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Daphne Cortese

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Daphne Cortese. Parental and environmental determinants of larval dispersal-associated traits and post-settlement physiology. Ecology, environment. Université Paris sciences et lettres, 2021. English. NNT : 2021UPSLP090 . tel-03982251

HAL Id: tel-03982251

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PSL UNIVERSITÉ PARIS

THÈSE DE DOCTORAT
DE L'UNIVERSITÉ PSL

Préparée à l'École Pratique des Hautes Études

Parental and environmental determinants of larval dispersal-associated traits and post-settlement physiology

Soutenue par
Daphne CORTESE
Le 12 mars 2021

École doctorale n° 472
École doctorale de l'École Pratique des Hautes Études

Spécialité
Biodiversité, génétique et évolution

Composition du jury :

Jean CLOBERT DR1, SETE (UMR5321)	*Président*
David J. MCKENZIE DR2, MARBEC (UMR9190)	*Rapporteur*
Peter M. BUSTON Professeur, University of Boston	*Rapporteur*
Delphine LEGRAND CR1, SETE (UMR5321)	*Examinateur*
Sinead ENGLISH Senior research fellow, University of Bristol	*Examinateur*
Suzanne C. MILLS Maître de conférence, EPHE-PSL	*Directeur de thèse*
Ricardo BELDADE Professeur, Univesidad Pontificia Católica de Chile	*Co-encadrant de thèse*
Shaun S. KILLEN Professeur, University of Glasgow	*Co-encadrant de thèse*

École Pratique des Hautes Études | PSL

ABSTRACT

Environmental changes induced by human activities impact marine and terrestrial ecosystems worldwide. While facing these perturbations, species may respond in a variety of ways, including migration to a more suitable habitat via dispersal, acclimation via phenotypic plasticity or adaptation via genetic changes. The main opportunity for dispersal in sedentary marine organisms such as coral reef fish is the pelagic larval phase, during which time larvae journey in open water before settling onto a suitable habitat. Marine larval dispersal depends on physical factors, such as ocean currents, but is also largely determined by larval biological traits, such as physiology, behaviour and morphology. However, in which way offspring traits associated to dispersal are influenced by either their local environment or their parental legacy, remains less well studied. After larval settlement, phenotypes can still be affected by environmental stressors and thus it is vital to also understand their ability to acclimate to environmental stressors induced by human activities. To answer these questions, I used the orange fin anemonefish *Amphiprion chrysopterus* as a model species and combined *in situ* and laboratory analyses. First, by using laboratory reared larvae of wild spawned eggs I explored the influence of maternal size on dispersal-associated traits throughout ontogeny (swimming performance and body size) and demonstrated that larger mothers produce larvae that swim faster (Chapter 2). Then, I investigated how anemonefish parental environmental conditions, such as anemone habitat size (proxy of habitat quality and degradation) affect offspring phenotype (i.e. egg size, larval size and larval swimming performance) and found that parents living in larger habitats produce larger eggs, while parents living in degraded habitats produce smaller offspring but with better swimming abilities (Chapter 3). In addition, I explored the transgenerational and developmental effects of water flow regimes on larval and juvenile morphology, physiology and survival. Results show that early life survival can be affected by the water flow regime that their parents live in, suggesting differential investment (Chapter 4). In the last part of the thesis, I focused on the direct effects of chronic exposure to environmental stressors, such as anemone bleaching (Chapter 5) and artificial light at night (ALAN, Chapter 6) on juvenile *A. chrysopterus* phenotypes and survival in the wild. I demonstrated that anemone bleaching affects the physiology and behaviour of fish but not survival, suggesting that wild fish may trade-off growth with survival, even though reduced growth at early stages may have life-long fitness effects. ALAN had direct effect on fish survival and also affected its growth. The results of this PhD provide a better understanding of the origin of variability in the factors influencing the dispersal process as well as the post-settlement phenotype in an iconic coral reef fish. This knowledge may help to better predict how fish populations will respond to climate change. Such predictions are key to mitigating the impacts of climate change and improving conservation strategies, for example by targeting the most important marine areas and fish phenotypes to protect.

KEYWORDS

Environmental stressors, larval traits, phenotypic plasticity, coral reef fish, parental effects, survival

RÉSUMÉ

Les changements environnementaux induits par les activités humaines impactent l'ensemble des écosystèmes marins et terrestres de la planète. Les espèces peuvent faire face à ces perturbations de différentes manières, par exemple en se déplaçant vers des habitats plus adaptés (migration et dispersion), en s'acclimatant (plasticité phénotypique) ou en s'adaptant (changements génétiques). Chez les organismes marins sédentaires comme les poissons des récifs coralliens, la principale opportunité de dispersion se situe lors de la phase larvaire, durant laquelle les larves se développent en milieu océanique avant de revenir s'installer dans les habitats récifaux. Cette dispersion larvaire dépend de facteurs physiques, tels que les courants océaniques, mais est également largement déterminée par les traits biologiques des larves, tels que leur physiologie, comportement et morphologie. Cependant, l'influence de l'environnement et des traits biologiques des parents sur ces traits larvaires associés à la dispersion reste moins bien étudiée. De plus, les jeunes poissons peuvent aussi être affectés par les stress environnementaux après s'être installés dans les récifs, soulignant l'importance de mieux comprendre leur capacité à s'acclimater à ces stress. Pour répondre à ces problématiques, ma thèse utilise le poisson clown à nageoires orange *Amphiprion chrysopterus* comme espèce modèle, et combine analyses *in situ* et en laboratoire. En collectant des œufs d'*A. chrysopterus* dans l'environnement puis en élevant les larves qui en découlent, j'ai d'abord étudié l'influence de la taille maternelle sur les traits larvaires associés à la dispersion (vitesse de nage et taille). J'ai pu observer que les mères les plus grandes produisent des larves qui nagent plus vite (Chapitre 2). J'ai ensuite examiné comment les conditions environnementales liées au milieu parental, telles que la taille des anémone (proxy de la qualité et de la dégradation l'habitat parental), affectent le phénotype des jeunes poissons. J'ai ainsi constaté que les parents vivant dans des habitats plus grands produisent des œufs plus grands. Au contraire, les parents qui vivent dans des habitats dégradés produisent des œufs et larves plus petites mais qui nagent plus vite (Chapitre 3). De plus, j'ai exploré les effets transgénérationnels et développementaux de la courantologie sur la morphologie, la physiologie et la survie des larves et des juvéniles, et observé qu'un fort courant autour des anémones influence négativement la survie des juvéniles. Ce résultat suggère notamment que l'investissement parental peut dépendre des conditions environnementales (Chapitre 4). Dans la dernière partie de ma thèse, je me suis intéressée aux effets d'expositions chroniques à deux stress environnementaux, le blanchissement des anémones (Chapitre 5) et la présence de lumière artificielle la nuit (ALAN, Chapitre 6), sur le phénotype et la survie des juvéniles d'*A. chrysopterus* en milieu naturel. J'ai démontré que le blanchissement des anémones affecte la physiologie et le comportement des poissons mais pas leur survie. Bien que des compromis entre croissance et survie peuvent se mettre en place, une réduction de croissance durant ces premières phases du cycle de vie pourrait avoir des effets à plus long terme sur leur fitness. De plus, j'ai observé que l'exposition à ALAN affect la survie des poissons et réduit leur croissance. Ma thèse permet donc de mieux comprendre comment et quels sont les facteurs et stress influençant le processus de dispersion et le phénotype post-installation chez un poisson emblématique des récifs coralliens. Ces connaissances aident à mieux prédire comment les populations de poissons réagissent aux changements climatiques. De telles prévisions sont essentielles aux stratégies de conservation, et permettent de cibler les zones marines et les phénotypes des poissons les plus importants à protéger.

MOTS-CLÉS

Facteurs de stress environnementaux, traits larvaires, plasticité phénotypique, poissons de récif coralliens, effets parentaux, survie

Acknowledgements

Many people have been involved in this exceptional journey, some are acknowledged at the end of each chapter but many others helped me throughout my thesis, it is with huge pleasure that I express my gratitude to all of them. I am very grateful to my external examiners Pr. Peter Buston (University of Boston) and Pr. David McKenzie (Marbec, UMR9190, Université de Montpellier) who reviewed this thesis and accepted to be part of the committee. I also thank Pr. Jean Clobert (SETE, UMR5321), Pr. Sinead English (University of Bristol) and Pr. Delphine Legrand (SETE, UMR5321) who accepted to be members of my PhD jury.

I would like to thank my supervisors, Suzanne C. Mills and Ricardo Beldade, for welcoming me in the team and offering the opportunity to do the PhD, but also for mentoring and trusting me during the entire course of my PhD. Thank you for involving me into this journey and for all the brilliant ideas that appeared like mushrooms! Thank you, Suzie, for all what I have learned from you, for having always believed in me, for your unwavering enthusiasm and determination in any project. Thank you for all the fun you always brought on the boat when coming in the field, it has been a great pleasure. Thank you, Ricardo, for your support, for your field experience and anemonefish knowledge that you passed to me. Thank you also for coming many times in the field with me and the students, and thank you for the confidence you placed in me from the beginning... I still remember the first time you asked me to drive the pickup and trailer in reverse in Papetoai, with the boat on the trailer!

I would like to express a huge thank to Shaun S. Killen for accepting to be my co-supervisor, for sharing your exceptional knowledge on fish physiology, for welcoming me in Glasgow as part of the team and for all the support and trust you have always given and you are giving to me.

Thank you to Annaïg LeGuen and Serge Planes for accepting and welcoming me at CRIOBE research station.

I would like to thank Tommy Norin for teaching me the first steps in fish physiology and with the swim tunnel. But thank you also for your important contribution in our paper and of course for the many beers we have shared. Thank you Amélie Crespel for your precious help in our collaborative works in Moorea as well as your mentorship at the University of Glasgow, although the project I carried out in Glasgow is not included in the thesis manuscript it has been an important step for my professional growth and a wonderful experience. Thank you, Steve Swearer, for your help and knowledge with the ALAN project, it has been a pleasure to collaborate with you.

I would like to especially thank all master students who have importantly participated in the projects involved in this PhD: Clara Diaz, Ignacio Pita Vaca, Marie-Louise Elian, Camille Vizon, Sam Manning and Jules Schligler. Your help in the field, free- and scuba-diving, driving the boat, catching anemonefish, swimming in the ocean with tiny larvae, being stinging by anemones, and many other adventures at any time of day, and even at night for some of you, has no price. All this work would have not been possible without you.

A great thank goes to Till Deuss and Robin Mannion for being the daddies of clownfish larvae, behind all this work there is your invaluable help with larval rearing. I would also like to thank all other CRIOBE technicians for their support in the field and at the research station. Thank you very much Marguerite Taiarui, Yannick Chancerelle, Anne Haguenauer, Frederic Zuberer, Frank Lerouvreur and Nao Nakamura. Thank you, Gilles Siu, for your help at CRIOBE and friendship, and for all the beautiful dives we shared in Tahiti. Thank you, Pascal Ung, for your generosity and ingenuity for solving any kind of mechanic problem.

Another important thank goes to all those people from the community of CRIOBE that helped me in the field but also at CRIOBE, for cooking, taking care of the community and for all the fun parties that we shared. Thank you, Zoe Scholz and Tara Cousin, for your important contribution in the field. I can't thank enough Camille Gache, Jason Vii, Jérémy Carlot and Yann Lacube, for your precious help in the field of course, but especially for being my family during my years in French Polynesia, I will never forget our hut. Chiara Pasqualetti and Lara Carosso, you are my family everywhere I go, thank you for coming on the other side of the world for helping me in the field, it has been a wonderful experience that I will remember forever, but thank you also for being with me in my best and worst moment during my writing period, despite the distance and the lockdown, without you and our sport sessions this thesis would not have been possible. Thank you also to Jennie Pistevos for your help during my difficult moments but also for being an exceptional housemate and for your love to animals that you bring in the house, a special think goes to Tupa, Aito and Honu. Thank you to Alex and Isla for starting this adventure in Moorea with me and for re-meeting us at the end this journey, this has been a beautiful unexpected surprise. Many other special people have been part of my PhD life with help both in the field and in the community life, thank you Nina, Ian, Mélissandre, Niké, Anne, Emma, Harry, Tim, Lisa, Pierrick, Shamy, Margaux, Quentin, Hendrikje, Philippa, Chérine, Maëlle, Caro and Alex, Gonzalo, Jeremie and Robin. I would also like to thank all those people who went at least one time in the field with me and those who lends me material for data collection, in few words thank you to all the CRIOBE Staff and Communities of the last three years! A big thank you also to all people from the Killen lab for the insightful

discussions and help on my side project I carried out there and which, although is not included in the manuscript, was part of my professional growth.

Thank you to Jan Lindstrom, David Bailey, Paul Johnson, David Pascall, Paolo Domenici, Bruno Frédérich and Jacob Johansen for the interesting scientific discussions.

Thank you Elina Burns for your generosity and big heart. Thank you to Nathalie and Aurelie for your availability and patience with all my bureaucratic questions.

A special thank also goes to Polynesian people, for their enthusiasm and smile. Thank you for the countless help I received whenever I had a problem with the boat, the trailer, my car, hitch-hiking and probably may other forgotten occasions. Marie-Jo, Maggy and Sandra you have a place in my heart, thank you for all the fun and laugh we had together.

And last but not least thank you to my family, you have always believed in me, this achievement is also yours. Thank you, Dario, Gabrielle, Noah, Aurelio, Taddeo, Edess, Sarina and Simina. Thank you also to Anne and Jean-Luc for welcoming me in your home and for bringing me in holiday, that has been an important break. A very unique thank goes to Marc, you are my anchor. Thank you for being part of my life, you have been my best encounter in my PhD. Thank you for your precious help in any of my steps, i.e. in the field, but also in the lab and in the process of my manuscript writing, and thank you for your immense support every time, everywhere. I simply love you.

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Chapter 1: General introduction

1.1. Context

Evolution is the change in organisms over time by the process of natural selection (Darwin 1859). Selection should act on favourable and heritable traits that increase fitness in a given environment. Evolution has led to the great diversity of living organisms and their adaptations to a large variety of environments. In the oceans, the majority of species have evolved to have a bipartite life cycle, which includes a larval dispersive stage and an adult stage (Thorson 1949; Cowen et al. 2000; Leis and McCormick 2002). These two stages are different ecologically (food resources, predation pressures and often habitat) and morphologically (Ebenman, 1992; Webb, 1999), due to millions of years of evolution under different selective pressures. The larval dispersive stage plays a major role in both the dynamics and evolution of spatially structured populations, in their global persistence despite local extinction, and in the tracking of favourable environmental conditions (Kokko and López-Sepulcre 2006; Ronce 2007). Today, under global change and novel environmental stressors, it is relevant to understand if and how dispersive stages are changing, as well as the post-dispersal repercussions to organisms.

1.1.1. Dispersal

Dispersal is defined as the movement of individuals that lead to gene flow, and natal dispersal refers to the movement of individuals from their birth place to a new location where they will reproduce (Ronce 2007). Dispersal is best seen as a multi-deterministic and plastic trait (Clobert et al. 2009). Several studies on a classic model organism, the common lizard, highlight how different factors spread across temporal scales that encompass ancestral, maternal and context (or environment), can change the dispersal outcome of young lizards. Dispersal in the common lizard can be affected by maternal condition (Meylan et al. 2004), parasitic load (Sorci et al. 1994), by the social context (Le Galliard et al. 2003) or the behaviour of conspecifics (Boudjemadi et al. 1999). The notion that dispersers are not individuals randomly drawn from a population, moving across a landscape at fixed rates, has arisen after a few decades of dedicated dispersal studies (Cote et al. 2017). Rather, dispersal depends on external factors (environments) and internal factors (individual phenotypes), i.e. context- and/or phenotype-dependent dispersal, at each of three dispersal steps: departure (emigration), transience, and settlement (immigration) (Holt 1987; Clobert et al. 2004, 2009; Armsworth and Roughgarden 2005; Bowler and Benton 2005; Benard and McCauley 2008; Delgado et al. 2010) (Fig. 1.1).

Examples of external factors include: parental influence (Meylan et al. 2004; Duckworth 2009), competition with conspecifics (Cote and Clobert 2007), habitat quality (Clobert et al. 2009; Cote et al. 2017), water currents or wind (Cowen and Sponaugle 2009). Internal factors, which are represented by the individual's phenotype and include a combination of morphological, physiological and behavioural traits that influence their dispersal, e.g. the morphology of insect wings (Harrison 1980; Zera and Denno 1997), flight metabolic rates (Hanski et al. 2004; Wheat et al. 2011) or the tiptoeing behaviour of wolf spiders that promotes wind dispersal (Bonte et al. 2006). Among internal factors influencing dispersal there are trade-offs between the costs and benefits of dispersal, which drive the evolution of dispersal (Bowler and Benton 2005). Furthermore, it is unclear the extent to which dispersal in nature arises from direct selection for dispersal or as a by-product of selection on traits with other functions (Burgess et al. 2016). The cost of dispersal may be direct or indirect. Direct costs include the loss of individuals during displacement (mortality), the metabolic energetic costs of movements, the energetic cost for development of specific organs or structure that enhance dispersal (such as muscle, wings or fins) and the risk of settling into an unfavourable habitat. Whereas, indirect costs of dispersal refer to the costs of expression of dispersal-associated phenotypic traits (Bonte et al. 2012; Addis et al. 2019). Nevertheless, dispersal-associated phenotypic traits (morphology, behaviour and physiology) are suggested to overall reduce the costs of dispersal (O'Riain et al. 1996; Sinervo et al. 2006; Bonte et al. 2012).

External determinants can directly act on dispersal, for example the environment can affect the vehicle of transport (e.g. wind or water current) or the quality and availability of habitat for settlement (Wilson et al. 2006; Gordon et al. 2018). But external factors can also indirectly influence dispersal via effects on internal factors. Several mechanisms may be involved in the determination of larval dispersal or dispersal-associated traits, such as heritability, parental effects and phenotypic plasticity throughout development. First, phenotypes related to dispersal may be transmitted across generations via genetic inheritance (Saastamoinen et al. 2018). Non-genetic parental effects through parental behaviour or cytoplasmic transfer (e.g. hormones, egg yolk, etc.) may also be involved in determining offspring dispersal propensity (Bernardo 1996a). Finally, the environment can indirectly affect individual's dispersal via phenotypic plasticity, e.g. the development of alternative phenotypes across environments without any change in the genotype (West-Eberhard 1989; Uller 2008).

The determinants of the dispersal process can act on each dispersal phase and are highly variable across taxa. For example, contrary to terrestrial animals, where the departure of dispersers is often determined by the decision to leave (Cote and Clobert 2007; Clobert et al.

2009; Bestion et al. 2015), in fish, departure is generally obligatory and partially determined by external factors, such as stochastic forces and environment (Cowen 2002), or by parents through parental effects (Nanninga and Berumen 2014). Indeed, parents are responsible for the timing and location of spawning (Cowen and Sponaugle 2009; Schilling et al. 2020) and via fecundity they can regulate the number of dispersing individuals (Hislop 1988). The effect of internal and external factors in each dispersal phase is described below (sections 1.2 and 1.3).

1.1.2. Post-settlement

At the end of the dispersal phase organisms settle into a new location. While phenotype-habitat matching is recognized in many terrestrial animals, there are many marine fishes for which post-settlement dispersal carries a considerable mortality risk (e.g. Morgan, 1995). Given this impediment, the fitness costs of habitat phenotype mismatch may be compensated for by organismal phenotypic plasticity (Edelaar et al. 2008). Evaluating the intraspecific ability to cope with environmental heterogeneity that characterizes many natural systems is vital, as well as determining such effects across different life stages (Dahlke et al. 2020). Furthermore, environmental effects on early life phenotypes can have carry-over effects on later stages and on subsequent generations (Burton and Metcalfe 2014). For example, in an African cichlid, individuals that experienced poor environments during their juvenile stage invest more in their own offspring, increasing their body size to prepare them for the adverse conditions they will experienced as juveniles (Taborsky 2006). Therefore, understanding how juvenile physiology and behaviour is affected by environmental heterogeneity, natural or anthropogenic, including whether they are able to acclimate to such variability and how the correlation among traits varies among environments is vital for a better understanding of human-induced global changes on future generations, and on populations dynamics (Pechenik et al. 1998; Burton and Metcalfe 2014).

