect on a wide range of phenotypic traits) than in previous meta-analyses on the impact of parasites, which focused on the magnitude of behavioural alterations (Nakagawa *et al.*, 2015: N = 202, including 92 effect sizes from nine acanthocephalan species), on body condition (Sánchez, 2018; N =553), on the relationship between infection and social status in vertebrates (Habig *et al.*, 2018, N = 128), or on the relationship between infection and group size (Patterson & Ruckstuhl, 2013; N = 70). A key feature of our study is that it incorporated all

phenotypic traits reported in order to (*i*) broaden our understanding of multidimensionality in manipulation by acanthocephalans, and (*ii*) avoid potential bias resulting from inclusion only of behavioural traits [as in previous studies (Poulin, 1994, 2000; Nakagawa *et al*., 2015)], given that they are more likely to be involved in parasite transmission. Second, we quantified the overall effect of these multiple phenotypic alterations induced by acanthocephalans on parasite transmission by assigning a benefit in terms of increased encounter rate with the final host or decreased energetic expenditure by the intermediate host. We also considered the magnitude of alterations independently from increased parasite transmission. Third, we addressed whether the effect size of infection differs according to trait category and parasite stage, as a way to address how fine-tuned PIPAs are.

(1) How strong is the general effect of infection, independent of parasite phylogeny?

We found little evidence for a phylogenetic signal. Relatedness between acanthocephalan species accounted for only a small proportion of the heterogeneity of all effect size estimates. The negligible effect of phylogenetic distance suggests that Acanthocephala is a homogeneous taxon in terms of phenotypic alterations induced in intermediate hosts. This conclusion must however be made with caution, as the class Archiacanthocephala is under-represented in the data set.

Overall, acanthocephalan parasites induce low to moderate alterations in their host phenotype, a result in agreement with Poulin (1994). The phylogenetic mean ranges from r = 0.23 to 0.40, depending on correction for phylogeny and on the use of absolute or signed effect sizes.

The meta-regression analysis on signed effect sizes revealed no effect of the type of infection (experimental or natural) on overall intensity of manipulation (Fig. 4). We can therefore confidently rely on results from experimental infections in studies investigating the

role of parasite stage (Dianne *et al.*, 2011), parasite age (Franceschi *et al.*, 2008), parasite and host populations (Franceschi *et al.*, 2010*b*), abiotic factors (Labaude, 2017*a*), and biotic factors (no to date, but see Fayard *et al*., 2019) in modulating the intensity of PIPAs. In addition, the intensity of PIPAs decreased with increasing sample size. This highlights the importance of the number of replicates within a study in estimating the magnitude of PIPA.

Among random factors, the meta-regression analysis on signed effect size revealed a negligible effect of parasite species on overall intensity of manipulation, but a more important effect of study. This study effect highlights the possible impact of differences in experimental designs in estimating the type and magnitude of PIPAs.

(2) Is there evidence for adaptive manipulation? Trophic transmission and parasite stage

For the mature parasite stage (cystacanth only), acanthocephalans do induce a moderate increase in traits affecting parasite transmission to the definitive host. However, there are still too few studies quantifying trophic transmission (only 5.4% of the cystacanth data set), in comparison to those reporting PIPAs. In addition, even fewer studies have actually attempted to estimate the contribution of a given altered trait to trophic transmission (Kaldonski *et al.*, 2009; Perrot-Minnot *et al.*, 2012; Jacquin *et al.*, 2014). This limitation should not be overlooked when reviewing evidence for adaptive manipulation.

Another line of evidence for adaptive manipulation is a trend for reversed parasite-induced alterations in the intermediate host between parasite developmental stages, predicted theoretically to enhance parasite infectivity to the final host (Parker *et al.*, 2009). The average magnitude of alterations induced by infection with acanthella was comparable to that induced by infection with cystacanth, but in the opposite direction (Fig.4; Fig. S3). This suggests that the acanthella stage could have a real and opposite impact on host phenotype compared to the cystacanth stage, in ways that are likely to decrease the vulnerability to predation of the infected

intermediate host (Parker *et al.*, 2009; Dianne *et al.*, 2011). This result must be considered with caution, however, given the low number of effect size estimates for the acanthella stage (n = 18) compared to the cystacanth stage (n = 261), and the low number of parasite species for which estimates were available (five). In addition, half of these effect sizes were estimated for behavioural traits (taxis/phobia, protection, and response to stimuli), which might lead to overestimated differences between acanthella infection and cystacanth infection. Indeed, while acanthella and cystacanths are theoretically likely to have opposing effects in terms of behavioural alterations (Parker *et al.*, 2009), energy-saving strategies, such as physiological or behavioural castration or immunosuppression, are expected to be shared by both parasite stages to some extent. However, while immunosuppression may allow energy conservation, it may also compromise the survival of infected hosts in response to other pathogens (Cornet *et al.*, 2009). Therefore, immunosuppression might represent a more costly strategy for the acanthella than for the cystacanth stage, given the longer developmental time required to reach the stage infective for the final host (Crompton & Nickol, 1985). Unfortunately, there have been no studies that quantify acanthella-infected host immunocompetence. Finally, there is only mixed evidence in support of an energy-saving strategy by depressing host reproduction at the acanthella stage (Bailly *et al.*, 2018).

(3) Is there evidence for multidimensional alterations?

Overall, all trait categories were impacted by cystacanth infection. Behaviour was the trait category that was most significantly impacted, and hence is the category expected to contribute the most to acanthocephalan transmission (Fig. 3). Taxis/phobia was the most strongly impacted subcategory, followed by response to stimuli. If reversed taxis can drive alterations in microhabitat preferences through alterations in reactions to light, gravity, air or water velocity, or substrate, the observed pattern is likely to increase the encounter rate of the

cystacanth with final hosts. These findings confirm that altering the host's microhabitat preference is an important feature of manipulation by acanthocephalans compared with other trophically transmitted parasites, whereas activity is not significantly affected (Lafferty & Shaw, 2013). Changes in responses to stimuli are also expected to modulate the encounter rate of infected prey and the final hosts. More surprising is the non-significant effect of cystacanth infection on protection behaviour (i.e. on exposure). However, we included studies that scored protection/exposure behaviour under simulated predation threat in the 'response to stimuli' subcategory, meaning that those in the 'protection/exposure' subcategory of behaviour reported alterations in protective behaviour solely in the absence of predation risk. The mechanisms by which acanthocephalans alter these context-dependent traits may thus rely on stimulus perception/response, rather than on avoidance or defensive behaviour itself.

Among physiological and life-history-related traits, only host immunity and reproduction were significantly impacted by infection with cystacanth stages (Fig. 3). We interpret immunosuppression and castration as part of an energy-saving strategy to support both host maintenance and parasite growth, thereby increasing host survival and indirectly, parasite transmission. Alternatively and non-exclusively, alterations in host reproductive and immune system traits could be linked to behavioural alterations, and thereby to parasite transmission. The immune and nervous systems are connected through several different pathways in animals (Dantzer *et al.*, 2008; Adamo, 2013). Neurological functions can be modulated by immune factors such as cytokines by means of specific neuronal receptors. Cytokines released by the immune system act as signalling molecules to the central nervous system, and can result in sickness behaviour: a set of physiological and behavioural alterations that promote the survival of infected individuals (Dantzer, 2004; Dantzer & Kelley, 2007). Adamo (2013) postulated that if parasites could alter the amount or the type of cytokines released by the host immune system,

then this could result in abnormal behaviour. Although highly interesting, this neuropsychoimmune hypothesis has not yet been addressed.

(4) Recommendations for future research

Our findings highlight several ways to improve our understanding of the adaptive significance of host manipulation. First, for future meta-analysis, researchers should attempt to increase the power and functionality of the metrics used to quantify phenotypic alterations. This could be achieved by increasing sample size, and by reporting effect sizes rather than statistical metrics. Indeed, 85 studies had to be excluded (Fig. 1) from the present analysis because suitable data were not provided or were no longer available. Second, as a consequence of the historical focus on behavioural trait alterations expected to increase trophic transmission of the infective stage, traits not directly related to predator–prey interactions have received little attention in acanthocephalans (Cézilly & Perrot-Minnot, 2010), including phenotypic alterations induced by acanthella. Yet, these remain crucial to developing a better understanding of whether PIPAs constitute a 'manipulation syndrome', and whether the adaptive value of PIPAs extends to developmental stages not infective to the final host (protective manipulation). Third, studies quantifying actual trophic transmission are still rare (Poulin & Maure, 2015). This is likely due to the fact that designing studies to quantify trophic transmission raises practical challenges, in particular under field conditions, as either prey choice or the diet of final hosts needs to be analysed [see Cézilly *et al*. (2010), for a recent review]. The study of proximate mechanisms, in particular the neuropsychoimmune hypothesis of parasite manipulation, also requires attention (Poulin & Maure, 2015). Finally, taxonomic bias may arise from focusing on only a small set of model species (Poulin & Maure, 2015). In our data set, the most diverse and derived class Palaeacanthocephala was over-represented, while the more ancient class Archiacanthocephala was represented by only two species (*Moniliformis moniliformis* and

Oncicola venezuelensis). This prevented a detailed comparison between these two classes, for example to investigate whether host manipulation increases over evolutionary time.

Finally, another stimulating area in the study of parasite manipulation from an evolutionary point of view is to investigate not only the magnitude of parasite manipulation (changes in trait means) but also alterations in trait variability. Behavioural variability is predicted to decrease in infected hosts, making them more susceptible to predators as part of the manipulation strategy (Nakagawa *et al.*, 2015). Alternatively, behavioural variability in infected hosts could increase as a consequence of parasite-induced disruption of regulatory pathways controlling behaviour. To our knowledge, only one meta-analytic study has quantified the effect size of infection on behavioural variability and they failed to find a significant effect (Nakagawa *et al.*, 2015). However, their study was not restricted to acanthocephalans, and it remains possible that other taxa of parasites could respond differently, both in mean host traits and also their variance.

V. CONCLUSIONS

(1) Overall, infection with acanthocephalans induces low to moderate phenotypic alterations in their hosts. The magnitude of alterations induced by the infective stage was highest for behavioural traits related to microhabitat choice and response to stimuli, and for immunity and reproduction. Although a trend for opposite effects of infection with acanthella was detected, a thorough analysis of the "predation suppression then predation enhancement" strategy is still limited by the lack of data at the acanthella stage of development. Furthermore, testing for publication bias showed that 48 data points were lacking, corresponding to negative effects (opposing parasite-induced transmission facilitation), although no significant publication bias was detected overall. Future studies should be careful not to censor negative evidence for the host manipulation hypothesis.

(2) Multidimensionality of parasite manipulation was indicated in the significant effect of infection on all trait categories. Questions remain regarding the links between behavioural, life-history, and physiological traits. For instance, testing of the neuropsychoimmune hypothesis has so far been restricted to establishing correlations between phenotypic responses (phototaxis and immunity) in few acanthocephalan species (Cornet *et al.*, 2009). Although informative from an ecological point of view, this is not a powerful mechanistic approach since the absence of a phenotypic correlation does not prove the existence of independent modulation of these traits. Manipulating the level of immunocompetence, and monitoring any resulting alterations in levels of brain neuromodulators, neurogenesis or neuronal apoptosis, would be a more promising way to decipher the interrelationships between the immune and neural systems, and any consequences on behaviour.

(3) Although we were able to detect low to moderate increases in traits promoting parasite transmission to the definitive host, there are still too few studies that actually quantify trophic transmission. Even fewer have attempted to understand the relationship between multidimensional phenotypic alterations and parasite transmission success (discussed in Cézilly & Perrot-Minnot, 2010; Thomas *et al*., 2010).

(4) To allow comparison of effect sizes between trait categories, we combined traits that were functionally comparable from an ecological viewpoint. The criteria used here to assign phenotypic traits to different categories may be more broadly applicable to a wide range of host species. As a theoretical approach to host manipulation by parasites is relevant across a diverse range of taxonomic groups (Thomas *et al.*, 2012; Lafferty & Shaw, 2013), our method may be applicable to many other parasites engaged in host manipulation.

(5) The past 10 years has seen a decreasing number of empirical studies relative to theoretical analyses and reviews, creating an "imbalance between facts and ideas" (Poulin & Maure, 2015). This review provides quantitative evidence that the fascinating phenomenon of host

manipulation has solid theoretical and empirical foundations, but also raises challenging questions about the underlying proximate and ultimate mechanisms that call for broader methodological and taxonomic coverage.

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Authorship: M.F. and M.-J. P.-M. designed project goals, trait categorization, and collected the data. M. F. and F.-X. D.-M. run the meta-analysis, R.W. and M.-J. P.-M. collected and analysed molecular data for phylogenetic reconstruction, and drafted the relevant section. M. F., F.-X. D.-M and M.-J. P.-M. drafted the manuscript. All authors revised the manuscript.

Data Accessibility Statement: Data and R script for the meta-analysis are available on Mendeley repository at DOI: 10.17632/k5y4g5nfwy.2

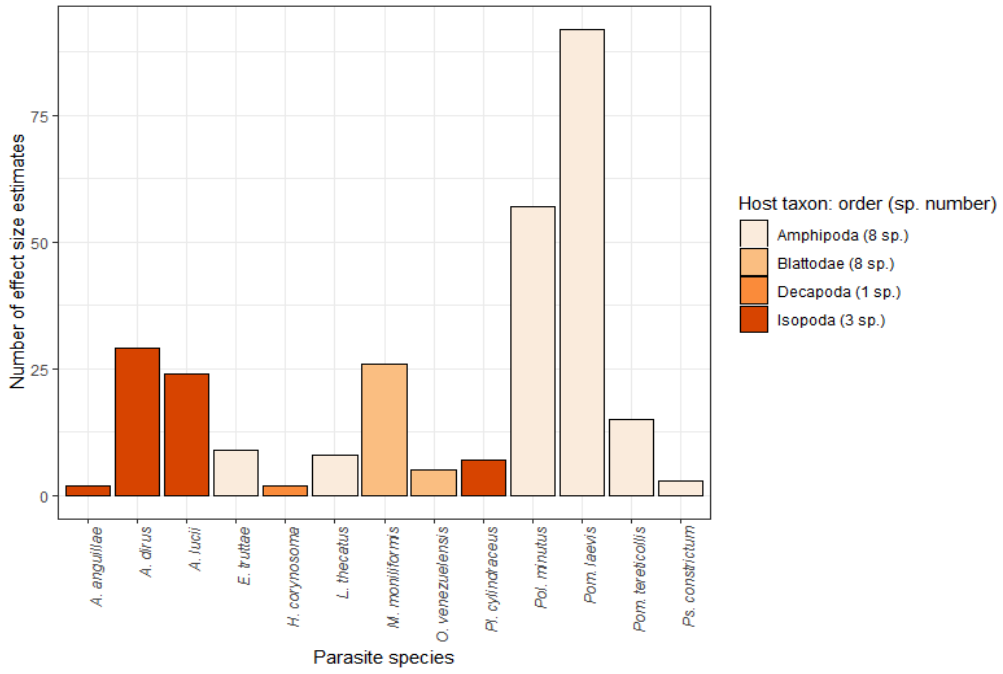
VIII. SUPPORTING INFORMATION

Table S1. List of acanthocephalan species included in the phylogenetic analysis ($N = 59$) and the outgroup species (*Seison nebaliae*, Order Seisonacea, Phylum Rotifera), with their accession numbers. The 18S sequences of *Acanthocephalus anguillae*, *Acanthocephalus ranae* and *Pomphorhynchus bulbocolli* were produced as part of the present study, all others were retrieved from Genbank. Accession number for the outgroup species *Seison nebaliae* is DQ089737.

Genbank accession no.	Species	Order	Class within Acanthocephala phylum
AF001844	*Macracanthorhynchus ingens*	Oligacanthorhynchida	Archiacanthocephala
AF001843	*Mediorhynchus grandis*	Gigantorhynchida	Archiacanthocephala
AF064816	*Mediorhynchus* sp.	Gigantorhynchida	Archiacanthocephala
HQ536017	*Moniliformis moniliformis*	Moniliformida	Archiacanthocephala
AF064817	*Oligacanthorhynchus tortuosa*	Oligacanthorhynchida	Archiacanthocephala
AF064818	*Oncicola* sp.	Oligacanthorhynchida	Archiacanthocephala
AF388660	*Polyacanthorhynchus caballeroi*	Polyacanthorhynchida	Polyacanthocephala
AF064811	*Floridosentis mugilis*	Neoechinorhynchida	Eoacanthocephala
AF001842	*Neoechinorhynchus crassus*	Neoechinorhynchida	Eoacanthocephala
U41400	*Neoechinorhynchus pseudemydis*	Neoechinorhynchida	Eoacanthocephala
AY830150	*Neoechinorhynchus saginata*	Neoechinorhynchida	Eoacanthocephala
AY830149	*Acanthocephaloides propinquus*	Echinorhynchida	Palaeacanthocephala
LS991432	*Acanthocephalus anguillae*	Echinorhynchida	Palaeacanthocephala
AY830151	*Acanthocephalus dirus*	Echinorhynchida	Palaeacanthocephala
AY830152	*Acanthocephalus lucii*	Echinorhynchida	Palaeacanthocephala
LS991433	*Acanthocephalus ranae*	Echinorhynchida	Palaeacanthocephala
JX460866	*Dentitruncus truttae*	Echinorhynchida	Palaeacanthocephala
JX014222	*Echinorhynchus gadi*	Echinorhynchida	Palaeacanthocephala
AY830156	*Echinorhynchus truttae*	Echinorhynchida	Palaeacanthocephala
AF064814	*Filisoma bucerium*	Echinorhynchida	Palaeacanthocephala
JX014229	*Filisoma rizalinum*	Echinorhynchida	Palaeacanthocephala
AY830154	*Gorgorhynchoides bullocki*	Echinorhynchida	Palaeacanthocephala
AY830157	*Koronacantha mexicana*	Echinorhynchida	Palaeacanthocephala
AF092433	*Koronacantha pectinaria*	Echinorhynchida	Palaeacanthocephala
AF001840	*Leptorhynchoides thecatus*	Echinorhynchida	Palaeacanthocephala
AY423346	*Pomphorhynchus laevis*	Echinorhynchida	Palaeacanthocephala
AY423347	*Pomphorhynchus tereticollis*	Echinorhynchida	Palaeacanthocephala
KY490051	*Pomphorhynchus zhoushanensis*	Echinorhynchida	Palaeacanthocephala
LS991434	*Pomphorhynchus bulbocolli*	Echinorhynchida	Palaeacanthocephala
EU090950	*Pseudoleptorhynchoides lamothei*	Echinorhynchida	Palaeacanthocephala
JX014224	*Rhadinorhynchus lintoni*	Echinorhynchida	Palaeacanthocephala
JX014226	*Rhadinorhynchus pristis*	Echinorhynchida	Palaeacanthocephala
JX014227	*Serrasentis sagittifer*	Echinorhynchida	Palaeacanthocephala

EU267802	*Andracantha gravida*	Polymorphida	Palaeacanthocephala
JX442165	*Arhythmorhynchus frassoni*	Polymorphida	Palaeacanthocephala
JX442166	*Bolbosoma turbinella*	Polymorphida	Palaeacanthocephala
JX014225	*Bolbosoma vasculosum*	Polymorphida	Palaeacanthocephala
U41399	*Centrorhynchus conspectus*	Polymorphida	Palaeacanthocephala
KM588206	*Centrorhynchus globirostris*	Polymorphida	Palaeacanthocephala
AF064813	*Centrorhynchus microcephalus*	Polymorphida	Palaeacanthocephala
JX442168	*Corynosoma australe*	Polymorphida	Palaeacanthocephala
AF001837	*Corynosoma enhydri*	Polymorphida	Palaeacanthocephala
EU267803	*Corynosoma magdaleni*	Polymorphida	Palaeacanthocephala
JX442169	*Corynosoma obtuscens*	Polymorphida	Palaeacanthocephala
EU267804	*Corynosoma strumosum*	Polymorphida	Palaeacanthocephala
JX442170	*Corynosoma validum*	Polymorphida	Palaeacanthocephala
EU267808	*Hexaglandula corynosoma*	Polymorphida	Palaeacanthocephala
GQ981436	*Ibirhynchus dimorpha*	Polymorphida	Palaeacanthocephala
AF001839	*Plagiorhynchus cylindraceus*	Polymorphida	Palaeacanthocephala
AF001838	*Polymorphus altmani*	Polymorphida	Palaeacanthocephala
JX442171	*Polymorphus brevis*	Polymorphida	Palaeacanthocephala
EU267806	*Polymorphus minutus*	Polymorphida	Palaeacanthocephala
JX442172	*Polymorphus obtusus*	Polymorphida	Palaeacanthocephala
JX442173	*Polymorphus trochus*	Polymorphida	Palaeacanthocephala
EU267805	*Profilicollis botulus*	Polymorphida	Palaeacanthocephala
JX442174	*Profilicollis bullocki*	Polymorphida	Palaeacanthocephala
EU267801	*Pseudocorynosoma anatarium*	Polymorphida	Palaeacanthocephala
EU267800	*Pseudocorynosoma constrictum*	Polymorphida	Palaeacanthocephala
EU267807	*Southwellina hispida*	Polymorphida	Palaeacanthocephala

A



B

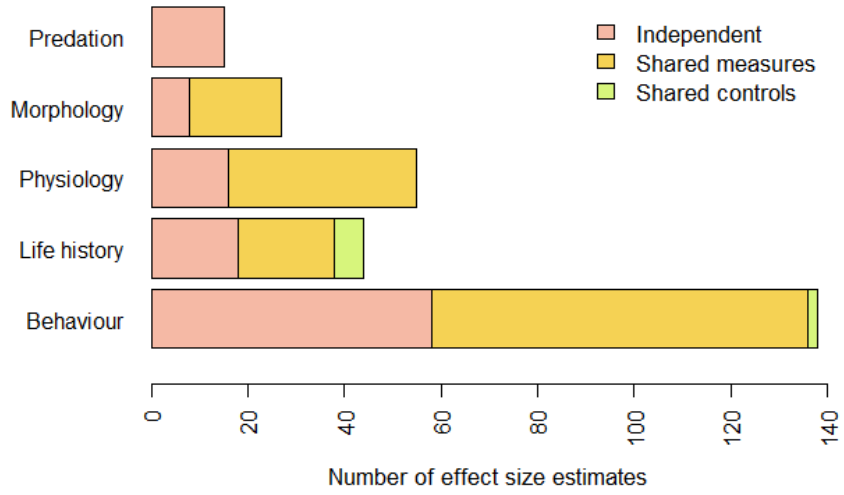


Fig. S1. Number of effect size estimates retrieved from published studies on the impact of acanthocephalan infection on their intermediate invertebrate hosts: (A) per host taxon (order) within each acanthocephalan species; (B) according to the source of non-independence between measures and for each host trait category. 'Shared measures' are effect sizes estimated on the same individuals but for different traits; 'shared controls' are effect sizes estimated for at least two parasite species using the same control group.

Appendix S1. Phylogenetic relationship of acanthocephalans based on 18S ribosomal gene sequences

We retrieved the distances between species from an ultrametric tree derived from a Bayesian inference based on 59 acanthocephalan 18S rRNA gene sequences and using a rotifer as an outgroup (Table S1).

18S rDNA gene sequences

Sequences from 56 acanthocephalan species were retrieved from GenBank. Sequences from three other species for which effect sizes were included in the data set were added (Table S1). Samples of *Acanthocephalus anguillae* and *Acanthocephalus ranae* were collected from the freshwater isopod *Asellus aquaticus*, in the river Ouche (La Colombière, Dijon) in 2004; samples of *Pomphorhynchus bulbocolli* were provided by Dr Spakulova. DNA extraction, amplification of a portion of 18S rDNA, purification of Polymerase Chain Reaction (PCR) products and sequencing, were done following Perrot-Minnot (2004). Three pairs of primers were used to obtain three overlapping sequences. These were assembled into a single sequence of approximately 1700 base pairs (bp) using BioEdit editor (Hall, 1999).

Phylogenetic analysis

Sequences were aligned using MAFFT7.388 software (Katoh & Stanley, 2013), with the E-IONS-I algorithm using the legacy gap penalty option. The best-fitting model of nucleotide substitution was determined using JModelTest-2.1.10. (Darriba *et al*., 2012) as being the General Time Reversible (GTR) with gamma-distributed rate heterogeneity (G) and a significant proportion of invariable sites (I) model. Bayesian phylogeny reconstruction was performed with MrBayes (Huelsenbeck & Ronquist, 2001). Four heated chains were run, each one million iterations long, sampled every 200 iterations. The runs reached satisfactory

effective sampling sizes (ESS > 200). The 50% majority-rule consensus tree was constructed after the removal of a 10% burn-in phase.

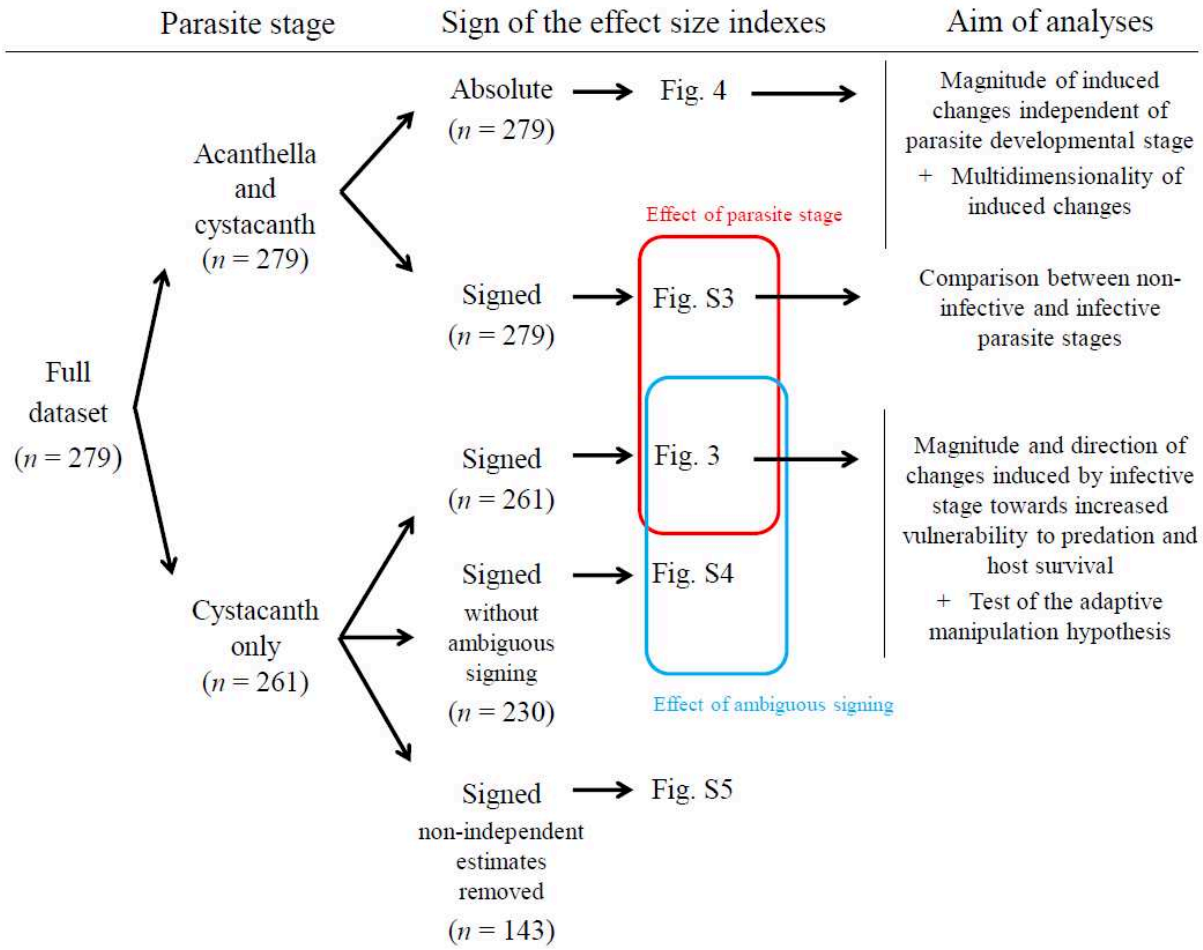


Fig. S2. Road map of the analyses. Diagram presenting the five analyses, using either signed effect sizes (the alteration of mean trait value, including the direction of change favouring parasite transmission) or absolute effect sizes (the magnitude of phenotypic alteration), and incorporating both parasite stages or only cystacanth infection. Two final analyses were run after excluding trait alterations for which the consequence on trophic transmission (sign) could not be unambiguously assigned, and trait alterations for which estimates were not independent (sensitivity analysis).

Table S2. Composition of the meta-analytic models in the complementary analysis run to explain variation in signed effect sizes (A) due to infection with acanthocephalans independent of parasite stage (incorporating effect size of both acanthella and cystacanth infection) and (B) due to cystacanth infection, excluding ambiguous traits (*cf*. Table 1). The corresponding deviance information criteria (DIC), and heterogeneity (I^2) arising from the random factors (parasite species, study and phylogeny) are provided. The best model, according to the lowest DIC, is shown in bold type.

A

Model	Moderators (fixed effects)	DIC	Heterogeneity I^2 (%) (random effects)		
			Parasite species	Study	Phylogeny
1	intercept only	250.96	10.41	29.33	19.11
2	category of traits	245.87	10.40	31.34	19.52
3	infection type	252.66	10.11	29.85	18.09
4	parasite stage	236.59	10.70	29.28	18.23
5	publication year	251.33	10.99	28.18	20.33
6	sample size	246.04	10.13	31.46	18.71
7	**category of traits + parasite stage + sample size**	**226.33**	**9.40**	**32.90**	**18.11**
8	category of traits + infection type + parasite stage + publication year + sample size	228.42	9.65	33.69	18.07

B

Model	Moderators (fixed effects)	DIC	Heterogeneity I^2 (%) (random effects)		
			Parasite species	Study	Phylogeny
1	intercept only	145.39	6.17	37.19	10.63
2	category of traits	133.49	6.74	40	12.54
3	infection type	146.55	6.18	36.58	11.47
5	publication year	146	6.24	37.59	10.50
6	sample size	136.19	6.18	39.68	10.43
7	**category of traits + sample size**	**124.19**	**6.94**	**41.88**	**12.44**
8	category of traits + infection type + publication year + sample size	126.04	6.95	42.22	13.08

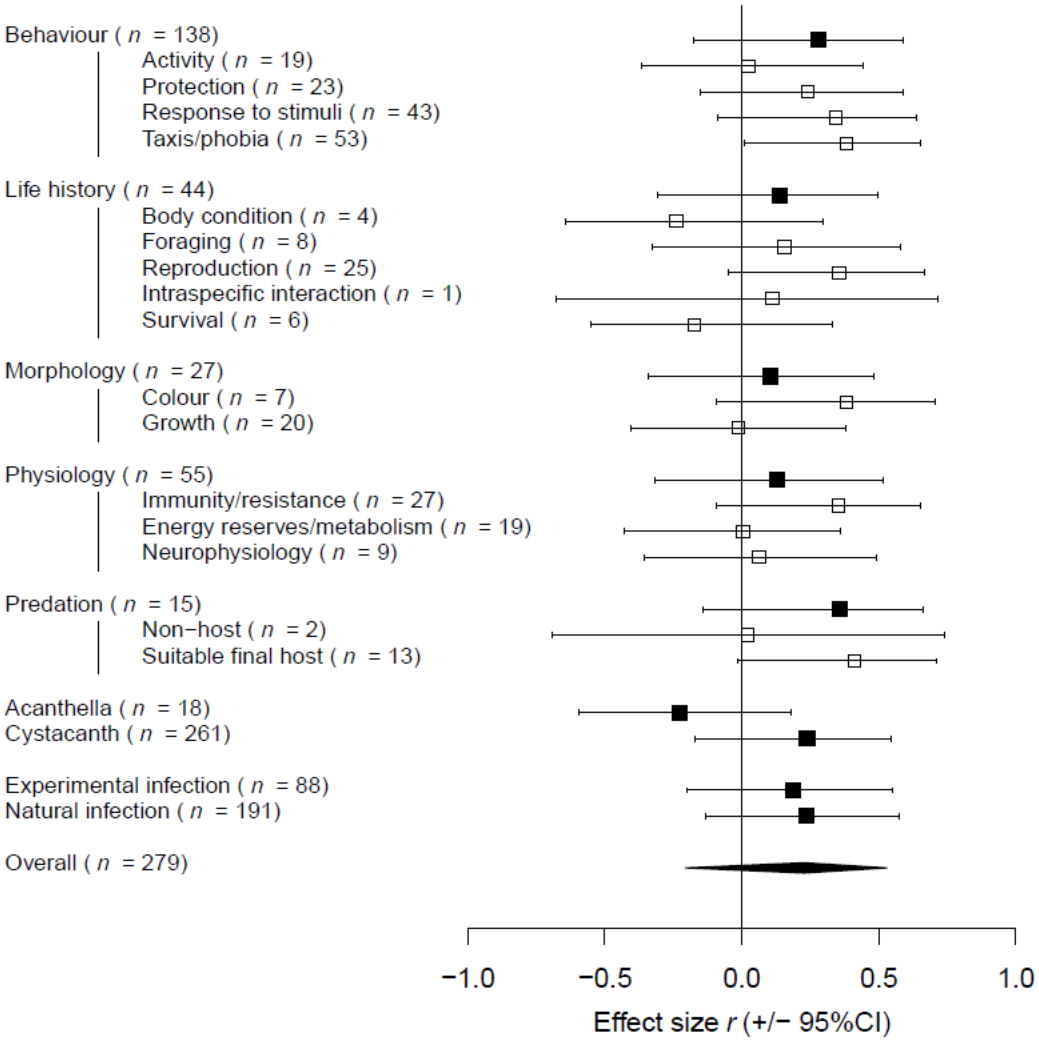


Fig. S3. Parasite-induced alterations of mean trait value from the complementary analysis, incorporating all parasite developmental stages: forest plot with global meta-analytic mean of signed effect sizes (overall), and the meta-analytic mean for each moderator (categories and subcategories of traits, parasite maturity and type of infection). Positive effect sizes represent infection-induced alterations expected to increase trophic transmission, whereas negative effect sizes represent infection-induced alterations expected to decrease trophic transmission. *n*, sample size.