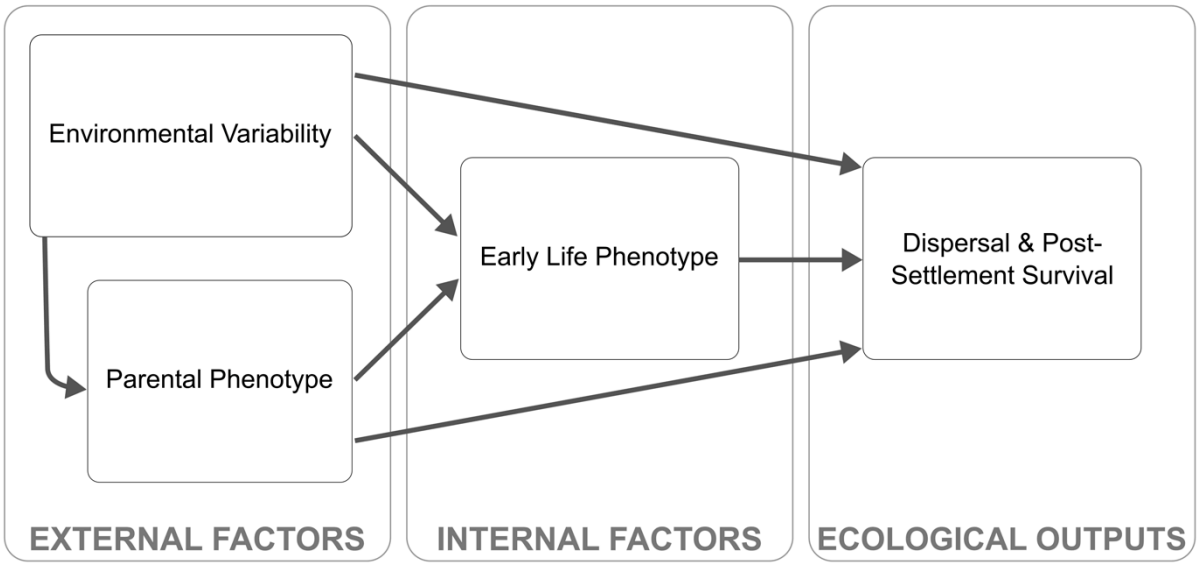

Figure 1. 1. Schematic representation of the relationships between the multiple determinants of traits associated with dispersal and post-settlement survival processes. Arrows indicate the effects of biological and ecological processes on one another.

1.2. External factors

1.2.1. *Natural* environmental changes

The natural environment is dynamic in space and time. Natural environmental changes are considered here to be those that do not directly depend on human action. Physical and biological factors can fluctuate generating variability. Ecosystems can be more or less stable (Pimm 1984) and species are continuously challenged by this variability and cope with it in a variety of ways. They can respond by adjusting their geographic distribution via dispersal (Hill et al. 2002) or by adapting via microevolution responses (Hofmann and Todgham 2010; Donelson et al. 2011). Species can also acclimate to these changing ecosystems via phenotypic plasticity (West-Eberhard 1989; Charmantier et al. 2008; Uller 2008). For example, aphids are able to develop dimorphic phenotypes, one adjusted to dispersal and the other to reproduction, depending on their environment (Braendle et al. 2006). Environmental cues, such as density of conspecifics, interactions with other aphid species, quality of the host plant and abiotic factors affect aphid morphology and physiology with the production of wings in the dispersal morph (Braendle et al. 2006). In a plant species, *Crepis sancta* (Asteraceae), mothers produce more seeds immediately after exposure to environmental stressors, and increased the proportion of seeds with dispersal structures (Imbert and Ronce 2001).

Habitat quality variation has for a long time been recognized as being linked not only to dispersal propensity, but also to (post-dispersal) fitness effects (e.g. Lin and Batzli 2001),

and finally to population persistence (Heinrichs et al. 2016). Habitat quality, with habitat loss and fragmentation, are now recognized as major threats to population persistence and biodiversity (Fischer and Lindenmayer 2007; Alvarez-Filip et al. 2015; Heinrichs et al. 2016). Yet, in the marine realm there are few examples of the impacts of habitat quality on fitness (but see e.g. Pereira and Munday 2016). The immediate effects of habitat quality change in fish, besides survival (e.g. McCormick, 2012; Wilson et al., 2008), are not yet completely clear.

Evidence of environmental variation in mechanisms aiding passive dispersal of mostly small life forms, such as wind and water flow, spans multiple taxa and global geographic locations. Examples encompassing global dispersal of microbes (Finlay 2002), resting stages of aquatic invertebrates over large expansions of dry land (Carroll and Viglierchio 1981; Incagnone et al. 2015), long-distance seed dispersal (Cain et al. 2000), etc., bear testimony to the relevance of natural environmental variability for dispersal outcomes and ensuing population dynamics, colonization of new habitats, etc.. In oceans, the small size of larvae, the main stage responsible for long distance dispersal in many fish, likely relates to the (predominantly) passive dispersive mode exhibited by the initial life-stages (eggs and larvae) of some fish. Variation in water current velocity induces phenotypic changes in many fish species (Sagnes et al. 2000; Svanbäck and Eklöv 2003; Ohlberger et al. 2006; Páez et al. 2008). Whether the changes induced are passed on to the next generation and whether that confers any survival advantage to the offspring living across different levels of the environmental spectrum is still to be determined. Indeed, processes such as post-dispersal selection or plasticity could play a role in determining the spatial distribution of such phenotypes (see Parental phenotype section).

1.2.2. Anthropogenic environmental changes

Over the last century increasing new threats associated with anthropogenic impacts are emerging, impacting marine and terrestrial ecosystems worldwide (Chapin et al. 2000; Halpern et al. 2008; Jenny et al. 2016; Hughes et al. 2017). Anthropogenic environmental changes are modifications that directly or indirectly depend on human intervention. One of the most diverse ecosystem in the world are coral reefs, but they are also one of the most susceptible to anthropogenic habitat modification. For example, coral cover has generally declined globally over the past few decades (Hughes et al., 2003; Walther et al., 2002). Predictions of warming oceans, ocean acidification and increases in the frequency and severity of storms suggest a further degradation of hard corals and other invertebrates such as sea anemones that provide essential resources for the organisms that live on coral reefs (Carpenter et al. 2008; Pandolfi et

al. 2011; Beldade et al. 2017). Several anthropogenic environmental changes are predicted to increase in frequency and severity (Donner et al. 2005; Anthony et al. 2011) and while major cascading changes in coral reef associated fish communities have been documented, the mechanisms underlying these changes are still poorly understood (e.g. McCormick, 2009).

Anthropogenic environmental changes may directly or indirectly affect animals at different stages of their life cycles. For example, it has been shown that climate change can cause a shift in the phenology of species (e.g. plants: Bradley et al. 1999; birds: Cotton 2003; butterflies: Kearney et al. 2010), or shift their latitudinal range (Stirling et al. 1999; Parmesan 2006). In coral reef ecosystems, global change causes increasing bleaching events, which in turns decreases fish fecundity (Beldade et al. 2017), and increases habitat degradation and fragmentation (Fahrig 2003). Larval dispersal-associated traits may be modified by phenotypic plasticity as a response to the local environment or through plasticity of parents affected by the environment they experienced (West-Eberhard 2003; Uller 2008). Finally, after settlement, juvenile organisms can still be affected by the changing environment. Therefore, understanding how the early life phenotype is affected by the environment directly or via parents and how they cope with such stressors in the wild is essential to better predict of the dispersal process and population dynamics under environmental change.

1.2.3. Parental phenotype

Dispersal-associated traits are highly variable within and among species and are generally heritable (Saastamoinen et al. 2018). Dispersal is the result of complex interactions among traits, each determined by one or more genes (Saastamoinen et al. 2018). Phenotypic variation may also arise from non-genetic parental effects and environmental variation, including transgenerational and epigenetic effects (Sinervo et al. 2006; Van Petegem et al. 2015). Parental effects include all non-genetic effects associated with parental phenotype and their surrounding environment (Mousseau and Fox 1998; Green 2008). Parental effects cascade in offspring, affecting offspring quality. Parental effects include hormonal transfer, immunological transfer, epigenetic modification of gene expression in germ cells, but also parental-offspring behavioural interactions (Mousseau and Fox 1998; West-Eberhard 2003; Badyaev and Uller 2009). For example, parental care can facilitate offspring development and survival (Bernardo 1996a; Green and McCormick 2005), growth (Dugas et al. 2016) or the development of social behaviour (Kolliker et al. 2012). In fish, increasing evidence suggests that maternal size enhances the quantity of offspring (Sogard et al. 2008; Barneche et al. 2018). Larger mothers

in wild fish populations have been shown to produce more self-recruits (Beldade et al., 2012), although the mechanisms by which this is achieved are not yet known.

Parental effects may be modulated by the environment. Environmental factors may include physical or biological factors. Biological factors may be nutrition resources or the interaction or competition with conspecifics or heterospecifics. While examples of physical factors are temperature, habitat complexity, the quality of their habitat in terms of pollutants and size, or for marine species, the water flow environment where they live. The first response to environmental stressors in vertebrates includes activation of the endocrine system with production of glucocorticoid hormones (e.g. cortisol) (Wingfield 2008). In wild anemonefish, prolonged anemone bleaching can increase fish cortisol levels, leading to reduced fecundity (Beldade et al., 2017). Effects of the stress response can also result in reduced offspring size (Saino et al. 2005; McCormick 2006). Parental reproductive traits may also be plastic (West-Eberhard 2003) and different reproductive strategies can arise in response to environmental variation (Barbasch et al. 2020).

1.3. Internal factors: Biological traits associated to dispersal

Increasing empirical and theoretical evidence have shown how internal factors (phenotype) and its interaction with external factors largely determine dispersal patterns and outcomes (Clobert et al. 2009; Cowen and Sponaugle 2009; Cote et al. 2010, 2017; Lowe and McPeek 2014; Nanninga and Berumen 2014). Not accounting for larval traits may lead to poor modelling estimation of dispersal (Staaterman and Paris 2014). Experimental studies have demonstrated the presence of dispersal dimorphisms, non-random variation in dispersal traits (Marshall & Keough, 2003, 2008; Raimondi & Keough, 1990; Ronce, 2007) and the existence of covariation patterns in morphological, behavioural and physiological traits described as dispersal syndromes (Ronce and Clobert 2012; Stevens et al. 2014). In the following paragraphs, I will discuss about larval traits associated with dispersal in fish and draw some comparisons from other biological models, ranging from plants to vertebrates.

1.3.1. Morphology

In both terrestrial and aquatic species, individuals that disperse may display specific morphological phenotypes in order to enhance dispersal capabilities. The variation in forms associated with dispersal is almost ubiquitous, for example some plants develop dimorphic propagules to ensure transport by specific vectors and reach different habitats (e.g. Sorensen 1978). Other species may develop forms that ensure dispersal by multiple vectors, including

water, wind and migratory animals (Nathan et al. 2008). Insects develop a wide range of movement associated features, such as wings (Harrison 1980; Zera and Denno 1997); spiders can produce ballooning silk that ensures dispersal (Schneider et al. 2001; De Meester and Bonte 2010); and mole rats can develop larger phenotypes with a higher percentage of body fat (O'Riain et al. 1996). In marine animals, larvae also have morphological adaptations that allow them to survive and disperse under very different environmental conditions to those experienced by adults. For example, larval fish have a transparent body, presumably to minimize their appearance to predators (Hobson and Chess 1976; Morgan 1995), some have relatively developed eyes for visual perception, and a gut and mouth adapted to their planktonic diet (Webb, 1999). Among morphological traits, larval size is particularly important as it generally correlates with predator avoidance and foraging (Pechenik and Cerulli 1991; Leggett and Deblois 1994; Leis and McCormick 2002; McCormick and Hoey 2004). Larger larvae may also have better swimming endurance (Videler 1993), settle earlier, and be more competitive for finite resources (Arendt 1997; Sogard 1997). During the transient phase, larvae experience tremendous mortality (e.g. Morgan, 1995), and size and growth may enhance survival and have important implications for the dispersal process. Small changes in larval mortality rate can also lead to significant difference in number of recruits (Houde 1989; Underwood and Fairweather 1989).

1.3.2. Behaviour

As opposed to plants, where, due to its passive nature, dispersal strategies focus on the diversity of a propagule's morphology coupled with dispersal vectors, e.g. wind and animals (Nathan et al. 2008; Ruxton and Martin Schaefer 2012), animal behaviour can significantly influence the departure, distance and the final destination of dispersal (Cote et al., 2010). Empirical studies suggest differences in exploration, boldness, aggressiveness and sociability between residents and dispersers within a species. Dispersers generally tend to be bolder in a wide range of taxa (Fraser et al. 2001; Cote et al. 2010) and more prone to take risks (Cote et al. 2010), often being also less social and aggressive (Cote et al. 2010; fish: Schradin and Lamprecht 2002; mammals: Blumstein et al. 2009; instects: Guerra and Pollack 2010). However, the correlations between behavioural traits and dispersal may change direction depending on the species, dispersal strategies or external factors, such as population density or habitat quality (Haughland and Larsen 2004; Cote and Clobert 2007; Cote et al. 2008, 2010; Clobert et al. 2009). In marine larvae, the behavioural propensity to move may have important consequences on dispersal outcomes (Cowen 2002; Fiksen et al. 2007; Paris et al. 2007; Cowen and Sponaugle 2009;

Nanninga and Berumen 2014; Faillettaz et al. 2018). Indeed, even small vertical movements of coral larvae, which have a limited capacity to move, can determine the vertical position in the water column, reducing the potential negative buoyancy that would cause larvae to sink (Guizien et al. 2020). The effects of movement on dispersal are exacerbated in fish larvae that display a greater mobility capacity, especially in coral reef fishes (Leis and McCormick 2002). Coral reef fish larvae are known to orientate (Leis et al. 2007; Irisson et al. 2009) and develop swimming capabilities early in their development (Fisher et al. 2000). In later larval stages, they are even able to move faster than average current speeds (Fisher et al. 2005). Swimming abilities may also reflect their ability to escape predators and to feed, which may indirectly affect dispersal by reducing its cost and increasing successful recruitment (Fisher et al. 2000; Leis and McCormick 2002; Fisher 2005; Leis et al. 2007; Nanninga and Berumen 2014). Active settlement behaviour requires both swimming ability and the development of sensory abilities to enhance the likelihood of finding a suitable habitat patches (Kingsford et al. 2002; Simpson et al. 2004).

1.3.3. Physiology

Organisms have a finite energy budget, with allocation trade-offs among processes including growth, activity, digestion, and reproduction (Weiner, 1992). The intake, processing, and dissipation of energy over time by an organism is its metabolic rate. In ectotherms, standard metabolic rate (SMR) is the lower limit of metabolic rate and represents the minimum energy necessary to sustain life. The upper limit of aerobic energy use is the maximum metabolic rate (MMR). The difference among MMR and SMR is the aerobic scope (AS), i.e. the capacity to perform simultaneous oxygen-consuming physiological functions above those required for maintenance. Metabolic rate varies with body-size and temperature (Hayes and Garland Jnr 1995; White and Seymour 2004; White and Kearney 2013), but it is also known to be highly variable among species, among individuals within species, and with ecological context, even after controlling for temperature and body mass (Burton et al. 2011; Killen et al. 2016).

Locomotor activity is energetically costly. Some organisms adjust their physiology to cope with the increased activity costs of dispersal (Haag et al. 2005). Thus, the ability of larval fish to swim rapidly, may be advantageous for survival but is also associated with an increased energetic demand (Boisclair and Tang 1993). Larval fish also grow rapidly during development, which again is energetically costly and environmentally mediated (Barry 2014). After settlement, the partitioning of energy to different functions is crucial, considering that competition, predation, and food acquisition all remain challenges for survival. Correlations

among traits may change under different environmental conditions (Killen et al. 2013) and understanding how traits and their correlation change under modified environments remains an important objective for predicting the effects of environmental change on natural populations.

1.4. The biology of coral reef fish

1.4.1. Coral reefs

Coral reefs ecosystems occupy less than 0.1% of the earth surface but are the most diverse ecosystem per unit area, hosting about a quarter of all marine fish species (Spalding and Grenfell 1997; Spalding et al. 2001). Coral reefs have tremendous economic value because of their large productivity, species richness, and aesthetic beauty. About 10% of fish consumed by human populations originate from coral reefs. They are also an important tourist attraction and protect the coastlines of hundreds of countries from erosion (Moberg and Folke 1999). On the one hand, coral reefs host a large variation in local environments, due to the topographic complexity, to the large diversity of corals that with their skeleton contribute to habitat complexity, to the temporal and spatial variability of water motion and current (Lenhardt 1991; Hearn 2011; Leichter et al. 2013), and to the diversity of species and biological interactions. On the other hand, other physical factors such as temperature and salinity are relatively stable in coral reefs compared to temperate areas where seasonal fluctuations are higher. Coral reefs are therefore particularly vulnerable to global climate change. Increasing ocean warming is causing widespread and more intense events of coral bleaching, i.e. the loss of symbiotic dinoflagellates (Hughes 2017) with associated mortality and habitat degradation (Graham et al., 2007; Wilson et al., 2006). Local disturbances and pollutants contribute to global change and affect the resilience capacity of coral reefs (i.e. the capacity to restore an ecosystem to initial levels after a disturbance) (Anthony et al. 2011). Species living in such ecosystems maybe at a higher risk from climate change.

1.4.2. The study species: *Amphiprion chrysopterus* and population dynamics in Moorea

Anemonefish live in symbiosis with sea anemones. The fish provide the anemone with protection from predators, oxygenation and nutrition; while the anemone provides protection, oviposition sites and food resources (Allen 1972; Fautin and Allen 1992; Verde et al. 2015). Indeed, anemonefish nutrition is based on plankton in the water columns, but also anemone waste products and symbiont algae (Verde et al. 2015). Due to adaptations arising from co-

evolution (Feeney et al. 2018), anemonefish can live among anemone tentacles without being harmed (Mebs 1994; Nedosyko et al. 2014). Anemonefish form social groups with size-dominance hierarchies within their territory (Buston, 2003b; Fricke, 1979). Territory size varies depending on the species, e.g. the territory of *A. percula* is generally represented by one anemone (Buston 2004a, 2004b; Seymour et al. 2018), while that of *A. chrysopterus* is generally made by more than one anemone (e.g. see Chapter 3).

Within each group, the alpha-dominant rank is occupied by the female, i.e. the largest individual, the male is the second largest individual and holds the beta-dominant rank. In addition to this, there are often 2-4 subordinates (gamma-rank) which are not sexually mature (Buston 2003a; Buston and Cant 2006). Fish growth is regulated by their social rank (Buston 2003a). Females and males live in monogamous breeding pairs and are protandrous hermaphrodites (Fricke and Fricke 1977; Moyer and Nakazono 1978). When the female dies, the breeding male changes sex and increases in size as growth suppression disappears (Hattori and Yanagisawa 1991), allowing it to take the alpha-rank, while the largest subordinate matures sexually and becomes the new breeding male (Buston, 2003b, 2003a).

The model species of this thesis is the orange-fin anemonefish, *A. chrysopterus,* the only anemonefish species in French Polynesia. I conducted a long-term monitoring of the *A. chrysopterus* population around Moorea (French Polynesia) which allowed me to assess its reproduction. Similar to other anemonefish species, *A. chrysopterus* reproduces throughout the year following a lunar cycle (0-2 and occasionally 3 spawns per lunar cycle). The spawning frequency is comparable to other anemonefish species (e.g. *A. percula*, Buston and Elith 2011; Seymour et al. 2018), while fecundity is generally higher (~1500 ± 660 eggs per clutch mean ± SD, n = 350). *Amphiprion chrysopterus* is one of the largest anemonefish species (Fautin and Allen 1992), which may explain the high number of eggs spawned per clutch (Hislop 1988). Before laying eggs, the male and female clear the nest site on a rock surface under the anemone tentacles. As the female lays the eggs, the male fertilises them, and tends and protects them throughout the incubation period (Buston and Elith 2011). The incubation period varies according to water temperature: in Moorea (French Polynesia) between 6 days in the summer and 7 days during the coldest months (Fig. 1.2). Egg developmental stages are easily recognizable by the change in colour and larval morphological characters (Fig. 1.3 and 1.4). After development eggs hatch into larvae that disperse for about 11-14 days in the open ocean (Schligler et al. *in prep*., Beldade et al. 2016).

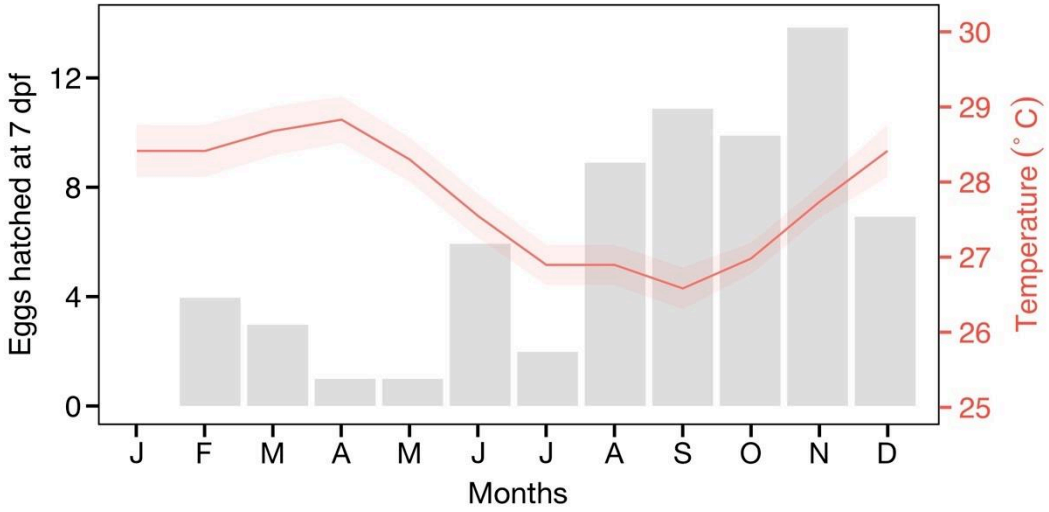

Figure 1. 2. Duration of the egg incubation period in *Amphiprion chrysopterus* throughout the year and as a function of lagoonal water temperature in Moorea (French Polynesia). Eggs observed to have hatched at 7 days post fertilization (dpf), over about ~720 nests spawned in 2 years.

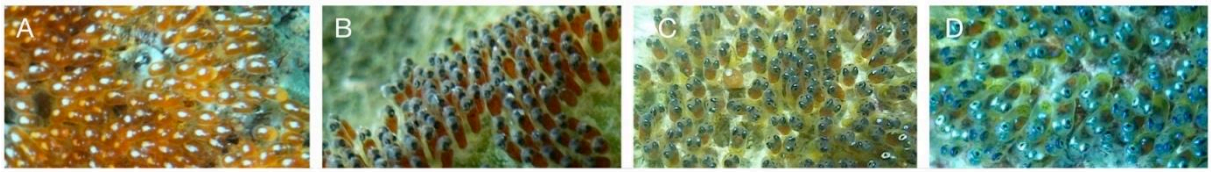

Figure 1. 3. Photographs of orange-fin anemonefish *Amphiprion chrysopterus* egg developmental stages at fertilization (A), 3-days post fertilization (B), 5-days post fertilization (C), and 6/7-days post fertilization, day of hatching (D).

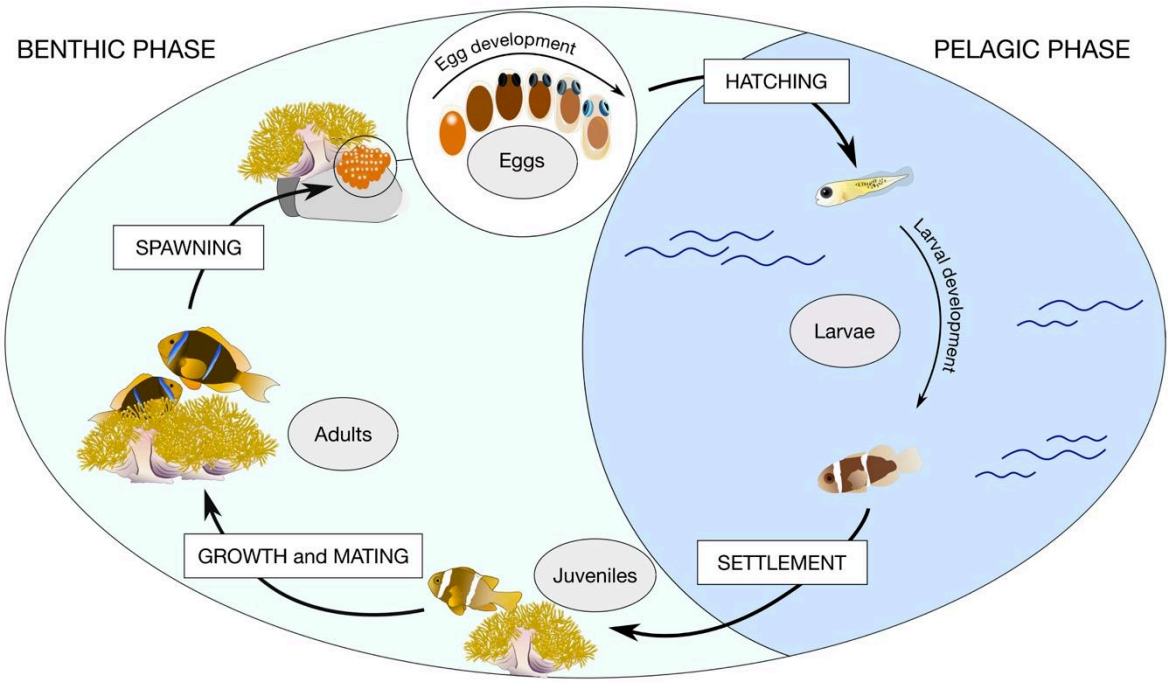

Figure 1. 4. Orange-fin anemonefish (*Amphiprion chrysopterus*) life cycle.

Anemonefish larvae are able to swim immediately after hatching (Fisher 2005) and they have been shown to settle at very different spatial scales, ranging from close to their nest location (Jones et al. 2009; Buston et al. 2012; Beldade et al. 2016), to hundreds of kilometers away from their origin (Simpson et al. 2014). After larval development in a pelagic environment, larvae metamorphoses and their acute sensory abilities allow them to locate a settlement habitat. After settlement, individuals are unlikely to move due to the high predation risk while outside of their anemone (Mariscal 1970; Elliott et al. 1995; Buston 2003b; Buston and Garcia 2007). In addition, they initially stay among anemone tentacles, and gradually venture outside the protection provided by the anemone in the following weeks (Ross 1978a).

During the monitoring of 70 anemonefish pairs around Moorea, two peaks of spawning were identified around the full moon (Fig. 1.5, Table 1.1), which result in two peaks of hatching, one around the full moon and a second peak around the new moon. As a consequence, settlement also resulted in two peaks, both around the new moon. Settlement around the new moon, during minimum intensity of moonlight is a strategy generally adopted for limiting predation (e.g., Acosta and Butler IV 1999). This strategy generally comes at the cost of exposing the benthic eggs and newly hatched larvae to potential predators during the brighter nights of full moon. However, during egg development they are generally protected by parents during the day and by anemone tentacles who can protect at night as well. When breeding pairs

lay twice per lunar month oogenesis is shorter, especially for the second clutch, and it is not clear if this may have an effect on fecundity or on offspring traits. In *Drosophila* , for example, an increase in egg size seems to be related to longer oogenesis (Fox et al. 1997). Therefore, we tested whether the moon peak of spawning (first or second) had an effect on fecundity and offspring traits, such as egg size, larval size and larval swimming speed. The test was performed on fecundity and offspring traits data gathered from breeding pairs who laid twice in the same lunar month (see chapters 2-3 for a detail description of the methods used for egg collection from the field, larval size measures and larval swimming speed trials). Results show that fecundity is not affected by moon peaks (LMER: peak(2); $t = 1.273$, $p = 0.224$, $mR^2 = 0.192$, $cR^2 = 0.804$, Fig. 1.6A), while egg size and larval swimming speed decrease and increase respectively with the second moon peak (LMER (egg size): peak(2); $t = -6.378$, $p < 0.001$, $mR^2 = 0.106$, $cR^2 = 0.673$; LMER (larval swimming): peak(2); $t = 1.793$, $p = 0.008$, $mR^2 = 0.186$, $cR^2 = 0.242$, Fig. 1.6B, 1.6D). On the contrary, larval size, i.e. total length, did not vary, but the sample size was very low making difficult generalization (LMER: peak(2); $t = 1.175$, $p = 0.249$, $mR^2 = 0.038$, $cR^2 = 0.094$, Fig. 1.6C).

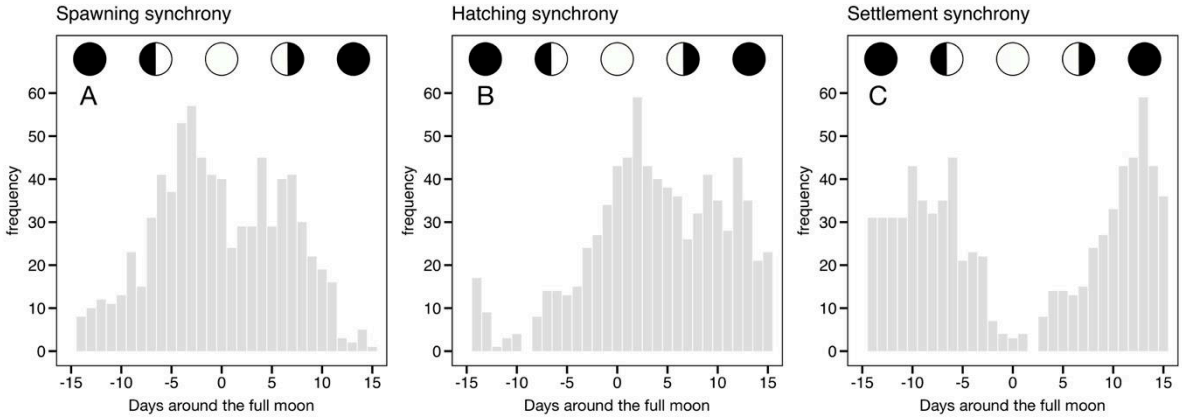

Figure 1. 5. Spawning (A), hatching (B) and settlement (C) synchrony of the wild population of orange-fin anemonefish in Moorea, French Polynesia. In total 772 spawning events from ~70 anemonefish pairs monitored over ~3 years were used.

Table 1. 1. Hartigans dip test of multimodality (deviance from unimodality)

	Peak 1		Peak 2		Hartigans dip test	
	Mean ± Sd	n	Mean ± Sd	n	D	p-value
Spawning	-5.36 ± 3.71	453	5.40 ± 3.06	319	0.033	< 0.001
Hatching	0.58 ± 4.04	454	11.25 ± 2.74	318	0.031	< 0.001
Settlement	-9.59 ± 3.57	397	11.09 ± 3.20	370	0.11	< 0.001

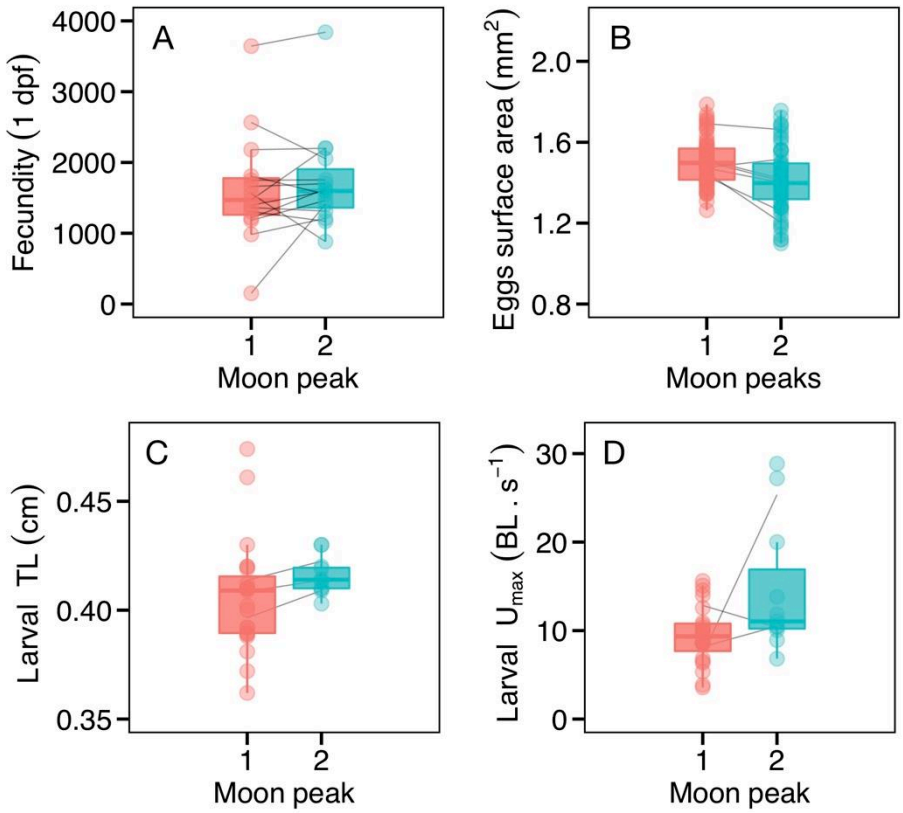

Figure 1. 6. Variation in anemonefish adult and offspring traits between the two moon peaks (1 = peak that occurs 5 days prior to the full moon; 2 = peak that occurs 5 days after the full moon): fecundity (A), eggs size (B), larval size, i.e. total length (C), larval swimming speed (D). Only data gathered from breeding pairs who laid twice within the same lunar month is shown. In offspring sites multiple measures within the same nest were collected. Dots indicates individual data points. Lines connect sites among the mean values of measures from peak1 and the mean value from measures on the peak 2 within the same site.

Anemonefish, being highly site-attached and with a size-dominance hierarchy, are good candidates for the study of parental and environmental determinants on larval phenotype in the wild. Indeed, females, males and recently-settled recruits can be identified by their size differences. In addition, due to the high predation risk outside the anemone's tentacles (Mariscal 1970; Elliott et al. 1995; Buston 2003b), fish are unlikely to move and individuals can be located and multiple measures can be taken on the same individuals over the course of weeks, months, and even years.

1.5. PhD Project presentation

Current environmental changes are damaging coral reefs ecosystems. Understanding of how populations may cope with this disturbance, including links with the larval dispersal phase of fish and the capacity of early-life stages to face environmental stressors is essential and timely. Dispersal-associated traits are important in determining dispersal outputs, and thus juvenile habitats, and are under selection by a variety of factors. However, how parents and the environment influence larval dispersal-associated traits in the wild has not yet been determined. Therefore, in this thesis, my main aims were to answer to these questions:

1) Can maternal phenotype (size) affect dispersal-associated larval traits? (Chapter 2)
2) Can environmental disturbance, via parental effects, modulate offspring dispersal associated phenotypes? (Chapter 3)
3) Are there developmental or transgenerational effects of water flow on *Amphiprion chrysopterus* early-life stages? (Chapter 4)
4) How do early life stages cope with human-induced environmental stressors, such as bleaching and artificial light at night in the wild? (Chapters 5 and 6)

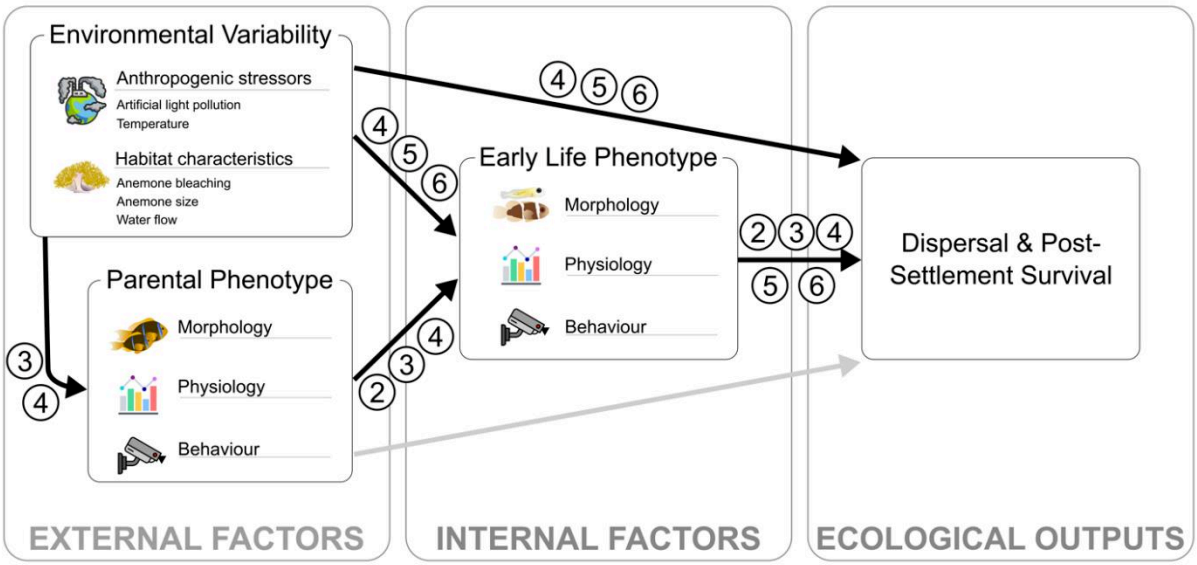

Figure 1. 7. Schematic representation of the relationships between the multiple determinants of traits associated with dispersal and post-settlement survival processes. Arrows indicate the effects of biological and ecological processes on one another. Black arrows indicate the effects that are studied in this thesis. Circled numbers indicate the thesis chapters that examine these effects.

In my thesis I used a combination of field and laboratory-based experiments, to answer the above listed questions. I was able to monitor anemonefish breeding pairs for a period of 3 years, to sample, collect, hatch eggs and rear larvae. The approach I used, entailing field-based experiments and wild individuals, integrated natural variability arising from selection and natural environmental variation offering a more realistic picture of natural processes. In chapters 2, 3, 4 and 6 I studied larvae and juveniles produced by a subset of breeding pairs belonging to the anemonefish population around Moorea. In Chapter 5, wild-caught juveniles were used instead. In addition, this thesis involved field manipulations of parental habitats (Chapter 3), and field-based experiments to take advantage of natural and manipulated environmental gradients (Chapter 3, 4 and 5). Finally, in each chapter laboratory measures were also carried out at CRIOBE research centre (Moorea, French Polynesia) on larvae, juveniles or adults. Different categories of data assessing morphology, physiology and behaviour allowed me to learn methodologies from different disciplines to answer questions related to ecology and evolution. The data gathered comprised laboratory measures of larval and juvenile swimming speeds using a swim tunnel system (Brett 1964), juvenile and adult metabolic rates using static intermittent-closed respirometry and swim tunnel respirometry (Clark et al. 2013; Svendsen et al. 2016), and morphological measurements from photographs under a binocular microscope.

Chapter 2: Larger mothers produce more and faster swimming offspring

2.1. Foreword

The objective of this chapter is to examine whether the parental phenotype determines larval traits associated to dispersal. Specifically, I investigated whether maternal size (standard length) affects larval size, swimming speed, and dispersion (active tracking) in a wild population of the orange-fin anemonefish *Amphiprion chrysopterus*, at Moorea, French Polynesia. For this chapter 35 breeding pairs were monitored two to three times per week, for a duration of up to three years. During these monitoring events, parents' sizes were measured, and the presence of eggs was surveyed, so that eggs could later on be collected on the day of hatching. Eggs were then brought back at the CRIOBE aquaria facilities, where hatching events occurred and dispersal-associated larval traits were measured (standard length and swimming speed). In addition, recently hatched larvae were released and tracked by scuba diving, to measure their dispersal patterns *in situ*.

This research therefore required intensive fieldwork and laboratory-based experiments. The project was conceptualised together with Dr. Ricardo Beldade and Dr. Suzanne C. Mills during my Master's Degree, when data collection also started (10 months prior to my PhD). This research was carried out in collaboration with Shaun S. Killen from the University of Glasgow and expert in fish ecophysiology. The project matured during my PhD and more than two years of my PhD were devoted to it (the first two years for fieldwork and laboratory analyses, and part of my third year for analyses and writing). I planned all the fieldwork and carried it out with help from Dr. Ricardo Beldade and two master students that I co-supervised with him and Dr. Suzanne C. Mills (Ignacio Pita Vaca and Marie-Louise Elian), and several other volunteers from the CRIOBE. The fieldwork involved the regular monitoring of breeding pairs, as well as larval release and tracking *in situ* in the lagoon of Moorea. Larval rearing was managed by Till Deuss and Robin Manion, with support from Ricardo Beldade, Marie-Louise Elian and me. I carried out most laboratory analyses (measurements of larval size and swimming speed) and I conducted all data analyses. I drafted the manuscript under the supervision of Dr. Ricardo Beldade.

2.2. Manuscript information

Title: Larger fish mothers produce more and faster swimming offspring

Authors: Cortese Daphne[1], Suzanne C. Mills[1,2], Ignacio Pita Vaca[1], Marie-Louise Elian[1], Shaun S. Killen[3], Ricardo Beldade[4]

[1] PSL Université Paris: EPHE-UPVD-CNRS, USR 3278 CRIOBE, BP 1013, 98729 Papetoai, Moorea, French Polynesia.

[2] Laboratoire d'Excellence "CORAIL"

[3] University of Glasgow, Institute of Biodiversity, Animal Health and Comparative Medicine, Graham Kerr Building, Glasgow, G12 8QQ, United Kingdom

[4] Estación Costera de Investigaciones Marinas, Departamento de Ecología, Facultad de Ciencias Biológicas, Pontificia Universidad Católica de Chile, Santiago, Chile

Manuscript status: submission planned for April 2021

Keywords: maternal size, maternal effects, larval traits, dispersal, larval swimming speed

2.3. Abstract

Fish population replenishment can be affected by the size of breeders. Beyond the hyperallometric relation between size and fecundity, it is not yet known what are the mechanistic reasons that sustain the disproportional importance of large breeding individuals in population replenishment. Differences in fecundity, spawning frequency and larval *quality* produced by a breeding size gradient can lend support to the outstanding value of large breeding fish in a population. In a wild population of the orange-fin anemonefish *Amphiprion chrysopterus*, an emblematic coral reef species, we explored the connection among fecundity, larval traits important for dispersal (size, swimming speed) and *in situ* dispersal in the wild. We show that (i) larger mothers not only produce more offspring resulting in a higher number of individuals that will hatch and disperse, (ii) offspring from larger mothers possess better swimming abilities for the most part of the larval dispersal phase; (iii) *in situ* movement of newly hatched larvae was not affected by maternal size but significantly different than that of a passive drifting larvae. The suit of differences found here in fecundity, larval swimming performance and dispersal reinforce the importance of larger fish in wild populations and are relevant for fisheries management and conservation policies.

2.4. Introduction

Global change and overharvesting cause fish sizes to decline (Sheridan and Bickford 2011; Garcia et al. 2012), and the size of breeding fish in a population is vital for management and conservation efforts (Le Bris et al. 2018). While the proportion of breeding individuals in a population or stock is a fundamental parameter in fisheries models the allometric increases in fecundity (Hixon, Johnson, & Sogard, 2014; Marshall, Needle, Thorsen, Kjesbu, & Yaragina, 2006) and increases in larval *quality* (Berkeley et al. 2004; Hixon et al. 2014) are not yet adequately considered in fisheries management and conservation strategies (Barneche et al. 2018). Here we relate fish maternal size to reproductive output (variation in fecundity, spawning frequency) and to offspring traits (egg size, larval size, locomotory abilities and dispersal in the wild) in the orange-fin anemonefish, to lend support to the outstanding value of large breeding fish to population replenishment (Hixon et al. 2014).