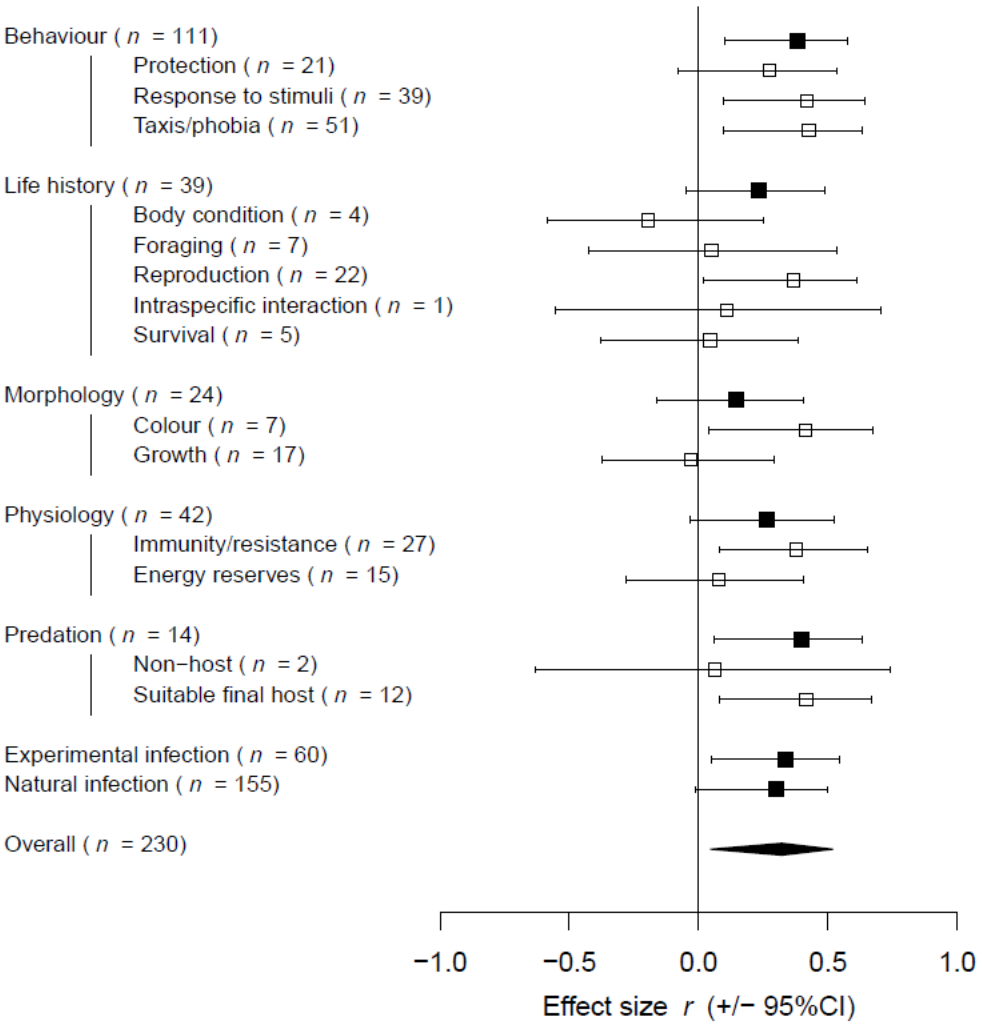


Fig. S4. Ambiguous traits: forest plot with global meta-analytic mean of signed effect sizes (overall), and the meta-analytic mean for each moderator (categories and subcategories of traits and type of infection). As the focus was on parasite transmission to final hosts, only effect size estimates for infection with cystacanths were included. The analysis was run after removing 31 ambiguous host traits with respect to their contribution to increased parasite transmission to final hosts [these traits were in the categories 'activity' (behaviour), 'energy reserves/metabolism' and 'neurophysiology' (both from physiology) and growth (morphology). *n*, sample size.

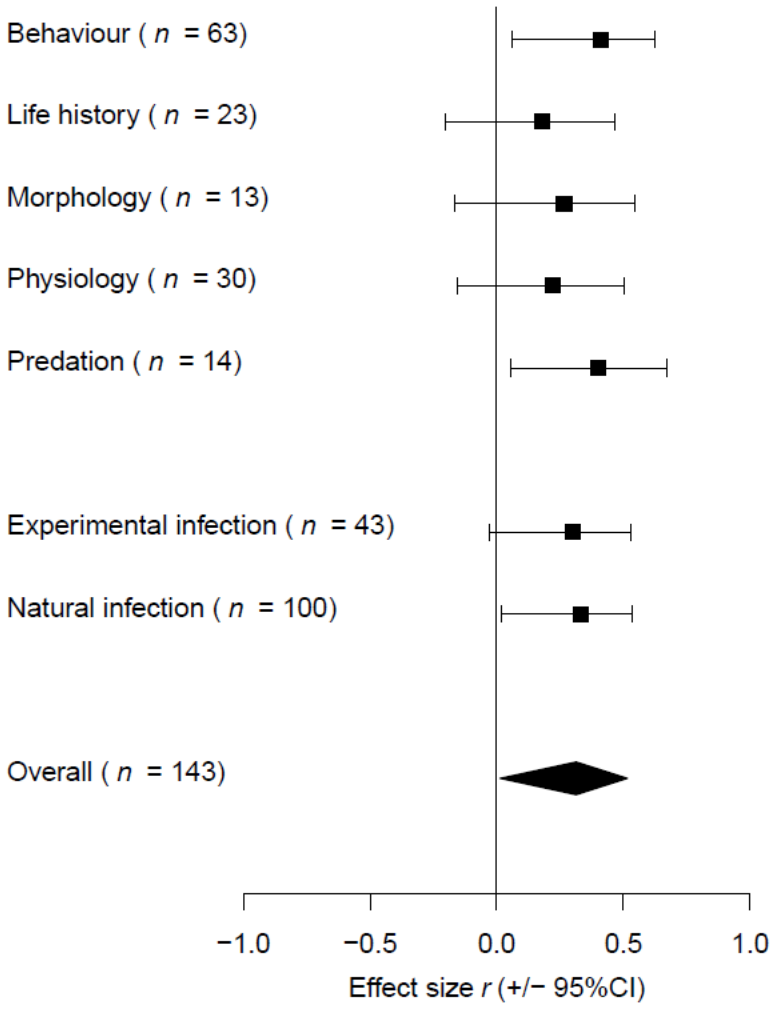


Fig. S5. Sensitivity analysis to account for the non-independence of effect sizes: forest plot with global meta-analytic mean of signed effect sizes (overall), and the meta-analytic mean for each moderator (categories of traits and type of infection). As the focus was on parasite transmission to final hosts, only effect size estimates of infection with cystacanths were included. The sensitivity analysis was performed by removing cases of pseudo-replication from the data set (mainly 'shared measures'). *n*, sample size.

Discussion-Transition
Variation liée au parasite ou à l'hôte

La variation dans l'intensité de la manipulation apparaît à plusieurs échelles et s'explique par différents facteurs. A l'échelle interspécifique, elle peut être liée aux différentes stratégies (changement d'habitat, utilisation de refuges, couleur) de complétion du cycle, que le cycle soit le même ou non (Poulin, 2010). La variation dans l'intensité de la manipulation se manifeste à travers son expression dans les différentes dimensions phénotypiques de leur hôte (Fayard *et al.*, 2020). Dans le cas de la manipulation multidimensionnelle, les traits qui sont les plus altérés (choix du microhabitat) semblent être bénéfiques à la transmission du parasite vers son hôte définitif. Ceci tend à penser qu'il y aurait eu sélection sur les gènes du parasite capables d'induire de tels changements comme étant la conséquence de l'inclusion de l'hôte définitif dans le cycle du parasite, initialement simple. Cependant, comme déjà discuté dans l'introduction, ce 'purposive design' ne pourrait être qu'apparent. En effet, parmi tous les traits altérés, certains ne semblent pas directement être bénéfiques à la transmission (reproduction, immunité). Ainsi, certains changements ne pourraient être que de simples effets secondaires négatifs pour l'hôte intermédiaire le rendant plus vulnérable à une éventuelle mort prématurée (soit à cause d'une condition trop faible, soit à cause de prédateurs non viables pour le parasite). Ceci aurait alors créé une pression de sélection vraiment forte sur le parasite. De ce fait, la réelle adaptation aurait consisté en l'inclusion d'un hôte définitif viable pour le parasite. Les changements phénotypiques en seraient alors la cause et non plus la conséquence. Cependant, il nous est encore impossible de trancher sur le lien de causalité entre apparition de la manipulation et inclusion de l'hôte définitif. La variation de l'intensité de la manipulation au niveau interspécifique ne nous est donc d'aucune aide dans le débat sur le caractère adaptatif de la manipulation.

Globalement, manipuler un hôte est associé à certains coûts et contraintes pour le parasite (Bakker *et al.*, 2017). Premièrement, il pourrait exister un coût à manipuler (coût de production) (Bakker *et al.*, 2017), via les mécanismes par lesquels le parasite induit les changements phénotypiques chez l'hôte. La majorité des changements produits serait due à une altération des systèmes neurologique et immunitaire, ce qui impliquerait que le parasite doive produire et secréter des substances spécifiques à certaines cibles chez l'hôte (Thomas, Adamo, & Moore, 2005 ; Poulin, 2010). Deuxièmement, une fois établi dans son hôte, le parasite n'est pas à l'abri d'une mort prématurée (Poulin *et al.*, 2005), soit par la prédation de l'hôte intermédiaire par un hôte définitif non approprié (Seppälä, Valtonen, & Benesh, 2008 ; Bakker *et al.*, 2017), soit par les mécanismes de défenses de l'hôte intermédiaire lui-même (Thomas *et al.*, 2000). Il y a donc aussi un coût relatif à la maintenance dans l'hôte intermédiaire (Bakker *et al.*, 2017).

Si elle existe à l'échelle interspécifique, cette variation est aussi présente à l'échelle intraspécifique. Comme pour tout trait qui s'exprime, la manipulation est elle aussi variable dans son intensité d'expression, tous les parasites en tant qu'individus, n'étant pas égaux (facteurs génétiques, âge, sexe) (Poulin, 2010). Il existe cependant des facteurs initialement indépendants aux parasites qui pourraient être à l'origine de la variation dans l'intensité des changements phénotypiques qu'ils induisent. Le contexte environnemental du parasite (génétique, réserves, résistance et expérience de l'hôte) et de l'hôte même (température, saison, pollution, compétition avec d'autres parasites, populations de prédateurs) peuvent jouer un rôle à ne pas négliger (Thomas *et al.*, 2011) (**Fig. 3**). En effet, certaines espèces de parasites présentent une gamme d'hôtes définitifs appropriés en conditions naturelles. Par exemple, *P.teretcollis* peut utiliser plusieurs espèces de poissons, dont les plus utilisées sont chevesne *Squalius cephalus* ou le barbeau *Barbus barbus* (Perrot-Minnot *et al.*, 2019). Considérons un

milieu où la communauté de prédateurs diverse et changeante est à l'origine de variation dans les défenses comportementales des hôtes. Un processus simple permettant d'expliquer la variation intraspécifique apparaît : le parasite modulerait les comportements antiprédateur de l'hôte, ces derniers étant déjà modulés par le patron local de prédation (intensité du risque, type et abondance des prédateurs).

Influence des facteurs environnementaux

Le pattern de prédation est susceptible de moduler l'activité des individus. Pour un risque élevé, les individus diminueraient leur activité liée à l'approvisionnement ou la reproduction et adopteraient un comportement de défenses antiprédateur plus marqué. Ceci pourrait se traduire par un changement de microhabitat par exemple ou l'utilisation de refuges. En supposant la plasticité des comportements défensifs, une diminution du risque résulterait d'une diminution de l'expression de ces derniers et un retour à la normale des activités liées à la survie ou la reproduction. L'expression de certains comportements défensifs reflète un état émotionnel bien défini (comme la peur) et prend sa source dans des circuits neurophysiologiques qui font intervenir des neurotransmetteurs spécifiques. Si le parasite était responsable d'un dérèglement dans le fonctionnement de ces circuits, il serait responsable indirectement d'une modification de l'état émotionnel de son hôte. Ainsi en changeant cet état, la perception et l'évaluation du risque de l'hôte dans son environnement pourrait être biaisées. Par ce biais l'hôte adopterait alors des comportements anormaux qui lui seraient préjudiciables jusqu'à augmenter sa vulnérabilité à la prédation.

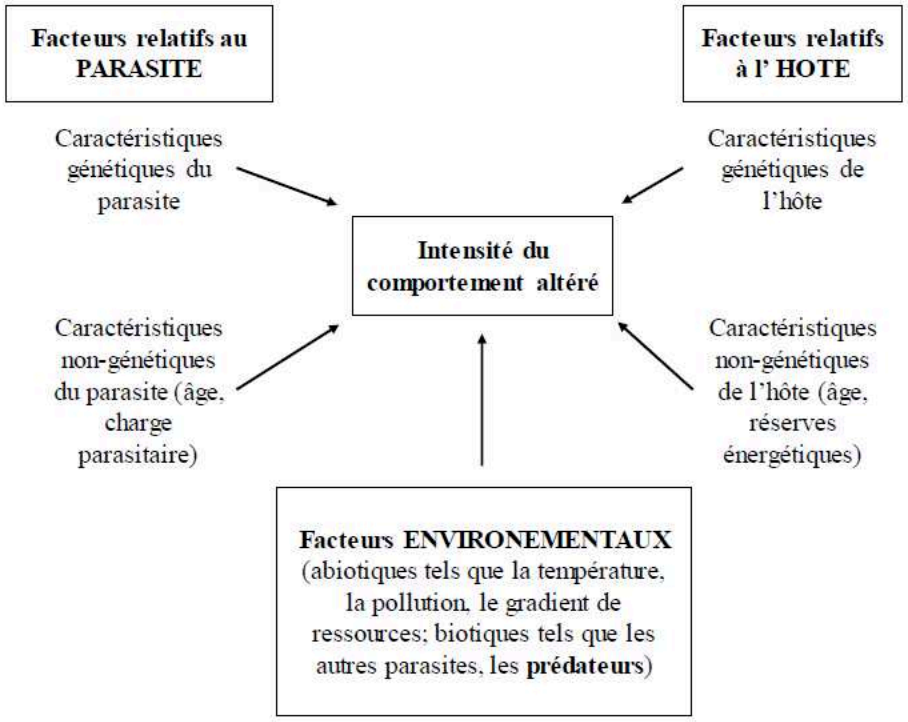


Fig.3 Facteurs influençant l'intensité de la manipulation de l'hôte par les parasites (de Thomas et al. 2011).

La manipulation parasitaire est donc influencée par plusieurs facteurs complexes et susceptibles d'interagir entre eux. Pour essayer de comprendre leur implication dans la variation que l'on observe dans l'intensité de la manipulation, il est nécessaire d'isoler certains de ces facteurs et de comprendre leur action indépendamment des autres. Premièrement, il s'agit de voir comment les facteurs relatifs à l'hôte lui-même, comme la génétique ou le régime alimentaire, peuvent moduler la manipulation de celui-ci. Par exemple, les populations de *G. pulex* naïves à l'infestation par *P.laevis* sont plus fortement manipulées (phototaxie inversée) que des populations qui sont naturellement infectées (Franceschi *et al.*, 2010a). La coévolution entre hôte et parasite semble avoir favorisé chez l'hôte une certaine résistance à la manipulation. Lorsque soumis à une alimentation réduite en protéines, les individus infectés montrent un taux métabolique plus faible, mais cette privation n'a cependant aucune répercussion sur la manipulation comportementale (Labaude *et al.*, 2015a). Deuxièmement, les facteurs environnementaux sont également susceptibles d'influencer l'intensité de la manipulation. En

effet, les températures élevées semblent responsables d'une augmentation de la consommation de feuilles par *Gammarus fossarum*. Cependant, l'infection par *P.tereticollis* diminue fortement la consommation, creusant davantage l'écart entre individus sains et infectés avec l'augmentation de la température (Labaude, Rigaud, & Cézilly, 2017b). Un des facteurs environnementaux les plus probables dans la modulation de la manipulation est le régime local de prédation. Autrement dit, quel est l'impact du patron local de prédation sur la manipulation de l'hôte ? Avec cette question, une difficulté supplémentaire apparaît alors : la perception du risque de prédation, soit des opportunités de transmission par le parasite à travers son hôte. Etant donné le stress que représente le risque de prédation, l'hypothèse la plus parcimonieuse serait que la perception se fait indirectement à travers l'état émotionnel de l'hôte, cet état modulant les comportements de défense antiprédateur. Dans un premier temps, il s'agit de voir comment la manipulation est influencée par le contexte local de prédation. L'impact du régime local de prédation sur la manipulation sera abordé dans le chapitre 2, et la variabilité des comportements antiprédateur des individus sains dans le chapitre 3.

PARTIE 2 –

VARIATION INTERPOPULATIONNELLE DE LA MANIPULATION COMPORTEMENTALE - LIEN ENTRE FLEXIBILITE COMPORTEMENTALE ET VULNERABILITE AUX FACTEURS EXTERNES DE MODULATION

Chapitre 2 -

Interpopulation variation in the intensity of host manipulation by the fish Acanthocephalan Pomphorhynchus tereticollis: are differences driven by predation risk?

Article publié dans PARASITOLOGY (2019)

RESUME

Beaucoup de parasites à transmission trophique sont responsables de changements comportementaux chez leurs hôtes intermédiaires, supposes augmenter la vulnérabilité à la prédation de ces derniers. Ainsi, si le pattern local de prédation et donc les opportunités de transmission varient, on peut alors s'attendre à une variabilité interpopulationnelle dans l'intensité des changements comportementaux. Cependant, cette hypothèse n'a pas encore été investiguée. Nous avons donc testé cette hypothèse pour quatre populations de gammares, *G. fossarum*, hôtes intermédiaires du parasite acanthocéphale *Pomphorhynchus tereticollis*, en utilisant la biomasse de poisons (utilisés en tant qu'hôtes définitifs) comme proxy d'opportunités de prédation. Nous avons relevé une variation dans l'intensité des changements induits par *P.tereticollis* dans la phototaxie et l'utilisation du refuge de *G. fossarum* pour ces quatre populations. Deux de ces populations, caractérisées par une faible biomasse de poisons, ont montré les plus forts niveaux de manipulation, ce qui était attendu pour une population où les opportunités de transmission sont faibles. Aussi, ces populations sont caractérisées par une faible prévalence. Enfin, ces populations montraient également sur le terrain une certaine ségrégation dans le microhabitat entre individus infectés et sains. Pour aller plus loin, deux systèmes de défense immunitaire, l'immunité (prophénoloxidase) et la capacité antioxydante sont affectés par l'infection. D'une façon générale, notre étude apporte un soutien partiel à la prédiction selon laquelle à la fois la manipulation et la prévalence devraient être élevées dans des populations où les opportunités de transmission sont faibles. Même si notre étude nécessite un plus fort soutien avec à travers une augmentation des réplicats des populations, nous mettons en évidence l'importance du contexte écologique, plus précisément la pression de prédation, dans l'étude et l'évolution de la manipulation chez les parasites à transmission trophique.

Interpopulation variation in the intensity of host manipulation by the fish acanthocephalan Pomphorhynchus tereticollis: are differences driven by predation risk?

Original Research Paper

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Running title: Host manipulation mediated by predator biomass

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SUMMARY

Many trophically transmitted parasites induce behavioural alteration in their intermediate hosts that tend to increase host vulnerability to predation. Interpopulation variability in parasite-induced alterations is expected to arise from variable local opportunities for trophic transmission. Yet, this hypothesis has not been investigated so far. We addressed the issue in four populations of the fish parasite *Pomphorhynchus tereticollis* (Acanthocephala), using variable fish biomass density as a proxy for transmission opportunities. We found variation in the intensity of parasite-induced changes in phototaxis and refuge use among populations. Two of the populations with the lowest predator biomass exhibited the highest levels of behavioural manipulation and prevalence, as expected at low transmission opportunities. They also exhibited microhabitat segregation between infected and uninfected gammarids in the field. In addition, infection had variable effects on two physiological defense systems, immunity and antioxidant capacity, and on total protein content. Overall, our study brings partial support to the prediction that host manipulation and prevalence should be higher at low predator biomass. Although stronger evidence should be sought by increasing population replicates, our study points to the importance of the ecological context, specifically transmission opportunities brought about by predation pressure, for the evolution of parasite manipulation in trophically transmitted parasites.

Key words: *Gammarus fossarum*, host manipulation, phenoloxidase, *Pomphorhynchus tereticollis*, prey-predator interaction, predation risk, antioxidant capacity, trophic transmission, variation.

KEY FINDINGS

- Interpopulation variation in an acanthocephalan prevalence was related to host phenotypic changes
- Host manipulation intensity ranged from non-significant to strong across populations
- Variation in prevalence and manipulation levels appeared to match differences in fish biomass
- Microhabitat segregation of infected prey in the field was found in highly manipulated populations
- Depressed physiological defense in infected gammarids was associated with lower protein content

INTRODUCTION

Some parasites have developed the ability to alter the phenotype of their intermediate host in ways that are supposed to increase their own fitness at the expense of that of their hosts, generally through increased parasite transmission (Thomas *et al.*, 2005). This phenomenon, known as "host manipulation by parasites" (HMP), is currently regarded as one of the most compelling examples of an extended phenotype (*sensu* Dawkins, 1982). In parasites with complex life cycles and trophic transmission, such changes are assumed to increase the vulnerability of infected intermediate hosts to predation by definitive hosts (Lafferty, 1999; Moore, 2002; Cézilly and Perrot-Minnot, 2005). For instance, the drifting behaviour of *Gammarus pulex* (Amphipoda: Crustacea) infected with *Pomphorhynchus laevis* (Acanthocephala) is increased compared to that of uninfected ones, a behavioural change supposedly contributing to the predation bias towards infected prey recorded in the field (Lagrue *et al.*, 2007).

Following a classical cost-benefit approach to understand the evolution of HMP, it has been predicted that the intensity of changes induced by parasites should vary according to transmission constraints and opportunities (Poulin, 2010; Thomas *et al.*, 2011). Such constraints may vary between host-parasite systems according to parasite transmission strategies. In trophically transmitted parasites, for instance, the probability for a parasite to pass from an intermediate host onto an appropriate final one strongly depends upon the pattern of predation. Transmission constraints may also vary between and within populations within a given host-parasite system (Poulin, 2010; Thomas *et al.*, 2011, Hafer-Hahmann, 2019). Consequently, the pattern and magnitude of HMP is expected to vary at multiple scales, between host-parasite systems but also between and within host populations.

Environmental factors may actually play a key role in the intensity of manipulation (Thomas *et al.*, 2012). For instance, seasonal fluctuations may be responsible for variations in HMP in relation to parasite requirements (to become mature), mainly because habitat quality varies according to season (Gotthard, 2001). Spatial variability in microhabitat features, and in the diversity and abundance of definitive hosts with different foraging strategies, may also contribute to variation in HMP (Thomas *et al.*, 2012). Indeed, parasite manipulation is not expected to be strong in populations where the abundance of definitive hosts is high, as parasites are likely to get transmitted by chance (Lafferty, 1992). Conversely, high intensity of HMP is expected under low abundance of definitive hosts. However, despite the diversity of host-parasite systems, and of the abiotic and biotic factors possibly modulating HMP, only a few studies have quantified variation in manipulation by trophically transmitted parasites (Franceschi *et al.*, 2010). Moreover, to the best of our knowledge, no study has related variability in manipulation by trophically transmitted parasites among natural populations to environmental factors.

The aim of the present study was precisely to investigate whether spatial variation in biotic factors modulate the magnitude of intermediate host manipulation by a fish acanthocephalan parasite. Acanthocephalans are well represented in studies on HPM (Moore, 2002; Fayard *et al*., in prep), including reported cases of interpopulation variation in the intensity of manipulation. For instance, the intensity of HMP in experimentally infected *G. pulex* has been shown to differ according to the geographical origin of *P. laevis* population (Franceschi *et al.*, 2010). Conversely, variation in parasite-induced mortality among populations of *G. fossarum* harbouring acanthocephalan parasites has been related to the differential susceptibility of host genetic lineages to HMP (Galipaud *et al.*, 2017). Here, we investigate the link between the magnitude of HMP in *G. fossarum* naturally infected with the fish acanthocephalan *P. tereticollis* and transmission opportunities to fish hosts at a local scale,

using local fish biomass density as a proxy for predation pressure (Maurer et al. 2014). The underlying assumption is that local fish biomass provides a proxy for transmission opportunities. This can be an approximation given that the host range of *P. tereticollis* includes both highly competent hosts and less competent ones (Perrot-Minnot *et al*., 2019). However, we considered here the whole fish community as a proxy for predation pressure and transmission opportunities, given theoretical evidence that trophic transmission to suboptimal or non-appropriate final hosts does not impose a cost high enough to constrain the evolution of HMP (Seppälä and Jokela 2008).

We recorded the magnitude of intermediate host manipulation by *P. tereticollis* in four populations from four rivers varying in local fish biomass. We first estimated the local prevalence of *P. tereticollis* cystacanths and the density of *G. fossarum*. We then quantified HMP on both behavioural and physiological traits. Host manipulation by acanthocephalans is known to be multidimensional (Cézilly *et al.*, 2013), notably involving traits related to taxis, protection and immune system (Fayard *et al*., in prep). Here, we recorded phototaxis and refuge use, two behavioural traits markedly altered by *P. tereticollis* (Perrot-Minnot *et al.*, 2007; Tain *et al*., 2006). Both traits are also altered under anxiety-like state, i.e. a state of sustained apprehension of the environment, as recently evidenced in two crustacean species, including gammarids (Fossat *et al*., 2014; Perrot-Minnot *et al.,* 2017). As defensive physiological traits, we estimated the level of host immunocompetence provided by the (pro)phenoloxidase system, and the level of antioxidant defenses. The prophenoloxidase (proPO) cascade is involved in melanization reactions accompanying innate immune responses and is a common response to infection in arthropods (Rigaud and Moret, 2003). Suppression of the proPO system has already been reported in *Pomphorhynchus*-infected gammarids (Rigaud and Moret, 2003; Cornet *et al*., 2009). In addition, oxidative stress has been associated with predation risk in several aquatic species (Slos and Stoks, 2008; Janssens and Stoks, 2013). We predict that the level of HMP

should be lower in localities where the high fish biomass enhances transmission probability independently of manipulation (and therefore relaxes selection for HMP) (Fig. 1a). Conversely, we predict that the level of HMP should be high in localities where fish biomass is low or intermediate, in response to selection on parasite for enhancing host vulnerability to predation (Fig.1). As a consequence, the prevalence of *P. tereticollis* cystacanths in *G. fossarum* could be either low, if strong manipulation leads to faster predation of infected individuals relative to uninfected ones (i.e. at intermediate fish biomass, Fig. 1b), or high, if low predator biomass and, hence, predation rate, leads to the accumulation of cystacanth-infected prey despite high HMP (Fig. 1c). Under the assumption that physiological changes are associated with infection, we also expect a decrease in host immunity (Cornet *et al.,* 2009; Fayard *et al.,* in prep.).

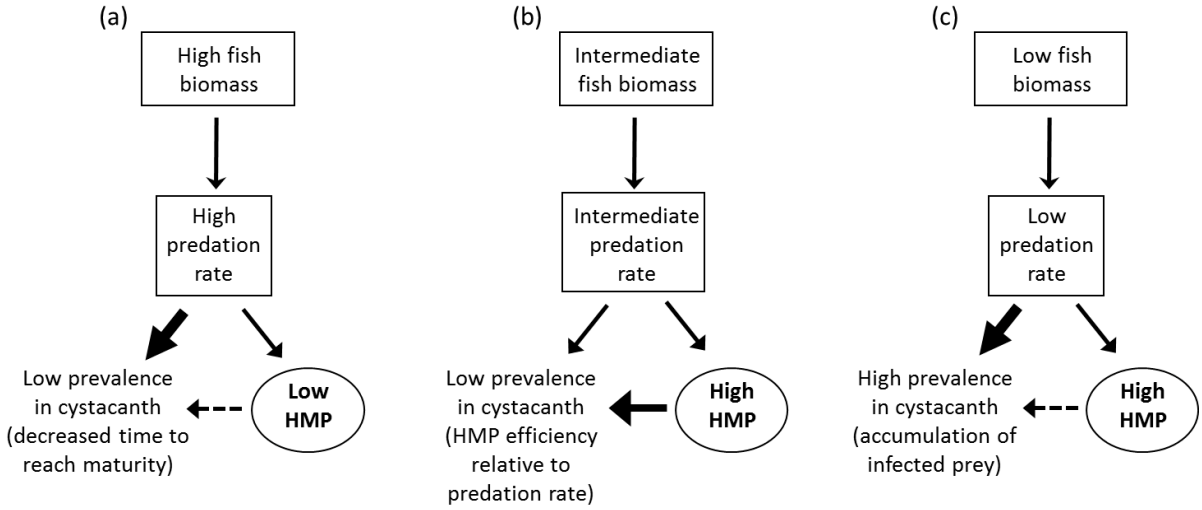

Fig. 1. Predicted consequences of local fish biomass on the level of host manipulation by parasite (HMP) through predation rate. Transmission opportunities for trophically transmitted fish parasite are assumed to increase with fish biomass when most fish predators are suitable as definitive hosts. Arrow thickness represents the impact of overall predation rate (independently of infection status) and of infected prey vulnerability to predation relative to uninfected ones (HMP); their relative impacts modulate the observed prevalence of infective parasite stages.

Table 1. Description of the localities with coordinates, temperature, descriptors of local fish assemblage structure, intermediate hosts (gammarid) density, and prevalence of *P. tereticollis* cystacanths in gammarids. The most competent fish species (*) are assumed to be five benthic feeders - barbel, dace, bullhead, loach, and gudgeon -, as reported in Perrot-Minnot et al. (2019). However, other species –notably the chub *Squalius cephalus*, ranging in relative biomass in these localities from 12% to 35%- also harbors gravid females of *P. tereticollis* albeit of smaller size (Perrot-Minnot et al., 2019). Gammarid density is only a semi-quantitative estimate, as the standardized protocol used provides relative abundance among populations rather than absolute abundance. Sampling replicates (nb repl.) are the number of kick-sampling replicates on a fixed area of 1.5 m^{-2}.

River (locality)	Coordinates (lat; long)	Average temperature (min-max) (°C)	Fish survey: nb and time period (sample size per sampling)	Fish species richness	Total fish biomass (g.100 m^{-2})	Shannon Index	Main fish host biomass (%) * (g. 100 m^{-2})	Gammarid density (ind.m^{-2}) (nb repl.)	Prevalence in gammarid hosts (%)
Bèze (Marandeuil)	47°20'52.30" N; 5°20'58.36" E	12.08 (3-18)	4x: 2008 to 2014	12	162.64	3.45	81.42 (50.2)	160.87 (4)	3.65
Cuisance (Vadans)	46°55'29.29" N; 5°42'10.65" E	9.50 (7-12)	4x: 2008 to 2014	9	401.55	2.18	138.4 (34.5)	146.85 (4)	1.67
Norges (Orgeux)	47°21'40.61" N; 5°9'30.53" E	11 (8-13)	1x: 2004	10	775.98	1.86	200.86 (25.9)	473.60 (3)	10.70
Vingeanne (Talmay)	47°20'51.51" N; 5°27'9.27" E	11.90 (2-23)	6x: 2001 to 2006	25	15507.12	3.44	2645.14 (17.1)	370.73 (3)	0.90

Four distinct rivers located in Bourgogne-Franche-Comté, eastern France, were prospected from mid-February to early-April 2017. We chose sampling localities based on previous records of *P. tereticollis,* and available information on fish communities, obtained through the fish-based ecological assessment achieved by the Office Français pour la Biodiversité (OFB) in the framework of EU Water Directive: Talmay on Vingeanne river, Vadans on Cuisance river, Orgeux on Norges river, and Marandeuil on Bèze river (Table. 1). We retrieved information on local fish biomass density (g.100 m²; thereafter fish biomass) from the OFB database. Fish survey was based on one to four replicates at different time periods (Table 1). At each locality, we sampled gammarids twice, at two-week intervals. We first randomly sampled gammarids in the benthos to estimate *P. tereticollis* prevalence, gammarid individual size, and gammarid density. Two weeks later, we collected uninfected and *P. tereticollis* -infected gammarids at the same place, for behavioural and physiological assays. Gammarids were kept in the lab for no longer than 48 hrs (prevalence estimates) or 24 hrs (behavioural assays). During this time, they maintained at 16°C under a 12:12 light regime in tanks filled with well-oxygenated water from the river, and fed with elm leaves.

Parasite prevalence and gammarid density

Both parasite prevalence and gammarid density were assessed over a six-week period, from mid-February to late March, to allow comparison between localities, thus avoiding the potential confounding effect of seasonality. We randomly sampled gammarids from the benthos in both the bank and the bed of the river, following the kick sampling procedure (Turner and Trexler, 1997) on fixed areas. The procedure consisted of moving the river substrate (gravel, plants, rocks and sand) by kicking, before harvesting using a fine mesh net downstream. Sampling was semi-quantitative: the surface area of benthos harvesting was standardized to 5 m² per sample replicate, and 3 or 4 replicates were collected in each locality. For each sample replicate, we

used a metal frame kick net (0.5 mm mesh size) to collect the benthos in three contiguous passes of 5 meters in length and approximately 0.3 meters in width. For each locality, the same experimenter collected at least 2000 individuals, to estimate gammarid density and *P. tereticollis* prevalence. Semi-quantitative estimates of gammarids density using this standardized protocol reflect relative rather than absolute abundance among populations (Davies *et al*., 2001). Gammarids from the bank and the bed were kept alive and brought back to the laboratory in two separate ice boxes. Assessment of prevalence, under a stereomicroscope, began the very same day the benthos was collected. Once the prevalence was assessed, individuals were pooled according to their status and the habitat they came from (bank or bed of the river), for further size measurement. Body size was measured for all infected individuals, and for a subset of 5% to 10% of uninfected individuals picked up at random. Body size was estimated from the height of the 4th coxal plate, following Bollache *et al.* (2002), using a Nixon SMZ 1500 stereoscopic microscope.

Pattern of HMP: behavioural assays

Behavioural assays were conducted within a short time period, from the 13th of March to the 19th of April 2017. We collected at least 60 infected and 60 uninfected individuals from each locality for behavioural and physiological tests. Phototaxis and refuge use were recorded by scan and time sampling, under a light intensity of 700 lux. Reaction to light was assessed by scoring the position of a single individual in a two-choice (light/dark) arena every 15 sec. for 5 min., following Perrot-Minnot *et al.* (2014). The two-choice (light/dark) arena consisted of a 23 cm long and 3 cm diameter closed glass tube, with half side painted in black while the other half was left translucent. A hole was made in the middle to allow the introduction of a single gammarid. Phototaxis score ranged from 0 (always in the lightened side: strongly photophilic) to 20 (always in the darkened side: strongly photophobic). Following Dianne *et al.* (2014),

refuge use was assessed using a 10.5 * 16 cm rectangular box where a refuge (half a terracotta saucer) was placed. The position of a single individual was registered every 30 sec. for 10 min. An individual was considered to be outside of the refuge when at least half of its body was. Refuge use score ranged from 0 (always out of refuge) to 20 (always under the refuge). Both phototaxis and refuge use scores were expressed as a proportion of the maximum score (20). To minimize handling stress between tests, phototaxis was scored first and then refuge use, as gammarids were more easily introduced from phototaxis tube to the refuge box than the reverse.