In many fish species, fecundity is known to increase hyperallometrically with maternal size (Barneche et al. 2018) but in which way spawning frequency depends on maternal size is not as clear. While some papers have found similar frequencies across maternal size classes e.g. (Farley et al. 2015); others have found an increase in spawning frequency for larger/older females e.g. (Claramunt et al. 2007). In which way the variation in larval traits can be explained by maternal effects such as energy allocation (Maddams and McCormick 2012; Stafford et al. 2014) on egg and larvae size (Berkeley et al. 2004; Green and McCormick 2005; Olin et al. 2012; Lim et al. 2014; Stafford et al. 2014) as well as offspring survival after settlement (Berkeley et al. 2004; Wright and Gibb 2005; Olin et al. 2012) are yet to be clarified. The concept of larval *quality*, linked often to their fitness, is thus multivariate and initially depends on their ability to successfully disperse to join a population.

Many marine fish have a pelagic larval phase during which they disperse. Dispersal is at the heart of population dynamics and species persistence (Kokko and López-Sepulcre 2006; Ronce 2007; Clobert et al. 2009) and understanding the ability to move is focal to its study (Wheat 2012). Fish ability to move is represented by its swimming capacities, which has a central role on fish performance, ultimately influencing its fitness and dispersal potential (Arnold 1983; Kingsolver and Huey 2003; Nanninga and Manica 2018; Majoris et al. 2019). Reef fish larvae have remarkably good swimming abilities, which at pre-settlement stages may even allow them to counter prevailing water currents (Leis and McCormick 2002; Fisher et al. 2005). Empirical studies of the larval phase in marine fishes are rare and particularly challenging given the small size of fish larvae at hatching (2.5-4 mm in length) (Bacchet et al. 2006). While common approaches used to track the movement of large animals cannot be

conveniently applied to such small larvae (Lett et al. 2010; Shima and Swearer 2016) alternative field-based tracking approaches have highlighted the variability in potential larval dispersal outcomes (Leis and Carson-Ewart 1997; Leis and McCormick 2002; Leis et al. 2007). However, in which way larvae disperse in the wild immediately after hatching is still poorly understood.

The model species, the orange fin anemonefish is part of the emblematic fish genus (*Amphiprion*) that after the dispersive phase live in symbiosis with sea anemones. Anemonefish are site attached, rarely change anemone host (Elliott et al. 1995; Buston 2003b), near which they lay their nests following a lunar or semi-lunar pattern (Seymour et al. 2018). In Moorea, incubation times last approximately a week while eggs undergo visible changes (Beldade et al. 2017), prior to hatch into larvae that disperse for approx. two weeks (Beldade et al. 2016). Wild laid eggs can be collected just prior to hatching and larvae easily reared in aquaria. This model species is used to specifically investigate the link between maternal phenotype (size) and reproductive traits (fecundity and spawning frequency) as well as offspring traits: eggs size, larval morphology and swimming performance throughout larval development (from hatching to nine days post hatching); and finally, in which way the maternal phenotype can influence larval dispersal in the wild.

2.5. Materials and methods

2.5.1. Field measurements and sampling

35 wild breeding orange-fin anemonefishes (*Amphiprion chrysopterus*) pairs, were monitored every 2-3 days in the lagoon of Moorea (17°30'S, 149°50'W) (Fig. 2.1), from November 2016 to February 2020. *A. chrysopterus* lay benthic eggs, following a lunar or semilunar periodicity, that hatch after 6 (±1) days and disperse for 9-14 days until they settle onto anemones (Beldade et al., 2016).

Parental traits and habitat characteristics. Both females and males from each pair were captured, photographed against an underwater slate with a scale bar and their total length (TL) determined with *Image J*. Female and male measurement were repeated at least a second time during the study period. As the period of the experiment is relatively short compared to the overall anemonefish life span (Buston and Garcia 2007) we considered fish growth as linear and linear regressed its size over time in order to obtain an approximation of the fish size at the period of measures of fecundity and offspring measurements. Fish TLs in a pair were highly correlated ($r = 0.672$, $t = 4.251$, $df = 22$, $p = 0.0003$), so only maternal total length (TL_M) was

used for subsequent analyses. At each site, both depth and anemone size were measured as they may influence fish size and reproductive success (Fautin 1992; Buston and Elith 2011) (Table S2.1). Depth was determined with a dive computer and the anemone size was determined as the sum of all the surface areas of the anemones used by the pair from photographs taken with a reference scale and using *Image J* software (following Holbrook and Schmitt 2005).

Fecundity and spawning frequency. Fecundity of each breeding pair, i.e. number of eggs laid, was calculated during the initial phase days 1, 2 or 3) and before hatching (final phase days 4, 5 and 6). The frequency of spawning was determined on a subset of anemonefish pairs (n = 15, Table S2.1) as the number of times each pair laid over a 6-month period (September 2017 - February 2018).

Egg traits. Eggs from each pair were collected a few hours before hatching and transferred to CRIOBE. In the lab, A subsample of eggs was photographed under a binocular microscope Leica EZ4W and egg surface area was measure with imageJ software considering them as ellipse (N = 10 per egg clutch measured in 22 breeding pairs, Table S2.1). The remaining eggs were put in a hatching chamber inside a rearing aquarium (30 L) where they hatched overnight at 27±1°C following a modified anemonefish rearing protocol (Cortese et al. 2021, in prep). By rearing larvae under controlled conditions, we avoided confounding environmental effects on larval traits (Hurst et al., 2019; Leis and McCormick, 2002).

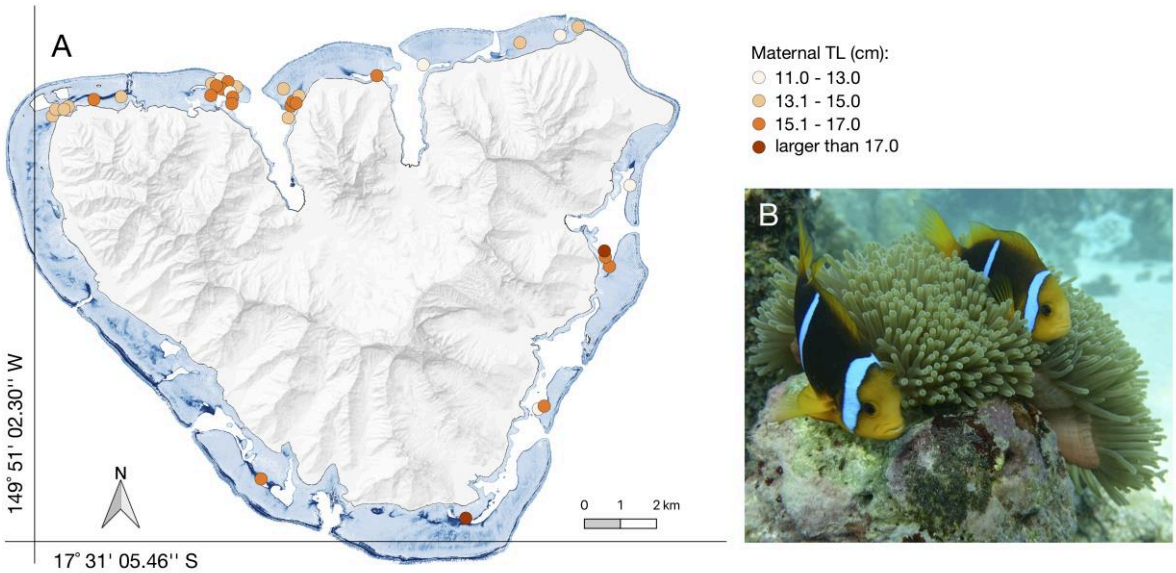

Figure 2. 1. Map of Moorea with location of *Amphiprion chrysopterus* breeding pairs categorized according to maternal total length (TL) (A). Photograph of a breeding pair in their anemone *Heteractis magnifica* tending a 6-days post fertilisation nest (B).

2.5.2. Larval laboratory measurements

Larval size and swimming performance were measured at the following developmental stages: 0 (within the first 24 hours), 1 (between 24 and 48 hours), 3 (72-96 h), 6 (144-168 h), and 9 (216-240 h) days post hatching (dph). Approximately 600 larvae from 18 anemonefish pairs were measured (TL: n = 602; U_{max}: n = 591).

Larval size was determined from photographs taken using a stereo microscope Leica EZ4W and the Leica Application Suite software (LAS) and *Image J*. Equally for each larvae, its swimming performance was determined by the constant acceleration test (Farrell 2008), which gives an indication of the maximum swimming speed (U_{max}) of a fish. Measurements were carried out in a 170 mL Blaza-style swim tunnel (Loligo System, Viborg, Danmark) immersed in a larger aquarium where temperature was maintained at 27.5 ± 1°C. The swim tunnel system consists of a two glass cylinders inside each other, one larger than the other. The inner cylinder (the swimming chamber) contains the larvae and an impeller at one end (separated from the fish by a honeycomb screen), which circulates water through the inner cylinder and return is through the space between the inner and outer cylinders. This ensures a one-way flow through the inner cylinder against which the fish swims (Brett 1964). The impeller velocity is regulated by a voltmeter. A larvae was introduced into the swim tunnel and kept at a water velocity of ~0.5-1 body length (BL) s^{-1} for an acclimation period (10 min) before the trials begun (Plaut 2001). Water flow was then gradually increased at a rate equivalent to approximately half a body length every 30 sec until the larvae was fatigued, could no longer maintain its position in the flow, and was swept towards a honeycomb screen located on the downstream end of the water tunnel (as described in Killen et al. 2015). The equation used to calculate U_{max} of larvae is (Brett 1964): $U_{max} = U_i + [U_j \times (T_i/T_j)]$; where U_i is the penultimate speed (the highest velocity maintained for the whole interval measured in cm s^{-1}), U_j is the velocity increment (0.5 BL, 0.25 cm s^{-1} in larvae of 0, 1, 3, and 6 dph and 0.5 cm s^{-1} in larvae of 9 dph), T_i is the time swum in the final velocity increment, and T_j is the prescribed interval time (30 sec). To account for larval size which is likely to affect swimming speed (Fisher and Hogan 2007), we calculated the larval swimming speed in body length per seconds. All larvae were kept unfed on the day of laboratory measurements in order to standardize swimming speed measurements and limit post-digestion energy absorbance. Larval swimming test were performed during the day-time. Generally 0, 1 and 3dph larvae were measured in the morning, while 6 and 9dph were measured in the afternoon.

2.5.3. Movement distance in the wild

Larval movement distance in the wild was measured within 24 hours after hatching (0 days-post-hatching, 0 dph). Unfed 0dph larvae from 18 anemonefish pairs were tracked in the wild (lagoon) and their speed, direction and dispersal distance was measured (n = 72). 0dph larvae were released, unfed, given that this is the moment lagoon spawned larvae are on the reef. Larvae were transported in plastic bags filled with water and air in coolers onto the release site. Equipped with buoys connected to GPS watches (POLAR M430), two scuba divers simultaneously tracked a live larva (called "active larva" afterwards) and a dead larva (called "passive larva" afterwards) for ~10 ± 6 minutes (mean ± SD) (methodology similar to that of Leis and Carson-Ewart, 1997). Divers were always located 1m away from the larvae, sideways to the larvae to avoid altering the prevalent flow. The passive larva was used to measure the effect of the current on a particle of similar size, shape and density as the live larva. All larvae were released in the morning (around 8am), during the same season (March to June 2019 and 2020) in a water mass with little temperature fluctuation (28 to 29˚C). The watches provided GPS points (latitude and longitude) at every second. The first and last 20 seconds of each GPS track were eliminated to avoid diver displacement. In addition, in order to reduce GPS precision errors and the movements of the buoy due to the swell, we kept only the GPS points every 10 seconds, which considerably smoothed the track. The larvae were released at approximately 2m deep over a sandy bottom to facilitate observation. To manipulate the geographic (spatial) data and calculated the distance traveled by each larvae a script was developed in R software, version 3.6.1 (R Core Team 2019) using the following packages: *geosphere* (Hijmans, 2017), *XML* (Duncan 2020), *lubridate* (Garrett Grolemund and Hadley Wickham 2011), *raster* (Hijmans 2020), *sp* (Pebesma 2005, Roger et al 2013), dplyr (Wickham 2020).

2.5.4. Data analyses

To determine the effect of maternal phenotype (size) on reproductive output we first analysed whether the fecundity varied according to maternal size, given the residual normality, we run an LMER with the number of eggs as response variable. The egg developmental stage (initial *vs* final), its interaction with maternal size, and environmental variables were initially included in the model and subsequently removed if not significant using the likelihood ratio test (LRT, Table S2.2A). In addition, breeding pair ID and month of trial were included as random factors to account for non-independence of data and potential temporal variation. To measure whether the frequency of spawning over a six-month period increased with maternal size we used a linear model (LM). We initially included log10-transformed anemone surface area and depth

as covariate and subsequently removed non-significant variables (LRT) to report the results of the best-fit model (Table S2.3A).

To explore the effect of maternal size on egg size we fitted a LMER with maternal size, log10-transformed anemone surface area and depth at parental sites and moon peak (i.e. the moon peak in which eggs were laid) as explanatory variables. We subsequently removed the non-significant variables using the likelihood ratio test (LRT) to report the results of the best-fit model (Table S2.4A). Breeding pair ID, clutch nested into pair ID and month of trial were included as random factors.

We tested the relationship between larval traits (size and U_{max}) across development (larval age). Using a linear mixed model (LMER) we explored whether larval swimming speed (in BL s^{-1}) depended on larval size. In the model we included larval size, larval age and environmental factors at parental site (depth, anemone surface and moon peak) as predictor variables, and random effects of breeding pair ID, egg clutch nested into pair ID and month of trial. We initially included an interaction among larval size and age and all variables in the model, and subsequently removed the interaction and non-significant variables (LRT) to report the results of the best-fit model (Table S2.5A).

To model maternal total length influence on larval size across development we used an LMER. Fixed effects included were environmental (depth, anemone surface and moon peak), as well as larval age, the interaction among larval age and maternal size, and random effects of breeding pair ID, clutch nested into pair ID and month of trial (Table S2.6A). Also, maternal influence on larval swimming performance across larval development was determined using the same LMER structure. Breeding pair ID, clutch nested into pair ID and month of trial were again included as random factors. The best-fit model was determined using the LRT (Table S2.7A).

The total distance travelled in the wild by each larva was determined by adding the distances between each point recorded every 10 seconds. To be able to compare the total distances traveled by the different larvae (active *vs* passive), we cut all the tracks to the same amount of time (7 minutes, minimum time recorded). First, with a LMER including all environmental variables (depth, anemone surface and moon peak) we investigated the difference between the distance travelled by the active and passive larvae (n = 72). Breeding pair ID, clutch nested into pair ID, month of trial and larval pair ID (i.e. active and passive larvae released at the same time) were again included as random factors. The best-fit model was determined using the LRT (Table S2.8A). Second, we tested whether maternal size influence larval distance movement in the wild using an LMER with the absolute value of the difference in distance of dispersal between the active

and the passive larvae as a response variable. The direction of dispersal was determined from the difference in distance between the active and passive larvae: positive value indicated that larvae went in the same direction of water current, while negative values indicated opposite direction. Maternal size, the direction of dispersal (with *vs* against the water current) and environmental variables (depth and anemone surface) were included in the model as predictor variables (Table S2.9A). We also tested whether maternal size influenced the variability of distance movement (Table S2.10A) using an LMER with maternal size, environmental factors and direction of dispersal as fixed factors, using the residuals of the LMER model on maternal influence on larval dispersal distance as responsible variable. Finally, using a generalised linear mixed model (GLMER) with a binomial distribution we tested if maternal phenotype affects the dispersal direction (with or against the water current). Maternal size and environmental variables (depth and anemone surface) were included in the model as predictor variables (Table S2.11A).

All statistical analyses were carried out in R software, version 3.6.1 (R Core Team 2019) using the following packages: *ggplot2* (Hadley Wickham 2016), *lmerTest* (Kuznetsova et al. 2017), *lmtest* package (Zeileis and Hothorn 2002), and *piecewiseSEM* (Lefcheck 2016).

2.6. Results

2.6.1. Maternal size effect on reproductive output: Fecundity and spawning frequency

Larger mothers have greater fecundity (LMER – TL_M : t = 3.768, p = 0.002, Fig. 2.2A, Table S2B). In addition, larger and smaller mothers may have similar spawning frequencies. According to our analyses there is no significant maternal length effect on spawning frequency (LM – TL_M: t = -0.454, p = 0.657, R^2 = 0.016, Fig. 2.2B, Table S2.3B). Finally, anemone surface area and depth had no effect on fecundity (LRT; depth: χ^2 = 0.043, p = 0.837; log10(anemone): χ^2 = 1.928, p = 0.165, Table S2.3A) and spawning frequency (LRT; depth: χ^2 = 4.721, p = 0.317; log10(anemone): χ^2 = 1.105, p = 0.293, Table S2.3A).

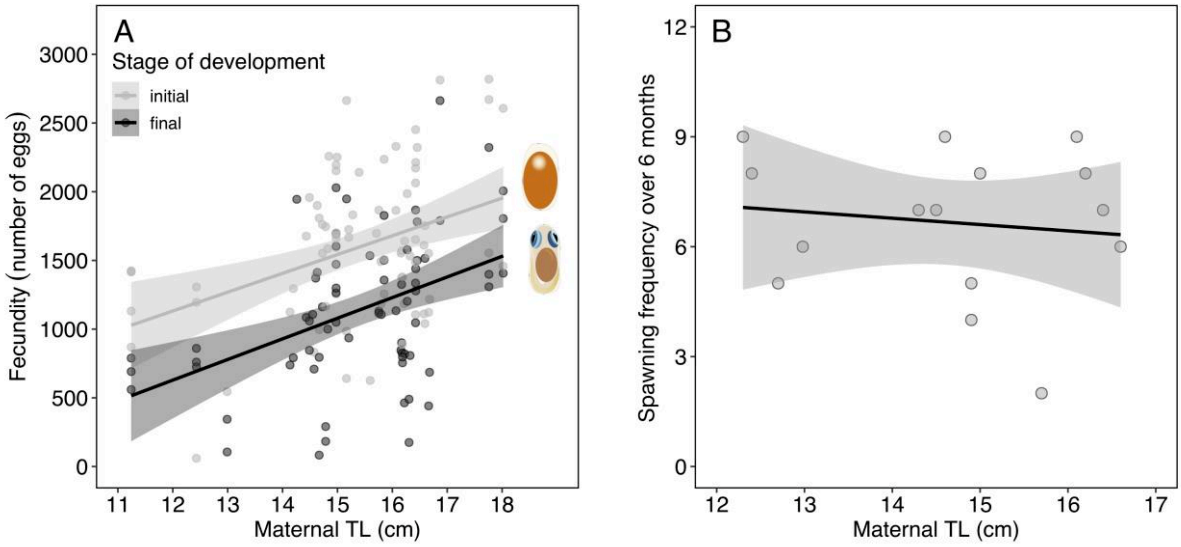

Figure 2. 2. (A) Fecundity (number of eggs per clutch) regressed with maternal total length (TL). The initial (0, 1, 3dph) and final (4, 5, 6dph) stage of egg developmental are shown. Regressions are surrounded by 95% confidence bands. (B) Spawning frequency, i.e. number of spawning events over 6 months, regressed with maternal total length (TL). Dots indicate individual data points.

2.6.2. Maternal size effect on egg size

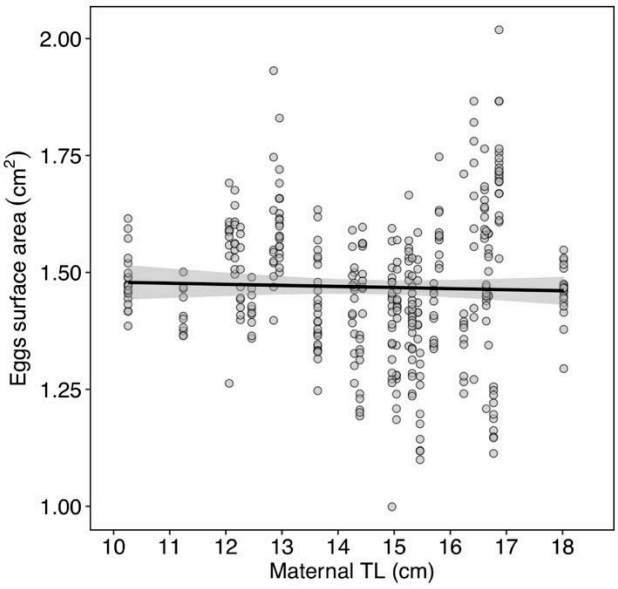

Figure 2. 3. Linear regression and 95% confidence bands of the egg size (surface area) and maternal total length (TL). Dots indicate individual data points.

Egg surface area is not linearly linked to maternal size (LMER: TL_M; t = -1.976, p = 0.066, mR^2 = 0.198, cR^2 = 0.670, Fig. 2.3, Table S2.4B). Site depth had a positive effect on egg size

(LMER: TL_M; t = 3.328, p = 0.004), while moon peak and anemone surface area had no effect (LRT; moon: χ^2 = 0.037, p = 0.848; log10(anemone): χ^2 = 1.113, p = 0.291, Table S2.4A).

2.6.3. Larval traits: Laboratory measures (larval size and swimming speed)

Larval swimming performance adjusted to fish body length increased with age but not with larval size (ANOVA(LMER) – TL_L: $F_{1,4}$ = 0.332, p = 0.565; Age: $F_{1,4}$ = 4.462, p = 0.002, Fig. 2.4, Table S2.5C). However, up to three days post hatching the swimming performance relative to larval body size did not change significantly from that at hatching. It is only between three- and six-days post hatching that larvae start to swim significantly faster (day 6: t = 3.060, p = 0.002; day 9: t = 3.007; p-value = 0.003, mR^2 = 0.107, cR^2 = 0.178), but highly variable in both size and U_{max} across ages (Table 2.1). Moon peak, depth and anemone surface area had no effect on larval swimming performance and were removed from the best-ft model (LRT; moon: χ^2 = 0.562, p = 0.453; depth: χ^2 = 2.069, p = 0.150; log10(anemone): χ^2 = 0.105, p = 0.746, Table S2.5A).

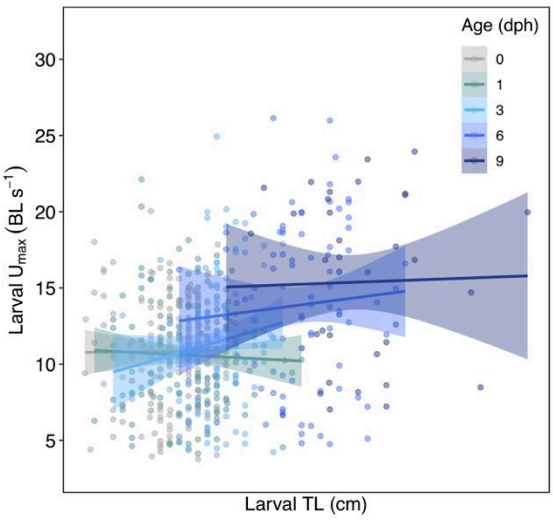

Figure 2. 4. Linear regression and 95% confidence bands of the larval swimming speed (U_{max}) and larval total length (TL). Dots indicate individual data points. Colours refer to different larval ages (0, 1, 3, 6 and 9 dph - days post hatching).

Table 2. 1. Variability in larval traits across ontogeny (from 0 to 9 days post hatching) with minimum, maximum values and sample size (n) indicated for each trait, i.e. total length (TL) and swimming speed (U_{max}).

Larval traits	Age 0dph		Age 1dph		Age 3dph		Age 6dph		Age 9dph	
	min	max	min	max	min	max	min	max	min	max
Larval TL (cm)	0.31	0.46	0.32	0.54	0.34	0.52	0.38	0.65	0.46	0.78
n (sample size)	185		163		129		87		38	
Larval U_{max} (BL s^{-1})	3.76	20.04	4.13	22.12	4.22	24.94	4.75	26.14	6.65	23.95
n (sample size)	185		162		126		80		38	

2.6.4. Maternal size effect on larval traits

Larval size

Maternal size did not affect larval size, neither at hatching nor during early development (LMER - TL_M: t = 1.270, p = 0.210, TL_M *Age (1 dph): t = -0.591, p = 0.555; TL_M *Age (3 dph): t = -0.798, p = 0.425, mR^2 = 0.705, cR^2 = 0.800, Fig. 2.5A, Table S2.6B). However, maternal size had an interacting effect with larval age. Indeed, after 3 days post-hatching the regression of larval size with maternal size had an opposite direction compared to earlier stages (TL_M *Age (6 dph): t = -3.377, p < 0.001; TL_M *Age (9 dph): t = -1.995, p = 0.047). However, data on larval size at older ages (6 and 9 dpf) is not available at extreme female sizes, suggesting that caution should be given to the effect at older ages. At age 6 and 9dph, larval size was significantly larger than at hatching (day 6: t = 6.190; p < 0.001; day 9: t = 3.842, p < 0.001). sizes. Moon peak, anemone surface area and depth had no effect on larval size (LRT; moon: χ^2 = 0.006, p = 0.936; log10(anemone): χ^2 = 0.853, p = 0.356; depth: χ^2 = 0.435, p = 0.509; Table S2.6A).

Larval swimming speed

Larger mothers produce larvae with better swimming performance across development (LMER: TL_M; t = 3.5178, p = 0.002; mR^2 = 0.143, cR^2 = 0.174, Fig. 2.5B, Table S2.7B). For each cm increase in female size the larval swimming speed increase by 0.57 BL cm^{-1}, which means that larvae produced from the largest mothers (18 cm) will swim 32% faster than larvae produced by the smallest mothers (11 cm). In addition, there is an age effect on larval swimming speed, with larvae at 6 dph starting to swim significantly faster than at hatching LMER: Age (6dph); t = 5.645; p < 0.001; Age (9dph); 5.778, p < 0.001). Moon peak, anemone surface area and depth

had no effect on larval swimming speed (LRT; moon: $\chi^2 = 0.037$, p = 0.847; log10(anemone): $\chi^2 = 0.964$, p = 0.326; depth: $\chi^2 = 0.032$, p = 0.859; Table S2.7A).

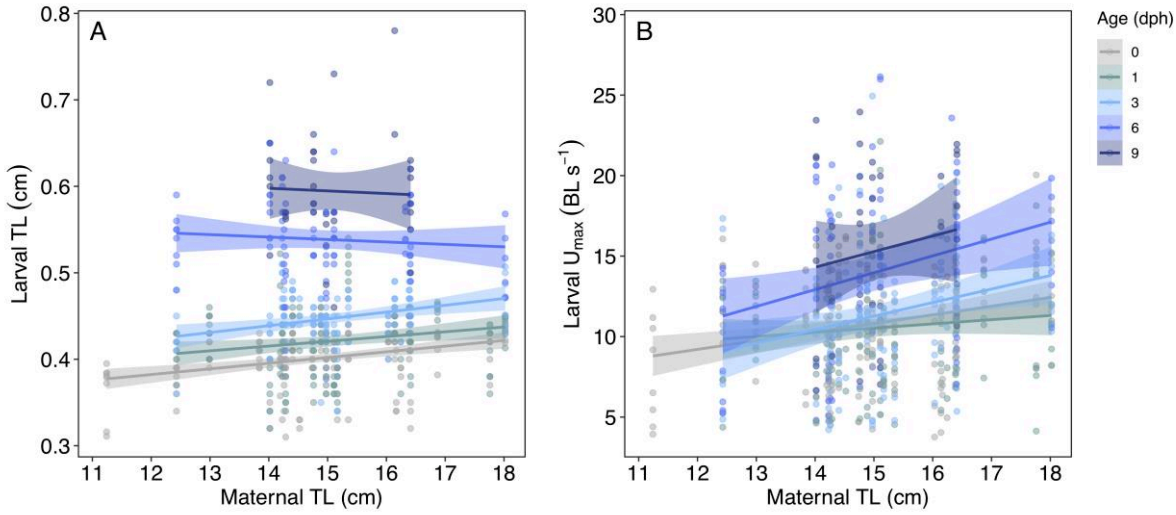

Figure 2. 5. (A) Maternal size (TL, total length) effect on larval size (TL) and (B) larval swimming speed (U_{max}) across larval development, from 0 to 9 days post hatching (dph). Linear regression and 95% confidence bands are shown. Dots indicate individual data points.

2.6.5. Larval traits: Field measure (movement distance)

The movement distance in the wild of active larvae was significantly different from that of passive larvae. Active larvae travelled more than 3 m further than passive larvae over only 7 min of tracking (LMER: status (passive); t = -3.113, p = 0.045; $mR^2 = 0.010$, $cR^2 = 0.847$, Fig. 2.6, Table S2.8B). Moon peak, depth and anemone surface area had no effect on movement distance (LRT; moon: $\chi^2 = 0.079$, p = 0.779; depth: $\chi^2 = 0.019$, p = 0.891; log10(anemone): $\chi^2 = 0.002$, p = 0.961; Table S2.8A).

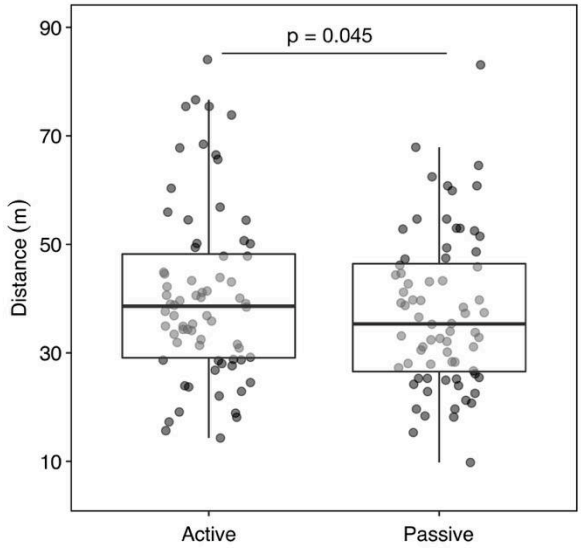

Figure 2. 6. Movement distance in the wild between active and passive larvae. Dots indicate individual data points. In the boxplot the median, the inter-quartile range (IQR) and whiskers are shown. The upper whisker extends to the largest observation less than or equal to upper hinge +1.5 IQR while the lower whisker extends up to the smallest observation greater than or equal to lower hinge -1.5 IQR.

2.6.4. Maternal size effect on larval distance movement in the wild

The distance travelled in the wild at hatching was not affected by maternal size (LMER – TL_M: t = 1.799, p = 0.084, mR^2 = 0.096, cR^2 = 0.418, Fig. 2.7, Table S2.9B) nor by the direction of dispersal, although there is a tendency to travel further when going in the same direction of the current (t = 1.903, p = 0.061). Moon peak, depth and anemone surface area had no effect on movement distance (LRT; moon: χ^2 = 1.358, p = 0.244; depth: χ^2 = 0.680, p = 0.410; log10(anemone): χ^2 = 0.174, p = 0.677; Table S2.9A).

The variability in larval distance movement was not linked to maternal size (LMER - TL_M: t = 1.189, p = 0.254, mR^2 = 0.103, cR^2 = 0.212, Table S2.10B). However, the direction of dispersal significantly affects larval variability: among larvae going in the same direction of the water current there is higher variability in the distance travelled, while those that go against the current travel approximately the same distance (LMER – direction(with): t = 2.743, p = 0.008). Finally, maternal size does not affect the swimming direction (i.e., going with or against the water current) (GMER - TL_M: t = -0.718, p = 0.473, mR^2 = 0.012, cR^2 = 0.107, Table S2.11B). Moon peak, depth and anemone surface area had no effect on movement distance (LRT; moon: χ^2 = 2.826, p = 0.093; depth: χ^2 = 2.235, p = 0.135; log10(anemone): χ^2 = 0.046, p = 0.831; Table S2.10A).

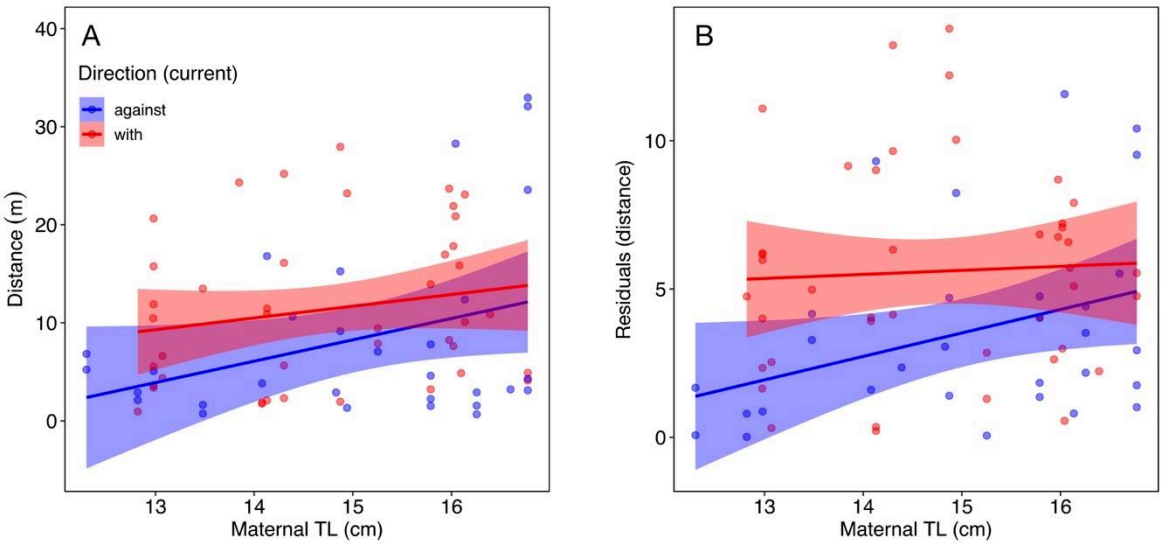

Figure 2. 7. Distance movement in the wild (A) and variability of distance (residuals of model on distance) (B) according to maternal total length (TL). Colours indicate the dispersal behaviour, i.e. whether the larvae moved with or against the water current.

2.7. Discussion

Here we show for the first time that larger females produce larvae with better swimming abilities for a large part of their pelagic dispersive stage, from hatching to nine days post hatching. Particularly, for each centimetre increase in maternal size, their offspring swim at 0.57 BL s^{-1} faster, which suggest that the largest individuals of *Amphiprion chrysopterus* (18 cm length) produce larvae that swim 32% faster than those produced by the smallest breeding females of the same species (11 cm length). These results may have important repercussions for the dispersal outcome of anemonefish larvae (Fisher 2005). The differences in swimming performance of larvae from large and small mothers, were clearer after the formation of the caudal fin (full torsion of the urostyle and formation of rays, Yasir and Qin 2007) which point 6dph larvae were significantly faster than larvae at hatching. Furthermore, larval swimming speed is extremely important for small movements such as predator evasion, as well as foraging, which may enhance survival (Fisher et al. 2000; Leis and McCormick 2002; Fisher 2005; Leis et al. 2007; Nanninga and Berumen 2014), and also at settlement (Kingsford et al. 2002). Differences in larval swimming abilities may be linked to changes in maternal anatomy or physiology (greater energy resources) with increasing maternal size or to the relationship between maternal phenotype and offspring environment (Marshall and Keough 2004; Green 2008; Marshall et al. 2010; Hixon et al. 2014).

Our study also shows that the active swimming behaviour of 0 dph larvae in the wild translate into a significant difference in the distance moved compared to a passive drifting larva after only 7 minutes of dispersal. Considering the whole pelagic larval duration (~11 days) and given that differences in swimming performance are accentuated throughout development, such results suggest a clear difference in dispersal between larvae from large and small mothers. Faster larvae, i.e. more capable swimmers, should have a survival advantage due to predator escape and foraging (Fisher et al. 2000; Leis and McCormick 2002; Fisher 2005; Leis et al. 2007; Nanninga and Berumen 2014) and may also directly affect the extent of dispersal (Majoris et al. 2019).

Larger mothers of the same population and during the same period of the year produced more offspring and had similar spawning frequency as smaller mothers. The overall reproductive output during the same period of time, also given that there were no differences in egg mortality throughout incubation, is ultimately superior in larger mothers. These results are compatible with a previous study on a different anemonefish species (*A. percula*) that found a positive correlation among parental size and egg laid (Barbasch et al. 2020). Interestingly, we did not find a clear relationship among maternal size and offspring size, unlike other studies (Green 2008). Large and small mothers both produce larvae with the same size range across development. However, offspring born from larger mothers are equally large but faster swimmers than larvae produced by smaller mothers.

The sum of these results agrees and reinforces the pleas made regarding the outstanding value of larger breeding fish in management and conservation. Maternal size can be extremely important for population replenishment and the fishery selective pressure on the larger individuals can have dire consequences for population dynamics, beyond those expected solely based on female fish size (Hixon et al. 2014). Evidence originating from self-recruitment studies (Beldade et al. 2012; Saenz-Agudelo et al. 2014) has underlined the importance of large female breeders to the natal population replenishment. Here we provide a putative explanation for such findings. On the other hand, it is also known that conservation efforts such as old/mature marine protected areas host larger fish that appear to spill over to surrounding areas (Di Lorenzo et al. 2020). Perhaps the differences in quality of offspring produced by these larger breeders might help explain such results. Finally, the integration of these findings into fisheries management models, might shed light into the sustainability of the few fisheries that impose maximum catch sizes (Le Bris et al. 2018; Pinsky 2018).

2.8. Acknowledgment

Financial support was provided by the Agence National de la Recherche to Glenn Almany, SCM, and RB (ANR-14-CE02-0005-01/Stay or Go) and to SCM (ANR-11-JSV7-012-01/Live and Let Die), and by LabEx "CORAIL" to RB and SCM ('Where do we go now?'). SSK was supported by Natural Environment Research Council Advanced Fellowship (NE/J019100/1) and European Research Council starting grant (640004). We particularly thank Till Deuss and Robin Mannion for their support and help in the CRIOBE anemonefish aquarium facility. We are enormous thankful to Tommy Norin for sharing his expertise in laboratory measures of larval swimming performance. We are very thankful to Zoe Scholz and Tara Cousins for support in the laboratory and in the field. We thank Jules Schligler, Sam Manning, Clara Diaz, Camille Vizon, Lisa Macera and Jennie Pistevos for support with larval release and tracking in the field. We are also thankful to Gilles Siu, Pascal Ung, Anne Haguenauer, Frederic Zuberer, Franck Lerouvreur and all the CRIOBE community for support in the field.

2.9. Supplementary materials

Table S2. 1. List of anemonefish pairs used in the study. Cross indicates which measures have been taken from each pair (e.g. site ID-1 was used for spawning frequency, eggs from site Id-1 were measured and larvae produced by site ID-1 were used for measures of larval dispersal in the wild). Maternal size or range of maternal size (when multiple measures were taken across months and years) are shown for each site ID.

Site (pair ID)	Fecundity	Spawning frequency	Eggs	Larval traits	Larval dispersal	Maternal size (cm)
1		X	X		X	14.4-16.4
2					X	14.1
3			X			15.0
4			X			13.6
5			X			15.7
6			X			14.4
7	X	X	X	X	X	15.2-16.3
8		X			X	12.3-13.0
9	X		X	X	X	14.7-16.0
10	X	X	X	X	X	13.8-14.9
11	X	X		X		14.975
12		X	X		X	15.3-16.6
13	X	X	X	X		16.0-16.2
14		X	X		X	12.5-13.1
15	X	X	X	X		16.39-16.58
16					X	16.135
17		X	X		X	12.4-13.9
18	X		X	X		16.14-16.32
19	X	X	X	X	X	14.4-16.2
20	X	X		X	X	14.2-16.3
21		X	X		X	15.0
22					X	14.3
23	X	X		X		16.4
24	X	X		X		12.4
25			X		X	13.5
26			X		X	12.8
27					X	14.1-15.3
28	X		X	X		11.2
29			X		X	16.4
30	X			X		15.4
31	X			X		17.8
32	X			X		13.0
33	X			X		15.3
34	X		X	X		18.0
35	X		X	X		16.1

Table S2. 2. Models and model outputs for fecundity analyses

Table S2. 2A. List of all LMER performed with *fecundity* (i.e. number of eggs) as response variable. TL_M is the maternal total length, *stage* is the egg developmental stage (initial *vs* final), *Anemone* is the anemone surface, which was log-10 transformed to account for model scaling problems, *depth*, is the depth at parental site. *ID* is the anemonefish pair ID and *time* is a variable that include the monthly temporal variability among measurements, which were included in the model as a random effect. Model selection was performed by sequentially dropping non-significant variables, starting with the interaction, and models were compared using likelihood ratio tests (LRTs) in order to identify the best-fit model (in bold) that included *maternal size* and *egg stage* as predictor variables.

Model	df	LogLik	χ^2	p-value
1: $fecundity \sim TL_M * stage + log10(Anemone) + depth + (1\|ID) + (1\|time)$	9	-1232.5		
2: $fecundity \sim TL_M + stage + log10(Anemone) + depth + (1\|ID) + (1\|time)$	8	-1232.6	0.152	0.697
3: $fecundity \sim TL_M + stage + log10(Anemone) + (1\|ID) + (1\|time)$	7	-1232.7	0.043	0.837
4: $\boldsymbol{frequency \sim TL_M + stage + (1\|ID) + (1\|time)}$	**6**	-1233.6	1.928	0.165

Table S2. 2B. Results of the best-fit model from Table S2. 2A. mR^2 is the marginal R^2, which describes the proportion of variance explained by the fixed factors alone. cR^2 is the conditional R^2, which describes the proportion of variance explained by both fixed and random factors.

Fixed effects	Estimate	SE	df	t-value	p-value	mR^2	cR^2
Intercept	-1354.99	680.4	15.56	-1.992	0.064	0.296	0.560
TL_M	164.74	43.7	15.27	3.768	0.002		
Stage	442.68	65.2	141.66	6.787	<0.001		

Table S2. 3. Models and model outputs for spawning frequency analyses

Table S2. 3A. List of all linear model (LM) performed with *spawning frequency* as response variable. TL_M is the maternal total length, *Anemone* is the anemone surface, *depth*, is the depth at anemonefish breeding site. Model selection was performed by sequentially dropping non-significant variables and models were compared using likelihood ratio tests (LRTs) in order to identify the best-fit model (in bold) that included *maternal size* as predictor variable.