Just after the completion of behavioural assays, we dissected each gammarid in 100 µL PBS - 0.2% Triton X100 pH 7.7 (reagents from Sigma-Aldrich) in an Eppendorf cap to remove the parasite, when present. After the addition of 100 µL PBS, samples were quickly frozen in liquid nitrogen, and stored at -80°C for subsequent physiological assays. Empty tubes were individually weighed prior to dissection, and then weighed again before biochemical assays, in order to estimate the fresh weight of individual gammarids.

Pattern of HMP: physiological assays

All biochemical assays were performed on batches of 36 samples within five days, after randomizing the samples with respect to population and infection status. Upon biochemical assay, samples were thoroughly grinded using a ball mill (RETSCH MM 400 Mixer Mill) during two rounds of 2 min. interspersed with 2 min. on ice. The homogenate was then centrifuged at 9000 g at 4°C for 15 min. Clear supernatant was collected and kept on ice to proceed to biochemical assays right away (detailed below). All dosages were conducted using a microplate spectrophotometer (Spectramax Plus384 Absorbance Microplate Reader; Molecular Devices LLC, Sunnyvale, CA, USA).

Phenoloxidase (PO) and prophenoloxidase (PPO) dosages were performed according to Cornet *et al*. (2009), with a few modifications, using 25 µL of supernatant for each. For PO

dosage, 20 µL of filtered PBS pH 7.4 0.1M and 120 µL of L-DOPA at 10 mM were added to the supernatant. The reaction was monitored by reading the optical density (OD) at 490 nm every 15 sec for 40 min., and PO enzyme activity was quantified as the slope (Vmax value) of the curve during the linear phase of the reaction. For PPO dosage, the sample was left for 10 min in 5 µL of chymotrypsin at 5 mg.mL ^{1} after addition of 20 µL of filtered PBS, prior to the addition of L-DOPA.

The antioxidant potential was measured using Trolox (6-hydroxy-2,5,7,8-tetramethychroman-2-carboxylic acid) equivalent antioxidant capacity (TEAC) assay, described in Re et al (1999). The antioxidant potential of a sample was estimated from its capacity to quench the free radicals of an oxidized ABTS ((2,29-azinobis-(3-ethylbenzothiazoline-6-sulfonic acid)) solution. The oxidized ABTS solution (ABTS+) was generated by reacting 7mM of ABTS solution in water with 2.45 mM of potassium persulfate at obscurity and ambient temperature for at least 14hs. Just before dosage, the absorbance at 734 nm of ABTS+ solution was adjusted to 0.7 by dilution in filtered PBS. Ten microliters of supernatant or of Trolox standard solution was then mixed with 240 µL of the ABTS+ solution in microplate well, and the absorbance at 734 nm was read every minute for 20 minutes, at 30°C. The range of standard Trolox concentrations (from 100 µM to 800 µM) was prepared by diluting a 2.5 mM stock solution in PBS triton X100 pH 7.7, in order to get from 20 to 80 % ABTS+ bleaching. TEAC was estimated by calculating the proportion of change in OD (debleaching) in 10 min. corrected by blanks, both in the samples and in Trolox standard, and by using the Trolox standard curve to derive the Trolox-equivalent antioxidant capacity of the sample in µM. To assess global investment in both immune system and antioxidant capacity, we corrected PPO-PO and TEAC raw values by the weight of individuals, using residuals from the linear regression between PPO-PO and TEAC raw values and the weight of individuals. All reagents for the PPO-PO and TEAC assays were purchased from Sigma-Aldrich.

Total protein concentration was estimated in 5 µL of supernatant using the DC™ Protein Assay kit (Biorad) and bovine serum albumin (BSA) as standard (from 0.15 to 0.30 mg. mL ^{1}), following the manufacturer's instructions.

Analyses

All analyses were conducted using the R software (v. 3.4.3, R Development Core Team, 2018).

We first calculated effect size, in order to quantify the magnitude of behavioural alterations and physiological changes induced by parasites, and then used non-parametric Cliff's delta effect size with 95% confidence interval (CI) (*effsize* R-package; Torchiano and Torchiano, 2017). An effect was considered as non-significant when its 95% confidence interval crossed zero. Negligible, small, medium and large effects correspond to an absolute value lower than 0.15, 0.33, 0.47 and higher than 0.47, respectively (Romano *et al.*, 2006).

To assess the contribution of predictor variables to variation in prevalence, behavioural and physiological traits, we used the information theoretical approach based on model comparison, as an alternative to traditional null hypothesis testing (Galipaud *et al.*, 2014). The risk of multicollinearity among two of the predictor variables - fish biomass and river locality - was avoided, by using the most relevant to variation in the dependent variable (fish biomass for prevalence and behavioural traits, locality for physiological traits). We used general linear model (GLM) with binomial-logistic (logit) regression (*lme4* R-package; Bates *et al.,* 2015) to analyze variation in prevalence according to environmental variables (gammarid density and total fish biomass) and gammarid size. We used beta-regressions (*beta-reg* R-package; Cribari-Neto and Zeilis, 2010) to assess the contribution of environmental variables (fish biomass and prevalence) and individual variables (physiology and infection status), to variation in phototaxis and refuge use (Ferrari and Cribari-Neto, 2004). Behavioural scores were transformed following Smithson and Verkuilen (2006) to exclude 0 and 1. We used GLMs to assess the

contribution of infection status, weight and population to variation in the levels of PO-PPO, TEAC and total proteins content. In all regressions, gammarid density, total fish biomass and PPO were log-transformed and TEAC was squared-transformed to meet normality requirements.

For all regressions, we performed model selection (*MuMIn* R-package, functions 'dredge' and 'subset'; Bartoń, 2016) based on the Akaike Information Criterion (AICc) (Akaike, 1973). There is currently no consensus about the best cut-off criterion to select models, more specifically to balance the risk of keeping spurious models with that of excluding biologically meaningful models. Here, we proceeded to model selection using as cut-off criterion an AICc weight above 0.01, or a large gap of delta AICc (at least 2) between two consecutive models, relatively to the delta AICc of lower ranking models (M. Galipaud and F.-X. Dechaume-Moncharmont, pers.comm.). Because both quantitative and qualitative predictors were used, we did not use model averaging to calculate averaged coefficients for predictors, because these coefficients would have had no meaning for qualitative predictors (F.X. Dechaume-Moncharmont, pers.comm.). However, we used the subset of 'best' models to identify predictors that more likely contribute to variation in the dependent variable, based on the above-mentioned cut-off criterion.

RESULTS

Prevalence

Prevalence of *P. tereticollis* cystacanth was high in the Norges river (above 10%) compared to the other three localities where it ranged from less than 1% (Vingeanne) to almost 4% (Bèze) (Fig. 2). Significant spatial segregation of infected and uninfected gammarids was observed in river Norges and river Bèze, with a higher proportion of infected individuals found on the river

bank compared to the river bed (Chi-squared tests: Bèze, $\chi^2 = 17.60$, $P < 0.0001$; Cuisance, $\chi^2 = 3.15$, $P = 0.08$; Norges, $\chi^2 = 17.59$, $P < 0.0001$; Vingeanne, $\chi^2 = 0.002$, $P = 0.96$) (Fig. 2).

The initial global model to analyze variation in prevalence included total fish biomass, gammarid density, their interaction, and gammarid body size, as predictor variables. Variation in prevalence was best explained by the model including total fish biomass, gammarid density and gammarid body size (Logistic regression: Log-likelihood = -1152.91; df = 4; AICc = 2313.80). Considering the subset of best models, local fish biomass and gammarid density appeared to play a key role in driving variation in prevalence in *P. tereticollis* cystacanth (see appendix, Table S1).

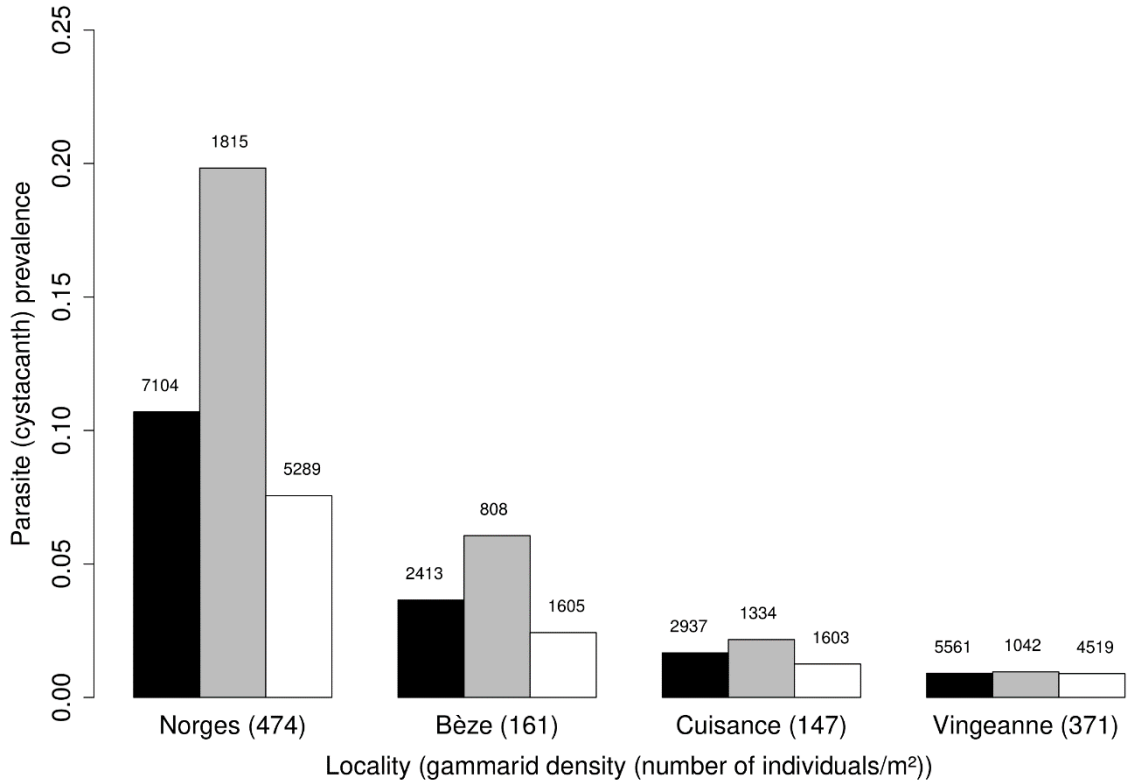

Fig.2 Prevalence (percentage of cystacanth-infected gammarids) in the study localities from four rivers ordered from the highest to the lowest prevalence of *P. tereticollis*. Numbers above the bars correspond to the number of gammarids sampled in three (Bèze, Norges and Vingeanne) or four (Cuisance) replicates. Density is expressed as the number of gammarids per m^2, and is only a semi-quantitative estimate, as the standardized protocol used reflects relative abundance among populations rather than absolute abundance. Black and grey bars correspond to bank and bed prevalence, respectively.).

Pattern of HMP: host behaviour

The magnitude of behavioural changes in infected individuals differed among the four localities (Fig. 3). Phototaxis and refuge use were strongly to moderately altered by infection in the Norges and Bèze rivers, while only phototaxis was altered in the Cuisance river. No parasite-induced behavioural change was evidenced in the Vingeanne river.

The global model prior to model selection included the status of individuals (infected or uninfected), total fish biomass, their interaction, physiological parameters (total PPO-PO activity and TEAC, corrected by the weight of individuals) and prevalence, as predictor variables. Variation in phototaxis was best explained by the model including infection status, total fish biomass, and their interaction (Beta-regression: Log-likelihood = 296.12, df = 6, pseudo R^2 = 0.22, AICc = -580) (Fig. 4; Table S2a). Parasite-induced change in phototaxis was significant in the three localities with the lowest fish biomass, but not in the locality with the highest fish biomass (Fig. 3; Fig. 4). For refuge use, the best model included infection status, total fish biomass and their interaction (Beta-regression: Log-likelihood = 185.27, df = 5, pseudo R^2 = 0.06, AICc = -360.40) (Fig. 4; Table S2b), with parasite-induced change in refuge use being significant only in two of the three localities with lower fish biomass (Fig. 4). However, effect sizes tended to be low for refuse use compared to phototaxis (Fig. 3), partly due to the overall weak use of refuge both in uninfected and infected gammarids (Fig. 4).

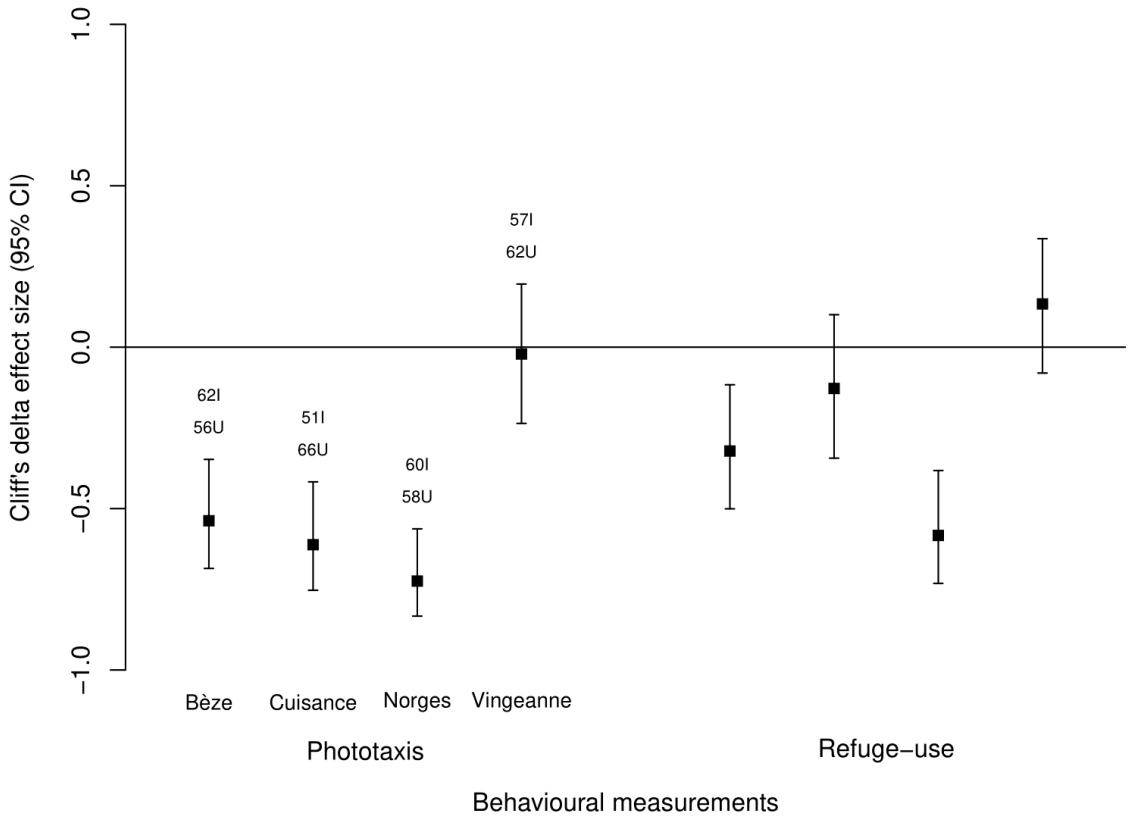

Fig.3 Cliff's delta effect sizes (with 95% CI) for changes in behaviour induced by infection with *P. tereticollis* in *Gammarus fossarum* (phototaxis and refuge use) for Bèze (Bèz.), Cuisance (Cui.), Norges (Nor.) and Vingeanne (Vin.) localities, respectively. The effect of infection was considered as non-significant when its 95% confidence interval crossed zero. Negligible, small, medium and large effects correspond to |d| lower than 0.15, 0.33, 0.47 and higher than 0.47, respectively (Romano et al. 2006). Numbers above the error-bars correspond to the numbers of gammarids used (I=infected, U=uninfected).

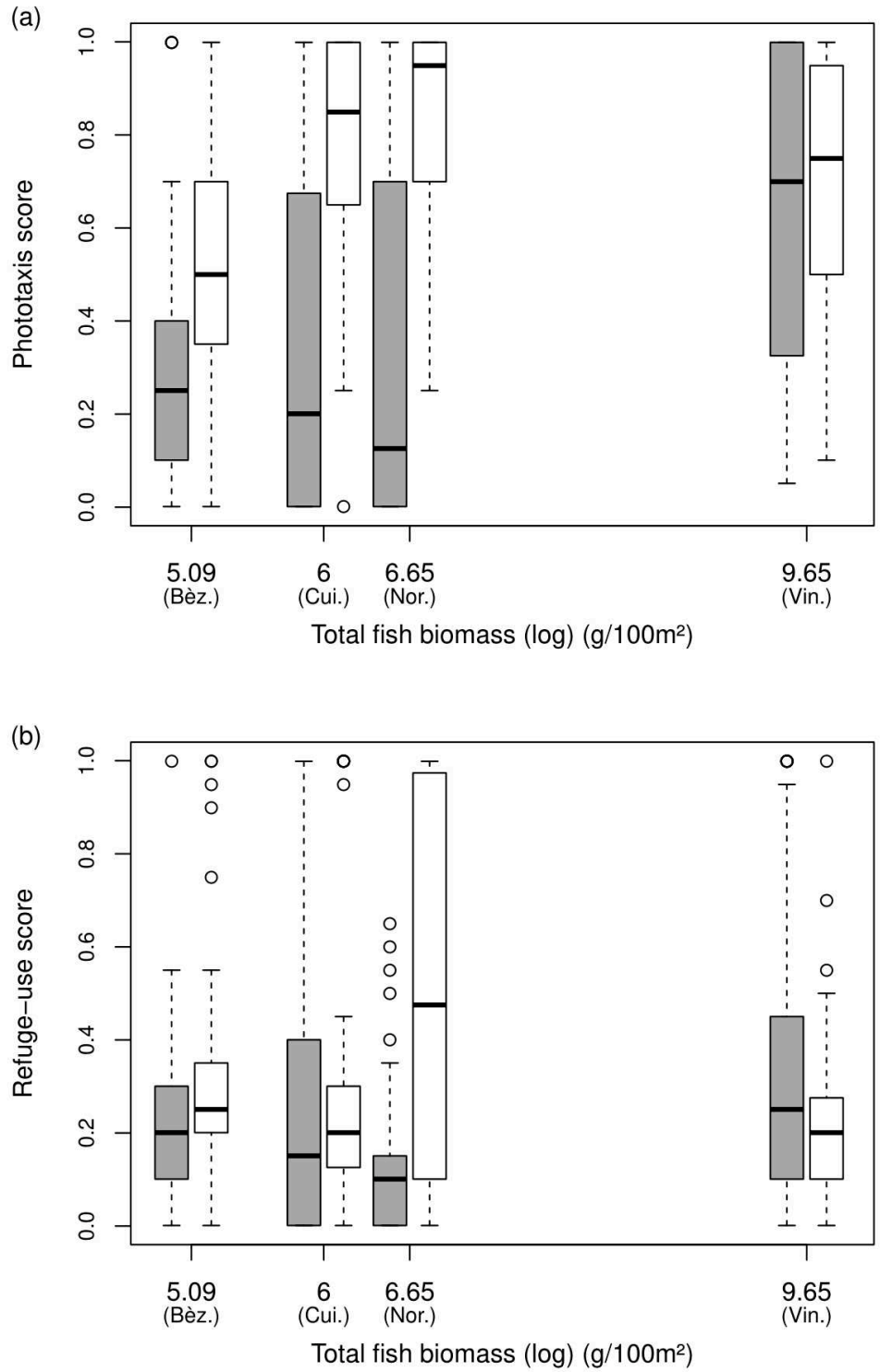

Fig.4 Behavioural scores: (a) phototaxis and (b) refuge use plotted against total fish biomass in interaction with the status of individuals (infected or uninfected). Phototaxis score ranges from photophilic (0) to photophobic (1), and refuge use score as always outside (0) to always under refuge (1). Grey and white boxes correspond to infected (Bèze, N=59; Cuisance, N=51; Norges, N=46 and Vingeanne, N=55) and uninfected (Bèze, N=52; Cuisance, N=63; Norges, N=44 and Vingeanne, N=60) individuals, respectively. Horizontal lines and vertical dashed lines correspond to median and interquartiles, respectively.

Pattern of HMP: host physiological state

Effect sizes of parasite-induced changes were medium to large for all three physiological parameters in river Bèze only. Depressed levels of PO-PPO activity, antioxidant capacity and protein concentration (all corrected for weight) were evidenced in infected individuals compared to uninfected ones from the Bèze river (Fig. 5). The effect of infection in Norges and Cuisance rivers was restricted to a moderate decrease in PO-PPO activity, while in Vingeanne river, infection moderately decreased antioxidant capacity and protein concentration (Fig. 5). Interestingly, these effects of infection status on PO-PPO activity and antioxidant capacity were no longer significant after correction with total protein content instead of gammarid weight, except for PO-PPO in the Cuisance river (Fig. S2, S3).

The initial global model for studying variation in physiological parameters included individual status (infected or uninfected), individual weight, their interaction and locality. The first-ranking model to explain variation in protein content included all four predictors (General Linear Model: Protein content: Log-likelihood = -1044.40, df = 8, $R^2 = 0.22$, AICc = 2105.10). The first-ranking model to explain variation in PPO-PO and TEAC included individual status, individual weight, and locality (Log-likelihood = -369.79, df = 7, $R^2 = 0.18$, AICc = 753.80; Log-likelihood = -5608.67, df = 7, $R^2 = 0.14$, AICc = 11231.60, respectively) (Fig. 6; Table S3). However, the interaction between individual status and weight should not be discarded, as it appears in the subset of best models (Fig. 6; Table S3). Protein content, antioxidant potential and PPO-PO increased with gammarid weight, and were lower in infected gammarids. The difference between infected and uninfected individuals in the three physiological parameters increased with gammarid weight (Fig. 6).

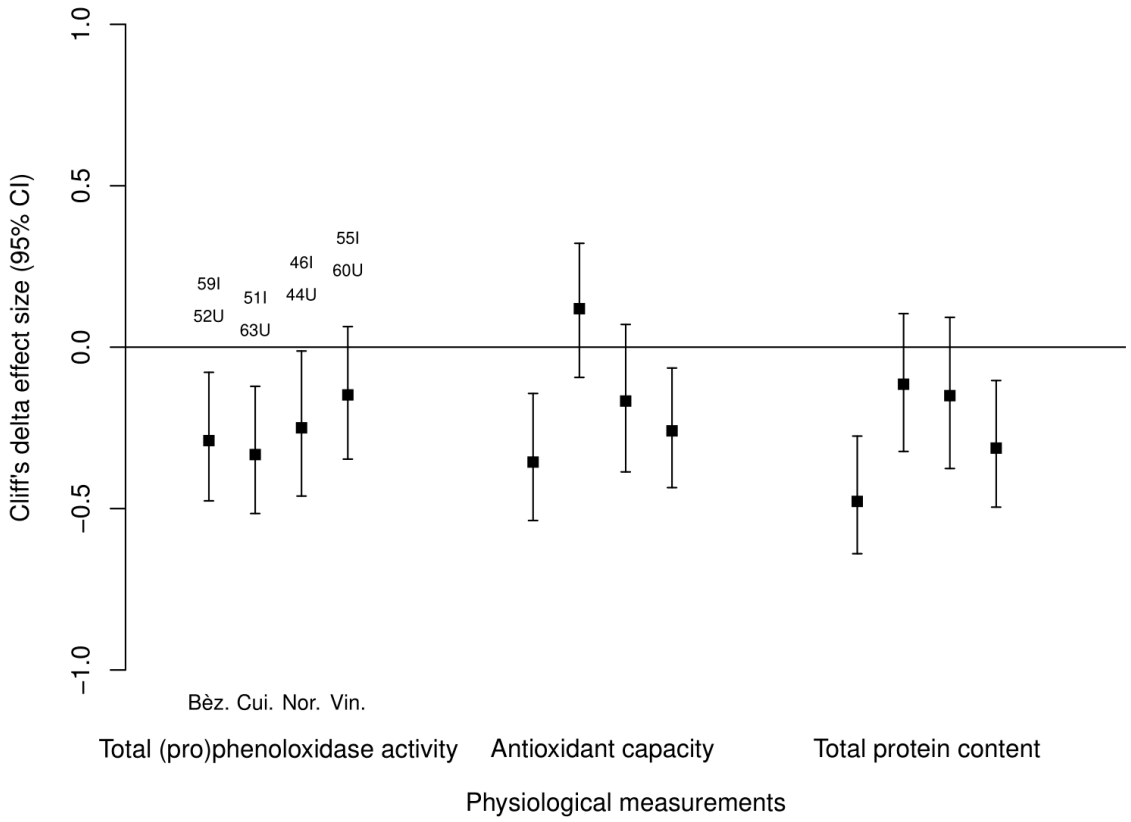

Fig.5 Cliff's delta effect sizes (with 95% CI) for changes in physiological state (total (pro)phenoloxidase activity (PPO activity), antioxidant capacity (TEAC) and total protein content (Proteins), all corrected by the weight of individuals) for Bèze (B.), Cuisance (C.), Norges (N.) and Vingeanne (V.) localities, respectively. Negligible, small, medium and large effects correspond to |d| lower than 0.15, 0.33, 0.47 and higher than 0.47, respectively (Romano et al. 2006). Numbers above the error-bars correspond to the numbers of gammarids used (I=infected, U=uninfected).

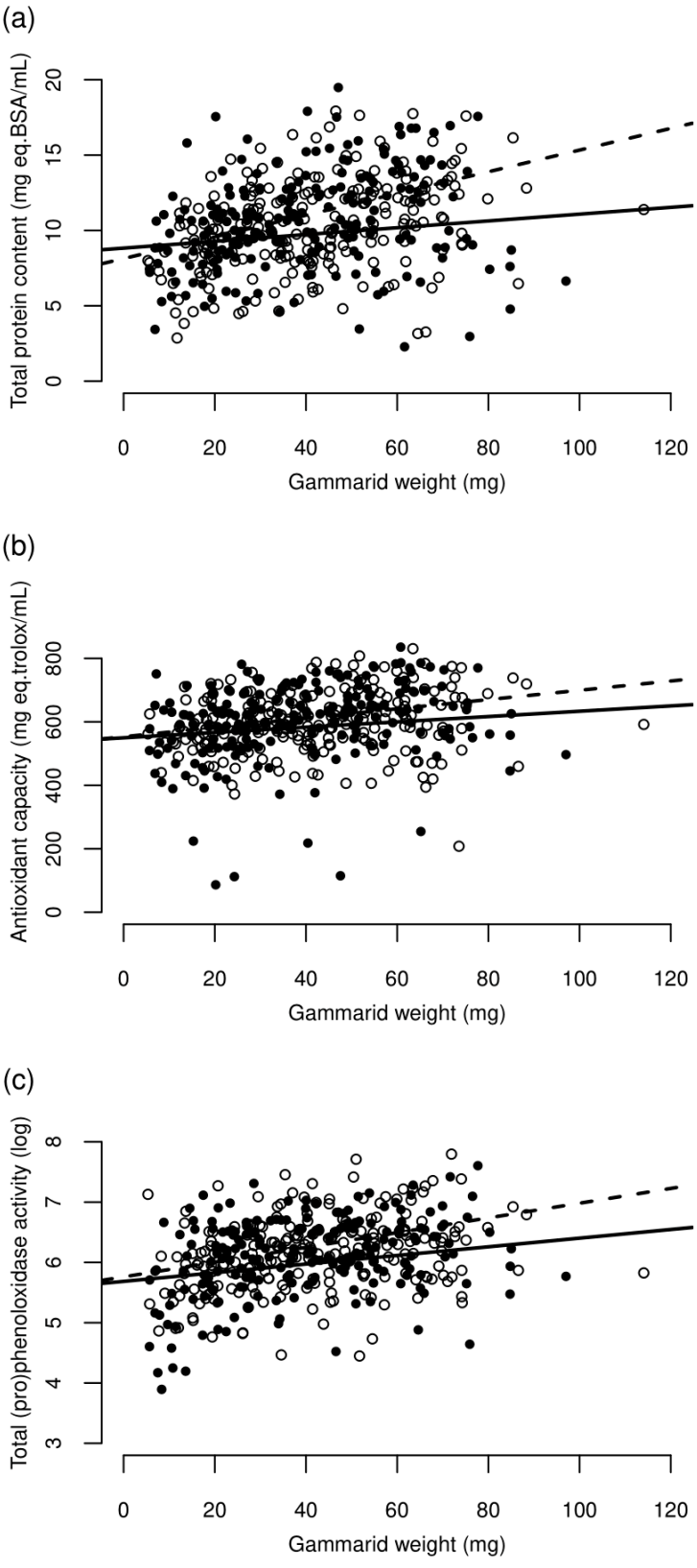

Fig.6 Physiological parameters: (a) total protein content, (b) antioxidant capacity and (c) total (pro)phenoloxidase activity, all plotted against the weight of individuals, in interaction with the status of individuals (infected or uninfected). Solid circles and lines correspond to infected individuals and empty circles and dashed lines to uninfected ones.

DISCUSSION

The aim of our study was to address the environmental causes of variation in prevalence and host manipulation by a trophically transmitted fish parasite, the acanthocephalan *P. tereticollis.* More specifically, we tested the hypothesis that high predation pressure would be associated with low cystacanth prevalence and low level of manipulation in intermediate host, by comparing four localities with contrasted fish biomass.

We first highlighted variation in both the prevalence of *P. tereticollis* cystacanth and behavioural alterations in infected gammarids among the four localities. Overall, infected individuals displayed lower photophobia and refuge use than uninfected ones, as previously reported (see Cézilly *et al*., 2013; Fayard *et al*., in prep.), with the exception of one locality. Interestingly, the two localities with the highest level of HMP (Norges and Bèze) also exhibited a significant spatial segregation between infected and uninfected gammarids, as previously observed in another fish acanthocephalan (MacNeil *et al.*, 2003). Our data on these two populations further suggest that lab estimates of HMP could match microhabitat segregation of infected hosts in the field. However, since the density estimates provided with kick-sampling on fixed area are only semi-quantitative (Davies et al., 2001), it should be emphasized that our conclusion relies on relative abundance for comparison between habitats. Further investigation should consider quantitative methods to specifically test the link between spatial segregation between infected and uninfected gammarids, and the level of HMP.

Strong HMP was associated with high cystacanth prevalence and low fish biomass (Norges river), and conversely, low and non-significant HMP was associated with low prevalence and high fish biomass (Vingeanne river). This result is in agreement with the hypothesis that the magnitude of HMP should partly match predation pressure, and should be associated with variable cystacanth prevalence. Two processes may explain the observed

pattern. First, low HMP in population with high predation pressure could be the expression of an optimal parasitic strategy balancing the costs and benefits of host manipulation, independently of cystacanth age. This optimal strategy could be shaped by plastic adjustment and/or adaptive response at a local scale. Alternatively, low HMP in naturally infected gammarids could be due to cystacanths being too young to manipulate. Indeed, in the course of behavioural switching from 'protection' by acanthella to 'facilitation' by cystacanth (Dianne *et al.*, 2011), young cystacanth may induce low level of manipulation as evidenced in *P.laevis* infecting *G. pulex* (Franceschi *et al.*, 2008). Under high predation pressure, cystacanth-infected gammarids could be rapidly removed from the population, such that those present would be, on average, younger than in populations with low predation pressure, where cystacanth-infected gammarids tend to accumulate and age. Under this hypothesis, gammarids from the Vingeanne river would have been infected with younger cystacanths, and therefore displayed a lower intensity of or even no parasite-induced manipulation. Because behavioural assays were performed right after sampling, it is still possible that differences in mean parasite age among populations were responsible for variable levels of HMP. Alternatively, under the first hypothesis, selection for high or low HMP would depend on whether manipulation is costly and whether such costs are compensated by the benefits of increased probability of transmission to final hosts. Since transmission probability is depending on predation pressure, predator density or biomass is likely to be a selective force shaping the pattern of HMP. Indeed, a recent model (de Vries and van Langevelde, 2018) showed that the selective advantage of two manipulative strategies –predation enhancement and predation suppression- depends on host density. Predation enhancement by mature parasites (i.e. infectious to final hosts) would be beneficial at low final host density, whereas at high host density, selection would rather favor predation suppression by immature parasites (i.e. not-yet infectious to final hosts; de Vries and van Langevelde, 2018). Apart from this ecological context, energetic costs associated with HMP

could limit its evolution, although evidence for such costs is still weak (Hafer-Hahmann, 2019). We cannot disentangle the two alternative hypotheses here, because the age of field-collected infected gammarids was unknown. To address this issue, a conditioning period during which parasites can grow older to reach their 'manipulation endpoint' could be applied, before assessing the level of HMP. In addition, the evidence for a link between predation pressure, HMP and prevalence should be considered with caution since only two populations matched the predicted pattern. The two other localities with low fish biomass presented intermediate to high levels of HMP (refuge use and phototaxis respectively) as expected, but prevalence was low (Cuisance and Bèze rivers). Therefore, stronger evidence should be sought by increasing population replicates, in order to cover a larger range of fish biomass.

One relevant question to the evolution of HMP in response to predator biomass is the mechanisms by which such fine-tuned response of parasites to variable transmission opportunities can evolve. The more parsimonious assumption would be that the increased predation risk associated with high predator biomass induces chronic stress in uninfected individuals, and thereby activate an anxiety-like state. Indeed, chronic stress might trigger a sustained apprehension of the environment and elevated vigilance, akin a 'mood-shift' towards anxiety-like state. Interestingly, phototaxis and refuge use have been recently associated with general anxiety-like state (Fossat *et al.*, 2014; Perrot-Minnot *et al.*, 2017). The parasite could thus reverse the behavioural response to predation risk by interfering with this modulatory pathway. One possible pathway involved both in anxiety-like state and parasite manipulation is the serotonergic neuromodulatory system (Tain *et al.*, 2006; Fossat *et al.*, 2014; Perrot-Minnot *et al.*, 2014). A topical injection of serotonin is indeed mimicking parasite-induced behavioural alteration of phototaxis but not that of refuge use (Perrot-Minnot *et al*., 2014). Both lab experiments reproducing the chronic effect of parasite and predation stress, and field studies

correlating the expression of anxiety-like behaviour to predator biomass across several localities, should therefore be undertaken to test these hypotheses.