Model	df	LogLik	χ^2	p-value
1: $frequency \sim TL_M + log10(Anemone) + depth$	5	-30.193		
2: $frequency \sim TL_M + log10(Anemone)$	4	-30.670	4.721	0.317
3: $\boldsymbol{frequency \sim TL_M}$	**3**	-31.222	1.105	0.293

Table S2. 3B. Results of the best-fit model from Table S2. 3A. R^2 is the multiple R-squared, which describe the proportion of the variance explained by the predictors variables included in the model. R^2_{adj} is the adjusted R-squared, which adjust the statistic based on the number of independent variables included in the model.

Fixed effects	Estimate	SE	t-value	p-value	R^2	R^2_{adj}
Intercept	9.1940	5.591	1.644	0.124	0.016	0.000
TL_M	-0.1726	0.380	-0.454	0.657		

Table S2. 4. Models and model outputs for egg size analyses

Table S2. 4A. List of all linear mixed model (LMER) performed with *egg size* (surface area) as response variable. TL_M is the maternal total length, *Anemone* is the anemone surface, *depth*, is the depth at parental site, *peak* is the lunar peak when egg where laid. *ID* is the anemonefish pair ID, *clutch* is the egg clutch and is nested into pair ID, and *time* is a variable that include the monthly temporal variability among measurements, which were included in the model as a random effect. Model selection was performed by sequentially dropping non-significant variables using likelihood ratio tests (LRTs) in order to identify the best-fit model (in bold) that included *maternal size* as a predictor variable.

Model	df	LogLik	χ^2	p-value
1: $y \sim TL_M + log10(Anemone) + depth + peak + (1\|ID/clutch) + (1\|time)$	9	323.23		
2: $y \sim TL_M + log10(Anemone) + depth + (1\|ID/clutch) + (1\|time)$	8	323.21	0.037	0.848
3: $\boldsymbol{y \sim TL_M + depth + (1\|ID/clutch) + (1\|time)}$	7	322.65	1.113	0.291
4: $y \sim TL_M + (1\|ID/clutch) + (1\|time)$	6	317.48	10.355	0.001

Table S2. 4B. Results of the best-fit model from Table S2. 4A. mR^2 is the marginal R^2, which describes the proportion of variance explained by the fixed factors alone. cR^2 is the conditional R^2, which describes the proportion of variance explained by both fixed and random factors.

Fixed effects	Estimate	SE	df	t-value	p-value	mR^2	cR^2
Intercept	1.8145	0.202	15.15	9.007	<0.001	0.198	0.670
TL_M	-0.0297	0.015	15.88	-1.976	0.066		
depth	0.02989	0.009	17.51	3.328	0.004		

n = 370, clutch = 38, families = 22

Table S2. 5. Models and model outputs for the analyses on the relationship between larval swimming speed (U_{max}) and size (TL) across ages

Table S2. 5A. List of all linear mixed model (LMER) performed with U_{max} as response variable. TL_L is the larval total length, *Age* is larval age in days post hatching, *Anemone* is the anemone surface, *depth*, is the depth at parental site, *peak* is the lunar peak when egg where laid. ID is the anemonefish pair ID, *clutch* is the egg clutch and is nested into pair ID, and *time* is a variable that include the monthly temporal variability among measurements, which were included in the model as a random effect. Model selection was performed by sequentially dropping non-significant variables, starting with the interaction, and models were compared using likelihood ratio tests (LRTs) in order to identify the best-fit model (in bold) that included *larval size* and *age* as predictor variables.

Model	df	LogLik	χ^2	p-value
1: $U_{max} \sim TL_L * Age + log10(Anemone) + depth + peak + (1\|ID/clutch) + (1\|time)$	17	-1641.8		
2: $U_{max} \sim TL_L + Age + log10(Anemone) + depth + peak + (1\|ID/clutch) + (1\|time)$	13	-1644.2	4.721	0.317
3: $U_{max} \sim TL_L + Age + log10(Anemone) + depth + (1\|ID/clutch) + (1\|time)$	12	-1644.5	0.562	0.453
4: $U_{max} \sim TL_L + Age + log10(Anemone) + (1\|ID/clutch) + (1\|time)$	11	-1645.5	2.069	0.150
5: $U_{max} \sim TL_L + Age + (1\|ID/clutch) + (1\|time)$	10	-1645.6	0.105	0.746

Table S2. 5B. Results of the best-fit model from Table S2. 5A. mR^2 is the marginal R^2, which describes the proportion of variance explained by the fixed factors alone. cR^2 is the conditional R^2, which describes the proportion of variance explained by both fixed and random factors.

Fixed effects	Estimate	SE	df	t-value	p-value	mR^2	cR^2
Intercept	9.8662	1.835	306.08	5.376	<0.001	0.107	0.178
TL_L	2.5617	4.437	345.72	0.576	0.565		
Age (1 dph)	-0.4831	0.433	576.72	-1.116	0.265		
Age (3 dph)	0.4371	0.506	542.05	0.864	0.388		
Age (6 dph)	2.6390	0.863	355.66	3.060	0.002		
Age (9 dph)	3.5181	1.170	249.65	3.007	0.003		

n = 591, clutch = 33, families = 18

Table S2. 5C. ANOVA of the best-fit model from Table S2. 5A.

Fixed effects	Sum sq	Mean sq	df	Den df	F-value	p-value
TL_L	4.944	4.944	1	345.72	0.3319	0.565
Age	265.873	66.468	4	435.32	4.4620	0.002

Table S2. 6. Models and model outputs for larval size analyses

Table S2. 6A. List of all LMER performed with *larval size* (TL = total length) as response variable. TL_M is the maternal total length, Age is the larval developmental stage (from day 1 to day 9 post hatching), *Anemone* is the anemone surface, *depth*, is the depth at parental site, *peak* is the lunar peak when egg where laid. *ID* is the anemonefish pair ID and *time* is a variable that include the temporal variability among measurements, which were included in the model as a random effect. Model selection was performed by sequentially dropping non-significant variables, starting with the interaction, and models were compared using likelihood ratio tests (LRTs) in order to identify the best-fit model (in bold) that included *maternal size* and *larval age* as predictor variables.

Model	df	LogLik	χ^2	p-value
1: $TL_L \sim TL_M * Age + log10(Anemone) + depth + peak + (1\|ID/clutch) + (1\|time)$	17	1162.9		
2: $TL_L \sim TL_M + Age + log10(Anemone) + depth + peak + (1\|ID/clutch) + (1\|time)$	13	1156.2	13.516	0.009
3: $TL_L \sim TL_M * Age + log10(Anemone) + depth + (1\|ID/clutch) + (1\|time)$	16	1162.9	0.006	0.936
4: $TL_L \sim TL_M * Age + depth + (1\|ID/clutch) + (1\|time)$	15	1162.5	0.853	0.356
5: $TL_L \sim TL_M * Age + (1\|ID/clutch) + (1\|time)$	14	1162.3	0.435	0.509

Model3 is compared to model1

Table S2. 6B. Results of the best-fit model from Table S2. 6A. mR^2 is the marginal R^2, which describes the proportion of variance explained by the fixed factors alone. cR^2 is the conditional R^2, which describes the proportion of variance explained by both fixed and random factors.

Fixed effects	Estimate	SE	df	t-value	p-value	mR^2	cR^2
Intercept	0.3451	0.043	47.65	8.093	<0.001	0.705	0.800
TL_M	0.0036	0.003	48.62	1.270	0.210		
Age (1 dph)	0.0433	0.041	586.38	1.061	0.289		
Age (3 dph)	0.0879	0.050	590.98	1.764	0.078		
Age (6 dph)	0.3220	0.052	585.74	6.190	<0.001		
Age (9 dph)	0.4366	0.114	552.48	3.842	<0.001		
TL_M * Age (1 dph)	-0.0016	0.003	586.34	-0.591	0.555		
TL_M * Age (3 dph)	-0.0026	0.003	591.41	-0.798	0.425		
TL_M * Age (6 dph)	-0.0116	0.003	584.57	-3.377	<0.001		
TL_M* Age (9 dph)	-0.0150	0.008	550.63	-1.995	0.047		

n = 602, clutch = 33, families = 18

Table S2. 6C. ANOVA of the best-fit model from Table S2. 6A.

Fixed effects	Sum sq	Mean sq	df	Den df	F-value	p-value
TL_M	0.001	0.001	1	43.76	0.836	0.366
Age	0.056	0.014	4	573.46	12.162	<0.001
TL_M*Age	0.016	0.004	4	573.67	3.554	0.007

Table S2. 7. Models and model outputs for larval swimming speed size analyses

Table S2. 7A. List of all LMER performed with larval *swimming speed* (U_{max}) as response variable. TL_M is the maternal total length, Age is the larval developmental stage (from day 1 to day 9 post hatching), *Anemone* is the anemone surface, *depth*, is the depth at parental site, *peak* is the lunar peak when egg where laid. *ID* is the anemonefish pair ID and *time* is a variable that include the temporal variability among measurements, which were included in the model as a random effect. Model selection was performed by sequentially dropping non-significant variables, starting with the interaction, and models were compared using likelihood ratio tests (LRTs) in order to identify the best-fit model (in bold) that included *maternal size* and *larval age* as predictor variables.

Model	df	LogLik	χ^2	p-value
1: $U_{max} \sim TL_M * Age + log10(Anemone) + depth + peak + (1\|ID/clutch) + (1\|time)$	17	-1637.3		
2: $U_{max} \sim TL_M + Age + log10(Anemone) + depth + peak + (1\|ID/clutch) + (1\|time)$	13	-1639.9	5.236	0.264
3: $U_{max} \sim TL_M + Age + log10(Anemone) + depth + (1\|ID/clutch) + (1\|time)$	12	-1640.0	0.037	0.847
4: $U_{max} \sim TL_M + Age + depth + (1\|ID/clutch) + (1\|time)$	11	-1640.4	0.964	0.326
5: $\boldsymbol{U_{max} \sim TL_M + Age + (1\|ID/clutch) + (1\|time)}$	10	-1640.5	0.032	0.859

Table S2. 7B. Results of the best-fit model from Table S2. 7A. mR^2 is the marginal R^2, which describes the proportion of variance explained by the fixed factors alone. cR^2 is the conditional R^2, which describes the proportion of variance explained by both fixed and random factors.

Fixed effects	Estimate	SE	df	t-value	p-value	mR^2	cR^2
Intercept	2.2651	2.473	21.33	0.916	0.370	0.143	0.174
TL_M	0.5704	0.162	21.33	3.517	0.002		
Age (1 dph)	-0.4346	0.421	583.49	-1.033	0.302		
Age (3 dph)	0.5148	0.454	576.03	1.133	0.258		
Age (6 dph)	3.0600	0.542	464.10	5.645	<0.001		
Age (9 dph)	4.1409	0.717	408.39	5.778	<0.001		

n = 591, clutch = 33, families = 18

Table S2. 8. Models and model outputs for larval dispersal in the wild analyses

Table S2. 8A. List of all LMER performed with distance of dispersal in the wild (*distance*) as response variable. Fixed factors were: *Status* is the larval status, i.e. alive (active larvae) or dead (passive drifter), *Anemone* is the anemone surface, *depth*, is the depth at parental site, *peak* is the lunar peak when egg where laid. Random effect*s* were: *siteID* is the anemonefish pair ID, *clutch* is the egg clutch and is nested into siteID, *time* is a variable that include the monthly temporal variability among measurements and *pairID* is the paired active and passive larvae that were released at the same time. Model selection was performed by sequentially dropping non-significant fixed effect variables and models were compared using likelihood ratio tests (LRTs) in order to identify the best-fit model (in bold) that included dispersal *status* as a predictor variable.

Model	df	LogLik	χ^2	p-value
1: $distance \sim status + log10(Anemone) + depth + peak + (1\|siteID/clutch) + (1\|time) + (1\|pairID)$	10	-568.66		
2: $distance \sim status + log10(Anemone) + depth + (1\|siteID/clutch) + (1\|time) + (1\|pairID)$	9	-568.70	0.079	0.779
3: $distance \sim status + log10(Anemone) + (1\|siteID/clutch) + (1\|time) + (1\|pairID)$	8	-568.71	0.019	0.891
4: $\boldsymbol{distance \sim status + (1\|siteID/clutch) + (1\|time) + (1\|pairID)}$	7	-568.71	0.002	0.961

Table S2. 8B. Results of the best-fit model from Table S2. 8A. mR^2 is the marginal R^2, which describes the proportion of variance explained by the fixed factors alone. cR^2 is the conditional R^2, which describes the proportion of variance explained by both fixed and random factors.

Fixed effects	Estimate	SE	df	t-value	p-value	mR^2	cR^2
Intercept	41.465	2.674	13.97	15.507	<0.001	0.010	0.847
Status(passive)	-3.113	1.528	71.00	-2.038	0.045		

n (pairs) = 72, clutch = 29, families = 18

Table S2. 9. Models and model outputs for distance of dispersal analyses

Table S2. 9A. List of all LMER performed with distance of dispersal in the wild (d) as response variable. d is calculated as the absolute value of the difference of distance among the active and passive larvae. TL_M is the maternal total length, *direction* is the larval dispersal direction (with *vs* against the water current), *Anemone* is the anemone surface, *depth*, is the depth at parental site, peak is the lunar peak when egg where laid. *ID* is the anemonefish pair ID, *clutch* is the egg clutch and is nested into ID, and *time* is a variable that include the monthly temporal variability among measurements, which were included in the model as a random effect. Model selection was performed by sequentially dropping non-significant variables, starting with the interaction, and models were compared using likelihood ratio tests (LRTs) in order to identify the best-fit model (in bold) that included *maternal size* and *direction* of dispersal as predictor variables.

Model	df	LogLik	χ^2	p-value
1: $d \sim TL_M * direction + log10(Anemone) + depth + peak + (1\|ID/clutch) + (1\|time)$	11	-248.66		
2: $d \sim TL_M + direction + log10(Anemone) + depth + peak + (1\|ID/clutch) + (1\|time)$	10	-248.72	0.130	0.718
3: $d \sim TL_M + direction + log10(Anemone) + depth + (1\|ID/clutch) + (1\|time)$	9	-249.40	1.358	0.244
4: $d \sim TL_M + direction + log10(Anemone) + (1\|ID/clutch) + (1\|time)$	8	-249.74	0.680	0.410
5: $\boldsymbol{d \sim TL_M + direction + (1\|ID/clutch) + (1\|time)}$	7	-249.83	0.174	0.677

Table S2. 9B. Results of the best-fit model from Table S2. 9A. mR^2 is the marginal R^2, which describes the proportion of variance explained by the fixed factors alone. cR^2 is the conditional R^2, which describes the proportion of variance explained by both fixed and random factors.

Fixed effects	Estimate	SE	df	t-value	p-value	mR^2	cR^2
Intercept	-17.2636	14.236	25.11	-1.213	0.237	0.096	0.418
TL_M	1.6952	0.942	24.40	1.799	0.084		
Direction (with)	3.6092	1.897	66.65	1.903	0.061		

n = 72, clutch = 29, families = 18

Table S2. 10. Models and model outputs for dispersal variability analyses

Table S2. 10A. List of all LMER performed with the variability of dispersal distance (residuals of the LMER model on maternal influence on larval dispersal distance) as response variable. TL_M is the maternal total length, *direction* is the larval dispersal direction (with *vs* against the water current), *Anemone* is the anemone surface, *depth*, is the depth at parental site, *peak* is the lunar peak when egg where laid. *ID* is the anemonefish pair ID, *clutch* is the egg clutch and is nested into ID and *time* is a variable that include the monthly temporal variability among measurements, which were included in the model as a random effect. Model selection was performed by sequentially dropping non-significant variables, starting with the interaction, and models were compared using likelihood ratio tests (LRTs) in order to identify the best-fit model (in bold) that included *maternal size* and *direction* of dispersal as predictor variables.

Model	df	LogLik	χ^2	p-value
1: $resid(d) \sim TL_M * direction + log10(Anemone) + depth + peak + (1\|ID/clutch) + (1\|time)$	11	-183.97		
2: $resid(d) \sim TL_M + direction + log10(Anemone) + depth + peak + (1\|ID/clutch) + (1\|time)$	10	-184.69	1.422	0.233
3: $resid(d) \sim TL_M + direction + log10(Anemone) + depth + (1\|ID/clutch) + (1\|time)$	9	-186.10	2.826	0.093
4: $resid(d) \sim TL_M + direction + log10(Anemone) + (1\|ID/clutch) + (1\|time)$	8	-187.22	2.235	0.135
5: $\boldsymbol{resid(d) \sim TL_M + direction + (1\|ID/clutch) + (1\|time)}$	7	-187.24	0.046	0.831

Table S2. 10B. Results of the best-fit model from Table S2. 10A. mR^2 is the marginal R^2, which describes the proportion of variance explained by the fixed factors alone. cR^2 is the conditional R^2, which describes the proportion of variance explained by both fixed and random factors.

Fixed effects	Estimate	SE	df	t-value	p-value	mR^2	cR^2
Intercept	-2.9430	5.419	14.53	-0.543	0.595	0.103	0.212
TL_M	0.4268	0.359	13.76	1.189	0.254		
Direction (with)	2.2096	0.806	68.42	2.743	0.008		

n = 72, clutch = 29, families = 18

Table S2. 11. Models and model outputs for dispersal behaviour analyses

Table S2. 11A. List of all generalized linear mixed models (GLMER) with binomial distribution performed with the direction of dispersal as response variable. TL_M is the maternal total length, *Anemone* is the anemone surface, *depth*, is the depth at parental site, *peak* is the lunar peak when egg where laid. *ID* is the anemonefish pair ID and time is a variable that include the temporal variability among measurements, which were included in the model as a random effect. Model selection was performed by sequentially dropping non-significant variables, starting with the interaction, and models were compared using likelihood ratio tests (LRTs) in order to identify the best-fit model (in bold) that included maternal size as predictor variable.

Model	df	LogLik	χ^2	p-value
1: $direction \sim TL_M + log10(Anemone) + depth + peak + (1\|ID/clutch) + (1\|time)$	8	-47.18		
2: $direction \sim TL_M + log10(Anemone) + depth + (1\|ID/clutch) + (1\|time)$	7	-48.02	1.662	0.197
3: $direction \sim TL_M + log10(Anemone) + (1\|ID/clutch) + (1\|time)$	6	-48.02	0.002	0.962
4: $\boldsymbol{direction \sim TL_M + (1\|ID/clutch) + (1\|time)}$	5	-48.02	0.025	0.960

Table S2. 11B. Results of the best-fit model from Table S2. 11A. mR^2 is the marginal R^2, which describes the proportion of variance explained by the fixed factors alone. cR^2 is the conditional R^2, which describes the proportion of variance explained by both fixed and random factors.

Fixed effects	Estimate	SE	z-value	p-value	mR^2	cR^2
Intercept	2.9148	3.600	0.810	0.418	0.012	0.107
TL_M	-0.1727	0.241	-0.718	0.473		

n = 72, clutch = 29, families = 18

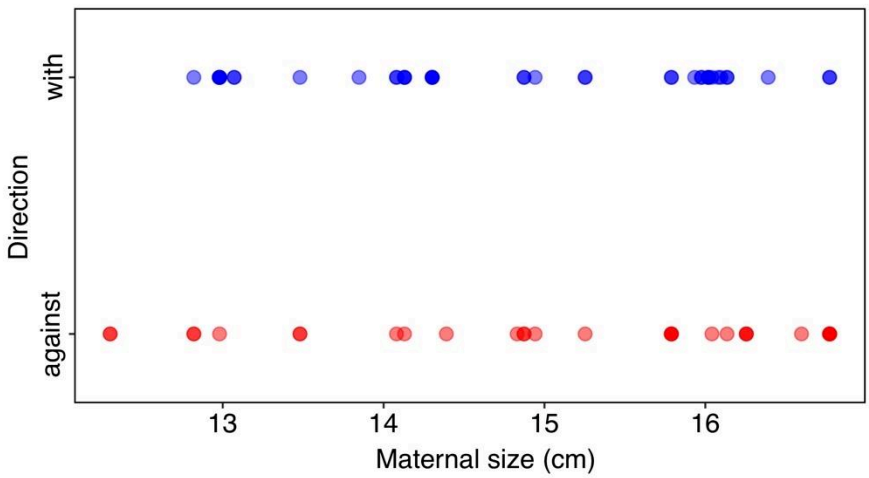

Figure S2. 1. Direction of dispersal (with, in blue, or against the water current, in red) according to maternal size (total length).

Chapter 3: Parental effects on offspring phenotype: larval dispersal in a changing world

3.1. Foreword

The aim of this chapter is to explore whether environmental disturbances can modulate, via parental effects, offspring dispersal associated phenotypes. Specifically, I investigated whether habitat size can affect egg size, larval size, and larval swimming speed, through parental hormonal levels and fecundity, in a wild population of the orange-fin anemonefish *Amphiprion chrysopterus*, at Moorea, French Polynesia.

This research project was conceptualised with Dr. Suzanne C. Mills and Dr. Ricardo Beldade, based on their previous study that showed that habitat degradation (i.e., anemone bleaching) can affect anemonefish reproduction. This project was carried out in collaboration with Shaun S. Killen from the University of Glasgow. Our objective was to evaluate whether environmental disturbances can have cascading effects on offspring, in particular on their dispersal-associated phenotype. To achieve this, I planned and carried out intensive fieldwork in which I monitored the reproduction of a wild population of anemonefish (sites were visited 2-3 days), measured their habitat size, collected adult fish blood samples, and collected eggs for further laboratory measures on offspring traits. I performed the fieldwork with Zoe Scholz and Clara Diaz, a master student that I co-supervised with Dr. Suzanne C. Mills and Dr. Ricardo Beldade. I carried out all laboratory measurements on offspring with the help of Clara Diaz. Hormonal measurements were carried out by Dr. Suzanne C. Mills, while larval fish husbandry was ensured by Robin Manion, Camille Vizon, Dr. Ricardo Beldade and me. I performed all data analyses, and drafted the manuscript under the contribution and supervision of Dr. Suzanne C. Mills. This project involved two year of my PhD (the second year of fieldwork and the third year for analyses and writing).

3.2. Manuscript information

Title: Parental effects on offspring phenotype: larval dispersal in a changing world

Authors: Daphne Cortese[1], Clara Diaz[1,2], Ricardo Beldade[3], Shaun S. Killen[4], Zoe Scholz[1], Suzanne C. Mills[1,5]

[1] CRIOBE USR 3278 EPHE-CNRS-UPVD PSL, BP 1013 Moorea, 98729 French Polynesia

[2] University of Plymouth, UK

[3] Estación Costera de Investigaciones Marinas and Center for Advanced Studies in Ecology and Biodiversity, Las Cruces, Pontificia Universidad Católica de Chile, Santiago de Chile.

[4] Institute of Biodiversity, Animal Health and Comparative Medicine, University of Glasgow, Graham Kerr Building, Glasgow G12 8QQ, UK

[5] *Laboratoire d'Excellence "CORAIL"*

Status: submission planned for March 2021

Keywords: plasticity, larval dispersal, offspring size, parental effects, environmental effects, habitat quality, reproductive output, larval swimming speed

3.3. Abstract

Natural populations are continuously challenged by variation in their habitat and threatened by anthropogenic-induced habitat loss and fragmentation. Non-genetic parental effects may be important determinants in the processes of re-location via dispersal or acclimation via phenotypic plasticity but have rarely been tested in marine fishes. Here, we carried out a field-experiment to determine if changes in habitat quality directly affect parental physiology (hormones) and reproduction, and trans-generationally, the phenotype and dispersal-associated traits of their offspring. By manipulating the territory size (anemone surface area) of orange-fin anemonefish, *Amphiprion chrysopterus*, we found that habitat quality decrease led to the production of smaller yet faster larvae right after the manipulation, while habitat quality increase led to the production of larger eggs and larvae, and increased the concentration of maternal 17β-estradiol. Longer-term effects suggest recovery from the manipulation over time. These results highlight the ability of populations to relocate after habitat change, with potential repercussions on population abundance, distribution and ecosystem diversity.

3.4. Introduction

Understanding the drivers of phenotypic variation in life-history traits is of paramount importance to evolutionary ecology due to links with survival and impacts at the population level (Sinervo 1990; Mousseau and Fox 1998; Venturelli et al. 2010). Evolutionary important life-history traits must refer to offspring phenotype, as it affects ontogeny and survival to adulthood and by extension maternal fitness (Roff 1992; Bernardo 1996a; Orr 2009). Parental genetic inheritance determines offspring phenotypes (Wilson et al., 2005), but environmentally induced parental effects, in particular nongenetic inheritance, may also influence offspring traits (Lacey 1998; Bonduriansky and Day 2009; Mousseau et al. 2009; Ducatez et al. 2012). Maternal effects, for example, represent an important non-genetic source of variation in offspring phenotype (Bernardo 1996a; Mousseau and Fox 1998), and, in response to environmental perturbations, enables mothers to influence offspring phenotype and potentially increase offspring fitness (Roff 1992; Mousseau and Fox 1998). The speed and persistence of adjustments to environmental change holds a central role in explaining species' persistence and maternal effects establish a link between adult and developmental acclimation (Marshall and Uller 2007). While many studies have demonstrated adaptive maternal effects on offspring phenotype in terms of egg size and number, juvenile size and survival (Einum and Fleming 1999; Allen et al. 2008; Jensen et al. 2014), fewer studies have examined whether parental effects translate into variation in offspring dispersal, nor whether these responses to environmental perturbations are adaptive (Gagliano and McCormick 2007).

Dispersal is one mechanism of coping with environmental change enabling offspring to disperse to more optimal habitats (West-Eberhard 1989; Beldade et al. 2011). Parental intergenerational effects alter the dispersive potential of offspring across a wide range of taxa enabling them to reach more optimal habitats, in particular depending on environmental conditions experienced by the mother (Sutherland 1969; Diss et al. 1996; Zera and Denno 1997; Donohue 1999; Massot and Clobert 2000; Krug et al. 2012). In many species, females respond to local conditions by modifying offspring development resulting in phenotypes with varying dispersal abilities (Sutherland 1969; Mousseau and Dingle 1991; Donohue 1999; Marshall 2008). In some species, fathers have also being shown to play a role in influencing offspring phenotype depending on their environmental experience (Bonduriansky and Day 2009; Jensen et al. 2014). Parental effects in species that have complex life-cycles, such as most fish that produce dispersive larval stages which are often distinct to adult forms (Cowen and Sponaugle 2009), are predicted to carry-over across life stages (Stoks and Crdoba-Aguilar 2012). However, despite plasticity being predicted to be common in the dispersive larval stage of fishes because

they live in an environmentally variable and unpredictable habitat (Warner 1997; West-Eberhard 2003), no studies have so far demonstrated the existence of plasticity in marine fish larval dispersal via parental effects as a response to changes in their environment.

In fishes, variation in egg size, which indicates parental investment, has consequences for survival (Chambers et al. 1989; Bernardo 1996b; Johnston and Leggett 2002), whereby larger eggs confer higher survival to offspring because they produce larger larvae at hatching (McGurk 1986), which may be better able to capture food, escape predators and be more resistant to starvation (Miller et al. 1988; Leggett and Deblois 1994; Fisher et al. 2000; Leis and McCormick 2002; McCormick and Hoey 2004; Leis et al. 2007). In natural populations, mortality is highest in the first hours and days after hatching suggesting that any variation in performance will be under intense selection (Elliott, 1989). Indeed, selection favours different offspring phenotypes in different environments (Einum and Fleming 1999; Green and Fisher 2004; Gagliano et al. 2007), and larval swimming ability may directly affect the outcome of offspring dispersal (Fiksen et al. 2007; Paris et al. 2007; Cowen and Sponaugle 2009; Nanninga and Berumen 2014; Faillettaz et al. 2018). Yet there is a paucity of studies that have demonstrated maternal effects on offspring traits associated with dispersal in fishes. Here we determine whether mothers are able to dynamically adjust the size of their offspring and their dispersal ability in the first 24 h after hatching in response to experimental manipulations of habitat quality. We will also examine the proximate mechanisms linking maternal phenotype, offspring phenotype and offspring dispersal potential.

Hormones provide an intergenerational mechanism through which environmental influences can be passed onto offspring. In vertebrates, environmental change induces hormonal responses including steroid hormones which are tightly linked to organismal fitness (Dufty et al. 2002; Remage-Healey and Bass 2006; Mills et al. 2009; Beldade et al. 2017) and can be carried-over intergenerationally via parental effects (McCormick 2009b). Environmental changes alter stress physiology and the production of corticosteroids benefitting immediate survival (Angelier and Wingfield 2013), but maternal cortisol may also be transmitted to eggs affecting their quality and development (smaller size and lower hatching: Saino et al. 2005). Environmental changes also directly, or indirectly via cortisol, impact reproductive hormones such as testosterone and 17β-estradiol, which themselves are linked to reproductive output (Dufty et al. 2002; Beldade et al. 2017) and may also alter offspring phenotype and fitness (Lindström 1999). We therefore measured maternal reproductive and stress hormones to determine the proximate mechanisms linking maternal effects with offspring phenotype and dispersal potential in a coral reef fish.

Here we determined whether parental effects contribute to variation in offspring phenotypes, through a manipulative field experiment on a wild population of *Amphiprion chrysopterus*, the orange-fin anemonefish. Anemonefish are sedentary and territorial, and live in an obligate association with sea anemones, which provide both protection from predators and access to resources (Fautin 1991; Feeney et al. 2018). Coral reef ecosystems, which include sea anemones and anemonefish, are nowadays threatened by a myriad of menaces (Graham et al. 2006; Bonin et al. 2009; Saenz-Agudelo et al. 2011) which may include the death and disappearance, as well as the bleaching of anemones (Hattori 2002; Beldade et al. 2017). Furthermore, environmental changes can stimulate sea anemone asexual reproduction whereby a large solitary sea anemone separates into two via longitudinal fission, impacting habitat quality for anemonefish (Brolund et al. 2004; Bocharova and Kozevich 2011; pers. comm. Cortese). Total anemone number and size are proxies of habitat quality with positive correlations with anemonefish size, reproductive success (number of eggs laid) and parental care (Buston and Elith 2011; Chausson et al. 2018; Barbasch et al. 2020). We used habitat size, i.e. the total number and size of anemones, as a proxy for habitat quality.

The objectives of this study are five-fold. First, we used observational data to correlate natural variability in habitat quality with parental reproductive success and offspring traits. Second, we use a manipulation study to examine how experimental changes in habitat quality impact parental phenotype, their reproductive output as well as the importance of parental effects in determining egg size, larval size and larval traits associated with dispersal ability (swimming performance). Third, we determine whether parental effects are immediate, consistent though time or whether they are delayed and/or plastic over subsequently reproductive events. Fourth, we assess the proximate mechanisms linking parental phenotype, offspring phenotype and offspring dispersal potential. Finally, we discuss how our results enhance our understanding of the role of parental effects in species persistence to environmental change.

3.5. Materials and methods

3.5.1. Study population

This study was carried out on a wild population of 35 adult breeding pairs of *Amphiprion chrysopterus* from 35 different anemone sites located in the lagoon of Moorea, French Polynesia (Table S3.1). Anemonefish, *Amphiprion* spp., are group living in which monogamous couples spawn repeatedly throughout the whole year, sometimes twice per month, in synchrony

with the moon (Seymour et al. 2018). Anemonefish lay benthic eggs that, after 6-7 days of development, hatch into larvae that disperse in the open water (Ross 1978b). The 35 groups consisted of a dominant pair and zero or 1 subordinate non-breeders. Reproduction was monitored every two days for 9 months, from February 1st 2018 to 31st October 2018. Adult reproductive success (number of eggs laid – fecundity; and number of eggs at hatching: see details below) was measured over one month before manipulation and within one to three months after habitat manipulation. Offspring traits (egg size, larval size and larval swimming speed: see details below) were measured in three periods: over one month before manipulation, within one to two months immediately after manipulation, and also after a longer period of time after manipulation (3-5 months) (Table S3.2). After the first spawning, each breeding pair was captured using hand nets and total length of both individuals were measured to 0.1mm using callipers.

3.5.2. Habitat quality measurements

The habitat quality of all sites containing breeding pairs was determined in terms of number of anemones and anemone surface area. Anemone area was calculated from photographs taken with a SONY camera (DSC-RX100M3) and subsequently measured with imageJ considering anemones as ellipses (Holbrook and Schmitt 2005). Given that anemones do not have a hard skeleton and thus are able to shrink and expand (Holbrook and Schmitt 2005), three replicate photographs were taken of 221 anemones from a subset of 28 anemone sites over two-three weeks to ascertain whether anemone size was repeatable over time (R), using the *rptR* package in R (Stoffel et al. 2017). Anemone number did not change over time, and anemone size calculated from photographs data was also repeatable over time ($R = 0.727$; $p < 0.0001$, $n = 627$ photographs).

3.5.3. Natural variability in reproductive success and larval traits

Data from all 35 breeding pairs were used to determine the influence of habitat quality (total anemone surface area) on reproductive success and larval traits (see below for details). Natural variation in parental and offspring traits was examined as a function of habitat quality (anemone surface area) using egg clutches laid by breeding pairs from all treatments before manipulation and only from *control* treatments after manipulation. For this analysis, a total of 86 egg clutches from 34 sites were used for reproductive success and a total of 265 eggs from 25 egg clutches

and 18 sites were used for egg size, and 205 larvae from 28 egg clutches and 21 sites provided larval traits.

3.5.4. Habitat quality treatments

Our experimental design consisted of measurements taken before and after three treatment manipulations, *control* (no change), *increased* and *decreased* habitat quality. Treatments were allocated randomly between the 35 anemone sites (Table S3.1). The *control* treatment consisted of 14 sites that were subjected to a similar level of diver disturbance as the other two treatments, but without any manipulation in anemone number or size (overall anemone size mean ± SD: 5412 ± 4565 cm^2; number of anemones mean ± SD: 9 ± 5). Measurements from the *control* treatment were used to account for variability in adult and offspring traits over time. In the two other treatments we manipulated habitat quality by changing the anemone number and surface area. The *increased* habitat quality treatment consisted of 12 sites in which anemones were added to double the initial anemone surface area (anemone size before habitat manipulation mean ± SD: 4541 ± 3927 cm^2; after habitat manipulation mean ± SD: 8465 ± 6309 cm^2; number of anemones before habitat manipulation mean ± SD: 6 ± 6; after habitat manipulation mean ± SD: 12 ± 8). The *decreased* habitat quality manipulation consisted of 9 sites in which anemone surface area was halved by removing anemones (anemone size before habitat manipulation mean ± SD: 6404 ± 3038 cm^2; after habitat manipulation mean ± SD: 3454 ± 1698 cm^2; number of anemones before habitat manipulation mean ± SD: 11 ± 5; after habitat manipulation mean ± SD: 7 ± 3).

As female size is the main predictor of reproductive success (Buston and Elith 2011; Barbasch et al. 2020) and larval traits (Cortese et al., Chapter 2), we tested for differences in female size between the three treatment groups but did not find any differences (LM; $F_{2,32}$ = 0.779, p = 0.468). Similarly, as female size is related to anemone size (Ross 1978a; Buston and Elith 2011; Barbasch et al. 2020), we tested for differences in anemone surface area and number between treatment groups. We found no significant differences in either anemone surface area (LM; $F_{2,32}$ = 0.556, p = 0.579) or number of anemones (LM; $F_{2,32}$ = 2.525, p = 0.096) among treatments before habitat manipulation. After habitat manipulation, the anemone surface area of sites where habitat was decreased tend to be smaller than in sites where the habitat was increased, but did not differ from control sites (LM; $F_{2,32}$ = 2.973, p = 0.065, post-hoc (*decrease vs increase*): t = -2.376 p = 0.060; post-hoc (*control vs increase*): t = -1.623 p = 0.251; post-

hoc (*control vs decrease*): t = 0.958; p = 0.608; Table S3.3), while the number of anemones was not different among treatments (LM; $F_{2,32}$ = 2.102, p = 0.139).

3.5.5. Adult reproductive success: Fecundity (number of eggs laid) and number of eggs at hatching

Fecundity was estimated as the total number of eggs that each anemonefish pair laid in a lunar month (~29 days) over the nine-month monitoring period. Anemonefish generally lay once or twice per lunar month (Seymour et al. 2018). The number of eggs in each clutch was determined by counting all eggs from photographs with ImageJ. All egg clutches were photographed at least twice and up to three times during development. Eggs suffer mortality during egg development, from either predation or cannibalism, according to a negative binomial distribution (Supporting Information 1, Fig. S3.1, S3.2). As egg clutch photographs were not always taken either on the first or the same day of development, we applied a correction factor to the number of counted eggs in order to obtain a fecundity measure on the same day of egg development, i.e. on the first (1dfp, day post fertilisation) day of egg development. The percentage of daily egg mortality was estimated at 5.86% of the total clutch of eggs alive on the previous day of development and this correction factor was applied to egg counts from photographs taken on other days of development. This correction factor was verified by comparing correcting egg counts to 1 dpf (or 6 dpf) for which we had observed egg counts and was found to be repeatable [(day1); R = 0.71, p < 0.001; (day6); R = 0.88, p < 0.01, Table S3.6]. No differences were found in daily egg mortality between treatments (GLMM: Treatment; χ = 0.405, p = 0.817; Table S3.4A, B and C). We also estimated the number of eggs at hatching on the last (6 or 7pdf) day of egg development using the same correction factor on egg counts from photographs taken on earlier days of development. Egg clutches that were found unhatched on the sixth (or rarely the seventh) day of development were considered as day of hatching. We collected estimates of both fecundity and number of eggs at hatching from a total of 172 nests, one month before and up to three months after habitat manipulation.

3.5.6. Adult's hormonal data

Adults were captured underwater by SCUBA divers. A blood sample of approximately 0.1 ml per fish for baseline measurement of cortisol and the androgens: 17β-estradiol, testosterone (T) and 11-ketotestosterone (11-KT) was immediately collected laterally from the caudal vein using heparinised 1 ml syringes and kept on ice until processing. The time lapsed from first

approaching the anemonefish until blood was flowing in the syringe was kept as short as possible (mean ± SE = 4:51 min ± 27 s; N = 57).

On return to the CRIOBE field station, blood samples were centrifuged (Sigma Centrifuge 1-14; http://www.sigma-zentrifugen.de) at 10,000 g for 5 min. The plasma was stored at -20°C until analysis. Plasma cortisol, 17β-estradiol, testosterone (T) and 11-ketotestosterone (11-KT) were measured using EIA kits (Cortisol EIA Kit, No. 500360; 17β-estradiol EIA Kit, No. 582251; T EIA Kit, No. 582701; 11-KT EIA Kit, No. 582751; Cayman Chemicals, SPI BIO, France) and a Beckman Coulter AD 340 Spectrophotometer at 405 nm as described in (Mills et al. 2010). The kits for cortisol, estradiol, T and 11-KT have already been validated for this species (Beldade et al. 2017; Mills et al. 2020, 2010).

Due to some difficulties in catching fish and the loss of some blood samples during centrifugation and defrosting, not all samples were available. Blood samples were preferentially used to measure baseline cortisol then 17β-estradiol and 11-KT; if enough of the sample was available for further measures, T was also measured. Therefore, out of the targeted 35 anemonefish pairs, hormone samples were analysed from a total of 34 fish (cortisol: N = 18 females and N = 16 males; 17β-estradiol: N = 8 females; T: N = 12 females and N = 15 males; 11-KT: N = 15 males). 17β-estradiol is synthesized from T in the ovarian follicles via the aromatisation of T using P450 aromatase (CYP19) (e.g. Lee et al. 2006) and 11-KT is biosynthesised primarily via the metabolism of T using 11b-hydroxytestosterone in the testes (Kime 1987). As both E_2 and 11-KT can be synthesised from T, the conversion ratios of T to 11-KT and E_2 were calculated as they indicate the physiological rate at which T is converted into each hormone and how this is impacted by environmental changes.

3.5.7. Offspring measurements (egg size, larval size, larval U_{max})

Unhatched eggs were collected from the field on the day of hatching (day 6 or 7) using a scalpel to detach them from the rock and a manual pump to collect them into a plankton bag and were transported in a cooler to CRIOBE. A subsample of eggs was photographed under a binocular microscope Leica EZ4W and egg surface area was measure with imageJ software considering them as ellipse (N = ~10 egg per clutch, 1 clutch per period for a total of 425 eggs measured from 18 sites). The remaining eggs were transferred into hatching chambers in aquaria (30L) at 27.5 ± 0.5 °C (mean ± SE) and kept in the dark overnight (Fig. S3.3).

Larval fish swimming performance and morphology were measured within 18 hours after hatching (N = ~8 larvae per clutch, 1 clutch per period for a total of 361 larvae from 21

sites). Larval swimming performance was measured using the constant acceleration test (Farrell 2008), which is a measure of the maximum swimming speed in fish (U_{max}). Measurements were carried out in a 170 mL Blaza-style swim tunnel (Loligo System, Viborg, Danmark) immersed in a larger aquarium where temperature was maintained at 27.5 ± 1°C. The swim tunnel system consists of a two glass cylinders inside each other, one larger than the other. The inner cylinder (the swimming chamber) contains the larvae and an impeller at one end (separated from the larvae by a honeycomb screen), which circulates water through the inner cylinder and return is through the space between the inner and outer cylinders. This ensures a one-way flow through the inner cylinder against which the fish swims (Brett 1964). The impeller velocity is regulated by a voltmeter. A larvae was introduced into the swim tunnel and kept at a water velocity of ~one body length (BL) s^{-1} for an acclimation period (10 min) before the trials begun (Plaut 2001). Water flow was then gradually increased at a rate equivalent to approximately half a body length every 30 sec until the larvae was fatigued, could no longer maintain its position in the flow, and was swept towards a honeycomb screen located on the downstream end of the water tunnel (as described in Killen et al. 2015). The equation used to calculate U_{max} of larvae is (Brett 1964): $U_{max} = U_i + [U_j \times (T_i/T_j)]$; where U_i is the penultimate speed (the highest velocity maintained for the whole interval measured in cm s^{-1}), U_j is the velocity increment (0.5 BL, 0.25 cm s^{-1}), T_i is the time swum in the final velocity increment, and T_j is the prescribed interval time (30 sec). After the swimming trial, we measured larval morphology (total length) under a binocular microscope Leica EZ4W after anesthetising the larvae with a solution of MS-222 (0.1 g diluted in 1L of salt water). To account for larval size which is likely to affect swimming speed (Fisher and Hogan 2007), we calculated the larval swimming speed in body length per seconds.