We found that three populations exhibiting moderate to high HMP were also immunosuppressed. However, we did not find any effect of immunocompetence on HMP, suggesting that the intensity of HMP was independent of parasite-induced immunosuppression, in agreement with Cornet *et al.* (2009). Furthermore, two populations had lower antioxidative defenses and protein concentration. Whereas a depressed protein content in then midgut glands of *G. pulex* infected with *P. laevis* has been previously reported (Bentley and Hurd, 1995), no evidence for an effect of infection with acanthocephalans on host antioxidant capacity has been published so far. Previous studies have focused on energetic metabolism, including lipid and carbohydrate reserves (see for instance Plaistow *et al.*, 2001; Caddigan *et al.*, 2017). Interestingly, total (pro)phenoloxidase activity and antioxidant capacity were no longer significantly affected by infection when corrected by individual total protein content instead of body weight. This suggests that immunosuppression and decreased antioxidant defenses in infected gammarids are the consequences of a more general depression in protein content. Such depression of protein content and physiological defenses likely contributes to the ecological and evolutionary cost of infection for the parasite (Cornet *et al*., 2009). Alternatively, depressed protein content and physiological defenses may be the consequences of resource reallocation to the growing parasite. The fact that depressed physiological defenses arise from a general depression in protein content instead of a specific effect of infection should therefore be considered when interpreting the evolutionary significance of such changes. For instance, immunosuppression should not be considered as an adaptive parasitic strategy to increase survival by controlling host immune response, but rather as a side-effect of resource acquisition.

In conclusion, our data indicate that among population variation in the intensity of HMP in both behavioural traits could be related to predator biomass. We postulate that the activation

of modulatory pathway of stress and anxiety-like behaviours in prey by predation risk might be exploited by parasites. Therefore, to understand variation in HMP, variation in the intensity of antipredatory and anxiety-like behaviours of uninfected individuals with respect to predation risk should be considered first. Overall our results suggest that considering the ecological context, particularly prey-predator interactions, might be essential for understanding the transmission strategies of trophically transmitted parasites.

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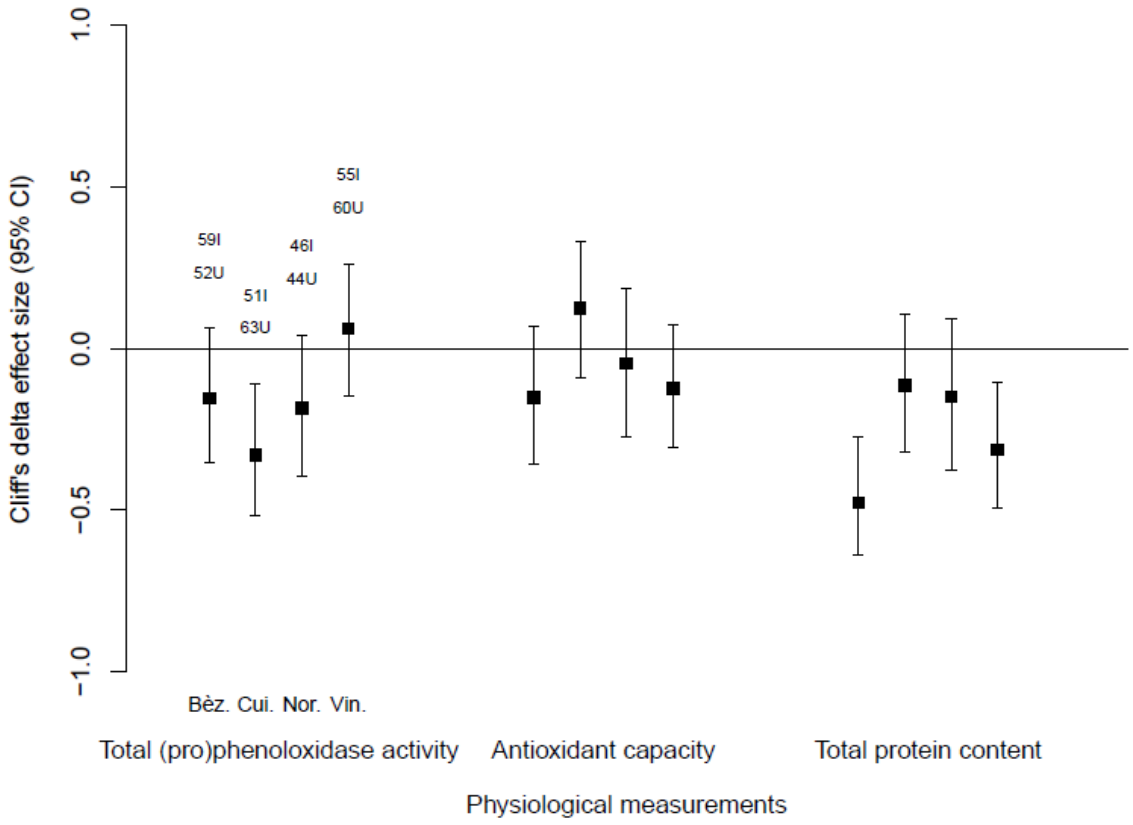


Fig.S1 Cliff's delta effect sizes (with 95% CI) for changes in physiological state (total (pro)phenoloxidase activity, antioxidant capacity both corrected by total protein content, and total protein content corrected by the weight of individuals) for Bèze, Cuisance, Norges and Vingeanne localities, respectively. Negligible, small, medium and large effects correspond to |d| lower than 0.15, 0.33, 0.47 and higher than 0.47, respectively (Romano et al. 2006). Numbers above the error-bars correspond to the numbers of gammarids used (I=infected, U=uninfected).

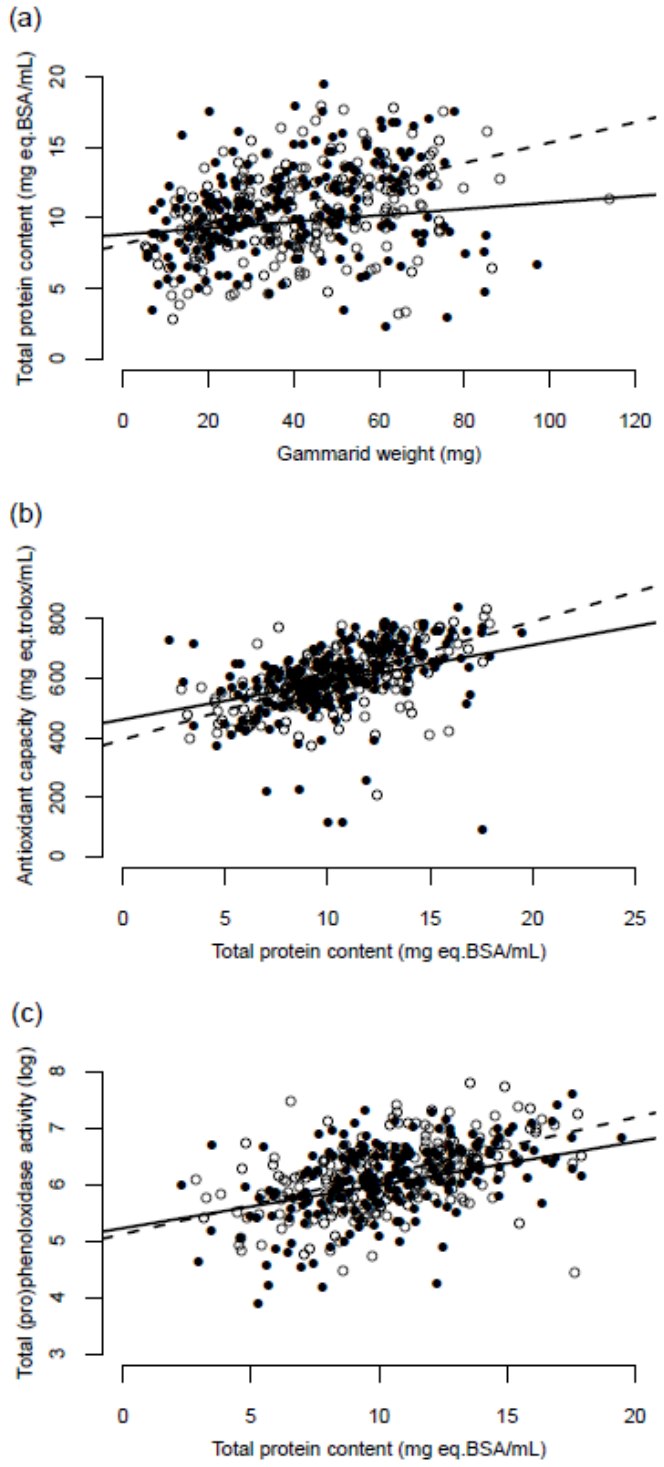


Fig.S2 Physiological parameters: (a) proteins plotted against the weight of individuals, (b) antioxidant capacity and (c) total (pro)phenoloxidase activity both plotted against total protein content, in interaction with the status of individuals (infected (N=211) or uninfected (N=219)). Solid circles and lines correspond to infected individuals and empty circles and dashed lines to uninfected ones.

Tab.S1. Models selection tables for General Linear Model (GLM) for the study of variation in prevalence. The table is shortened to the first 10 models and are ranked from the best one (lowest AICc, on the top) to poorer ones (higher AICc, at the bottom). Complex model is represented in bold. The line represents the threshold that separates the set of best models (above the threshold) that can be used to assess the contribution of each predictor. The threshold is chosen according to a significant gap in delta AICc from one model to another.

Model	Intercept	Total fish biomass	Gammarid density	Gammarid size	Total fish biomass : Gammarid density	R^2	Df	Log Likelihood	AICc	Delta	Weight
1	-9.32	-0.67	1.95	-2.74e-04		1.77e-02	4	-1152.91	2313.80	0	0.38
2	-10.22	-0.69	2.06			1.75e-02	3	-1153.99	2314	0.18	0.35
3	**-11.60**	**-0.27**	**2.35**	**-2.80e-04**	**-0.07**	**1.77e-02**	**5**	**-1152.88**	**2315.80**	**1.94**	**0.14**
4	-10.79	-0.60	2.16		-0.02	1.75e-02	4	-1153.99	2316	2.18	0.13
5	-0.27	-0.27		-8.88e-04		8.63e-03	3	-1206.04	2418.10	104.25	0
6	-1.78	-0.28				6.18e-03	2	-1220.35	2444.70	130.88	0
7	-1.93			-1.03e-03		3.03e-03	2	-1238.73	2481.50	167.65	0
8	-1.82		-0.02	-1.03e-03		3.03e-03	3	-1238.72	2483.40	169.63	0
9	-3.77					0	1	-1256.38	2514.80	200.94	0
10	-4.07		0.05			1.39e-05	2	-1256.30	2516.60	202.78	0

Tab.S2. Models selection tables for Beta-regressions for the study of variation in (a) phototaxis and (b) refuge-use. Total PO-PPO activity and antioxidant capacity (TEAC) are corrected by the weight of individuals. Tables are shortened to the first 10 models and are ranked from the best one (lowest AICc, on the top) to poorer ones (higher AICc, at the bottom). Complex models are represented in bold. The lines represent the thresholds that separate the set of best models (above the thresholds) that can be used to assess the contribution of each predictor. The thresholds are chosen according to a significant gap in delta AICc from one model to another.

(a)

Model	Intercept	Total fish biomass	Total PO-PPO activity	Prevalence	Status	TEAC	Total fish biomass : Status	Df	Log Likelihood	AICc	Delta	Weight
1	-2.80	0.38	0.16		+		+	6	296.12	-580	0	0.29
2	-2.78	0.37			+		+	5	294.94	-579.70	0.29	0.25
3	-2.78	0.37	0.17		+	-1.98e-07	+	7	296.19	-578.10	1.93	0.11
4	-2.84	0.38	0.16	0.52	+		+	7	296.17	-578.10	1.97	0.11
5	-2.79	0.37		0.11	+		+	6	294.95	-577.70	2.34	0.09
6	-2.79	0.37			+	2.99e-08	+	6	294.95	-577.70	2.34	0.09
7	**-2.82**	**0.38**	**0.17**	**0.47**	**+**	**-1.87e-07**	**+**	**8**	**296.23**	**-576.10**	**3.92**	**0.04**
8	-2.80	0.37		0.12	+	3.44e-08	+	7	294.95	-575.60	4.41	0.03
9	-1.62	0.21	0.18		+			5	284.39	-558.60	21.40	0
10	-1.59	0.20			+			4	282.90	-557.70	22.34	0

(b)

Model	Intercept	Total fish biomass	Total PO-PPO activity	Prevalence	Status	TEAC	Total fish biomass : Status	Df	Log Likelihood	AICc	Delta	Weight
1	-1.73	0.14			+		+	5	185.27	-360.40	0	0.31
2	-1.68	0.14			+	-5.31e-07	+	6	185.76	-359.30	1.08	0.18
3	-1.73	0.14	-0.05		+		+	6	185.38	-358.60	1.84	0.12
4	-1.67	0.14		-0.65	+		+	6	185.35	-358.50	1.91	0.12
5	-1.60	0.13		-0.90	+	-5.73e-07	+	7	185.91	-357.50	2.86	0.07
6	-1.68	0.14	-0.02		+	-4.99e-07	+	7	185.78	-357.30	3.11	0.07
7	-1.66	0.14	-0.05	-0.75	+		+	7	185.49	-356.70	3.70	0.05
8	**-1.60**	**0.13**	**-0.03**	**-0.94**	**+**	**-5.36e-07**	**+**	**8**	**185.94**	**-355.50**	**4.87**	**0.03**
9	-0.74				+			3	179.74	-353.40	6.97	0.01
10	-0.76				+	-6.54e-07		4	180.51	-352.90	7.48	0.01

Tab.S3. Models selection tables for General Linear Models (GLMs) for the study of variation in (a) total protein content, (b) antioxidant capacity and (c) total (pro)phenoloxidase activity. Tables are shortened to the first 10 models and are ranked from the best one (lowest AICc, on the top) to poorer ones (higher AICc, at the bottom). Complex models are represented in bold. The lines represent the thresholds that separate the set of best models (above the thresholds) that can be used to assess the contribution of each predictor. The thresholds are chosen according to a significant gap in delta AICc from one model to another.

(a)

Model	Intercept	Locality	Status	Gammarid weight	Status : Gammarid weight	R^2	Df	Log Likelihood	AICc	Delta	Weight
1	**9.22**	**+**	**+**	**0.02**	**+**	**0.22**	**8**	**-1044.40**	**2105.10**	**0**	**0.95**
2	8.59	+	+	0.04		0.20	7	-1048.36	2111	5.84	0.05
3	10.24	+	+			0.16	6	-1058.89	2130	24.83	0
4	9.09	+		0.04		0.16	6	-1059.80	2131.80	26.65	0
5	8.83		+	0.02	+	0.16	5	-1060.88	2131.90	26.76	0
6	7.98		+	0.04		0.13	4	-1066.95	2142	36.86	0
7	10.89	+				0.11	5	-1071.17	2152.50	47.33	0
8	8.56			0.05		0.09	3	-1077.92	2161.90	56.75	0
9	9.72		+			0.05	3	-1085.52	2177.10	71.95	0
10	10.45					0	2	-1097.37	2198.80	93.62	0

(b)

Model	Intercept	Locality	Status	Gammarid weight	Status : Gammarid weight	R^2	Df	Log Likelihood	AICc	Delta	Weight
1	310200	+	+	1446		0.14	7	-5608.67	11231.60	0	0.62
2	**319400**	**+**	**+**	**1200**	**+**	**0.14**	**8**	**-5608.16**	**11232.70**	**1.06**	**0.36**
3	323000	+		1515		0.12	6	-5613.38	11239	7.35	0.02
4	369300	+	+			0.10	6	-5616.96	11246.10	14.51	0
5	313800		+	944	+	0.08	5	-5622.20	11254.50	22.94	0
6	386300	+				0.08	5	-5622.30	11254.70	23.14	0
7	297100		+	1367		0.07	4	-5623.64	11255.40	23.77	0
8	312400			1423		0.05	3	-5628.36	11262.80	31.17	0
9	351000		+			0.02	3	-5634.95	11276	44.36	0
10	370300					0	2	-5640.38	11284.80	53.20	0

(c)

Model	Intercept	Locality	Status	Gammarid weight	Status : Gammarid weight	R^2	Df	Log Likelihood	AICc	Delta	Weight
1	5.78	+	+	0.01		0.18	7	-369.79	753.80	0	0.57
2	**5.84**	**+**	**+**	**0.01**	**+**	**0.19**	**8**	**-369.04**	**754.40**	**0.59**	**0.43**
3	5.69		+	0.01	+	0.15	5	-378.38	766.90	13.07	0
4	5.60		+	0.01		0.14	4	-379.91	767.90	14.08	0
5	6.11	+	+			0.15	6	-379.26	770.70	16.88	0
6	5.89	+		0.01		0.13	6	-382.48	777.10	23.31	0
7	5.72			0.01		0.09	3	-391.94	789.90	36.09	0
8	6.25	+				0.09	5	-392.79	795.70	41.89	0
9	5.97		+			0.06	3	-400.30	806.70	52.81	0
10	6.12					0	2	-413.18	830.40	76.55	0

Transition
Etudier la modulation des comportements des individus sains pour mieux comprendre la manipulation

Pour tenter de comprendre la variation dans l'intensité des changements comportementaux induits par le parasite, il est nécessaire de comprendre comment les comportements antiprédateur des individus non-infectés sont modulés en condition naturelles. Plus particulièrement, les comportements de réponse au stress représentent une piste à ne pas négliger. D'après Fayard *et al.* (2020), les comportements de réponses à des stimuli sont très fortement impactés par l'infection par les parasites acanthocéphales, et pourraient être une voie d'action intéressante pour des parasites à transmission trophique. En modifiant les comportements de réponse au stress, le parasite pourrait indirectement jouer sur la probabilité de rencontre avec des prédateurs et sur les réactions de défense, et donc de vulnérabilité face à d'éventuels prédateurs. Il nous appartient ainsi de comprendre en priorité comment ces comportements de défense sont modulés en condition naturelle chez des individus sains et d'évaluer leur flexibilité. Le stress de la prédation reste une expérience forte qui entraîne la mise en place de stratégies d'évitement ou de défense (Lima & Dill, 1990 ; Adamo, Kovalko, & Mosher, 2013). Pour évaluer l'importance du contexte local de prédation sur les comportements antiprédateur, nous avons mené une étude en milieu naturel visant à mettre en relation la variabilité dans l'expression des comportements antiprédateur de *G. fossarum* à la variation dans la pression de prédation. Tout ceci sera présenté et détaillé dans le chapitre suivant de cette même partie.

Chapitre 3 -

Effect of predator biomass and prey density on the magnitude of non-consumptive effects and reproductive investment in a freshwater amphipod: a correlational study

Article en préparation

ABSTRACT

Non-consumptive effects (NCEs) of predators have non negligible consequences on the phenotype of prey in different ways, particularly on behaviour. The effect of predation risk on prey behaviours has been relatively widely studied in invertebrates but most of these studies focused on immediate responses after direct exposure to predation threat. However, antipredatory behaviours may be shaped as the result of a sustained predation risk pattern experience. We addressed this question with an in-situ approach by assessing the variability in two antipredatory (phototaxis and refuge use) and mating (homogamy for size) behaviours in *Gammarus fossarum* with respect to local predation risk pattern. We also tested whether *G. fossarum* showed flexibility in their response to different concentrations of predator signal. We found that both antipredatory behaviours were modulated by the complex interaction of predator biomass and conspecific density. To go further, individuals from populations characterized by high predation risk showed a flexible behaviour, increasing their use of a refuge as the concentration of predator signal increased. We found no effect of the local predation pressure on mating behaviour. Our results show that the effect of predation risk on antipredatory behaviours can be evidenced in natural populations. We thus suggest that laboratory experiments are needed to confirm our results, more specifically by testing the effect of chronic stress on NCEs.

Introduction

Predator-prey interaction has long been an active field of research in evolutionary ecology, since predation represents one of the major selective force acting on organisms (Lima & Dill, 1990; Lind & Cresswell, 2005; Walzer & Schausberger, 2011). Trophic interactions between predator and prey are mainly studied for the consumptive effects of predation (Preisser, Bolnick, & Benard, 2005). By consumptive effects we mean direct effects as predators capture, kill and consume their prey. The prey either dies or survives. However, indirect effects, *i.e.* non-consumptive effects caused to prey, are generally considered of little importance (Clinchy *et al*., 2013). Yet, these effects should not be neglected as prey responses to predation risk is likely to strongly affect predator-prey interactions, and their ecological and evolutionary outcome (Brown *et al.*, 1999). Therefore, prey are likely to be real active participants in such interactions (Lima, 2002). Non-lethal effects include changes in prey phenotypic traits, such as morphology, physiology, but mainly behaviour in response to predation threat (Hill & Weissburg, 2013; Buchanan *et al.*, 2017). Indeed, individuals are expected to change habitat use, foraging and reproductive strategies in response to predation risk (Preisser & Bolnick, 2008; Hill & Weissburg, 2013; Buchanan *et al.*, 2017).

Predator-induced stress induces immediate responses in prey but also long-lasting changes in life-history strategies, behaviours and physiology. This widely supported observation has led to a new field of research, the Ecology of Fear (Clinchy *et al.*, 2013). Ecologists have generally viewed stress as a time-limited stimulus, such as the imminent threat of a predator. The idea of a sustained psychological stress was restricted to humans and some primates. Hence, the Ecology of Fear has for a long time been focused on vertebrates, particularly mammals, considered as more 'behaviorally sophisticated' (Brown *et al*., 1999). Yet, many studies have shown that predation pressure can result in sustained effects, affecting

multiple phenotypic traits (behaviour and physiology) and strategies at large-time scales similar to 'sustained psychological stress', in fish (Galhardo & Oliveira, 2009) or invertebrates such as crickets (Adamo & Baker, 2011).

Whether predator-induced stress can be either topic or chronic, it is never static (Mitchell, Bairos-Novak, & Ferrari, 2017). As there is variation in both predation risk (predators community and number), resources availability and habitat structure in the environment, behavioural changes in prey is likely to be context-dependent (Hill & Weissburg, 2013). According to temporal variation in predation risk, prey must have evolved a set of strategies based on the trade-off between defense and activity (Lima & Dill, 1990). Dunn, Dick, & Hatcher (2008) reported that the investment in mating behaviours in *Gammarus duebeni* changes according to perceived predation risk. This refers to as the '*risk allocation*' hypothesis. It states that allocation to antipredatory behaviours should vary with the frequency and duration of high-risk periods (Lima & Bednekoff, 1999; Higginson *et al.*, 2012). As predation pressure acts on animals during most of their lives, not only perception of predation risk must have evolved for a better detection and avoidance of predators (Brown, Ferrari, & Chivers, 2011), but also efficient escape/defensive strategies must have been shaped (Lind & Cresswell, 2005)

As predator avoidance is likely to be costly, individuals facing predation threats are expected to reduce their investment in energy for other activities (feeding) (Sih, 1992), even morphological traits such as growth (Preisser, Orrock, & Schmitz, 2007). In addition, one can expect intense predator cues to cause strong prey defensive behaviours if antipredatory behaviours are plastic. The '*threat-sensitive predator avoidance*' hypothesis states that prey should adapt the intensity of their defensive response according to current predation risk (Brown *et al.*, 2006). The riskier the situation is at a time, the stronger antipredatory behaviours

are likely to be. This was reported in the threespot damselfish, *Stegastes planifrons* (Helfman, 1989) and the juvenile convict cichlid *Archocentrus nigrofasciatus* (Brown *et al.*, 2006). Therefore, plasticity in defensive behaviours appears to be essential so that individuals can respond properly to local predation risk. In a changing environment, individuals with plastic strategies are thus expected to survive better or have a greater reproductive success. Although some studies have reported the role of local predation patterns in the response to predator cues concentrations (see for instance Brown *et al.* (1999)), the link between interpopulation variability in baseline level of antipredatory behaviours and predation pressure remains poorly addressed.

In the present study we used a correlational approach to address whether the background level of predation risk (approached by fish biomass, see Hill & Weissburg (2013) modulates antipredatory behaviours, and reproductive strategy of *Gammarus fossarum* (Crustacea, Amphipoda). We related individual variation in antipredatory behaviours (refuge use and phototaxis) and reproductive strategy (mate-guarding and fecundity) across 15 populations, to local predation risk. To assess local predation risk, we considered both fish density biomass (thereafter fish biomass), as a proxy for the background level of predation (Maurer, Stewart, & Lorenz, 2014; Barbosa *et al.*, 2018), and either conspecific density (Peacor, 2003; McCoy *et al.*, 2015), or invertebrate prey biomass density (thereafter prey biomass). Both fish biomass and conspecific density (or prey biomass) were categorized into three levels (low, moderate and high), and each of the 15 populations was assigned to one of these categories for predation level and prey density. We first tested whether interpopulation differences antipredatory behaviours and reproductive strategy (size homogamy in precopula pairs, fecundity) are explained by local predation risk. Then, we tested the flexibility of these antipredatory behaviours in response to immediate predation cues of varying intensities, on a subset of 6 populations. We expected

individuals from populations with high fish biomass, *i.e.* high predation risk, to show stronger antipredatory behaviours even in absence of immediate predation threat. According to the '*threat-sensitive predator avoidance*' hypothesis, we expected individuals from populations with different background level of predation risks to differ in the flexibility of their responses. More precisely, individuals from low-risk populations should respond to lower concentrations of predator cues and increase their response as concentrations increase too. On the other hand, we predicted that individuals from riskier populations should display a high response threshold with less flexible responses as predator concentrations vary. We also expected pre-copula pairs from riskier populations to show weaker homogamy as individuals should be less selective. In these populations, we also predicted a higher female investment in fecundity. Increasing their reproductive efforts as a response to high predation risk would increase their reproductive success (see Grégoir *et al.* (2018)).

Material and methods

Biological model

Gammarus spp. are freshwater amphipods present in most of french hydrosystems. They exhibit a sexual dimorphism with males generally larger than females and with larger gnathopods (prehensile maxilliped), resulting from intrasexual selection (Bollache & Cézilly, 2004). Gammarids mating system includes a guarding phase called amplexus during which the male grabs a female via its gnathopods (Piscart & Bollache, 2012).

Sampled populations and information on fish biomass density

We sampled 15 gammarids populations in Bourgogne-Franche-Comté (France) classified into three fish biomass categories (referred as "predation risk"): Low (280 – 610 g/100m^2), Medium (1330 – 1850 g/100m^2), High (3490 – 15000 g/100m^2). We retrieved information on fish

biomass densities from the database of the Agence Française pour la Biodiversité (Table S1). Fish biomass density was used as a proxy for predation pressure as gammarids are one of the main resources for a large variety of fish. By contrast to other macroinvertebrates, they are present annually in their environment (Macneil *et al.,* 1999). Four of the 15 localities were chosen according to a relatively small variance in phototaxis and refuge use and to the presence of trout (predation signal, see below) for flexibility tests (Table S1).

Field sampling

Males and amplexus sampling

Two hundred males and 50 amplexus were randomly collected by kick-sampling in each of the 15 localities. Amplexus were placed upon collection in separated bottles containing ethanol (70%) (1 pair in each bottle). Bottles were then put in a 4°C room until they are measured. One month later, 200 males were collected in the same way in the four localities chosen for flexibility tests. Tests were done on uninfected males to control for the effect of sex and parasitism.

Macroinvertebrates biomass

For each population, we performed semi-quantitative macroinvertebrates biomass sampling by the mean of three 1-meter-square-samples using kick sampling in a way to absorb gammarids habitats heterogeneity (excluding microhabitats where gammarids would not be found). On the day of collection, the biomass samples were sort out to keep only macroinvertebrates. The biomass of macroinvertebrates was assessed by weighing individuals without water with a precision balance (Sartorius Quintix35-1S). Prey biomass was classified into three categories: Low (0 – 2 g/m^2), Medium (2 – 4 g/m^2), High (4 – 6 g/m^2) (thereafter prey biomass; Table S1). The samples were also used to determine adult gammarids densities and sex-ratio for

homogamy and fecundity analyses. The density of gammarids (number of individuals/m²) was expressed as an ordinal variable, by 'cutting' the distribution into three categories of equal range, for each population.

Behavioural tests

Male individuals collected in the field during the morning were brought back to the 15°C room where behavioural tests were performed. They were put in a container with rocks and water from the river of collection and oxygenated. Tests were run 2h later. Gammarids were kept fasting to standardize for motivation during tests.

Refuge use and phototaxis

For each of the 15 populations, 80 individuals (males) are tested for phototaxis and 80 other males for refuge use (unless local gammarid abundance was not large enough to reach this sample size). Four series of 20 individuals were performed to record refuge use. Each series consisted in putting individually 20 males in boxes containing around 200 mL of their river water and an opaque shelter (half a terracotta saucer of 8.5 cm on its larger side) whose 1 cm² - entrance was completely submerged (the shelter was not totally covered to prevent the individual to lay on it). After a 5 min acclimation period, the position of each individual was recorded by time sampling according to Fayard, Cézilly, & Perrot-Minnot, (2019). The position was recorded every 30 s for 10 min and scored as 1 (outside) and 0 (under the shelter). For phototaxis, eight series of 10 individuals were performed. Each series consisted in putting individually 10 gammarids in transparent glass tubes (around 200mL of water from their own river), half of which was covered with thick black tissue to provide obscurity. After a 3 minutes acclimation period, the position of each individual was recorded by time-sampling every 15 s for 5 min and scored as 1 (in the lightened area: visible) and 0 (in the obscurity: not visible).

For both phototaxis and refuge use, scores were then added to obtain a binomial score (visible, not visible) bounded between 0 (always in the dark area: photophobic / under the refuge) and 20 (always in the lightened area: photophilic / outside the refuge). Both tests were performed under a homogeneous illumination at 550 lux.

Fecundity and homogamy

Fecundity was assessed by counting the number of eggs of each female under a binocular loupe (Nikon SMZ-745 Stereo Microscope) by opening the marsupium and spreading its content. Homogamy assessment was done by measuring the height of the 4th coaxial plate (dorsal-ventral length) under the binocular loupe and using an image acquisition and analysis software (Nikon NIS-Elements v. 4.10.00, Digital sight DS-U3) for both males and females.

Flexibility

On a subset of four populations (two 'high' and two 'low' predator biomass), we recorded refuge use for 60 individuals before and after signal addition, following the same protocol. The signal was obtained from trout guts grinded in approximately 10 mL water per fish mixed with fresh gammarids to signal for both the fish odours and consumed prey 'alarm cues'. Trouts were bought in Cordier-Gand fish farm, Corgoloin, France. Individuals were first tested in 150mL of water from their river, and tested again after the addition of 50mL of one of three treatments: control (river water), low predation signal (1/400 dilution of shredded tissues in river water) or high (1/50 dilution in river water).

Statistical analyses

Analyses were performed on RStudio software (v. 1.2.1335, (RStudio Team, 2016)).

Phototaxis and refuge use

For both refuge use and phototaxis analyses we ran generalized linear mixed models (*lme4* R package (Bates *et al.*, 2015); *lmerTest* R package (Kuznetsova, Brockhoff, & Christensen, 2017)) with a negative binomial and binomial distribution, respectively, in order to obtain the best residuals distribution for both models. Consequently, the response variable for refuge use was the number of times under the refuge out of 20 observations per individual, and the response variable for phototaxis was the proportion of time spent in the dark. Independent variables were the categories of fish biomass (*i.e.* predation risk), prey biomass categories or prey density categories, and their interactions. Predator and prey categories were ordinal variables. The same models were made replacing the prey biomass variable by the density of adult gammarids (*i.e.* conspecifics) (number of individuals/m^2) of the populations. To deal with non-independent observations, we used gammarid localities as a random effect nested within the predation risk variable and within the prey biomass or conspecific density variables. We estimated the best model explaining the dependent variable (behaviours) by performing model selection, using AICc values (Akaike Information Criteria corrected)) of all possible nested models from the full model described above (*MuMin* R package (Bartoń, 2019). This procedure was preferred to a stepwise method whose use of *p*-value is criticized (Halsey, 2019). We then performed model averaging on the subset of models with a **Δ**AICc below 4, following the 'rule of thumb' of Burnham et al. (2011), to keep only significant variables and interactions.

Fecundity

To analyze female fecundity, the number of eggs was used as the dependent variable in a generalized linear mixed model. The data showed a large number of zeros. Zeros were considered as either true zeros (*i.e.* females were actually not gravid) or false zeros (*i.e.* eggs were not counted because not seen or lost in the tube of alcohol). In that regard, we therefore choose to perform a zero-inflated mixture model, rather than a two-step approach consisting in a binomial model for the presence or absence of eggs followed by a zero-truncated model (*i.e.* Hurdle model - in the case where we would have considered all zeros as false zeros). We chose a negative binomial family with zero-inflation (*glmmADMB* R package, (Fournier *et al.*, 2012; Skaug *et al.*, 2016)) to compensate for overdispersion of egg counts (Hartig, 2019) and the large number of zeros. The choice of a type 1 or type 2 negative binomial was made comparing AICc of the two models and the type 2 distribution was the best (AICc=4116.5, df=11, **Δ**AICc with type 1=9.2). Our model included female size to consider the strong relationship between fecundity and female size (Bollache & Cézilly, 2004). We also included predation risk, gammarids density (as density can have effects on fecundity (Peters & Barbosa, 1977), their interaction, sex-ratio, and localities as random effect nested within the predation risk variable. We then proceeded to select significant variables using AICc comparison and model averaging in the same way as detailed above.

Homogamy

The level of homogamy (size assortative pairing) across populations was assessed by using Spearman correlation coefficients between the size of male and female in precopula (non-normality of size distribution in some localities) according to Bollache & Cézilly (2004). The Spearman correlation coefficient was then used as dependent variable in a GLM (gaussian family). We used predation risk, adult gammarids density, their interaction, and sex-ratio (as it

can impact mate choice mechanism: Dechaume-Moncharmont, Brom, & Cézilly, 2016) as covariables to explain variability in size assortative pairing. No random effect was included as we have one observation per locality (correlation coefficients). Spearman correlation coefficients 95% confidence intervals were calculated by bootstrapping (1000 iterations). We then proceeded as detailed above, using AICc comparison and model averaging.

Flexibility

The magnitude of changes in refuge use following signal addition (control, 1/400 or 1/50) compared to refuge use without signal was quantified using effect size and its confident interval. We estimated the non-parametric Cliff's delta with 95% confidence interval (*effsize* R package, (Torchiano, 2018)). Due to small sample size (n = 4) we did not perform statistical analyses but only a verbal interpretation of the results.

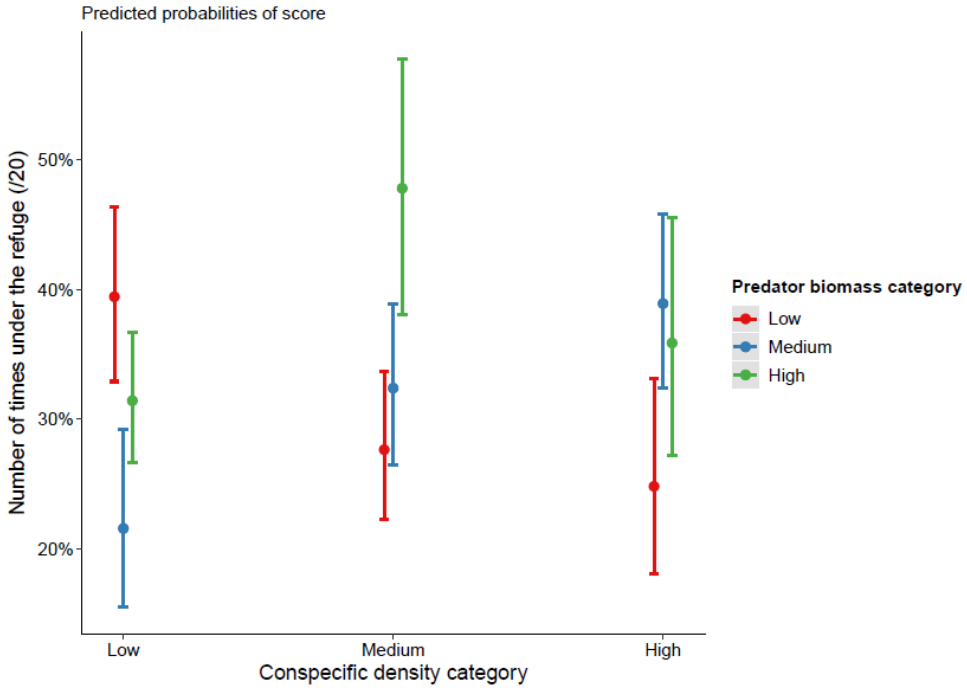
Results

There was no collinearity between the logarithm of prey biomass density and the logarithm of fish biomass density ($r = 0.12$, $n = 15$, $p = 0.66$). There was no collinearity between the logarithm of the predator biomass and the density of adult gammarids ($r = 0.163$, $n = 15$, $p = 0.6$) or gammarids sex-ratio ($r = 0.252$, $n = 15$, $p = 0.4$) and no collinearity between sex-ratio and adult density ($r = 0.008$, $n = 15$, $p > 0.9$).

Refuge use

The results of the conditional model averaging evidenced a significant interaction between predation risk and prey biomass (Table.S2A). We observed a trend for individuals from populations with low predation to decrease their refuge use with increasing prey biomass.

A



B

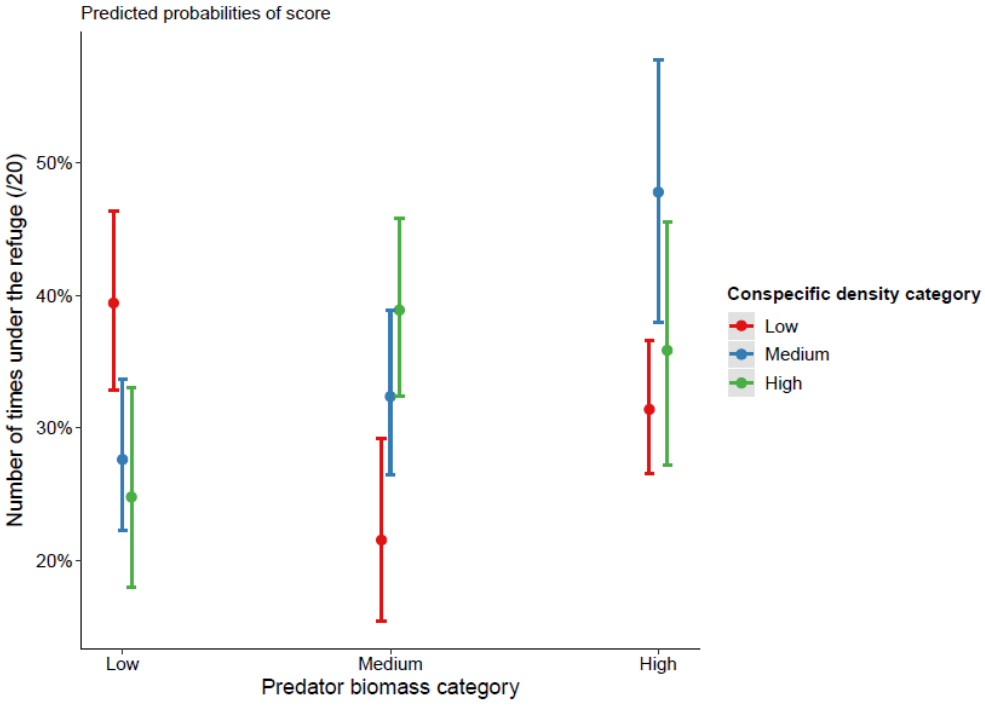


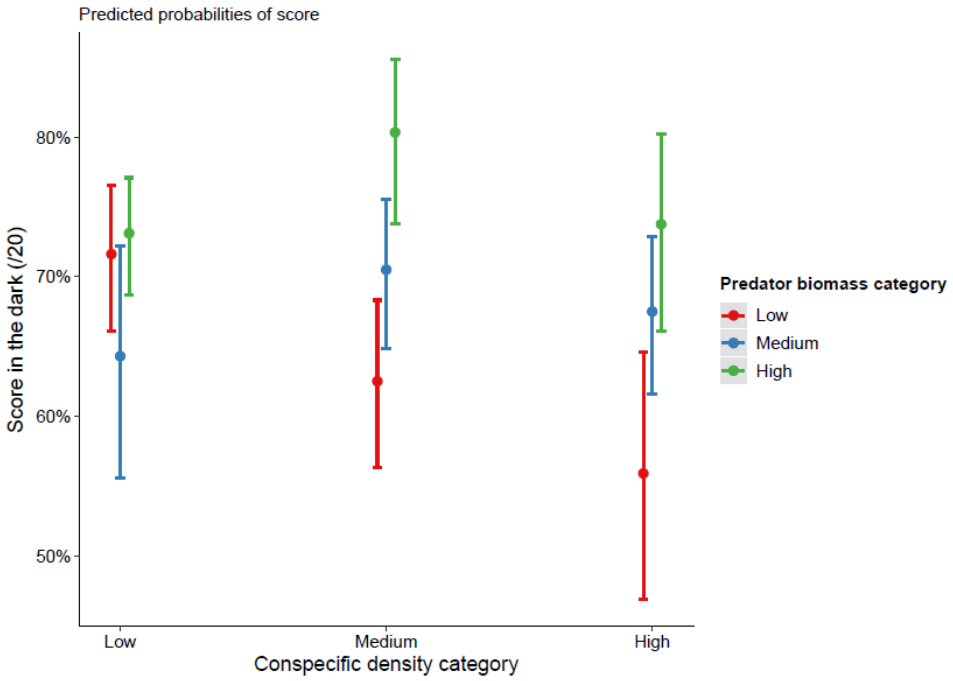
Fig.1 Model predictions for score of refuge use with 95% confidence interval according to A) conspecific density (number of individuals/m²) in interaction with predator biomass (g/100m²) and B) predator biomass in interaction with conspecific density. Red: low predation risk, blue: medium predation risk, green: high predation risk. A score of 0 is an individual always outside of the refuge, a score of 20 is an individual always inside the refuge. For each locality n = 80, except Grozonne (n = 60) and Morthe (n = 79), both in the low predation risk category.

On the other hand, we observed a trend for an increased refuge use followed by a plateau for individuals facing medium predation risk, with increasing prey biomass. Finally, individuals from highly predated populations display a quadratic refuge use with a greater refuge use under medium prey biomass. When replacing prey biomass by gammarid density, there was still a significant interaction between predator biomass and gammarid density (Table.S2B): high predator biomass induced increased refuge use, but only at medium gammarid density (Fig.1A). In addition, gammarids at low density were more under refuge, particularly at low predator biomass (Fig.1A, B).

Phototaxis

The results of model selection revealed that only predator biomass was significant (Table.S3A). Indeed, the model explaining phototaxis by predator biomass showed a positive linear effect, with photophobia increasing with predator biomass. When replacing prey biomass by the density of gammarids, there was still a significant effect of predator biomass (Table.S3B): high predator biomass induces increased photophobia, but only at medium gammarid density (Fig.2A). In addition, gammarids at high density were more photophobic, in particular at high and medium predator biomass (Fig.2A, B).

A



B

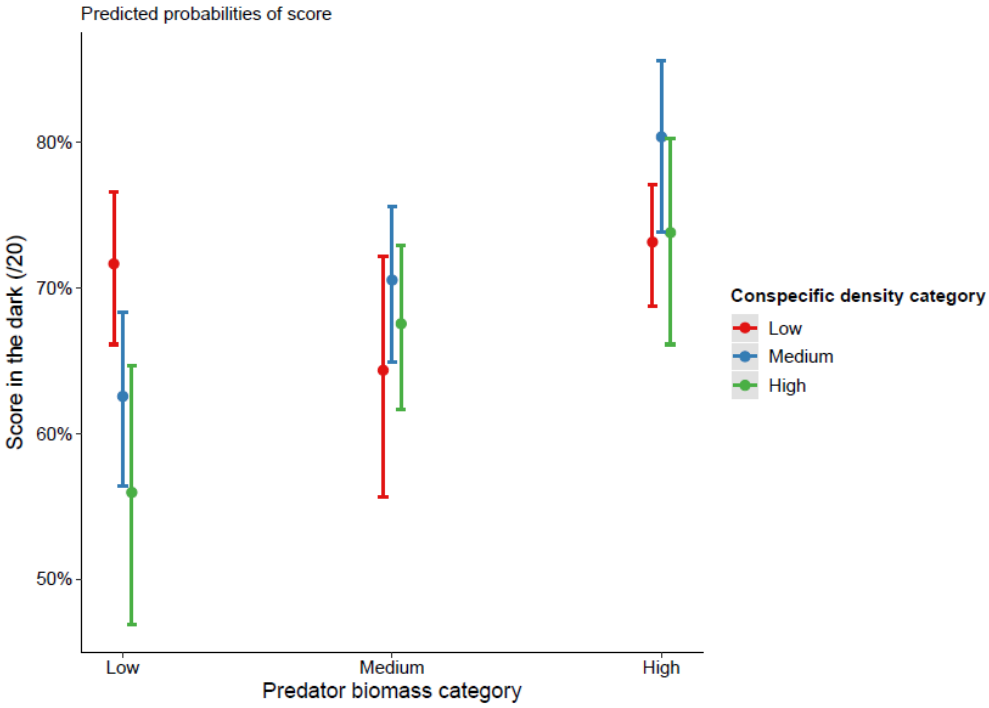


Fig.2 Model predictions for score of phototaxis with 95% confidence interval according to A) conspecific density (number of individuals/m²) in interaction with predator biomass (g/100m²) and B) predator biomass in interaction with conspecific density. Red: low predation risk, blue: medium predation risk, green: high predation risk. A score of 0% is an individual always in the lightened area (strongly photophilic), a score of 100% is an individual always in the obscurity (strongly photophobic). For each locality n = 80, except Grozonne (n = 60).

Fecundity

Only female size significantly explained the number of eggs being incubating by females (GLMMADMB: Log-Likelihood=-2049.83, AIC=4109.70, beta=4.25*10^{-4}, se=8.34*10^{-5}, p<0.0001) (Table.S4A), with larger females having more eggs as expected (Fig.3). Despite female size was significantly explaining the number of eggs, the large number of zeros tended to reduce the slope of the relationship (zero-inflation=0.28, se=0.02).

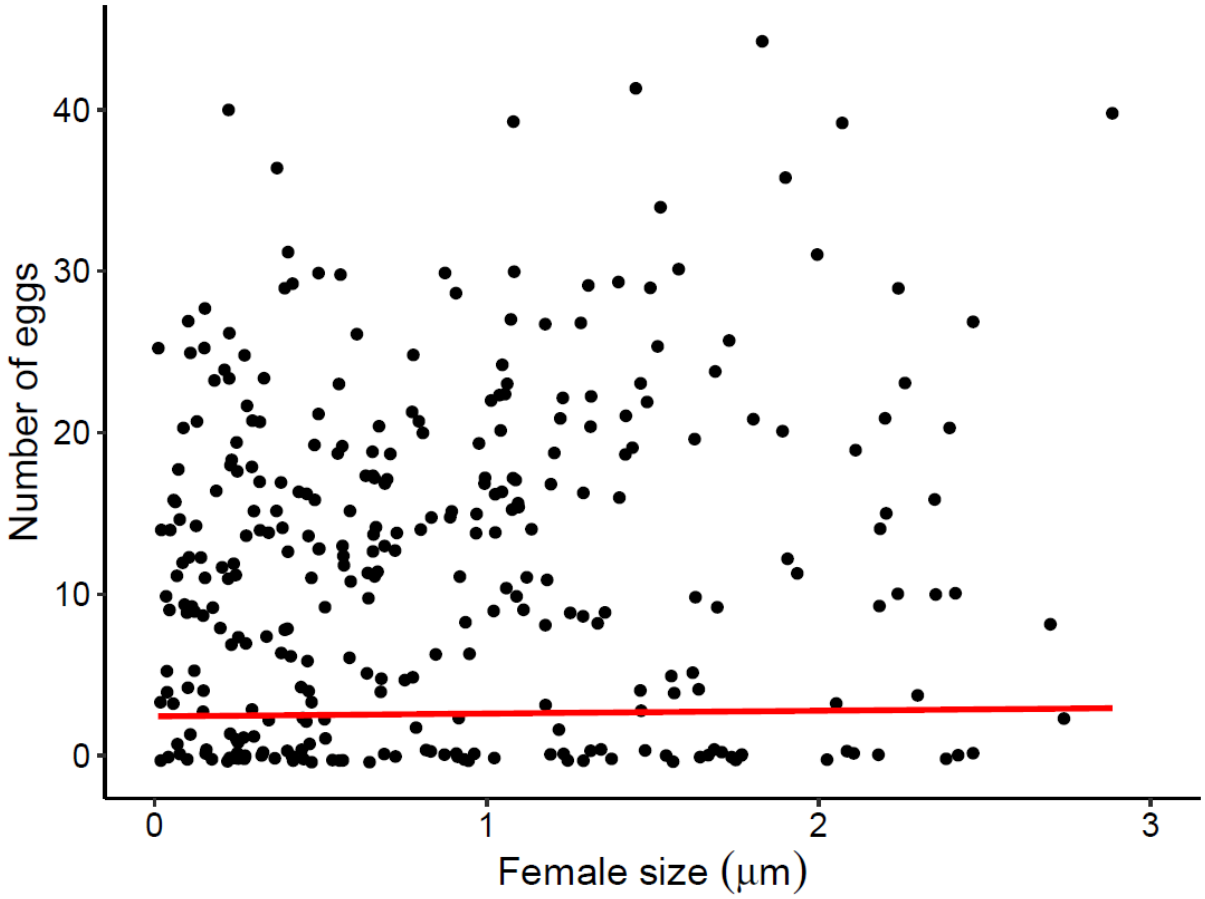


Fig.3 Number of eggs per female according to female size (µm) with prediction line from the model explaining the number eggs by female size and with populations nested within predation risk as random factor. For each locality n = 50 amplexus except Grozonne, n = 20 and Linotte, n = 26.

Homogamy

No variables were significantly explaining variation in homogamy for size across populations (Table.S4B). The coefficient of size assortative pairing (Spearman correlation coefficient) ranged from -0.18 to 0.62 across the 15 populations, with median and interquartiles at 0.33 [0.23 – 0.45].

Flexibility

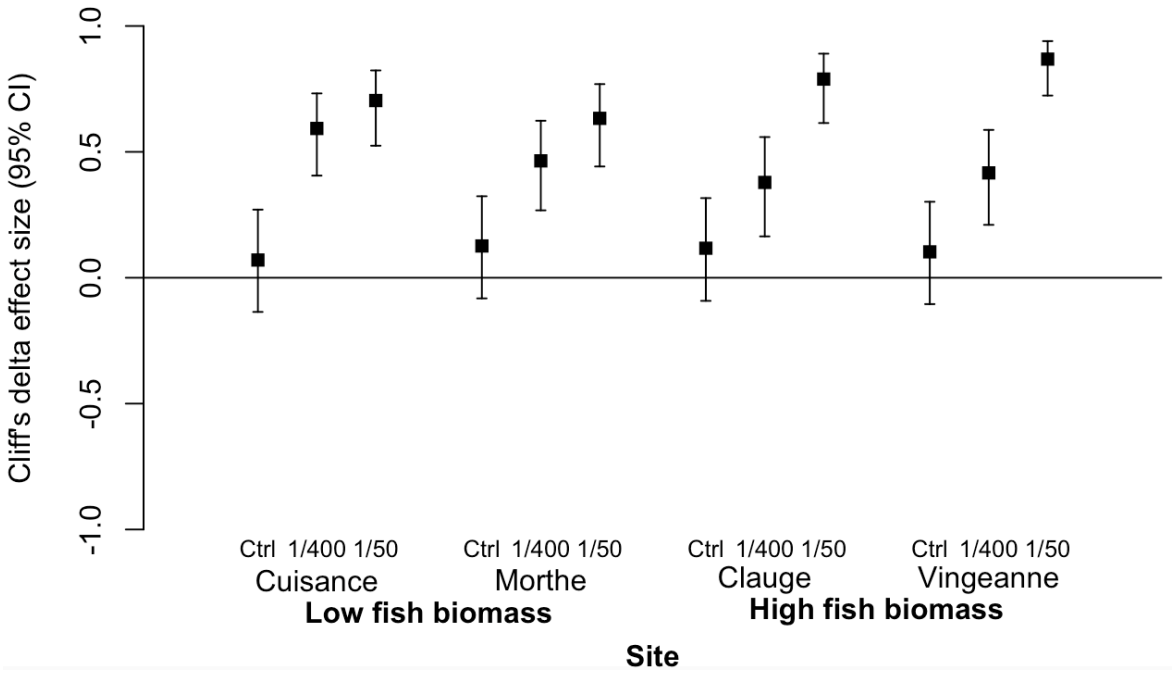


Fig.4 Cliff's delta effect sizes with 95% CI for changes in refuge use in *Gammarus fossarum* induced by a signal compared to the absence of signal (Ctrl: control, 1/400: grinded trout guts diluted 400 time in river water, 1/50: grinded trout guts diluted 50 time in river water) for Cuisance, Morthe, Clauge and Vingeanne localities. The effect of the stimulus was considered as non-significant when its 95% confidence interval crossed zero. The magnitude is assessed using the thresholds provided in Romano et al. (2006), *i.e.* |d| < 0.147: "negligible", |d| < 0.33: "small", |d| < 0.474: "medium", |d| > 0.474: "large". For each treatment n = 60 males.

Control individuals did not respond to the addition of water (Fig.4). We found an effect of the addition of both concentrations of predator signal on the use of a refuge of *G. fossarum*: individuals increased their use of a refuge in response to the addition of the predator signal.

Gammarids from high-risk rivers responded gradually to an increase in predation threat by increasing their use of a refuge (no overlapping of effect sizes' CI between 1:400 and 1:50 treatments). By contrast, individuals from low-risk rivers did not behave differently under low and high concentrations of predation cues (Fig.4).

Discussion

We aimed at testing the effect of predation risk on different phenotypic traits in natural populations of *G. fossarum*. We assessed the variability of antipredatory behaviours with respect to local predation pattern. We also tested the effect of predation risk on mating and reproductive traits. Finally, we assessed the flexibility of antipredatory responses using different predator cues concentrations. We evidenced a complex interplay between predator biomass and conspecific density in the variability of both refuge use and phototaxis. Neither homogamy nor female fecundity were affected by predation risk. In addition, refuge use appeared to be more flexible and sensitive to differences in cue concentration in individuals from populations with high predation risk.

Some patterns fit the general expectation of enhanced protective behaviours at elevated background level of predation risk: stronger photophobia and to a lesser extent enhanced use of refuge were observed at high predator biomass. However, their expression seemed to be more or less dependent from conspecific density, particularly for refuge use, as evidenced in the interaction between predator biomass and conspecific density. Individuals from populations facing low predation risk at high gammarids density, showed decreased defensive behaviours. However, individuals facing high predation risk at high or medium conspecific density increased their refuge use. Conspecific prey density may therefore convey another information than the 'dilution effect' (i.e. a decrease in individual predation probability with increasing

conspecific density) in particular at high gammarid density. Several nonexclusive hypotheses may be put forward, some related to unmet assumptions of the dilution effect hypothesis in this predator-prey association. First, from a methodological point of view, assessing refuge use of gammarids in isolation might actually represent a stressful context for individuals living at high conspecific density, and thus result in higher defensive behaviour (refuge use a an 'anxiety-like' behaviour). However, under this hypothesis, we should have observed a similar response on reaction to light (higher photophobia in gammarids from high conspecific density-population). Second, predators may respond to prey density by switching diet towards the most abundant ones (Waraniak, Valentine, & Scribner, 2017). Therefore, high gammarids density would be associated with high prevalence of gammarid prey in fish diet; hence high predation signal (alarm cues) from conspecifics. In the coevolutionary arm race between predator and prey, gammarids could associate conspecific density with a higher baseline risk of predation even in absence of alarm cues. Third, alternative behavioural tactics may match to different predation context / conspecific density, because protective behaviours also trade-off with other functions, specifically foraging and mating. If the shape of this trade-off depends on conspecific density, this could explain the counter-intuitive observation that refuge use increases at high conspecific density under high predator biomass. Noticeably, the trade-off between foraging for mate and sheltering might be stronger at low conspecific density, unless gammarids (males) are less selective at low density or at high predator biomass, but we didn't evidence any effect of these factors on size assortative pairing. Since refuge use may compromise foraging activity more than photophobia, this would also be in agreement with the lower dependency of photophobia to conspecific density than refuge use.

Although the overall pattern of variation in refuge use and reaction to light according to predator biomass was similar, best models explaining variation in these two defensive

behaviours are slightly different. The interaction between predator biomass and conspecific density may be stronger for refuge use as no clear trend emerged compared to phototaxis (particularly modulated by predator biomass), suggesting that the use of a refuge may be a more complex behaviour. This tends to support previous data showing that these two behaviours are differentially modulated by environment and by internal state. David, Salignon, & Perrot-Minnot (2014) showed that the relationship between phototaxis and refuge use is dynamically affected by both sex and predator cues. In addition, these two behaviours have already been reported to differentially contribute to protection from predation in microcosms experiments. Indeed, phototaxis does not seem to play a direct role in the vulnerability to predation (Perrot-Minnot *et al.*, 2012) but refuge use does (Dianne *et al*., 2011; Perrot-Minnot *et al*., 2014). The use of a refuge may thus involve more complex cognitive processes than phototaxis, and may not depend on predator biomass solely, but on many more factors.

Predation pressure is an acute stress that comes with direct (prey either die or survive) and indirect outcomes. The indirect effects of predation pressure are called non-consumptive effects. Individuals are expected to change their habitat use, foraging and reproductive strategies in response to predation risk (Preisser & Bolnick, 2008; Hill & Weissburg, 2013). These changes resulting from a 'sustained psychological stress' induced by predator stress have recently open a new field of research, the Ecology of Fear (Clinchy *et al*., 2013). This sustained psychological stress would induce an emotional state that might affect the expression of antipredatory behaviours even in absence of a threat. For instance, Fossat *et al.* (2014) reported the involvement of phototaxis in anxiety-like state in the crayfish *Procambarus clarkii*. In another crustacean, *G. fossarum,* the use of refuge is increased under anxiety-like state. Taken together, our results suggest that the anxiety-like state of individuals differ among populations varying in the background level of predation risk, as no predator cue was added during the tests.

Our results on mating behaviours did not match the expectations of Dunn *et al*. (2008) who suggested that under high predation risk, males should be less choosy and mate with females regardless of their size. Indeed, size homogamy was not affected by predation risk. However, Dunn *et al*. (2008) used laboratory experiments. They found an effect of predation risk on mating behaviour after the presentation of predator cues, *i.e.* imminent threat, resulting in an immediate response. In our approach, homogamy was estimated on precopula pairs formed *in situ*, under natural conditions, and not as the immediate reponse to an acute risk of predation. The overall strength of size-assortative pairing was also slightly lower than the one reported by Bollache and Cézilly (2004) in a transversal survey over 18 different localities (from 0.35 to more than 0.8), but with a larger variability across populations (from -0.18 to 0.62).

As expected, females' fecundity was positively correlated to size. However, fecundity was not modulated by predation risk. We expected that under high predation risk, females might invest more in reproduction as a response to a high probability of dying. Females with high fecundity would have a greater reproductive success. However, Buchanan *et al.* (2017) reported that while non-consumptive effects have negative effects on prey phenotypic traits, some of them are more impacted than others. Indeed, prey activity, and more generally behavioural responses are more strongly affected than physiology or life-history traits (Preisser & Bolnick, 2008). This could be the result of the costs related to changes, as behaviour is energetically less costly to modulate than fecundity.

The pattern of responses to predator cues were similar to those reported by Brown *et al.* (2009). The highest responses were exhibited by individuals from populations with high predation risk. In addition, there is an increase in response intensity with increasing predator

cues concentration. This is in accordance with the '*threat-sensitive predator avoidance*' hypothesis (see Brown *et al.* (2006, 2009)). However, and regardless of predator cues concentrations, individuals from populations with low predation risk exhibited less-graded responses. Behavioural responses to predator threats are expected to be shaped according to long-term predation experience (Brown *et al*., 2009). Predator avoidance is costly and is part of a trade-off between defenses/protection, and general activity (foraging or reproduction) (Brown, 2003; Elvidge, Ramnarine, & Brown, 2014). The level of antipredator behaviour and amount of behavioural flexibility should therefore be adjusted with respect to predation risk so that this trade-off can be optimized (Brown *et al.*, 2009, 2014). We showed that individuals that usually experience high predation risk exhibited more finely tuned responses, in agreement with the hypothesis that high flexibility in defensive behaviours and accurate risk assessment capacities are adaptive.

Our study is the first one to evidence interpopulational variability in antipredatory behaviours in relation to the background level of predation risk in *G. fossarum*. Interestingly, we evidenced complex modulation of refuge use and phototaxis according to both direct predation risk (predator biomass) and relative individual vulnerability (depending on conspecific density or prey biomass). The absence of results for mating behaviour and fecundity could come from recording reproductive strategy under natural conditions, as compared to previous experimental studies. However, it is consistent with the hypothesis that such traits are likely to be more costly to change, contrary to defensive behaviours. We showed that behavioural strategies are partly shaped by local predation risk but are also flexible, as the magnitude of behavioural response can be modulated by the intensity of threat. To complement this correlational study, laboratory experiments are needed to confirm sources of variation in antipredatory behaviours. More precisely, laboratory experiments should address the effects of

both temporal variation in predation risk and chronic stress on these phenotypic traits / strategies. We think that testing the risk allocation hypothesis might be a complementary approach, to address the role of temporal variability in predation risk on the modulation of behaviours. In addition, it would be interesting to estimate physiological costs in gammarids related to non-consumptive effects, such as increased oxidative stress as predation risk increases, and immune reconfiguration (Slos & Stoks, 2008; Janssens & Stoks, 2013; Adamo *et al.*, 2017). Finally, multiple factors may affect the impact of predation pressure (combining predator biomass and prey density), including predator foraging behaviour (sit-and-wait benthic fish *versus* chasing benthopelagic fish), and density-dependent flexibility in predators' preference for certain species of prey. These questions should be addressed in future studies by comparing individual defensive/protective behaviour in isolation and in group, by undertaking a detailed analysis of the invertebrate communities (to assess opportunities for diet switch in fish predators).

Supporting information

Table S1. Description of the localities with fish biomass and predation risk associated, macroinvertebrates biomass and prey category associated, gammarids density and sex-ratio. Localities in bold are the localities used for flexibility tests.

Localities	Fish biomass ($g/100m^2$)	Predation risk	Macroinvertebrates biomass (g/m^2)	Prey category	Gammarids density ($adults/m^2$)	Sex-ratio (female proportion)
Bèze à Marandeuil	281.34	Low	4.08	High	91.08	0.59
Cuisance à Vadans 2	**341.72**	**Low**	**0.86**	**Low**	**149.94**	**0.49**
Morthe à Bucey-Les-Gy 3	**365.42**	**Low**	**2.45**	**Medium**	**101.45**	**0.46**
Seille de Beaume à Cosges	607.73	Low	4.33	High	114.33	0.38
Tille à Cessey-Sur-Tille 1	449.66	Low	1.47	Low	170.69	0.51
Bièvre à Brazey-en-Plaine 1	1 335.62	Medium	4.38	High	140.03	0.50
Doulonne à Plumont 2	1 652.93	Medium	2.12	Medium	119.17	0.46
Lacanche à Vievy	1 851.34	Medium	2.66	Medium	52.43	0.50
Ruisseau des Vieilles Granges à Sorans-Les-Breurey	1 478.94	Medium	1.82	Low	86.09	0.47
Vallière à Savigny-en-Revermont	1 566.89	Medium	5.30	High	108.51	0.46
Arne à Lavans-Les-Dole 3	3 493.51	High	1.11	Low	137.93	0.37
Clauge à La-Loye	**3 706.09**	**High**	**1.61**	**Low**	**195.11**	**0.51**
Grozonne à Neuvilley	5 318.06	High	1.74	Low	180.92	0.38
Linotte à Loulans-Verchamp	7 514.92	High	3.08	Medium	91.94	0.51
Vingeanne à Talmay	**15 000**	**High**	**4.70**	**High**	**39.18**	**0.38**

Table.S2 Models selection tables for analyses of variation in refuge-use. Fixed factors are predation category, A) prey biomass category or B) conspecific density category and their interaction. Tables are shortened to the models characterized by A) delta AICc < 4 or B) delta AICc < 7, and are ranked from the best one (lowest AICc, on the top) to poorer ones (higher AICc, at the bottom).Complex models are represented in bold. Best models are represented in italics.

A

model	intercept	predation category	prey biomass category	interaction	df	logLik	AICc	delta	weight
1	*0,70*				*3*	*-7626,65*	*15259,30*	*0,00*	*0,52*
8	**0,43**	**+**	**+**	**+**	**11**	**-7619,09**	**15260,40**	**1,09**	**0,30**
2	0,78	+			5	-7626,33	15262,70	3,38	0,10
3	0,75		+		5	-7626,58	15263,20	3,88	0,07

B

model	intercept	conspecific density category	predation category	interaction	df	logLik	AICc	delta	weight
8	***-0,43***	**+**	**+**	**+**	***11***	***-7618,16***	***15258,50***	***0,00***	***0,52***
1	-0,70				3	-7626,65	15259,30	0,79	0,35
3	-0,78		+		5	-7626,33	15262,70	4,17	0,07
2	-0,75	+			5	-7626,56	15263,20	4,63	0,05

Table.S3 Models selection tables for analyses of variation in phototaxis. Fixed factors are predation category, A) prey biomass category or B) conspecific density category and their interaction. Tables are shortened to the models characterized by delta AICc < 4 and are ranked from the best one (lowest AICc, on the top) to poorer ones (higher AICc, at the bottom).Complex models are represented in bold. Best models are represented in italics.

A

model	intercept	predation category	prey biomass category	interaction	df	logLik	AICc	delta	weight
2	*-0,62*	+			*5*	*-5629,70*	*11269,40*	*0,00*	*0,52*
8	**-0,93**	**+**	**+**	**+**	**11**	**-5624,41**	**11271,00**	**1,60**	**0,23**
1	-0,82				3	-5632,94	11271,90	2,46	0,15
4	-0,66	+	+		7	-5629,62	11273,30	3,89	0,07

B

model	intercept	conspecific density category	predation category	interaction	df	logLik	AICc	delta	weight
3	*0,62*		+		*5*	*-5629,70*	*11269,40*	*0,00*	*0,48*
8	**0,93**	**+**	**+**	**+**	**11**	**-5624,41**	**11271,00**	**1,59**	**0,22**
1	0,82				3	-5632,94	11271,90	2,46	0,14
4	0,64	+	+		7	-5629,04	11272,20	2,72	0,12

Table.S4 Models selection tables for analyses of variation in A) female fecundity and B) homogamy. Fixed factors are predation category, conspecific density, their interaction, sex-ratio and A) female size. Tables are shortened to the models characterized by delta AICc < 4, and are ranked from the best one (lowest AICc, on the top) to poorer ones (higher AICc, at the bottom). Best models are represented in italics.

A

model	intercept	conspecific density category	predation category	sex-ratio	size	interaction (predation* conspecific)	df	logLik	AICc	delta	weight
5	*2,44*				*0,17*		*5*	*-2049,83*	*4109,70*	*0,00*	*0,27*
7	2,43	+			0,18		6	-2049,25	4110,60	0,87	0,18
13	2,44			-0,08	0,17		6	-2049,43	4111,00	1,23	0,15
6	2,44		+		0,17		7	-2048,54	4111,20	1,50	0,13
15	2,43	+		-0,08	0,17		7	-2048,79	4111,70	2,00	0,10
8	2,44	+	+		0,17		8	-2048,27	4112,70	3,00	0,06
14	2,44		+	-0,05	0,17		8	-2048,39	4113,00	3,24	0,05

B

model	intercept	conspecific density category	predation category	sex-ratio	interaction (predation*conspecific)	df	logLik	AICc	delta	weight
1	*0,31*					*2*	*0,94*	*3,10*	*0,00*	*0,53*
5	-0,23			1,16		3	1,70	4,80	1,67	0,23
2	0,16					3	1,37	5,50	2,33	0,17

Discussion - Transition
Flexibilité des comportements antiprédateur

La flexibilité des comportements face au risque de prédation est susceptible de varier selon le pattern de danger de prédation auquel les individus sont exposés en condition naturelle. Deux hypothèses peuvent expliquer la variabilité dans les réponses antiprédateur. Premièrement, l'approche coût / bénéfice nous permet de comprendre que les individus ne peuvent qu'optimiser leurs défenses à travers un compromis d'investissement entre protection et autres fonctions, tels que l'approvisionnement ou la reproduction (Lima & Dill, 1990 ; Brown *et al.*, 2006). En effet, dans un contexte local de faible risque de prédation, nous ne nous attendons pas à ce que les individus montrent des comportements marqués de protection mais plutôt une activité liée à l'approvisionnement ou la reproduction. En revanche, si le risque de prédation est élevé, les individus diminueraient ces activités au profit d'une augmentation des défenses. Deuxièmement, la variabilité dans les réponses de défenses peut provenir d'une variabilité dans la capacité et / ou l'efficacité à percevoir le risque. Cette perception dépendrait d'un apprentissage supposé se faire sur la base d'évènements de rencontre répétés, et donc favorisant une certaine mémoire du risque. Que ce soit dans l'optique coût / bénéfice ou de l'apprentissage, la perception du risque est primordiale puisque c'est elle qui influencera les comportements adoptés et qui éventuellement permettra de contrôler ce risque (Lima & Dill, 1990).

Reconnaître des prédateurs et exprimer des réponses défensives requièrent généralement un apprentissage chez la plupart des animaux (Griffin, 2004 ; Ferrari *et al.*, 2007 ; Ferrari, Messier, & Chivers, 2008). Premièrement, le risque de prédation n'est jamais fixé mais fluctue dans le temps (Sih & McCarthy, 2002) et l'espace (Creel & Winnie, 2005). Ainsi, à travers un apprentissage et une évaluation fiable du risque, l'intensité des réponses des individus est censée être ajustée au pattern local de prédation (Griffin, 2004 ; Brown *et al.*, 2006). Deuxièmement, tout au long de sa vie, un individu est susceptible de rencontrer de nouveaux

stimuli relatifs à des prédateurs inconnus. L'ajustement des réponses à ces nouvelles menaces n'est alors possible que si l'individu apprend à les reconnaître (Griffin, 2004) ou si l'apprentissage passe par la reconnaissance d'une situation similaire déjà expérimentée (McLean *et al.*, 2000). Dans tous les cas, l'apprentissage dépendra alors des expériences vécues (Griffin, Evans, & Blumstein, 2001). L'intensité de la réponse au stimulus peut aussi augmenter avec la répétition de ces évènements, ce que l'on appelle 'sensitisation' (Gotz & Janik, 2011). Chez les organismes aquatiques, cet apprentissage passe principalement par la détection d'indices provenant de composés chimiques relâchés dans l'environnement par les conspécifiques (alarme) et/ou par les prédateurs (Ferrari *et al.*, 2007 ; Ferrari, Manek, & Chivers, 2010). On suppose qu'il existe un seuil de perception au-delà duquel les organismes vont répondre par un comportement antiprédateur et en dessous duquel ils ne répondront pas. Par exemple, Mirza *et al.* (2006) ont montré que les crapauds américains *Bufo americanus* étaient sensibles à un seuil de concentration d'alarmes de conspécifiques blessés au-dessus et en dessous duquel ils montraient des réponses élevées ou nulles, respectivement. Pour aller plus loin, les organismes sont capables d'ajuster l'intensité de leur réponse en fonction de celle du risque de prédation (Mirza & Chivers, 2003). C'est pour cela que même s'ils perçoivent un risque, les organismes peuvent ne montrer aucune réaction comportementale si l'intensité du signal est inférieure au seuil de réaction (Brown *et al.*, 2001).

Stress de la prédation et états émotionnels

La présence de prédateurs est un facteur reconnu comme responsable d'un stress accru chez les organismes (Boite.1). De ce stress s'en suit une réponse qui est censée favoriser la survie des individus (en réinstaurant l'homéostasie précédemment perturbée par la source de stress) sur le moment présent et éventuellement sur le long terme, en aidant les individus à faire face ultérieurement à d'autres sources de stress (Suri & Vaidya, 2015). L'expérience d'évènements

est susceptible de façonner chez les individus des états émotionnels bien spécifiques (Mendl, Burman, & Paul, 2010) qui pourraient renseigner sur les caractéristiques de ces évènements. Ainsi, comme les facteurs environnementaux, l'état émotionnel propre aux individus est susceptible de moduler les comportements des animaux. Plus précisément, des individus avec un état émotionnel négatif auront un jugement plus négatif des stimuli ambigus, comparé à des individus avec un état émotionnel positif (Mendl *et al.*, 2009). Avec une appréciation du risque dépendant de l'état émotionnel, on peut alors supposer que la réponse comportementale pourrait aussi différer soit par son intensité (réponse faible ou forte), soit par sa nature (fuite, affrontement, …). Par exemple, lorsque rendus dans un état similaire à l'anxiété par impulsions électriques, les individus *G. fossarum* adoptent un comportement de protection plus marqué que des individus non choqués, ce qui se traduit, en microcosme, par une meilleure survie à la prédation (Perrot-Minnot *et al.*, 2017a). Dès lors que les individus choqués sont traités au préalable avec une molécule anxiolytique ciblant la voie glutaminergique (LY354740), ils se comportent tels que des individus non choqués. Ceci suggère l'existence de tout un système complexe de régulation de l'état d'anxiété y compris chez les invertébrés, qui modulerait certains comportements. Cette implication de l'état émotionnel dans les réponses comportementales au risque sera abordée dans la troisième partie, qui sera dans un premier temps introduite par un état des lieux sur les états émotionnels chez les invertébrés.

PARTIE 3 –

ETATS EMOTIONNELS MODULATEURS DU COMPORTEMENT ANTIPREDATEUR ?

Introduction

Bien que la simple présence d'émotions chez des animaux, autres que l'homme, ait longtemps débattue au sein de la communauté scientifique (Panksepp, 2011), leur étude a récemment et ouvertement suscité un grand intérêt des chercheurs en comportement animal (Paul & Mendl, 2018). Plusieurs raisons peuvent expliquer une telle évolution. Premièrement, un nouveau regard s'est posé sur l'animal non-humain, associé à la notion de bien-être intimement liée au fait que les animaux vivent des sensations et expriment des émotions (Mendl, Paul, & Chittka, 2011). Deuxièmement et sans doute le plus important, des mécanismes et structures physiologiques à la base de procédés cognitifs tels que les émotions ou la mémoire, longtemps étudiés chez l'homme et quelques groupes de mammifères "modèles", sont retrouvés chez d'autres animaux y compris invertébrés (Panksepp, 2011 ; Arbilly & Lotem, 2017).

L'émotion a longtemps été vue comme un état se définissant et se mesurant grâce à sa composante subjective (liée à la conscience, voir Boite.1) chez l'homme, à travers un retour (verbalisation) caractérisant cet état et nécessitant une conscience de ce dernier (Clore & Ortony, 2000). Cependant, nous ne disposons d'aucune connaissance, aucune méthode qui nous permettrait de démontrer l'existence d'une telle composante chez les animaux non-humains. Même s'il n'est pas possible d'étudier les émotions d'une telle façon chez les animaux non-humains, plusieurs travaux ont montré que les émotions ne se conceptualisent pas seulement par cette composante subjective. Par exemple, la douleur chez l'homme est une interprétation émotionnelle d'une perception déplaisante associée à des dommages (Sneddon *et al.*, 2014). Chez l'animal non-humain, la douleur fait référence à une expérience sensorielle aversive causée par une blessure et qui va induire une réaction de l'animal (Sneddon *et al.*, 2014) (Boite.1). On suppose alors chez un animal endolori un rapide apprentissage d'évitement du stimulus causant cette expérience à travers la mise en place de comportements de protection.

De ces deux définitions de la douleur ressortent des critères communs mis en avant par Elwood (2011, 2012) et repris par Sneddon *et al.* (2014**)**. Premièrement, l'animal doit absolument présenter des nocicepteurs, récepteurs sensoriels de la douleur, pour sa perception. Deuxièmement, il doit avoir un système nerveux central permettant les procédés cognitifs d'intégration. Troisièmement, ces capacités cognitives ne doivent être trop limitées : elles doivent permettre analyse, mémoire, apprentissage et prise de décision comme chez les vertébrés. Enfin, l'administration d'analgésiques est sensée diminuer les réponses au stimulus aversif. Bien que l'on ne parle pas de retour émotionnel chez l'animal non-humain, la partie cognitive reste commune, car on a déjà pu démontrer chez ces animaux leur habilité à détecter un stimulus physique négatif et d'y répondre en l'évitant Par exemple, après avoir reçu des impulsions électriques, les bernard-l'hermite *Pagurus bernhardus* quittent leur coquille, indispensable à la survie, contrairement aux individus qui n'ont pas reçu de choc, ce qui démontre de la perception du stimulus aversif (Elwood & Appel, 2009). Suite à la mise à disposition d'autres coquilles, les individus choqués ont tendance à approcher et à entrer rapidement dans la nouvelle coquille en investiguant bien moins la coquille que les individus non choqués, ce qui va dans le sens d'une plus grande motivation à changer de coquille.

Plus objectivement, l'émotion peut se définir comme une réponse à une situation ou un stimulus, intégrant des composantes cognitives (perception et intégration de l'information), physiologiques et comportementales (réponse via une action) (Clore & Ortony, 2000 ; Anderson & Adolphs, 2014) (Boite.1). On l'appelle dans ce cas « affect » (Paul, Harding, & Mendl, 2005). Ainsi, c'est uniquement sur la partie cognitive faisant appel à la perception, l'intégration et la réponse que nous discuterons des émotions puisque la conscience de celles-ci n'a jamais été démontrée chez les animaux non-humains.

Boîte.1

Anxiété : Etat émotionnel qualifié d'« humeur » négative qui se traduit par un état d'appréhension constant de l'environnement et qui ne nécessite pas obligatoirement un stimulus pour être exprimé.

Composante objective d'un état émotionnel : composante n'intégrant pas la conscience de l'intervention de celle-ci dans l'état émotionnel et qui ne peut être soumis à l'interprétation de l'individu.

Composante subjective d'un état émotionnel : composante liée à la conscience de l'individu dans le ressenti de l'état émotionnel lui permettant de faire un retour sur ce dernier quant à son interprétation.

Douleur : Expérience sensorielle aversive causée par un dommage ou dégât physique, et qui va induire une réaction d'évitement de l'animal.

Emotion : Etat émotionnel discret (à court-terme) et de forte intensité.

Etat émotionnel : Réponse à une situation ou un stimulus intégrant des composantes cognitive, physiologique et comportementale. L'émotion se visualise dans un espace à deux dimensions que constituent la valence (positive / négative) et l'intensité (faible / fort).

Humeur : Etat émotionnel qui se prolonge dans le temps (à long-terme) et dont l'intensité est amoindrie.

Peur : Etat émotionnel qualifié d'émotion discrète négative dont la réponse est immédiate (fuite ou affrontement) et qui fait suite à l'exposition à un stress identifié par l'individu.

Réponse au stress : Tout changement de type physiologique et / ou comportemental permettant à un individu de faire face à un stress.

Stress : Stimulus aigu auquel un individu est exposé et qui va provoquer chez ce dernier une réponse.

L'émotion, état à caractère adaptatif (Nettle & Bateson, 2012 ; Anderson & Adolphs, 2014), est ainsi supposée jouer un rôle crucial dans la survie des organismes, en leur permettant de faire face à certaines situations critiques dans leur environnement (Nesse & Ellsworth, 2009 ; Nettle & Bateson, 2012). En effet les émotions auraient pour rôle la mise en place de réponses comportementales appropriées face à un stimulus particulier et dont la conséquence du comportement adopté peut être négative (« punition ») ou positive (« récompense ») (Martin & Delgado, 2011). Par exemple, la peur représente une émotion ressentie en présence d'un danger, potentiellement de mort, et qui va s'exprimer par une réponse, souvent de type fuite, permettant d'augmenter les chances de survie. Les états émotionnels se visualisent objectivement dans un espace à deux dimensions, par leur valence (positive ou négative) et leur intensité (faible à forte) (Russell, 2003 ; Paul *et al.*, 2005 ; Mendl *et al.*, 2010 ; Bliss-Moreau, 2017) (Fig. 3). La peur est ainsi caractérisée par une valence négative et une intensité relativement forte (Mendl *et al.*, 2010) (Fig.4). Pour aller encore plus loin, il est possible de distinguer différents types d'états émotionnels en fonction de leur durée d'expression et de leur lien éventuel avec un stimulus externe. On parlera ainsi d'émotion discrète (peur) pour un état de plus forte intensité à court-terme, en lien direct avec un stimulus identifié. Lorsque l'état émotionnel se prolonge dans le temps avec une intensité amoindrie, indépendamment d'un stimulus déclencheur, on le qualifiera alors d'« humeur » (Russell, 2003 ; Mendl *et al.*, 2010 ; Nettle & Bateson, 2012). Dans la littérature, la peur et l'anxiété sont les deux exemples phares de l'émotion discrète et de l'état émotionnel à long-terme ("humeur") respectivement, à valence négative (Boite.1). D'après Mendl *et al.* (2010**)** et Nettle & Bateson (2012), les états émotionnels qualifiés d'humeurs résulteraient de la répétition d'expression d'émotions discrètes. Par exemple, l'animal qui fait l'expérience répétée d'épisodes de rencontre de menaces est susceptible de développer, à plus long-terme, un état d'anxiété (Nettle & Bateson, 2012). Cependant, il n'est

pas exclu que les « humeurs » puissent affecter en retour les réactions émotionnelles discrètes et donc les comportements (Reefmann *et al.*, 2012).

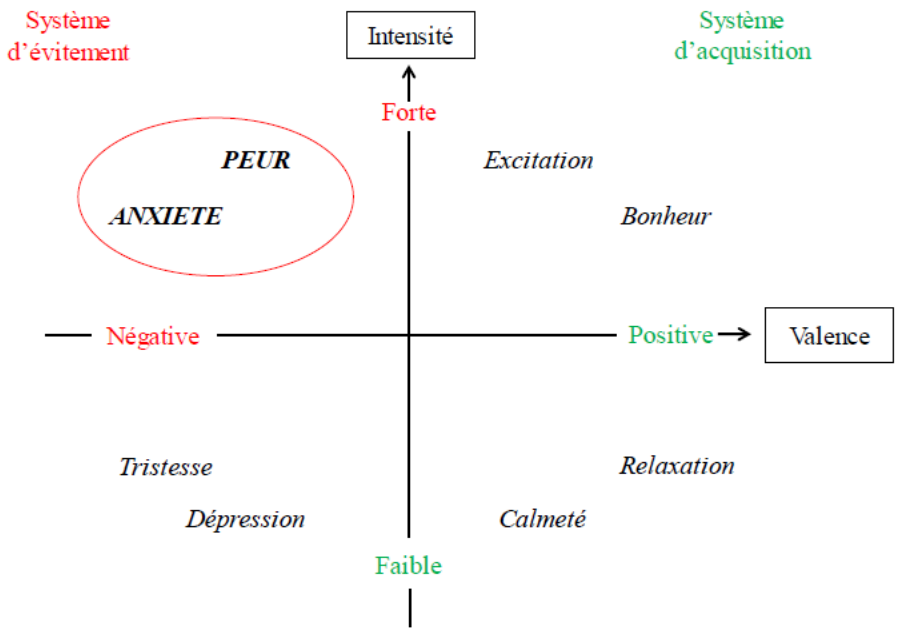


Fig.4 Les états émotionnels représentés dans un espace à deux dimensions, d'après Mendl et al. (2010). Les états émotionnels positifs et négatifs sont représentés dans les espaces à valence positive et négative, respectivement. Les états émotionnels négatifs et positifs de haute intensité se traduiront par la mise en place de comportements de type évitement (punition) et facilitation de l'acquisition (récompense), respectivement, à travers un processus cognitif menant à une association entre stimulus inconditionnel (répulsif ou attractif) et conditionnel.

Plusieurs travaux ont montré l'influence des états émotionnels sur les procédés cognitifs des animaux (humains et non-humains) (Paul *et al.*, 2005) et donc sur leur comportement (Vögeli *et al.*, 2015). Les tests dits de biais cognitif représentent une très bonne façon objective de mettre en évidence un état émotionnel influençant la cognition chez un individu, et même de qualifier cet état, positif ou négatif. Le principe est simple : un individu optimiste aura plutôt tendance à juger un stimulus ambigu, auquel il n'a jamais été confronté, de façon plus positive qu'un individu pessimiste (Roelofs *et al.*, 2016). Dans le prochain chapitre, nous aborderons brièvement la mise en évidence d'états émotionnels chez les animaux non-humains jusqu'à certains invertébrés, notamment à travers les tests de biais cognitif. Ce prochain chapitre ne sera pas expérimental mais basé sur la littérature. Dans un second temps, nous verrons quelle

influence les états émotionnels peuvent avoir sur le comportement chez ces mêmes organismes, et quelles possibles voies d'action pourraient être sollicitées. Pour terminer et après avoir énoncé les contraintes méthodologiques liées à l'étude des émotions chez les invertébrés, le dernier chapitre sera consacré à la différenciation peur / anxiété via une approche expérimentale.

Chapitre 4 –

Mise en évidence d'états émotionnels chez les invertébrés et leur rôle sur le comportement

L'émotion chez les animaux non-humains est étudiée via l'expression des composantes physiologique et comportementale (Mendl *et al.*, 2010). Plus précisément, les biais de cognition représentent un très bon indicateur de l'état émotionnel animal (Mendl *et al.*, 2009, 2010 ; Bateson & Nettle, 2015 ; Roelofs *et al.*, 2016), procédure généralisée déjà démontrée chez l'homme et d'autres animaux non-humains (Eysenck *et al.*, 1991 ; Bateson *et al.*, 2011 ; Mendl *et al.*, 2011 ; Deakin *et al.*, 2018). Un biais de jugement est défini comme une réaction à un stimulus ambigu, cette réaction reflétant l'interprétation qu'en fait l'individu vis-à-vis de la conséquence de cette réaction (Bateson & Nettle, 2015). Par exemple, des rats présentant un état de type anxiété sont plus susceptibles d'évaluer un stimulus ambigu comme étant source d'un retour négatif (‘*punishment’*) (Enkel *et al.*, 2010), ce qui caractérise un biais cognitif pessimiste. L'existence de biais cognitif pessimiste reflétant un état émotionnel anxieux a également été mise en évidence chez les oiseaux (Salmeto *et al.*, 2011).

Si la majorité des travaux sur les états émotionnels des animaux non-humains concerne les mammifères (Perry & Baciadonna, 2017), l'idée que les invertébrés puissent montrer des états émotionnels primitifs est largement en cours d'acceptation (Anderson & Adolphs, 2014 ; Perry & Baciadonna, 2017). Les premiers travaux réalisés pour montrer l'existence de biais de cognition chez les invertébrés ont été menés sur les abeilles. A travers l'induction d'un stress simulant une attaque de prédation, Bateson *et al.* (2011) ont montré que les abeilles *Apis mellifera* stressées étaient plus susceptibles d'interpréter négativement un stimulus ambigu, ce qui serait le reflet d'un état émotionnel à valence négative. Les abeilles stressées présentaient également une concentration en sérotonine dans l'hémolymphe réduite (Bateson *et al.,* 2011). Ainsi, les invertébrés et les vertébrés partageraient plus que ce que nous pensions en termes d'émotions. Plus particulièrement, les gènes et voies d'action liés à certains états émotionnels pourraient avoir été conservés. Les gènes impliqués dans la régulation de l'activité

sérotonergique, modulatrice des états d'anxiété et de dépression (Neumeister, 2003) seraient partagés entre les rongeurs et les drosophiles. La manipulation des gènes impliqués dans la signalisation sérotonergique chez la drosophile *Drosophila melanogaster* présente en effet des résultats concordants avec ceux obtenus chez les rongeurs, en influençant des comportements de défense (Mohammad *et al.*, 2016). La dopamine, reconnue chez la souris pour être impliquée dans la régulation de l'apprentissage des évènements négatifs et positifs à travers des systèmes de punition et récompense (Matsumoto *et al.*, 2016), pourrait alors aussi avoir son rôle à jouer chez les invertébrés. D'après Weiss *et al.* (2015), la dopamine serait impliquée dans la mise en place des défenses morphologiques, et ainsi dans la plasticité phénotypique, chez la daphnie *Daphnia pulex*.

Depuis peu de temps, les travaux se sont élargis à d'autres invertébrés pour s'intéresser aux crustacés. Fossat *et al.* (2014) a mis en évidence l'existence d'un état d'anxiété chez l'écrevisse *P.clarkii* qui peut se moduler avec l'injection de sérotonine, neuromodulateur impliqué dans des comportements liés au stress et à l'anxiété chez les vertébrés (Ellison & Bresler, 1974 ; Heisler *et al.*, 1998) voire la dépression chez l'homme (Owens & Nemeroff, 1994 ; Neumeister, 2003). L'état émotionnel de type anxiété, partagé à la fois par les vertébrés et invertébrés, pourrait avoir quelques mécanismes sous-jacents en commun. Plus récemment, Perrot-Minnot *et al.* (2017) ont montré un comportement de type anxiété chez l'amphipode *G. fossarum.* Plus surprenant encore, l'injection topique de sérotonine chez une espèce proche *G. pulex* a eu pour effet de mimer partiellement l'infestation par le parasite *Pomphorhynchus laevis*, en abaissant le niveau de certains comportements liés à la taxie (Perrot-Minnot *et al.*, 2014), ce qui pourrait être perçu comme une diminution d'un état d'anxiété général. Cependant dans cette même étude, l'utilisation du refuge ne semble pas affectée par l'injection topique de sérotonine, alors que l'on suppose une importante contribution des refuges dans les comportements de

protection vis-à-vis du risque de prédation (Dianne *et al.*, 2011 ; Perrot-Minnot *et al.*, 2017). Ceci soulève donc une limite à l'administration topique de sérotonine. Reproduire un changement dans l'utilisation du refuge qui reflèterait alors davantage un état émotionnel de plus longue durée nécessiterait sans doute un stress chronique et la manipulation expérimentale chronique du système sérotonergique.

Transition
Peur – anxiété : différenciation comportementale

Les réponses comportementales des individus face à un risque de prédation ("*fight or flight*") sont souvent vues comme l'expression de la peur des individus. Cependant, d'autres états émotionnels, comme l'anxiété, peuvent venir moduler le comportement des individus. Peur et anxiété, termes souvent confondus, sont des états émotionnels relativement distincts. Peur et anxiété se distinguent sur deux plans, la nature de la menace (connue ou non) et la durée d'expression de l'état (courte ou prolongée) (Grillon, 2008). La peur représente un état émotionnel ressenti par un animal après la perception d'une menace connue et identifiée (prédateur). S'en suit alors une réponse comportementale de type fuite, cache, ou agression. L'anxiété est plutôt perçue comme un état d'appréhension de l'environnement prolongé, sans nécessité de la présence d'une menace ou de son identification (stimulus inconnu). Elle se traduit davantage par une vigilance accrue. Chez l'homme principalement et certains mammifères tels que les rongeurs, la différenciation peur / anxiété se perçoit sur les plans comportemental et neurologique (Grillon, 2008). La peur se traduit par un comportement réponse de type réflexe donc phasique, souvent la fuite ou l'affrontement tandis que l'anxiété implique un comportement persistant d'évitement, de surveillance et d'évaluation (Grillon, 2008).

Bien que des études se soient récemment intéressées à l'expression de l'anxiété chez les invertébrés, le (dé)couplage sur le plan comportemental peur – anxiété n'a toujours pas été investigué. Est-ce que la peur et l'anxiété s'expriment différemment au niveau comportemental chez les invertébrés ? Mettre en évidence cette éventuelle distinction entre peur et anxiété chez les invertébrés s'avère être un réel défi. En effet, chez les vertébrés il semble plus aisé de distinguer différents comportements en lien avec un stress car la méthodologie et les protocoles sont plus développés et le panel de comportements est plus étendu (pour les rongeurs, voir

Sestakova *et al.*, 2013 ; Schöner *et al.*, 2017). Mais comment démontrer une appréhension ou une vigilance accrue chez les amphipodes qui sont de base très peu « à découvert » ? Pour tenter de distinguer peur et anxiété, nous identifions deux approches. Premièrement, il s'agit de manipuler de façon pharmacologique un des deux états, et d'en estimer la conséquence sur le deuxième. L'anxiété, qui pourrait être définie comme la « peur de la peur », représenterait alors une cible privilégiée de cette manipulation pharmacologique. Des individus traités par anxiolytiques exprimeraient-ils des comportements de peur atténués en comparaison avec des individus non traités ? Pour cela, plusieurs molécules, citées plus tôt, sont connues pour leurs effets anxiolytiques chez les vertébrés et chez certains invertébrés. Deuxièmement, nous pouvons utiliser une approche corrélationnelle en exploitant la variabilité interindividuelle dans l'expression de ces deux états. Le principe est de mettre en relation, au niveau individuel, l'expression d'un état de peur et d'un état de type anxiété au travers d'un même comportement, afin de tester l'hypothèse de la covariation entre comportement de peur et comportement anxieux. Dans le cas où ces comportements ne seraient pas corrélés, peur et anxiété pourraient avoir évolué sous la forme de deux stratégies alternatives de réponse au risque. Cette deuxième approche sera présentée dans le chapitre suivant.

L'approche corrélationnelle semble l'approche la plus abordable compte tenu du modèle biologique. La difficulté de travailler avec les amphipodes sur les émotions réside principalement dans le fait que la méthodologie et les protocoles ne sont pas développés. L'étude des biais cognitifs nécessite en amont un processus d'apprentissage / entraînement avant de faire les tests de choix. Ces tests d'apprentissage associatifs sont très importants puisqu'ils démontrent une certaine sensibilisation et mémoire. Cependant chez les amphipodes, il n'existe actuellement aucune méthodologie / protocole qui permettrait de s'engager dans des

tests d’apprentissage. Par contre, les comportements défensifs / de protection en réponse au stress, sont mieux connus.

Chapitre 5 –

Anxiety and fear in a freshwater amphipod: two overlapping emotional states?

Etude en laboratoire

RESUME

Anxiété et peur sont souvent confondues. Elles modulent les comportements défensifs des individus mais représentent deux états émotionnels distincts. La peur est une émotion de courte durée, dépendante du contexte dans lequel elle est exprimée tandis que l'anxiété représente un état de longue durée qui ne nécessite pas la présence ou l'identification d'une menace pour être exprimée. L'anxiété est alors exprimée sous forme d'une appréhension prolongée de l'environnement. Bien que ces deux états émotionnels aient été mis en évidence chez les animaux non-humains et récemment chez quelques invertébrés, nous ne savons pas si les mécanismes neurophysiologiques les modulant sont partagés. Dans cette étude, nous abordons cette question en évaluant la corrélation intra-individuelle entre anxiété (induite par impulsions électriques) et peur (induite par odeur de prédateur) chez l'amphipode *G. fossarum*. Nous avons estimé le niveau d'expression de l'anxiété et de la peur à travers un comportement défensif, l'utilisation d'un refuge. Dans un premier temps, nous avons mis en évidence la répétabilité intra-individuelle des réponses à l'anxiété et à la peur, sur un pas de temps de 24 heures. Puis nous avons testé la corrélation intra-individuelle entre ces deux états. Le niveau d'anxiété induit par impulsions électriques était positivement corrélé au niveau de peur exprimé 24 heures plus tôt, mais la réciproque n'était pas significative. Nous discutons des aspects proximaux (mécanismes) et de la signification adaptative de la répétabilité de ces deux états émotionnels, et de l'asymétrie de leur association.

Anxiety and fear in a freshwater amphipod: two overlapping emotional states?

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ABSTRACT

Anxiety and fear are often confused. Both modulates defensive behaviours, but they represent distinct emotional states. Fear is a context-dependent phasic emotion while anxiety is a long-lasting emotional state that does not require an identified threat to be expressed. Anxiety is thereby expressed through a sustained apprehension of the environment. Although both emotional states have been evidenced in many animals including invertebrates, it has not yet been established whether their underlying neurophysiological and behavioural mechanisms overlap. Here, we addressed this question by assessing the intraindividual correlation between anxiety-like state (induced by electric shocks) and fear reaction (induced by predator odour) in the freshwater amphipod *Gammarus fossarum*. We estimated the level of anxiety and fear reaction by recording refuge use. We first evidenced that anxiety-like state and fear responses were repeatable within individuals, on a 24 hours interval. We then tested whether they were correlated at the individual level. The level of anxiety induced by electric shocks was positively correlated to the level of fear expressed the day before, but the reciprocal was not significant. We discuss the proximate and ultimate mechanisms possibly involved in this asymmetrical association between these two emotional states.

INTRODUCTION

Anxiety and fear represent two defensive emotional states that are commonly exhibited by animals (Lang, Davis, & Öhman, 2000). They are adaptive responses helping individuals dealing with conflicts (Ekman, 1999) and expected to increase the probability to survive to potential or actual risk-prone contexts (Frijda, 1986; Steimer, 2002; Blanchard *et al.*, 2008; Giske *et al.*, 2013). For instance, when facing cat in an open area, the first explicit behavioural response of rats is to immediately flee in a proximate tunnel (Blanchard & Blanchard, 1989). Besides, they tend to display an inhibition of non-defensive behaviours during several hours (up to 24 hours) following exposure to this predation threat, such as eating, drinking or grooming, suggesting a long-lasting distress (Blanchard & Blanchard, 1989).

Although the distinction between anxiety and fear is still actively discussed, some characteristic features have been proposed. Fear is an emotional response to an imminent threat, which has been perceived and identified by an individual and which will generate an immediate reaction, such as 'fight or flight' (Lang *et al.*, 2000; Blanchard *et al.*, 2008; Grillon, 2008; Perry & Baciadonna, 2017). Anxiety is a more complex emotional state which does not involve exposure to a clearly defined threat (Blanchard *et al.*, 2008). Anxiety is conceptualized as an anticipatory state (Steimer, 2011) associated with avoidance (Grillon, 2008), a state of sustained apprehension of the environment with high vigilance level (Grillon, 2008). Contrary to fear, anxiety is a context-independent emotional state that does not involve specific stimuli (Davis, 1998). Regardless of the context and specificity of the threat, anxiety also stands out from fear by the duration of response over time (Davis, 1998). Indeed, fear represents a short-term emotional state (immediate response) whereas anxiety is a long-term one (Lang *et al.*, 2000), called 'mood' (Mendl *et al.*, 2010). For instance, Cohen *et al.* (2004) reported that, after a single exposure to a cat, rats exhibit anxiety-like behaviours for 30 days even in the absence of the cat.

Anxiety and fear are well documented in vertebrates and especially in humans. The expression of fear in birds and mammals is usually quantified through escape-related behaviours, such as the flight initiation distance (FID) (Stankowich & Blumstein, 2005). This metric refers to the minimal distance between a prey and a predator that will induce the flight of the prey (Ydenberg & Dill, 1986). The use of FID to study fear responses is supported by the fact that FID is easily quantifiable and is also repeatable. Fear of humans, measured through FID, is highly repeatable in both rural and urban owls *Athene cunicularia* (Carrete & Tella, 2013). Regardless of the use of FID, fear behaviours in general, seem to be repeatable. For instance, laying hens displayed consistent fear levels over three days (Jones, 1988). This is not surprising given that defensive behaviours, that appear to be the main target of fear to be expressed, are usually repeatable. This is reported for aggressive defensive behaviours in yellow-bellied marmots *Marmota flaviventris* (Blumstein, Petelle, & Wey, 2013) or nest defense in Eastern bluebirds *Sialia sialis* (Burtka & Grindstaff, 2013). Fear is also reported in invertebrates where fear behaviours seem to be repeatable. For instance, Asian honeybees *apis cerana* reduce their foraging activity when a hornet is in the patch (Tan *et al.*, 2013). Amphipods *Gammarus pulex* increase their use of a refuge when exposed to predator cues (Perrot-Minnot *et al.*, 2007). In addition, *Gammarus fossarum* that increase their use of a refuge under predation risk also show consistency in this behaviour (David *et al.*, 2014).

Anxiety is largely studied in rodents (Steimer, 2002) where anxiety-like behaviours seem to be reduced after the use of anxiolytics. For instance, rodents spend significantly more time in open areas of elevated plus-maze tests when treated with an opioid receptor agonist (Saitoh *et al.*, 2004) or diazepam (Kapus *et al.*, 2008). However, the evidence for anxiety-like state in invertebrates is still scarce, since only few species have been shown to express anxiety-

like states or behaviours (Perry & Baciadonna, 2017). For instance, unstressed crayfish *Procambarus clarkii* injected with serotonin exhibited a strong avoidance of aversive light similar to individuals that received negative nociceptive stimuli (Fossat *et al.*, 2014). Also, Mohammad *et al.* (2016) reported that the avoidance behaviour of fruit flies *Drosophila* was driven by the same neurogenetic pathways as the ones modulating anxiety in mammals. Modulation of anxiety-related neurotransmitters known in mammals seems to produce similar patterns in invertebrates. More recently, the amphipod *Gammarus fossarum* have been shown to display anxiety-like behaviours when stressed with electric shocks (Perrot-Minnot, Banchetry, & Cézilly, 2017).

To our knowledge, despite several examples of altered defensive behaviours by predator cues or unknown stressors, no study has addressed the question of the relationship between anxiety and fear in invertebrates. Yet, these two emotional states could correspond to two alternative strategies to cope with threat, either as a flexible state responsive to current and identified threat, or as an elevated basal defensive state independent of the context. Alternatively, fear and anxiety may be the two sides of the same coin, both of which revealing interindividual differences in threat sensitivity or responsiveness. From an adaptive point of view, the relationship between these two emotional states at the individual level may thus broaden our understanding of individual strategies to cope with threats.

In the present study, our aim was to investigate whether anxiety and fear could be two overlapping emotional states in the freshwater amphipod *G. pulex* (Amphipoda: Crustacea). Gammarids are photophobic benthic invertebrates exhibiting threat-sensitive antipredatory behaviours, including negative phototaxis and refuge use, which are repeatable across context (David, Salignon, & Perrot-Minnot, 2014). When stressed with both familiar (predator scent)

and unknown (electric shocks) stressors, they tend to increase their use of refuge (Perrot-Minnot, Banchetry, & Cézilly, 2017). Here, we investigated the link between anxiety-like and fear behaviours, induced by electric shocks and predator scent respectively, in *G. pulex* individuals. This link was assessed through recording the magnitude and repeatability of anxiety-like state and fear, and testing for a correlation between these two emotional states, at the individual level. We first estimated the repeatability of refuge use after exposure to one of the two stressors, electric shocks and predator scent, at a 24 h interval. Refuge use is an altered defensive behaviour under anxiety-like state, as recently evidenced in gammarids (Perrot-Minnot, Banchetry, & Cézilly, 2017). We expect that individuals that strongly respond to a stressor the first day, regardless of its nature, should respond in the same way 24 h later. Then, we assessed the intraindividual correlation between anxiety and fear at 24 h interval, controlling for order effect of exposure to anxiety- and fear-eliciting stimuli. If fear and anxiety are the expression of an individual sensitivity or responsiveness to threat, either actual (fear) or by anticipation (anxiety), then we predict that individuals that are more anxious (stressed with electric shocks) should display stronger fear behaviour (stressed with predator scent) and reciprocally. Alternatively, if fear and anxiety are two alternative strategies to cope with threat, a negative correlation between these two states at the individual level should be found.

MATERIALS AND METHODS

Biological model and maintenance

G. pulex individuals were collected in the river Val Suzon, Bourgogne-Franche-Comté, France (47°24’13.94” N, 4°52’59.241” E) in February, 2019. To control for a potential sex-effect, only males were used for the experiments. Individuals were maintained in a large tank filled with oxygenated water taken from the river and fed with elm leaves. They were placed in a room where both photoperiod (12:12 light regime) and temperature (15°C) were controlled.

Twenty-four trouts, *Salmo trutta*, were collected in February 2019 (Cordier-Gand fish farm, Corgoloin, France).

Experimental induction of anxiety and fear

To induce anxiety-like state, gammarids were exposed to electric shocks (thereafter ES) corresponding to short pulses of 10 V direct current. Electric shocks are nociceptive stressors, easy to produce and standardize by controlling pulses frequencies and voltage. Electric shocks were delivered by a portable device that surrounded individuals in the 'refuge box', thus preventing additional stress due to handling of individuals (Fig.S1, portable device). Gammarids were individually exposed to 3 shocks of 2 sec. at regular intervals during 10 min., after an acclimatization time of 5 min. following the introduction of the electric device. Control individuals experienced the same conditions but were not exposed to electric shocks.

To induce fear, gammarids were exposed to predator scent (thereafter PS) produced by mixing guts and bile pouches from trouts. Trouts were euthanatized by cutting off the head, and immediately dissected to collect gut and bile pouch. After mixing in water (approx. 1:20 vol.), the homogenate was coarsely filtered, and stored at -20°C until use. Upon behavioural assay, one mL of trout mixture was added to the 200 mL of water in the refuge box where individuals were tested (see below). The final concentration of trout mixture used as predator scent was therefore 1/2000. For control individuals, 1 mL of neutral water was introduced instead of 1 mL of PS.

Behavioural assays

Refuge use was recorded by time-sampling, following Perrot-Minnot *et al.* (2014). Refuge use was assessed in a 10.5 * 16 cm rectangular and opaque box with half a terracotta saucer placed at one side, filled with 200 mL of mix of water from the river and filtered water, and placed under a light of 0.7 K lux. The position of a single individual (0 = inside or 1 = outside the refuge) was recorded every 30 sec. for 10 min, after an acclimatization time of 5 min. The score of refuge use ranged from 0 (always under) to 20 (always outside). Behavioural assay was run in the same room as for the maintenance of gammarids.

To measure the response to anxiety- and fear- eliciting stimuli (ES or PS), refuge use of each individual was scored twice in a row, before and after exposure to ES or PS. Between the two measures, individuals were stressed in the refuge box either by introducing the electric device or by adding 1 mL of predator scent (see above). The magnitude of the response to stressor was quantified using the formula: [(score after – score before) + 20) / 40], thereafter referred to as "Response index, RI". This response index therefore ranged from 0 (decrease in refuge use after exposure to stimuli) to 1 (increase in refuge use after exposure to stimuli) and was correcting for the initial tendency of individuals to use refuge in absence of stimuli. A response index of 0.5 was associated to an absence of response after exposure.

Repeatability and correlation of anxiety-like and fear behaviours at the intraindividual level: repeated measure of response to stimuli

The repeatability of the response to PS or ES, and the correlation between responses to PS and ES, were assessed at a 24-hour interval. Two separate set of males were used for repeatability

and correlation measurements. Individuals were isolated 24h prior to the first day of experiment in 50 mL glass vial with a 1 cm² piece of elm leaf and a small rock, and then isolated again during the 24-hour interval between the two repeated measures.

To control for order effect in the correlation between anxiety-like and fear behaviours, we exposed one half of gammarids to ES on the first day and PS on the next day, and the other half in the reverse order.

Analyses

The effect of electric shocks and predator scent at the intraindividual level was represented using effect size (Fritz, Morris, & Richler, 2012). We estimated cliff delta effect sizes and 95 % confidence intervals, using measures of refuge use before and after the exposure to both stimuli. We compared the scores of refuge use before and after exposure using Wilcoxon paired tests. We also tested for the remanence of the effect of stimuli by comparing the scores of refuge use before exposure between day 1 and day 2 using Wilcoxon paired tests. We compared the response indexes between day 1 and day 2 using Wilcoxon paired tests.

Repeatability of refuge use at 24 h interval was estimated using the intraclass coefficient (ICC) and its 95 % confidence intervals, using scores as proportions (number of times in and out of the refuge) with the rpt function in rptR package (Stoffel, Nakagawa, & Schielzeth, 2017). We used the day of experiment as a fixed effect and the individual as a random one. Repeatability of response index at 24 h interval was estimated using non-parametric Spearman correlation coefficient.

The correlation between response index to electric shock and response index to predator scent was assessed at 24 h interval, using non-parametric Spearman correlation coefficient.

All analyses were conducted with RStudio software (v. 1.2.1335, (RStudio Team, 2016)).

RESULTS

Refuge use in response to exposure to anxiety- and fear- eliciting stimuli

Both electric shocks and predator scent had a significant effect on refuge use: individuals were more under refuge after exposure to stimuli (Wilcoxon paired tests on behavioural scores before and after exposure to stimuli: ES, day 1, $n = 45$, $V = 104.5$, $p < 0.01$, day 2, $n = 45$, $V = 104$, $p < 0.01$; PS, day 1, $n = 48$, $V = 39$, $p < 0.01$, day 2, $n = 48$, $V = 51$, $p < 0.01$) (Fig.1). However, control individuals of ES treatment were also more under the refuge after setting up the electric device, on the first day only ($n = 27$, $V = 19$, $p < 0.01$) (Fig.1).

Refuge use before exposure to stimuli on day 2 was higher than before exposure on day 1 for both types of stimulus (Wilcoxon paired tests on behavioural scores before exposure to stimuli between day 1 and day 2: ES, $n = 45$, $V = 636$, $p < 0.01$; PS, $n = 48$, $V = 612$, $p < 0.01$) (Fig.2). Actually, refuge use by gammarids on day 2 before exposure was still high and comparable to the one after exposure to ES 24 hours before (Fig. 2). This trend was also found for control individuals of ES treatment, experiencing the introduction of the portable device in the refuge box ($n = 27$, $V = 147$, $p < 0.01$) (Fig.2).

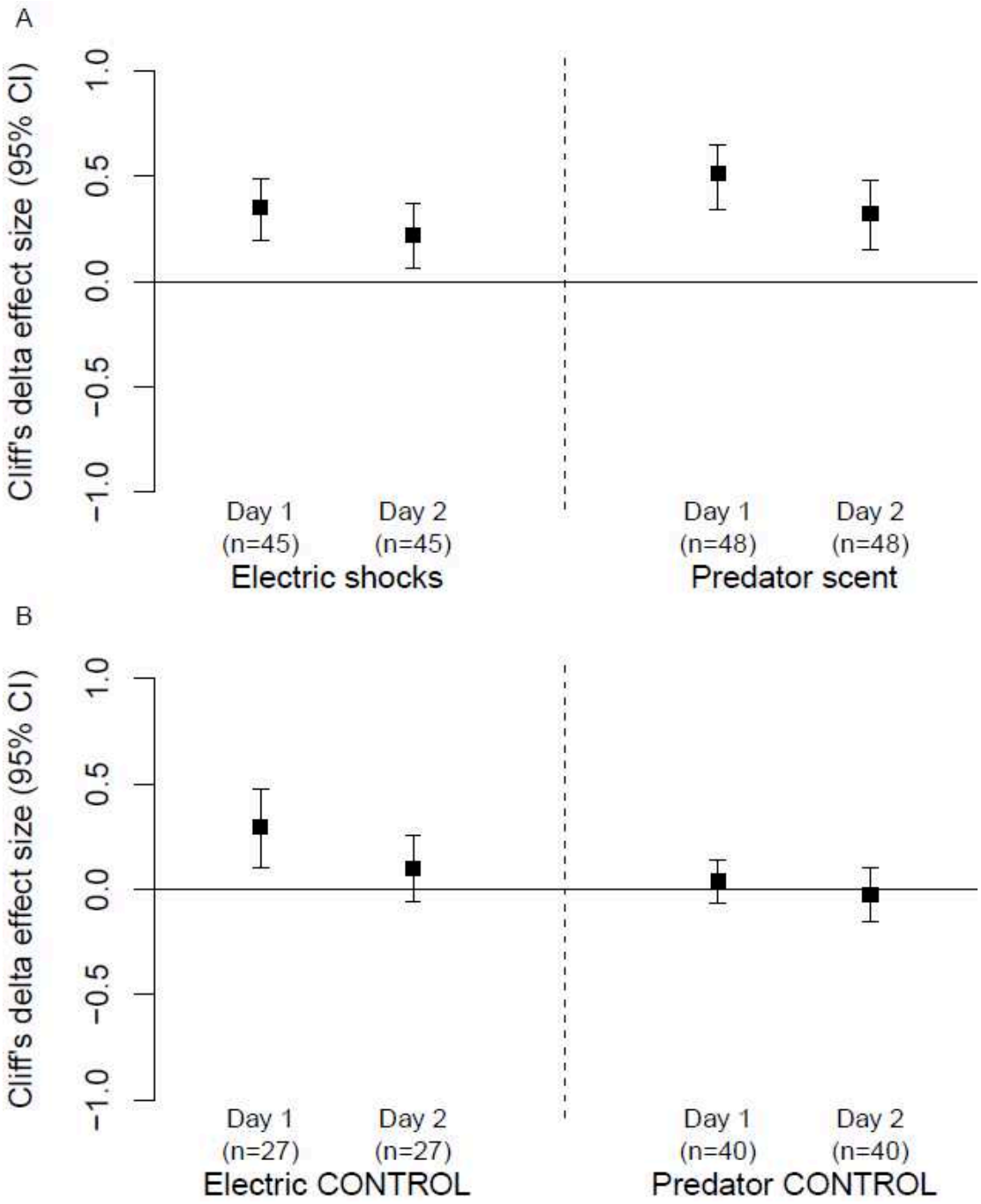

Fig.1. Cliff's delta effect sizes (with 95% CI) for changes in refuge use induced by A) anxiety- and fear- eliciting stimuli (electric shocks and predator scent, respectively) and B) their controls, with repeated measures on two consecutive days. The effect of stimuli was considered as non-significant when its 95% confidence interval crossed zero. Negligible, small, medium and large effects correspond to |cliff's delta| lower than 0.15, 0.33, 0.47 and higher than 0.47, respectively (Romano *et al.*, 2006). Sample sizes are given into brackets.

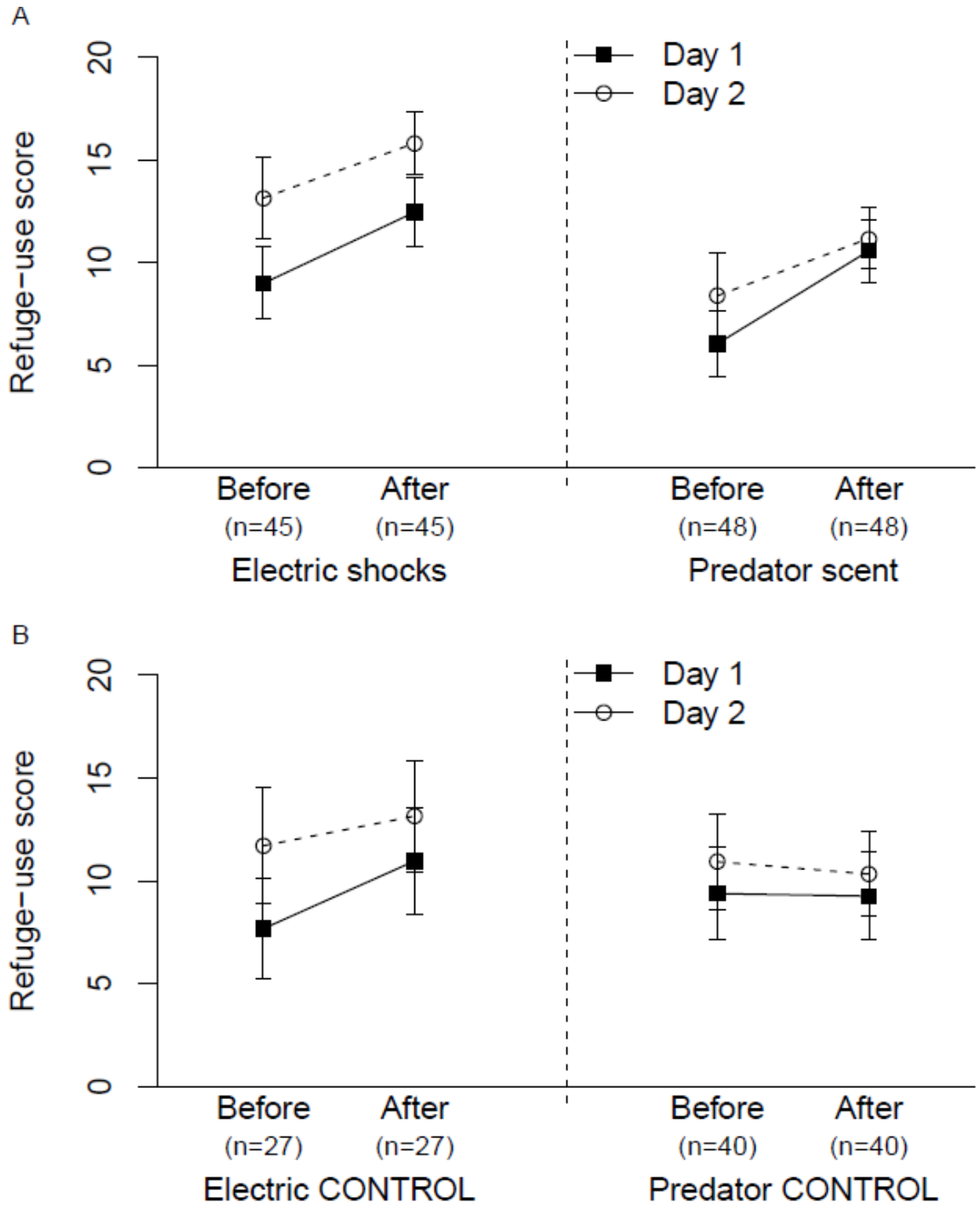

Fig.2. Mean scores of refuge use with 95 % confidence intervals according to A) the stimulus (electric shocks and predator scent) and B) their controls. Repeated measures at the intraindividual level were done within trial before and after exposure to stimulus, and on two consecutive day) of the experiment. The higher the score, the higher the refuge use. Bold squares linked with solid lines and empty circles linked with dashed lines represent the evolution of behavioural scores on day 1 and 2, respectively. The magnitude of response to both stimuli is given by the slope of the lines. Sample sizes are given into brackets.

Response to anxiety- and fear- eliciting stimuli

Responses to electric shocks at 24 hours interval were not significantly different, while responses to predator scent between day 1 and day 2 were almost significantly different (Wilcoxon paired tests on response index RI between day 1 and day 2: ES, $n = 45$, $V = 403$, $p = 0.64$; PS, $n = 48$, $V = 632$, $p = 0.06$) (Fig.2). The response to predator scent tended to be lower on day 2.

Repeatability

The repeatability of refuge use at a 24 h interval was significant for both anxiety- and fear-eliciting stimuli, before and after exposure (Table.1). Overall, individuals that were more under the refuge the first day were also more under the refuge 24 h later. Refuge use after exposure to fear-eliciting stimulus was less repeatable than refuge use after exposure to anxiety-eliciting stimulus, and less repeatable than refuge use by the same individuals before exposure to predatory cues (Table.1). The response (RI) to both stimuli was not repeatable at a 24 h interval (Table.1). Individuals that were the most responsive on the first day were not the most responsive 24 h later, despite the fact that their rank in refuge use (scores) was repeatable.

Table.1. Intra-class coefficients with their 95 % confidence intervals for repeatability at 24 hours interval of refuge-use before and after the induction of anxiety- and fear- eliciting stimuli and Spearman correlation coefficients for repeatability of response indexes associated with sample sizes (ES = electric shocks and PS = predator scent). Bold values represent significant measures of repeatability.

	Anxiety		Fear	
	ES treatment	ES control	PS treatment	PS control
Before stimulus	R = **0.39** [0.19 - 0.68] (n = 45)	R = **0.51** [0.25 - 0.99] (n = 27)	R = **0.57** [0.34 - 0.95] (n = 48)	R = **0.35** [0.19 - 0.68] (n = 40)
After stimulus	R = **0.36** [0.21 - 0.52] (n = 45)	R = **0.30** [0.11 - 0.60] (n = 27)	R = **0.18** [0.07 - 0.29] (n = 48)	R = **0.29** [0.14 - 0.51] (n = 40)
Response to stimulus	Rho = -0.04 p = 0.78 (n = 45)	-	Rho = 0.23 p = 0.12 (n = 48)	-

Intraindividual correlation between response to ES and response to PS

The response to electric shocks was positively correlated to the response to predator scent expressed the day before, but the reciprocal was not significant (Fig.3 and Table.2). Overall, individuals that were more responsive to predator scent the first day were also more responsive to electric shocks 24 h after.

Table.2. Spearman correlation coefficient and sample size for correlations between response indexes to electric shocks (ES) and predator scent (PS) behaviours controlling for order effect. Bold values represent significant correlations.

	ES (day1) - PS (day2)	PS (day1) - ES (day2)
Correlation	Rho = 0.11 p = 0.47 (n = 46)	Rho = 0.51 **p < 0.01** (n = 50)

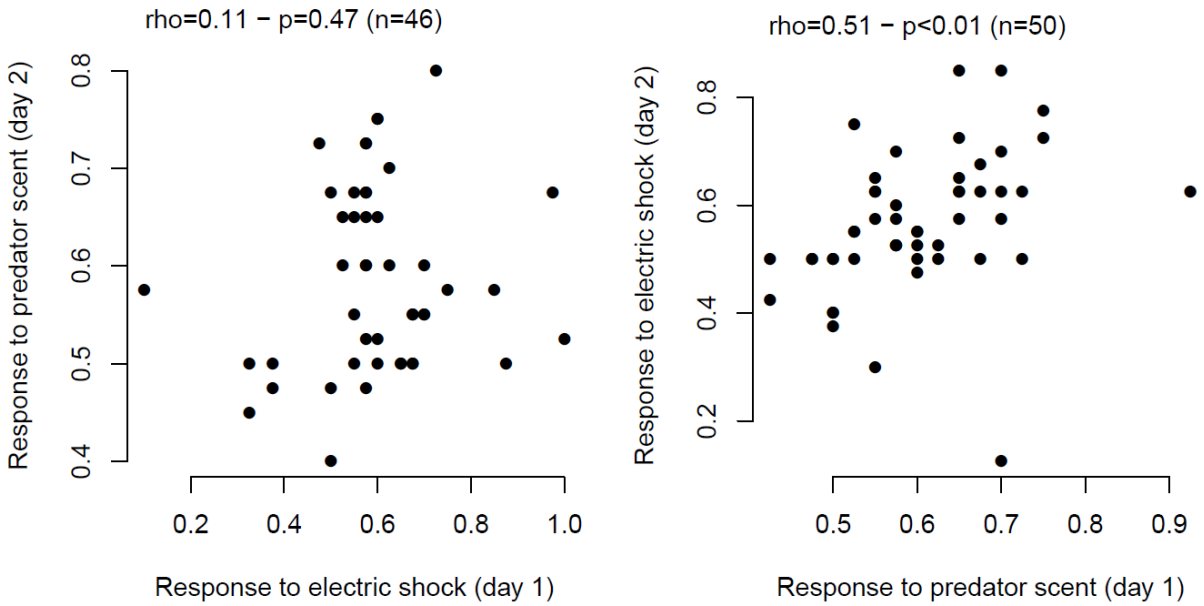

Fig.3. Correlation of (a) responses to predator scent (PS) expressed the second day plotted against responses to electric shocks (ES) expressed the first day and of (b) responses to ES expressed the second day plotted against responses to PS expressed the first day, with associated Spearman correlation coefficients and sample sizes, respectively.

DISCUSSION

We aimed at addressing the possible overlap between anxiety-like state and fear in the freshwater amphipod *G. pulex*. More specifically, we tested the hypothesis of a correlated behavioural response to anxiety- and fear-eliciting stimuli at the individual level.

We first demonstrated that exposure to two stimuli, one eliciting anxiety (electric shocks)- and one eliciting fear (predator scent) increased antipredatory behaviour in *G. pulex*. Overall, individuals used more the refuge after exposure to the stimulus than before, regardless of the nature of the stimulus. This has been previously reported in *G. fossarum* (Perrot-Minnot *et al.*, 2017). In addition, the effect of both stimuli on the first day was of the same order of magnitude as those reported by Perrot-Minnot *et al.* (2017). Interestingly, the effect of both stimuli was weaker on the second day. This suggests that gammarids could have 'habituated' to anxiety- and fear- eliciting stimuli. However, the strong effect of anxiety- and fear- eliciting stimuli on the first day was associated with a significant increase in refuge use on the second day prior to exposure to stimulus. This would be suggestive of a modifying, or a retention effect, or an effect of remanence of at least 24 hours for both stimuli, but stronger for ES than PS. The modifying effect of exposure to electric shocks could be related to physical damages, however, Perrot-Minnot *et al.* (2017) found no evidence for decreased locomotor activity following exposure to ES. Surprisingly, controls individuals of ES treatment more or less behaved as shocked individuals did on day 1. Indeed, they were more under the refuge after setting up the device solely, without electric shocks. This suggests an important sensitivity to handling considered as a major source of stress for gammarids. However, this was non-significant on day 2. Stress-evoking exposure to ES device itself (and associated handling) might be responsible for anxiety-like state on day 1, whether it delivered shocks or not. This could explain why individuals were under the refuge on day 2 before eliciting shocks or setting up ES device.

Control individuals might have associated ES device with no negative consequences on day 1, explaining the weaker response to the setting of ES device on day 2. Therefore, this suggests an involvement of both the manipulation associated with electric device and nociceptive response to electric shocks, more than a retention effect of electric shocks per se. This hypothesis is relying on the assumption of short-term memory in gammarids. However, cases where memory was reported in invertebrates such as honeybees involved conditioning periods (Menzel, 2001; Lockett, Helliwell, & Maleszka, 2010).

Our results showed that, regardless of the treatment (ES, PS or control), individuals were repeatable in their refuge use both before and after eliciting stimulus. Indeed, individuals that were more under refuge on day 1 were also more under refuge on day 2. This means that gammarids showed some consistency in this defensive behaviour, in agreement with David et al. (2014). We went further by showing consistency in refuge use after exposure to anxiety- and fear-eliciting stressors, suggesting that anxiety-like and fear behaviours are consistent. However, the magnitude of stress-evoking change in refuge use was not repeatable, meaning that individual response to these stressors is plastic, or, alternatively, that the response index chosen to quantify it is masking individual's consistency. Plasticity may be advantageous as it allows individuals to optimize the trade-off between securing safety and foraging for food or mates. Such trade-off may set an upper limit to the use of refuge, thereby explaining a lower magnitude of response on day 2 due to a higher level of refuge use before exposure to ES or PS. To test this hypothesis, we could change this trade-off by expetimentally manipulating the need/motivation to feed or mate in males: increasing or decreasing access to food or mates during an appropriate conditioning period should induce a higher or lower upper limit to refuge use, respectively, and thereby increased or decreased the magnitude of response to ES or PS.

Our study also contributes to clarifying the difference between anxiety-like state and fear. While response to anxiety-eliciting stimulus was correlated to response to fear-eliciting stimulus expressed the day before, the reverse was not observed. First, individuals that experienced predation-related event on the first day may become more sensitive to nociceptive stimulus thereafter. Mendl et al. (2010) stated that "mood" states expressed by individuals, such as anxiety, could be the result of the accumulation of episodes of discrete negative emotions, such as fear. Thus, anxiety-like state could result from several / chronic fear experiences. However, in our study, individuals were exposed to predation threat only once, and were coming from a "naïve" population with respect to fish predators. More likely, acute stress, such as fear, can exert modulatory effects on nociception (Butler & Finn, 2009). More specifically, fear could induce hyperalgesia, i.e. an exaggerated response to noxious stimuli, as repeatedly evidenced in rodents (Itoga *et al.*, 2016). Under this hypothesis, being more responsive to predation cue on the first day would have resulted in an enhanced perception and response to electric shocks the day after. However, at least one other empirical study addressing the link between stress and nociception, has actually reported an opposite effect, known as stress-induced analgesia. In zebrafish larvae, fear-eliciting stimulus (predator odor) actually activated antinociceptive mechanisms, thereby inhibiting response to anxiety-eliciting situations (Lopez-Luna *et al.*, 2017).

In conclusion, our data showed that *G. pulex* individuals can expressed both anxiety-like state and fear behaviours. In addition, we suggest that handling (setting up ES device) represents a non-negligible source of unknown stress, resulting in a negative emotional state. Our results also suggest that *G. pulex* individuals could associate their environment with a discrete negative stimulus, which is likely to shape a negative emotional state. Particularly, we hypothesize that the environment in which strong and known negative events occur,

independently of the physical/olfactory effects of stimuli themselves, plays a key role in shaping the emotional responses. The involvement of short-term memory should be investigated to better understand the individual consistency in behavioural responses to (manipulation) stress, fear- and anxiety-stimuli, and the correlation between fear and anxiety evidenced here. The present study should also be replicated, by performing these on more than only two consecutive days. A larger time scale, at least five days, remains necessary to do conditioning to test for (ii) habituation, (ii) memory, (iii) sustained emotional state resulting from episodes of discrete emotions and (iv) cognitive bias, in amphipods. In addition, investigating the consequence of experiencing fear on the subsequent perception and response to noxious stimuli, either as hyper- or hypo-analgesia, is of paramount importance to clarify the link between fear and anxiety, and more generally of individual strategies to cope with threats.

SUPPORTING INFORMATION

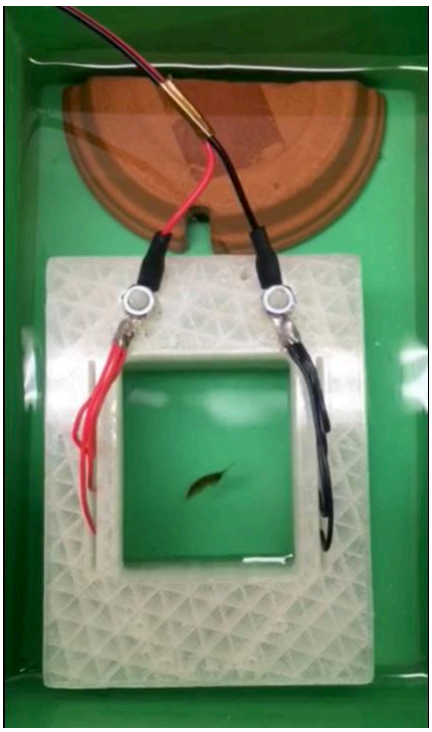

Fig.S1 Portable device delivering electric shocks (10V). The device is placed at the center of a 10.5 * 16 cm rectangular and opaque box filled with 200 mL, and with half a terracotta saucer placed at one side as a refuge for the tests. A gammarid is placed at the center of the device.

Discussion

La perception et l'évaluation d'une information par un animal, humain ou non, peut dépendre de son état émotionnel, qui peut alors biaiser sa future décision (Harding, Paul, & Mendl, 2004 ; Mendl *et al.*, 2009). En effet, des individus dans un état anxieux auront tendance à juger un stimulus ambigu plutôt comme négatif via une réponse « pessimiste » (Harding *et al.*, 2004 ; Mendl *et al.*, 2009). C'est ce qu'on appelle le biais cognitif. L'utilisation des biais cognitifs sur les animaux non-humains permettrait d'avoir une idée de leur état émotionnel et de pouvoir prédire comment ils se comporteraient en milieu changeant et incertain. En ce sens, il serait intéressant de voir si la présence du parasite serait responsable d'un biais cognitif. L'hypothèse que le parasite modulerait l'état émotionnel d'anxiété de son hôte pourrait passer par l'étude de biais cognitifs chez les hôtes infectés. En abaissant l'état d'anxiété basal de son hôte, le parasite pourrait biaiser le jugement de tout stimulus dans l'environnement de façon moins « pessimiste », et ainsi impacter indirectement les décisions de son hôte à travers des changements de comportement. Pour aller encore plus loin, nous pouvons nous demander sur quel axe caractéristique des émotions le parasite jouerait-il. Abaisse-t-il l'intensité de l'état d'anxiété basal, module-t-il sa valence en rendant cet état moins négatif ou intervient-il sur ces deux axes ? Le parasite serait-il donc capable de changer la nature de l'état émotionnel de son hôte ?

Rappelons que les changements dans le comportement de l'hôte semblent opposés selon le stade parasitaire, infectieux ou non (Fayard *et al*. 2020). De plus, que les changements soient induits par l'acanthelle ou le cystacanthe, ils semblent aussi bien différents des comportements des individus sains (Dianne *et al*., 2011 ; Fayard *et al*. 2020). De façon globale, les individus infectés avec l'acanthelle adopteraient un comportement plus protecteur vis-à-vis de la prédation, à l'opposé des individus infectés avec le cystacanthe (Dianne *et al*., 2011). Dans le

cas où le comportement de l'hôte dépend de son état émotionnel, et que le parasite module cet état, cette inversion dans le comportement de l'hôte pourrait résulter du changement de valence et/ou de l'intensité de cet état. Ainsi, au stade acanthelle le parasite augmenterait ces deux caractéristiques jusqu'à ce que l'hôte rentre dans un état de peur/anxiété très profond, et au stade cystacanthe, l'inverse se produirait pour tendre vers un état plus « calme/relaxé ». Le parasite jouerait donc sur l'axe correspondant au système d'évitement de la punition du '*core affect*' proposé par Mendl *et al.* (2010). Faire appel au biais cognitif prend encore un peu plus de sens quant au stade parasitaire, afin de savoir si en effet il existe un changement d'état émotionnel entre ces deux stades. Ainsi, on peut supposer que des individus infectés par une acanthelle auraient tendance à juger un stimulus ambigu comme négatif alors que des individus infectés par un cystacanthe le jugeraient davantage comme positif. Ceci permettrait alors de prédire des comportements plus appréhensifs et défensifs pour des individus infectés par une acanthelle, et plus laxistes, moins défensifs pour des individus infectés par un cystacanthe. Le volet état émotionnel et tout ce qui en découle semble représenter une piste très sérieuse à prospecter, si nous voulons comprendre les mécanismes sous-jacents à la manipulation parasitaire.

DISCUSSION GENERALE ET PERSPECTIVES

Lors de ces années de thèse, nous espérons avoir approfondi l'étude la manipulation parasitaire et précisé les pistes à privilégier. Nous avons ainsi fait le bilan quantitatif et qualitatif de plus de 50 ans d'études sur la manipulation par les parasites acanthocéphales, pour en tirer des conclusions et perspectives autant sur le plan biologique que méthodologique qui pourraient s'étendre et s'appliquer à beaucoup de systèmes hôte-parasite. Nous proposons également certaines pistes d'étude non explorées jusqu'à présent chez notre modèle d'étude pour tenter de mieux comprendre les voies d'action de ces parasites et construire de façon plus pertinente les futures études de la manipulation.

Manipulation parasitaire : bilan et perspectives

Globalement, les changements phénotypiques, multidimensionnels, semblent « profiter » au parasite en termes de transmission, même si certains traits sont plus fortement affectés que d'autres, tels que les comportements de défense (Fig.6). Il semblerait que le parasite ait pour effet d'interagir avec les processus cognitifs de décision liés à la survie de son hôte. Pour éclaircir le débat sur le caractère adaptatif de la manipulation il s'agirait d'essayer de mettre en relation ces changements avec la probabilité de transmission et quantifier ainsi le bénéfice pour le parasite. Ce défi s'avère cependant plus compliqué que le simple fait d'établir une corrélation entre les changements et la vulnérabilité à la prédation en micro- ou mésocosme. En effet, l'interaction hôte-parasite s'opérant dans des milieux très complexes, le seul fait d'estimer les changements phénotypiques et cette vulnérabilité dans des conditions écologiquement réalistes afin de pouvoir les mettre en relation représente déjà un sérieux défi.

Nous avons mis en évidence une altération du système immunitaire qui pourrait venir s'insérer dans le débat sur le caractère adaptatif de la manipulation. En effet, si les changements

phénotypiques observés ne sont pas le résultat d'une manipulation directe par les parasites, ceux-ci ne pourraient être qu'un effet secondaire de l'infection. L'hypothèse neuropsychoimmune (Adamo, 2002) selon laquelle les changements phénotypiques observés ne seraient que le résultat d'un dérèglement physiologique et plus précisément de certains neuromodulateurs, est restée théorique bien que proposée depuis plusieurs années, et doit être testée expérimentalement. Les pistes de l'immunodépression et du rôle de la sérotonine (5-HT) semblent très prometteuses, et ont besoin d'être creusées davantage surtout que jusqu'à aujourd'hui elles n'ont été qu'abordées de façon indépendante. Adamo (2008) a déjà relaté le lien entre neuromodulateurs intervenant dans la réponse au stress et l'immunité à travers plusieurs phylums, y compris les invertébrés. En effet, la noradrénaline (chez les mollusques, voir Lacoste *et al.* (2001)) et l'octopamine (chez les insectes, voir Roeder (2005)) sont libérées en réponse au stress, et ceci serait accompagné d'une immunosuppression mise en évidence au travers d'une diminution de la résistance face aux pathogènes (Adamo, 2008).

Même si globalement les changements phénotypiques, et plus particulièrement comportementaux, semblent favoriser la transmission des parasites, il existe une variabilité à ne pas négliger dans l'intensité de l'expression de ces changements. En ciblant les comportements antiprédateur, nous avons pu montrer que ces comportements n'étaient pas affectés avec la même intensité par les acanthocéphales selon le risque de prédation dans le milieu. La manipulation serait donc plus forte dans un milieu où le risque de prédation, et donc l'opportunité de transmission, est plus faible.

Effets combinés de facteurs environnementaux (pression de prédation) et individuels (état émotionnel), et intérêt complémentaire des études in situ et au laboratoire

De nos jours, les données proviennent en grande partie de tests de laboratoire, et même si elles sont obtenues dans un cadre très contrôlé, celui-ci ne reflète pas la variabilité des conditions naturelles. En effet, même si les conditions standardisées au mieux de l'expérimentation en laboratoire permettent de faire des comparaisons et de tester directement certains facteurs en en controlant d'autres, il est peu envisageable de conclure sur de tels résultats obtenus avec une extrapolation en milieu naturel. Ainsi, un comportement enregistré en laboratoire ne peut que partiellement être comparé au comportement qui serait adopté en milieu naturel. Recourir à l'approche in situ semble approprié pour mettre en évidence la variabilité. Certaines limites restent tout de même à prendre en compte. Les individus prélevés in situ ne sont pas tous « comparables » (âge, état corporel, stade de développement, expérience) au moment de leur prélèvement. La complexité de l'approche in situ réside dans la maîtrise du compromis entre la prise en compte de toute la fluctuation des paramètres du milieu qui sont d'intérêt et la mise à l'écart de ceux qui constitueraient des facteurs confondants pouvant biaiser les résultats. Cependant et malgré leurs contraintes respectives, les approches in situ et en laboratoire peuvent être complémentaires.

L'étude menée in situ sur la variabilité des comportements antiprédateur en lien avec le risque de prédation, et détaillée dans le chapitre 3 représente alors un premier pas. Bien que notre approximation du risque de prédation, à travers la biomasse de prédateurs (poissons), soit grossière, nous avons pu montrer une certaine variabilité dans deux comportements antiprédateur (phototaxie et utilisation d'un refuge) en lien avec ce risque. Les comportements antiprédateur semblent donc modulés par l'interaction complexe de la biomasse de prédateurs

et de conspécifiques dans le milieu. La phototaxie semble davantage liée à la biomasse de prédateurs, tandis que l'utilisation d'un refuge serait plus dépendante des conspécifiques desquels les individus dépendent pour l'investissement dans certaines fonctions (reproduction). Ces deux comportements seraient alors modulés différemment et contribueraient à la protection contre les prédateurs également de façon différente sur la base de l'investissement différentiel entre défenses et reproduction/approvisionnement. De plus, nous avons montré une flexibilité dans le comportement d'utilisation du refuge en réponse à l'odeur de prédateur différente selon l'intensité du risque de prédation. Même si plusieurs facteurs peuvent entrer en interaction dans le milieu naturel (biomasse et régime alimentaire des prédateurs, saisonnalité, …), il est donc possible de mettre en évidence certaines réponses et leur variabilité avec une approche in situ. Il nous appartient ensuite avec l'approche en laboratoire de tester les réponses de façon plus fine, avec discrimination des facteurs et après avoir sélectionné les traits les plus pertinents mis en évidence par l'approche in situ.

Dans notre cas, une nouvelle étude visant à tester en laboratoire l'effet du stress chronique de la prédation sur le niveau des défenses comportementales de base (avant stress) et réponses (après stress) s'avèrerait complémentaire (Fig.6). Elle permettrait de mieux comprendre l'effet du risque de prédation sur la modulation des comportements de défenses et d'évaluer leur plasticité en fonction des patterns de risque. Pour cela, l'approche 'risk allocation' développée par Lima & Bednekoff (1999) et reprise plus tard par Sih & McCarthy (2002) semble appropriée. L'hypothèse est que le comportement des individus s'ajuste en fonction du pattern temporel du risque de prédation (Sih & McCarthy, 2002) (Fig.5). Cela repose sur un compromis crucial entre l'effort que les individus doivent allouer au comportement d'approvisionnement en ressources alimentaires par exemple et au comportement antiprédateur. Concrètement, plus le contexte environnemental présente un risque de prédation élevé, moins

les individus vont s'approvisionner et plus ils adoptent un comportement antiprédateur marqué (Lima & Bednekoff 1999). Sih & McCarthy (2002) vont plus loin en proposant d'ajouter les facteurs « incertitude » et « constance » du risque.

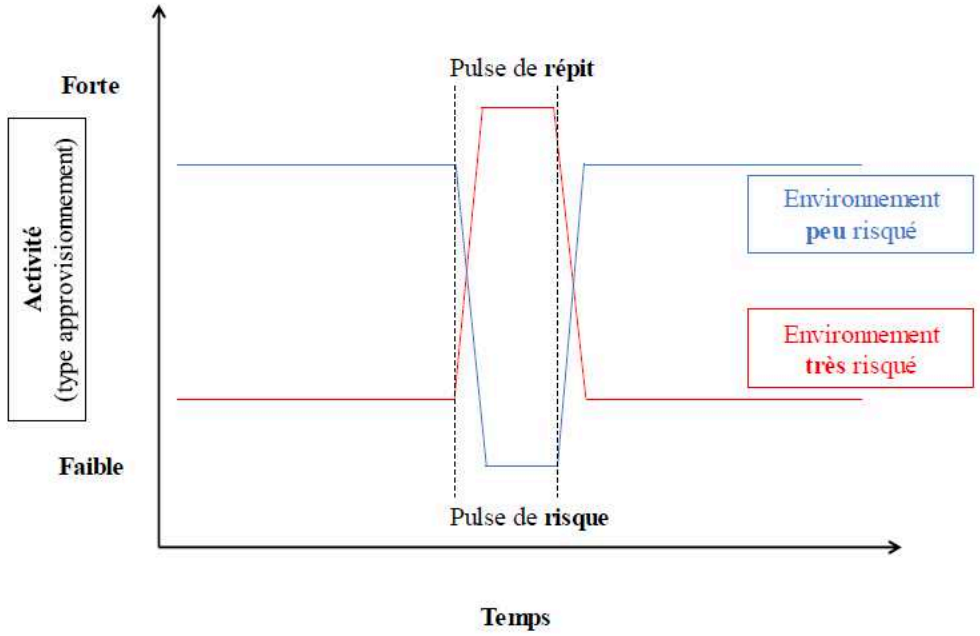

Fig.5 Prédictions de l'hypothèse « risk allocation » de Lima & Bednekoff (1999). Modulation de l'activité (approvisionnement alimentaire) en fonction du temps et de l'environnement (peu ou très risqué) dans lequel l'individu se trouve. S'il se trouve dans un environnement peu risqué, l'individu devrait exprimer une activité élevée (et faible comportement antiprédateur) mais devrait la réduire au moment d'un bref pulse de risque. Inversement, s'il se trouve dans un environnement très risqué, l'individu devrait exprimer un niveau faible d'activité (et fort comportement antiprédateur) mais devrait l'augmenter lors d'un bref pulse de répit.

Ainsi, on suppose que le comportement antiprédateur des individus est d'autant plus fort que le risque de prédation est intense et non constant (pulses brefs). Inversement, il est au plus bas quand le risque de prédation est faible constamment. Dans leur étude, Sih & McCarthy (2002) démontrent que, lorsque les escargots sont soumis à un environnement constant dans le risque perçu (faible ou fort), ils expriment des comportements antiprédateur et d'activité stables. Inversement, quand ils sont soumis à un bref pulse de répit interrompant un risque constamment élevé de prédation, ils présentent une grande plasticité dans les deux types de comportement.

L'incertitude d'un évènement a pour effet d'augmenter la variation temporelle dans l'expression des comportements. La constance créée alors une « habituation » des proies qui apprennent davantage à gérer leurs comportements.

Tester l'effet chronique du stress lié au risque de prédation permettrait alors de voir comment les individus investissent dans les deux parties du compromis défense / activité, à travers l'ajustement de leurs comportements en fonction du pattern de prédation. Si l'expression des défenses comportementales dépend d'un état émotionnel de type anxiété, il serait pertinent en parallèle de tester l'effet chronique d'un stress induisant cet état, tel que les impulsions électriques (comme détaillé dans le dernier chapitre de ce manuscrit). Dans ce chapitre, nous avons proposé que le niveau de l'état s'apparentant à l'anxiété pouvait dépendre de l'intensité des épisodes de peur vécus. Procéder de façon expérimentale à un stress chronique (odeur de prédateur et impulsions électriques) nous permettrait donc de tester plus finement la modulation de l'expression de la peur et de l'anxiété ainsi que de mieux percevoir le lien entre peur et anxiété sur le plan comportemental.

A travers une approche expérimentale, nous avons pu mettre en évidence l'expression de comportements de peur et de type anxiété chez un amphipode à travers l'augmentation d'utilisation d'un refuge, après l'exposition topique à un stimulus connu (odeur de prédateur) et inconnu (choc électrique). Nous avons pu mettre en évidence l'importance de la manipulation comme un stress pouvant induire un état de type anxiété, comme le choc électrique. Il semble également qu'il existe un effet de rémanence du stress perçu puisque 24 heures après la première exposition, les individus montrent un comportement de défense avant la présentation du stimulus (quelle que soit sa nature, connu ou non), plus élevé que la veille. Ceci pourrait suggérer l'existence d'une mémoire de l'expérience. Pour aller plus loin, la réponse à un

stimulus inconnu semble déterminée par la réponse faite à un stimulus engendrant la peur le jour précédent. Ainsi, un état de type anxiété pourrait être la conséquence de la répétition d'épisodes de peur. Tout ceci reste hypothétique et à vérifier à travers des expériences complémentaires. Ainsi, il semble pertinent de prolonger la durée de cette expérience afin d'exposer de façon chronique les individus au stress, en prenant soin de préciser le stress induisant l'état de type anxiété. De plus, l'apprentissage et la mémoire se doivent d'être testés chez les amphipodes à travers le développement de méthodologies appropriées afin de comprendre comment les comportements sont forgés (Fig.6). La découverte de la capacité d'apprentissage associatif permettrait ainsi de procéder, comme chez d'autres invertébrés, aux tests de biais cognitifs, révélateurs d'états émotionnels.

Etat émotionnel comme "cible" du parasite : le parasite peut-il moduler un niveau basal d'anxiété de son hôte ?

Dans un environnement où les rencontres avec les prédateurs sont fréquentes, les épisodes de peur que vivent les proies sont répétitifs. Par conséquent, ces proies sont susceptibles de développer un état émotionnel de type anxiété. Cet état s'associerait à des comportements antiprédateur plus marqués qui tendraient à diminuer la probabilité de prédation. Cependant, c'est bien sur cette probabilité de prédation que repose la transmission des parasites à transmission trophique. Si l'intensité des comportements antiprédateur découlent d'un état d'anxiété basal et optimal pour les proies, les parasites induisant une atténuation de cet état seraient mieux transmis (Fig.6). L'infection pourrait alors être responsable d'un biais cognitif. Ainsi, les tests de biais cognitif mentionnés dans le chapitre 4 s'avèreraient utiles en comparant individus sains et infectés. Pour un conditionnement similaire, on attendrait ainsi à ce que des individus infectés jugent un stimulus ambigu de façon différente comparé aux individus sains. Plus précisément, des individus infectés auraient tendance à juger un stimulus ambigu de façon

moins pessimiste que des individus sains. Ceci serait révélateur d'un état émotionnel sans doute moins négatif et interprété comme un niveau d'anxiété abaissé.

Cette hypothèse du lien entre infection et état émotionnel serait d'autant plus pertinente qu'il existerait un niveau d'anxiété optimal de l'hôte impliquant différents traits et non pas seulement les comportements antiprédateur, tels que survie (immunité), reproduction, approvisionnement). Se pose alors la question de l'existence d'un syndrome d'anxiété (multidimensionnalité), comme celui de la manipulation parasitaire. L'anxiété se manifeste-t-elle hors des comportements défensifs ? On sait par exemple que chez d'autres animaux, humains ou non, l'anxiété agit sur plusieurs types de traits, tels que les interactions sociales (Henniger *et al.*, 2000; Zheng *et al.*, 2011), la mobilité (Overstreet *et al.*, 2003; Zheng *et al.*, 2011), les défenses / la vigilance (Ohl, Arndt, & van der Staay, 2008; Lee *et al.*, 2016), l'exploration (Mallo *et al.*, 2007; Bourin *et al.*, 2007) et l'immunité (Rammal *et al.*, 2008, 2010). Ainsi, si ce niveau peut être modifié par le parasite, alors celui-ci manipulerait son hôte de façon plus globale, non plus via une inversion des comportements antiprédateur, mais via un changement des décisions liées à la survie de l'hôte au sens large. En ce sens, il semble intéressant de comparer pour certains types de fonction (défenses, approvisionnement et reproduction), des individus sains anxieux et des individus infectés non anxieux afin de voir s'il existe une relation entre les altérations dont les fonctions diffèrent. Ce type d'approche par "comparaison de syndromes" a été adopté pour comparer l'infection par *P. laevis* et l'augmentation transitoire de sérotonine, chez *G. pulex/fossarum* (Perrot-Minnot *et al.,* 2014). Cependant, certains traits pouvant être affectés par l'anxiété pourraient de pas être bénéfiques à la survie des hôtes. Sur le plan de l'immunité, des individus anxieux sont-ils aussi immunodéprimés et si oui, quelle est la similarité avec les individus infectés non anxieux ? Pour voir si l'état d'anxiété est indépendant de l'infection, comparer la réponse à l'induction d'un

état d'anxiété entre individus sains et infectés semble tout aussi pertinent. Si après induction d'un état d'anxiété, les individus ne montrent aucune différence dans leur comportement de défense (défenses augmentées) qu'ils soient infectés ou non, cela irait dans le sens d'un effet de l'infection indépendant de l'état d'anxiété.

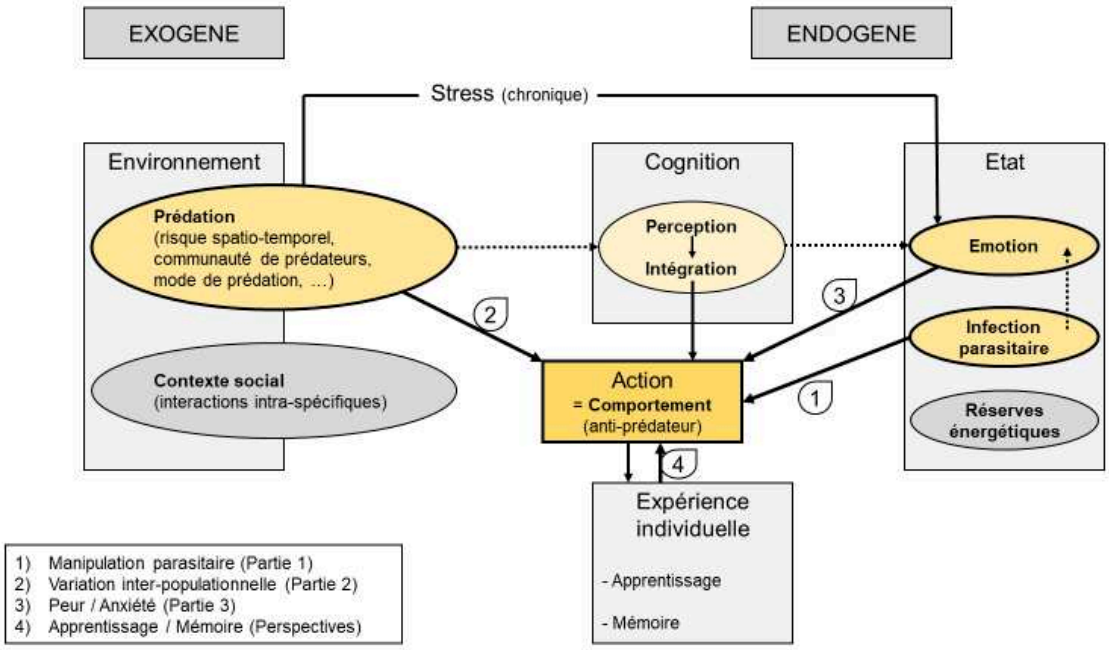

Fig.6 Implication et rôle des facteurs endogènes (état émotionnel, infection parasitaire) et/ou exogènes (pression de prédation) ainsi que leur interaction dans la modulation des comportements (antiprédateur). L'infection parasitaire diminuerait l'expression des comportements de défenses (1), éventuellement de façon indirecte à travers la modulation d'un état émotionnel de type anxiété. Le risque de prédation qui représente un stress aigu va moduler les comportements (2), directement après perception du risque. Le risque de prédation va sur le long-terme créer un stress chronique qui est supposé moduler l'état émotionnel. Cet état émotionnel (de type anxiété) va conditionner l'expression des comportements antiprédateur (3). Par ce stress chronique, à travers la répétition des épisodes de rencontre avec les prédateurs, l'individu va mettre en place de stratégies antiprédateur basés sur la mémoire des expériences passées et donc sur l'apprentissage (4).

Pour aller encore plus loin, les changements dans le phénotype de l'hôte semblent opposés en fonction du stade du parasite (Dianne *et al.,* 2011, mais voir Fayard *et al.,* 2020). La question de la relation entre l'expression de l'anxiété et l'ontogénèse du parasite reste tout

aussi pertinente. Si le parasite non mature a pour effet de protéger son hôte, le fait-il en augmentant le niveau basal d'anxiété de son hôte ?

Les mécanismes : le rôle des neurotransmetteurs sérotonine et dopamine

L'expression de l'anxiété chez les crustacés utilise principalement les voies sérotonergique et GABAergique (Fossat *et al.,* 2014). Elle peut être inhibée via certaines molécules anxiolytiques comme la benzodiazépine qui agit sur les récepteurs GABA (Fossat *et al.,* 2014), la fluoxétine (principe actif du Prozac®) qui augmente la concentration synaptique de sérotonine via l'inhibition de sa recapture (Hamilton *et al.*, 2016), ou le LY354740, une molécule ciblant la voie glutaminergique (Perrot-Minnot *et al.,* 2017). Par exemple, la fluoxétine, inhibiteur de la recapture de sérotonine, atténue les comportements anxieux chez le crabe *Pachygrapsus crassipes* (Hamilton *et al.*, 2016). Des individus *G. fossarum* rendus anxieux par impulsions électriques mais préalablement traités par LY354740 utilisent bien moins le refuge que des individus anxieux qui n'ont pas été traités, et se comportent alors comme des individus non anxieux (Perrot-Minnot *et al.,* 2017).

La sérotonine (5HT) représente le neurotransmetteur principal impliqué dans les voies de l'anxiété. C'est aussi un neuromodulateur dont le rôle et les effets sont débattus car contrastés. La sérotonine a déjà été montrée comme responsable d'une augmentation de l'agressivité chez le crabe *Chasmagnathus granulatus* (Pedetta, Kaczer, & Maldonado, 2010), le homard *Homarus americanus* (Huber *et al.*, 1997) et l'écrevisse *P. clarkii* (Tricarico & Gherardi, 2007). Chez l'écrevisse *P. clarkii* (Fossat *et al.,* 2014), l'injection de sérotonine a un effet anxiogène, inversement comparable à celui de la benzodiazépine anxiolytique (Graeff, 2002). Cependant, une simple injection topique de sérotonine mime partiellement le syndrome de manipulation

parasitaire chez *G. pulex*, en diminuant certains comportements antiprédateur (Perrot-Minnot *et al.,* 2014) et qui pourrait traduire une diminution du niveau d'un état s'apparentant à l'anxiété. De plus, Bacqué-Cazenave *et al.* (2018) ont récemment montré que l'injection de sérotonine peut avoir des effets opposés sur l'agressivité du mâle écrevisse *P. clarkii* en fonction de la taille d'un compétiteur perçu. Ils suggèrent alors que la sérotonine ait un rôle dans les processus de perception du risque et/ou de décision qui vont moduler en conséquence les comportements. Pour aller plus loin, des éventuels effets dose et chronicité pourraient faire l'objet d'études.

Parmi les monoamines, la dopamine semble également intervenir dans la régulation d'états relatifs au stress comme l'anxiété (Steiner, Fuchs, & Accili (1997) chez la souris). Chez les invertébrés, les neurones dopaminergiques sensibles aux informations nociceptives, sont impliqués dans le codage sensoriel de l'information comme étant positive ou négative (Adamo, 2019). Chez la drosophile *D. melanogaster*, la dopamine est impliquée dans l'apprentissage associatif avec récompense / punition (Waddell, 2013). Chez ce même animal, l'intensité des comportements, caractérisée par le terme 'arousal' (axe des états émotionnels), semble être modulée par les variations de dopamine dans le cerveau (Andretic, van Swinderen, & Greenspan, 2005). Toujours chez la drosophile, la dopamine semble jouer un rôle très important en étant responsable du développement de tissus diverses (sensoriels par exemple) et dans le comportement (Neckameyer *et al.*, 2001). En effet une déplétion en dopamine serait responsable de tissus sensoriels non développés et de comportements reproducteurs anormaux. La dopamine interviendrait alors sur la morphologie et le comportement. Par exemple, chez la daphnie *D. pulex* et *Daphnia longicephala*, l'administration de dopamine induit des défenses morphologiques (Weiss *et al.*, 2015). Cependant, même si la concentration en dopamine dans le cerveau de l'écrevisse *P. clarkii* semble être augmentée après un stress, sa fonction exacte reste encore inconnue car son injection ne suffit pas à induire directement un comportement de

type anxieux (Fossat *et al.,* 2014). Tout comme la sérotonine dont le fonctionnement est débattu, la dopamine représente une voie à investiguer car ces deux neurotransmetteurs ne semblent pas indépendants. Chez la drosophile par exemple, une déficience dans l'action de l'enzyme tryptophane-phénylalanine hydroxylase (DTPH) serait responsable de modifications dans les voies de signalement et de production de la dopamine et de la sérotonine (Coleman & Neckameyer, 2004).

Conclusion

Depuis plusieurs années, la communauté de chercheurs en parasitologie est restée « coincée » dans un schéma fermé d'études relativement redondantes, avec une tendance à n'étudier que les effets des parasites sur les mêmes traits phénotypiques (comportements de défense antiprédateur) de l'hôte. A travers cette thèse, nous invitons les chercheurs à s'engager sur des pistes moins « confortables » et sans doute plus périlleuses nécessitant de développer de croiser plusieurs disciplines et tester de nouvelles hypothèses et situations.

Nous insistons ainsi sur l'importance de travailler en parallèle sur deux volets. Premièrement, il nous faut cibler les travaux sur la manipulation sur l'existence et la quantification d'une éventuelle causalité entre changements phénotypiques et transmission ainsi que la relation entre changements comportementaux et physiologiques tout au long de l'ontogénie du parasite. Ces deux pistes sont primordiales et alimenteraient le débat sur le caractère adaptatif de la manipulation. Deuxièmement, il nous faut mieux comprendre comment sont modulés les comportements défensifs des individus sains en milieu naturel, sur les plans écologique, comportemental et physiologique. Pour comprendre ces changements et donc la manipulation, nous devons absolument comprendre comment les différents traits s'expriment chez des individus sains avant d'être altérés. Sur le plan comportemental, la piste des

comportements antiprédateur modulés par le stress que représente le risque de prédation reste à privilégier. C'est ici que la complémentarité des approches in situ et en laboratoire prend son sens. Les études in situ, comme celle présentée dans ce manuscrit, permettent de voir si les comportements défensifs sont différents en fonction de la structure de la communauté des prédateurs au sens large. Cependant, nous ne pouvons isoler aucun facteur relatif au risque de prédation tel que la densité de prédateurs, leur espèce, leur régime de prédation, le ratio proie-prédateur voire la durée de l'exposition. L'approche en laboratoire permettrait d'isoler et contrôler certains paramètres liés à la prédation qui varient en populations naturelles et qui pourraient nous permettre de mieux comprendre leur effet.

Enfin, nous avons amené l'importance de la perception et de l'intégration du stress, plus particulièrement lié à la prédation chez les animaux non-humains, notamment chez les invertébrés. Dans la dernière partie de ce manuscrit, nous sommes allés un peu plus loin encore en faisant intervenir la notion d'état émotionnel, lié au risque et donc affectant les comportements. Sans doute que pour amorcer l'études des états émotionnels chez les amphipodes, il serait pertinent de s'intéresser aux capacités d'apprentissage de ces derniers. Les états émotionnels liés au risque de prédation pourraient représenter un pont entre l'étude de la manipulation et des comportements défensifs chez les individus sains. Ainsi il nous semble important d'essayer de comprendre comment l'interaction entre état émotionnel et infection s'établit et évolue tout au long de l'infection, afin de pouvoir éclaircir les voies d'action et mécanismes sur lesquels le parasite agit.

Ainsi à travers ces principaux objectifs, nous invitons les chercheurs en écologie, comportement, neurophysiologie et parasitologie, entre autres, à travailler ensemble pour répondre de façon la plus efficace et pertinente possible à toutes les hypothèses et questions

posées dans ce manuscrit. Il n'est pas envisageable d'étudier le comportement des individus sans y intégrer les dimensions physiologique et neurologique qui y sont associées. Aussi, tout en connaissant le rôle des interactions entre organismes dans le façonnement des comportement, l'écologie des interactions et donc l'étude des relations proie-prédateur ne peut se dissocier de l'étude de la manipulation parasitaire. La manipulation parasitaire représente un champ d'étude et un phénomène très complexe qui se doit de faire intervenir, pour la comprendre, une coopération la plus fine qui soit entre ces différents domaines de recherche.

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ANNEXES

ANNEXES

Liste des travaux

FAYARD, M., CÉZILLY, F. & PERROT-MINNOT, M.-J. (2019) Interpopulation variation in the intensity of host manipulation by the fish acanthocephalan Pomphorhynchus tereticollis: are differences driven by predation risk? *Parasitology* **146**, 1296–1304. Cambridge University Press.

FAYARD, M., DECHAUME-MONCHARMONT, F., WATTIER, R. & PERROT-MINNOT, M.-J. (2020) Magnitude and direction of parasite-induced phenotypic alterations: a meta-analysis in acanthocephalans. *Biological Reviews*, brv.12606.

LALANDE, L., MOSER, T., FAYARD, M. & PERROT-MINNOT, M.-J. (IN PREP)
Effect of predator biomass and prey density on the magnitude of non-consumptive effects and reproductive investment in a freshwater amphipod: a correlational study

Listes des communications

2019, Dijon	International Colloquium on Amphipoda (ICA) **Anxiety and Fear in a freshwater amphipod : two overlapping emotional states ?** Communication affichée
2019, Lille	Société Française pour l'Etude du Comportement Animal (SFECA) **Anxiety and Fear in a freshwater amphipod : two overlapping emotional states ?** Communication orale
2018, Liverpool	European Conference on Behavioural Biology (ECBB) **Magnitude and direction of parasite-induced phenotypic alterations : a meta-analysis in acanthocephalans** Communication affichée
2017, Besançon	Forum des Jeunes Chercheurs (FJC) **Magnitude and direction of parasite-induceed phenotypic alterations : a meta-analysis in acanthocephalans** Communication orale

Activités complémentaires

Enseignements

2019-2020 L3 Biologie des Organismes + L3 Sciences de la Vigne
Biostatistiques (TP + TD) – 64h

2018-2019 L3 Biologie des Organismes + L3 Sciences de la Vigne
Biostatistiques (TP + TD) – 44h
L3 Biologie des Organismes
Comportement Animal (TP + TD) – 8h
M1 Behavioural Ecology
Ecologie comportementale – 12h

2017-2018 L3 Biologie des Organismes + L3 Sciences de la Vigne
Biostatistiques (TP + TD) – 44h
L3 Biologie des Organismes
Comportement Animal (TP + TD) – 8h
L3 Biologie des Organismes
Ecophysiologie (TP) – 12h

Formations

2019-2020 Initiation au métier d'enseignant dans l'enseignement supérieur – 12h

2018-2019 Matlab – 8h

Premiers secours – 3h

2017-2018 Ethique et intégrité scientifique – 1h

Usage des extincteurs – 3h

Encadrement de stagiaires

2018-2019 M1 (2 stagiaires) - Effect of predator biomass and prey density on the magnitude of non-consumptive effects and reproductive investment in a freshwater amphipod: a correlational study

2017-2018 M1 (1 stagiaire) – Anxiety in an amphipod crustacean, *Gammarus fossarum*
L3 (2 stagiaires) – Effet de la supplémentation en précurseur et inhibiteur de la sérotonine sur la phototaxie des gammares
L2 (1 stagiaire) – Effet du MS222 comme anesthésiant sur les gammares

ÉCOLE DOCTORALE
PRES I Bourgogne I Franche-Comté
Environnement – Santé

Titre : Anxiété et manipulation parasitaire chez un invertébré aquatique : approches évolutive et mécanistque

Mots clés : acanthocéphale, amphipode, comportement, état émotionnel, manipulation parasitaire, prédation

Les parasites à transmission trophique sont connus pour les changements phénotypiques qu'ils induisent chez leurs hôtes. Ces changements sont supposés favoriser la transmission des parasites vers un hôte définitif à travers la prédation de l'hôte intermédiaire. Ce phénomène de « manipulation parasitaire » a longtemps été vu comme un trait adaptatif des parasites. La manipulation reposant sur des interactions proie-prédateur, il est nécessaire de comprendre comment les comportements antiprédateur sont modulés par des facteurs exogènes (pression de prédation) et endogènes (infection parasitaire, état émotionnel, …) aux individus. Au cours de cette thèse, nous avons tenté d'approfondir la compréhension de ce phénomène, chez les crustacés amphipodes, en répondant à plusieurs questions : (1) quelle est l'étendue de la multidimensionnalité de la manipulation parasitaire par les Acanthocéphales, quantifiée au travers d'une méta-analyse ; (2) l'amplitude des changements de comportements antiprédateur varie-t-elle selon le contexte local de prédation ? ; (3) les comportements antiprédateur présentent-ils une flexibilité en lien avec le contexte local de prédation ? ; (4) le parasite "exploite" t-il la flexibilité comportementale des gammares sains, notamment en lien avec leur régulation émotionnelle de type anxiété ?. Au niveau interspécifique à l'échelle du phylum des Acanthocéphales, nous avons mis en évidence une altération marquée des comportements en lien avec la défense antiprédateur des hôtes (changement de microhabitat, protection et réponse à des stimuli). Au niveau du couple hôte-parasite *Gammarus fossarum, Pomphorhynchus tereticollis*, nous avons montré que l'intensité des changements de comportements antiprédateur induit par l'infection (phototaxie et utilisation du refuge) présentait une variabilité interpopulationnelle, en lien avec le risque de prédation : la manipulation semble d'autant plus forte que la pression de prédation locale, i.e. les opportunités de transmission, est faible. Chez les individus sains, nous avons mis en évidence, par une approche corrélationnelle, une variabilité interpopulationnelle de l'intensité des comportements antiprédateur en lien avec le risque local de prédation, et la densité de gammares conspécifiques. Nous avons également montré une flexibilité de la réponse au stimulus de prédation chez des individus provenant de populations où le risque de prédation était élevé, la réponse augmentant avec l'intensité du stimulus. En revanche, les individus provenant de populations à faible risque de prédation semblent montrer une réponse relativement forte indépendamment de l'intensité du stimulus, ce qui suggère une hypersensibilité. Ces études corrélationnelles, portant sur l'analyse de la variabilité interpopulationnelle selon les pressions de prédation locales, nous ont amenés à supposer que ces différentes stratégies seraient intimement liées à l'expérience d'un stress chronique de la prédation. Nous suggérons alors l'existence d'un état de long-terme de type anxiété qui pourrait être la résultante de la répétition d'épisodes de court-terme de peur. Nous avons effectué un premier pas en montrant expérimentalement l'existence de comportements de peur et de type anxiété chez *G. fossarum*. L'ensemble de ces travaux a démontré la place centrale des interactions proie-prédateur dans l'étude de la manipulation parasitaire. Plus précisément, nous avons mis en évidence une variabilité et une modulation complexe des comportements antiprédateur des hôtes en lien avec le contexte local de prédation, et qui pourraient trouver racine dans un état émotionnel lié au stress chronique de la prédation. Ces travaux ouvrent alors quelques pistes à investiguer telles que les mécanismes sous-jacents de cet état et l'éventuel rôle des parasites dans la modulation de cet état de type anxiété qui viendrait modifier l'expression des comportements antiprédateur.

Title : Anxiety and parasite manipulation in an aquatic invertebrate : evolutive and mechanistic approaches

Keywords : acanthocephala, amphipoda, behaviour, emotional state, parasite manipulation, predation

Trophically transmitted parasites induce changes in their host's phenotype. These changes are supposed to increase transmission probability to definitive hosts through the predation of intermediate hosts. This phenomenon is known as 'parasite manipulation' has been hypothesized to be an adaptive trait of parasites for a long time. As manipulation involves predator-prey interactions, it is therefore necessary to understand how antipredatory behaviours are modulated by exogenous (predation pressure) and endogenous (infection, emotional state) factors. We tried to go into this phenomenon in depth, in amphipods, by responding to several questions : (1) what is the extent of the multidimensionality in parasite manipulation by Acanthocephalan, quantified through a meta-analysis ; (2) is there variation in the magnitude of antipredatory behaviours according to local predation risk? (3) are antipredatory behaviours flexible with respect to local predation risk? (4) Do parasites exploit behavioural flexibility in uninfected individuals, in relation to an emotional state such as anxiety-like state? Within the Acanthocephala phylum, we evidenced notable changes, more particularly of host antipredatory behaviours (microhabitat changes, protection and responses to stimuli). Considering *Gammarus fossarum, Pomphorhynchus tereticollis* host-parasite couple, we showed that there was variation in the magnitude of antipredatory behavioural changes induced by infection (phototaxy and refuge use), with respect to local predation risk : host manipulation seemed as strong as predation risk, i.e. transmission opportunities, is low. With a correlational approach, we also evidenced variation in the magnitude of antipredatory behaviours according to local predation risk and conspecifics density, in uninfected individuals. In addition, we emphasized flexibility of behavioural responses to predator cues: individuals from populations experiencing high predation risk exhibited increased responses as predator cues concentrations increased. In contrast, individuals from populations experiencing low predation risk appeared to exhibit strong responses independent of predator cues concentrations, suggesting hypersensitivity. We supposed that these strategies would be closely related to the exposure to chronic predation risk. We therefore suggest the existence of an anxiety-like state that could result from the repetition of acute stress, i.e. fear, episodes. We made a first step through an experimental approach, evidencing the existence of both anxiety-like and fear behaviours in *G. fossarum*. Overall, these works pointed out the importance of predator-prey interactions in the study of parasite manipulation. More particularly, we evidenced variation and complex modulation of host antipredatory behaviours in accordance with local predation risk, and which may be closely related to an emotional state stemming from chronic predation stress. We therefore suggest some future directions to investigate, such as the underlying mechanisms of anxiety-like state, and the role of parasite in the modulation of this state that would modify the expression of antipredatory behaviours.

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