3.5.8. Data analyses

To analyse the role of habitat quality on reproductive and larval traits, we ran a linear mixed effect model (LMER) with the log-transformed anemone surface area and female size as explanatory variables, site as a random factor, and fecundity at 1dpf per lunar month, the number of eggs at hatching at 6 or 7dpf per lunar month, egg size, larval size and larval U_{max} as response variables. In the offspring traits models we and clutch nested into site was also added as a random factor. The interaction among anemone surface area and female size, and the female size were subsequently removed if not significant via the likelihood ratio test (LRT) (Table S3.7A, S3.8A, S3.9A, S3.10A, S3.11A).

To analyse the effect of habitat manipulation on parental hormones we ran LMERs with "treatment" (*control*, *decrease*, *increase*) and "period" (*before* and *after* habitat manipulation) as predictor variables, "site" as a random effect and the basal plasma cortisol concentration, the conversion ratios of T to E_2 and T to 11-KT as response variables (Table S3.12A, S3.13A, S3.14A).

To analyse the temporal and treatment effects on fecundity (1dfp) and on the number of eggs at hatching (6 or 7dfp) per month, we performed a LMER. Fecundity and the number of eggs at hatching were used as response variables, the explanatory variables included "treatment" as a categorical variable (*control*, *decrease*, *increase*), "period", categorical variable (*before* and *after* habitat manipulation), and "site" as a random effect to account for non-independency of data within each site (Table S3.15A, S3.17A). Using a linear model (LM) we also explored the relationship between fecundity (1dpf) and 17β-estradiol.

To explore the effect of habitat manipulation on offspring traits we performed a model per offspring trait, including the explanatory variables "treatment" as a categorical variable with three levels (*control*, *increase*, *decrease)*, and "period" as a categorical variable with three levels (*before*, *after 1* as the first spawning event after manipulation and *after 2* as a subsequent spawning event after habitat manipulation). "Time of treatment exposure" was initially added as a covariate to account for differences in time of treatment exposure and subsequently removed if it did not contribute significantly to the model fit, tested via the LRT. Egg size, larval size and larval swimming speed were used as response variables (Table S3.18A, S3.19A and S3.20A).

In all LMER the interaction between "treatment" and "period" was initially included, whose significance was tested through a likelihood ratio test and removed if not significant. When the interaction was maintained we performed "Tukey" post-hoc tests to explore the pairwise effects on each level of the categorical variables included in the models. In each model, residuals were visually inspected to ensure that all assumptions were met.

We ran all statistical analyses in RStudio (version 1.2.1335) (R Core Team 2019) using the following packages: *lmertest* (Kuznetsova et al. 2017), *ggplot2* (Wickham 2016), *emmeans* (Lenth 2020), *piecewiseSEM* (Lefcheck 2016), *lmtest* (Zeileis and Hothorn 2002), *Rmisc* (Hope 2013), *car* (Fox and Weisberg 2019), *mgcv* (Wood 2017).

3.6. Results

3.6.1. Observational study of natural variability

Natural variability in adult reproductive success: fecundity and number of eggs at hatching:

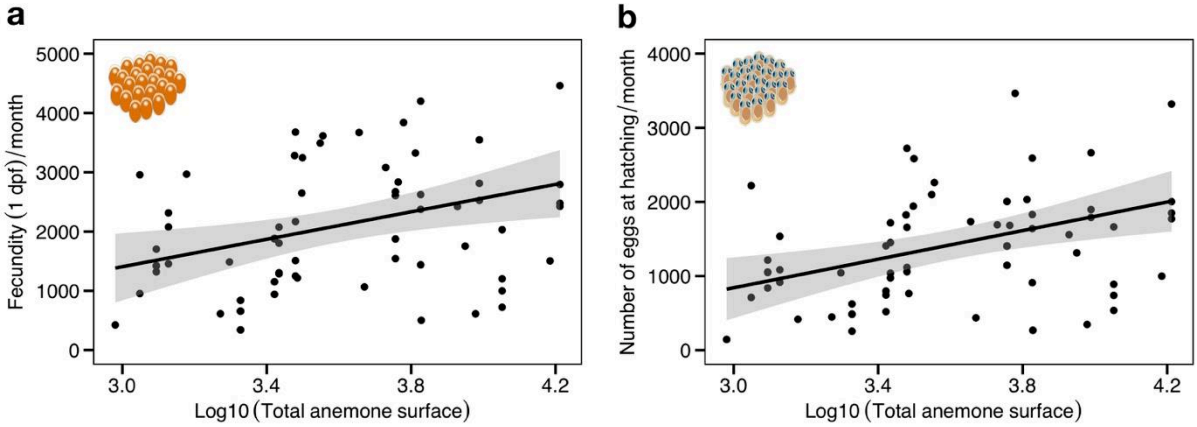

Figure 3. 1. Natural variability in anemonefish reproductive success: (**a**) fecundity at day 1 post fertilization (dpf) and (**b**) number of eggs at hatching at 6 or 7 dpf according to anemone surface area.

Anemonefish living at sites whose anemones have a naturally larger surface area tend to lay more eggs (LMER: log10(Anemone); t = 1.929, p = 0.066, mR^2 = 0.097, cR^2 = 0.692; Table S3.7, Fig. 3.1a) and have more eggs at hatching (LMER: log10(Anemone); t = 2.358, p = 0.027, mR^2 = 0.139, cR^2 = 0.707; Table S3.8, Fig. 3.1b). Female size had no effect on the number of egg laid and those who hatched (LRT; eggs laid: χ^2 = 0.526, p = 0.468; egg at hatching: χ^2 = 0.599, p = 0.439, Table S3.7 and S3.8).

Natural variability in offspring traits

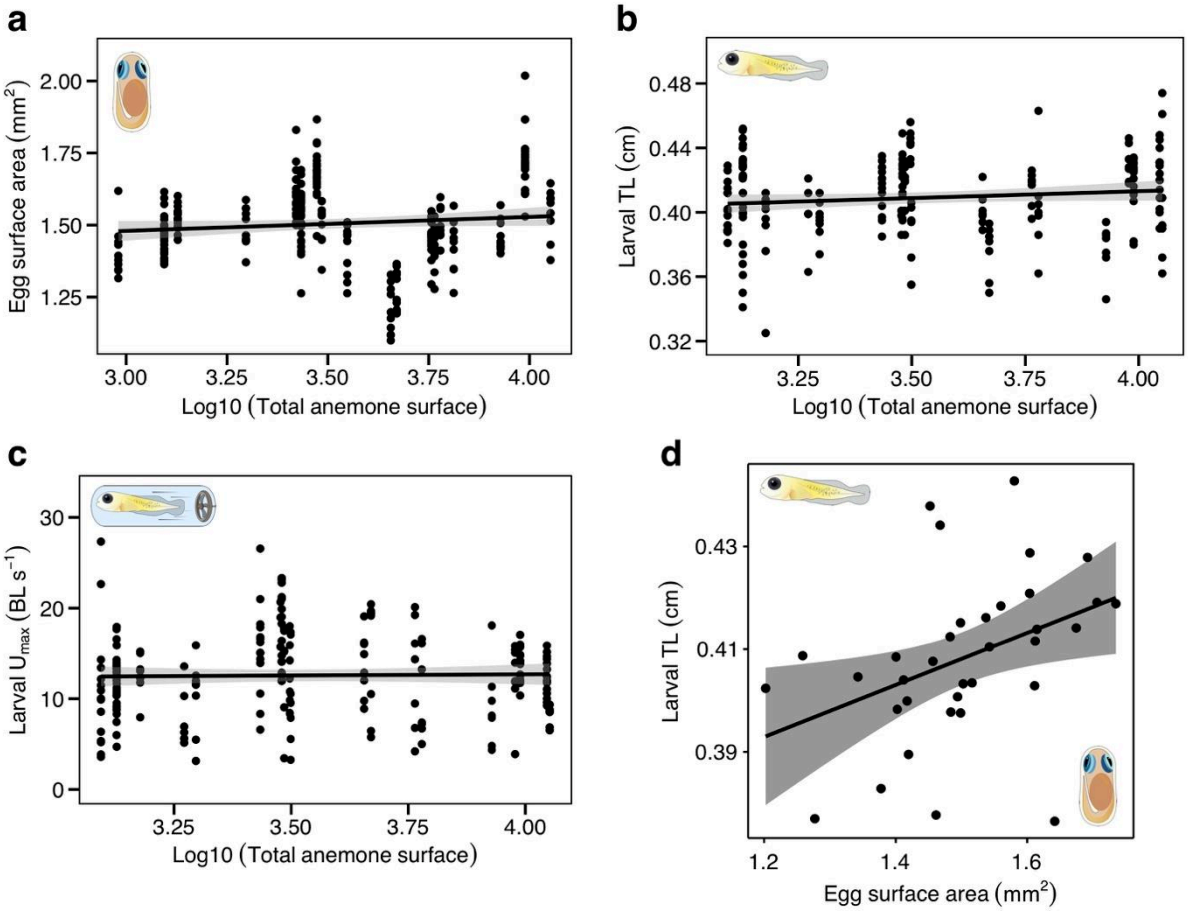

Figure 3. 2. Natural variability in anemonefish (**a**) egg size, (**b**) larval size and (**c**) larval U_{max} according to anemone surface area. (**d**) Correlation between anemonefish larval size (TL) and egg size (surface area). In (d) Each data point represents the mean larval size and the mean egg size from the same clutch (n = 8-10 eggs per clutch and n = 6-8 larvae per clutch; Pearson correlation: R = 0.4, p = 0.018).

Overall, despite large variability in egg and larval metrics, there is no effect of anemone surface area on either egg surface area (LMER: log10(Anemone); t = 0.255, p = 0.802, mR^2 = 0.003, cR^2 = 0.735; Table S3.9, Fig. 3.2a) or larval traits (LMER: Larval TL - log10(Anemone); t = 0.684, p = 0.503, mR^2 = 0.011, cR^2 = 0.364; Larval U_{max} - log10(Anemone); t = 0.071, p = 0.944, mR^2 < 0.001, cR^2 = 0.212; Table S3.10 and S3.11, Fig. 3.2b, c). Larval size is positively correlated to egg size (R = 0.4, p = 0.018, Fig. 3.2d). Female size of fish used in this study had no effect on any offspring trait (LRT; egg size: χ^2 = 1.219, p = 0.270; larval TL: χ^2 = 0.713, p = 0.399; Larval U_{max}: χ^2 = 0.691, p = 0.406, Table S3.9, S3.10 and S3.11).

3.6.2. Habitat manipulation

Adult hormonal data (cortisol, 17β-estradiol, testosterone and 11-ketotestosterone)

Despite the low sample sizes, the conversion ratios of T to 17β-estradiol in females increases when habitat quality is increased (*increase* treatment, LMER- post-hoc: B vs A; t = -3.002, p = 0.022, Table S3.12, Fig. 3.3), whilst it does not change in either the *control* or *decrease* treatments (LMER- post-hoc in control: B vs A; t = 0.618, p = 0.558; decrease: B vs A; t = 0.441, p = 0.674, Table S3.12, Fig. 3.3).

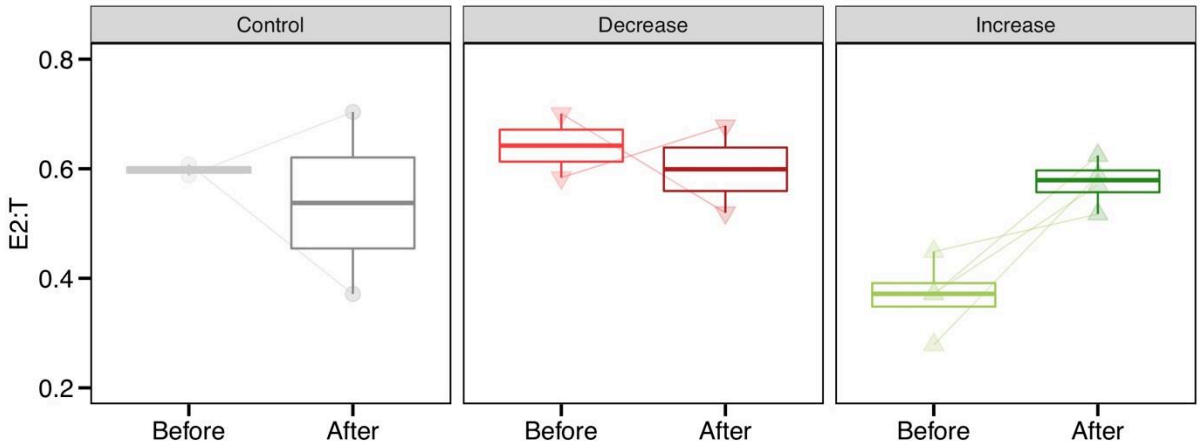

Figure 3. 3. Index of conversion of testosterone to 17β-estradiol of individual adult anemonefish females on the day of hatching in each treatment condition (*control*, *decrease* and *increase* habitat size), before and after habitat manipulation. Thin lines connect individual females.

Similar results were found in males for the conversion ratios of T to 11-KT, but they were non-significant (interaction LRT: χ^2 = 3.101; p = 0.212; LMER: treatment (decrease); t = 0.478, p = 0.636, treatment (increase); t = -0.120, p = 0.905, Table S3.13, Fig. S3.4). However, there were no differences in baseline cortisol levels for both sexes combined (interaction LRT: χ^2 = 0.910; p = 0.233; LMER: treatment (decrease); t = -1.502, p = 0.138, treatment (increase); t = -0.267, p = 0.790, Table S3.14, Fig. S3.5).

Adult reproductive success

a) Fecundity at one-day post fertilization

The number of eggs at one-day post fertilisation (fecundity linked to pre-zygotic effects) produced per month did not change across treatments and periods (ANOVA (LMER): period; $F_{3,2}$ = 2.437, p = 0.070, treatment; $F_{3,2}$ = 0.754, p = 0.479, Table S3.15C, Fig. 3.4a). However,

fecundity significantly correlated with concentration of maternal 17β-estradiol (LMER: Estradiol; t = 4.155, p < 0.001, Table S3.16, Fig. 3.5).

b) Number of eggs at hatching

Fecundity at six- or seven-days post fertilisation, i.e. linked to a combination of pre- and post-zygotic effects, does not change across treatments (ANOVA(LMER): $F_{3,2} = 0.478$, p = 0.624, Table S3.17, Fig. 3.4b). However, we observed an increase in fecundity on the third spawning event after habitat manipulation in all treatments, including controls, which indicates that fecundity increased with time (LMER: period(A3); t = 2.075, p = 0.041, mR^2 0.050, cR^2 = 0.554, Table S3.17, Fig. 3.4b).

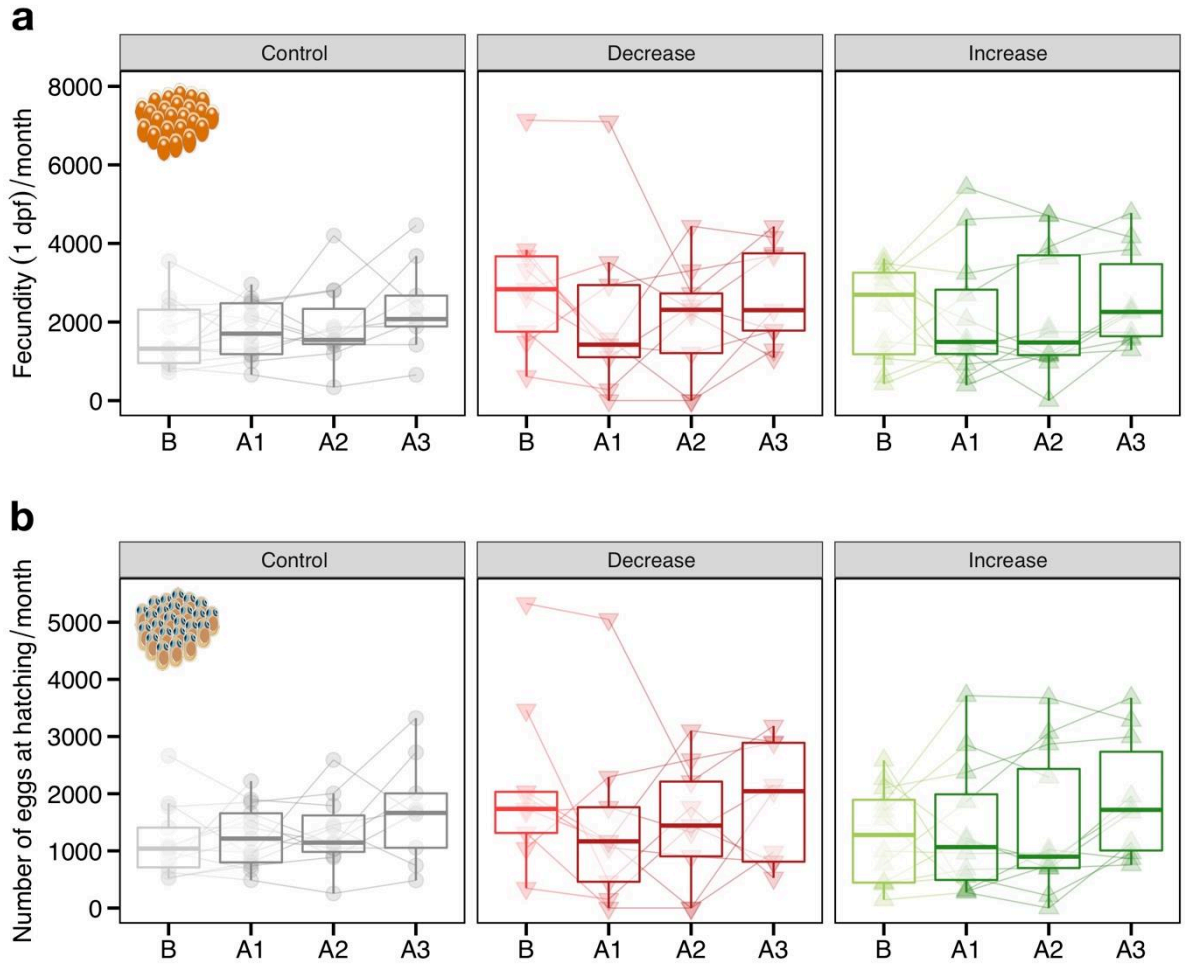

Figure 3. 4. Fecundity at (**a**) one-day post fertilization (dpf) per month and at (**b**) hatching per month in each treatment condition (control, decrease and increase habitat quality), before (B) and after habitat manipulation: A1 = first spawning event after manipulation, A2 = second spawning event after manipulation, A3 = third spawning event after manipulation. Dots and triangles represent individual data points and lines connect each site between two spawning events.

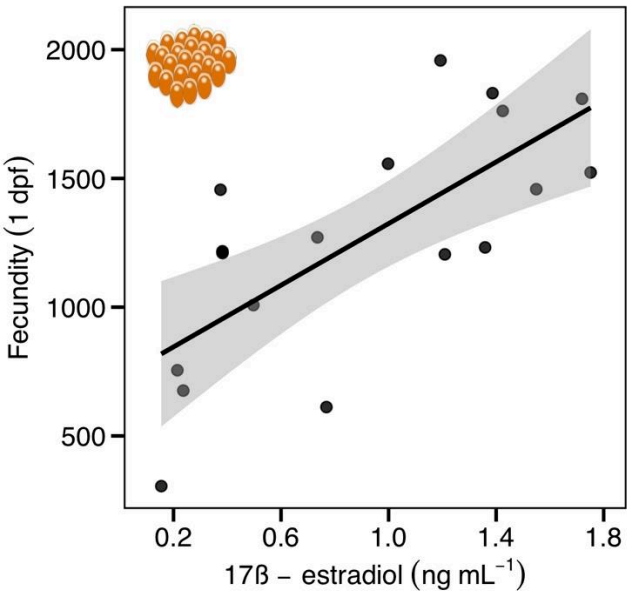

Figure 3. 5. Correlation between anemonefish maternal 17ß-estradiol and fecundity.

Offspring traits

a) Egg size

Anemonefish egg size varies over time with different effects depending on treatment (LRT: interaction period*treatment; χ^2 =7.363; p = 0.007, LMER: $mR^2 = 0.294$, $cR^2 = 0.676$; Table S3.18, Fig. 3.6a). Whilst we observed no difference in egg size before and after manipulation at the *control* sites (post-hoc test LMER: *control* (B *vs* A1); all p > 0.05, Fig. 3.6a, Table S3.18C), egg size decreases immediately after habitat size was decreased (post-hoc test on LMER: *decrease* (B *vs* A1); t = 3.406, df = 403, p = 0.002, Table S3.18C) and at the *increase* habitat quality sites eggs were larger immediately after habitat manipulation (post-hoc test on LMER – *increase* (B *vs* A1): t = -6.352; df = 402; p < 0.001; Table S3.18C).

In terms of changes a longer period after manipulation, egg size did not change at the *control* sites (post-hoc test LMER: *control* (B *vs* A2, A1 *vs* A2); all p > 0.05, Fig. 3.6a, Table S3.18C) and did not change at the sites where habitat quality *increased*, the size was still larger than before the manipulation (post-hoc test on LMER – *increase* (B *vs* A2): t = -5.075; df = 415; p < 0.001; (A1 *vs* A2): t = -1.090; df = 414; p = 0.521; Table S3.18C). On the other hand, egg size in the *decrease* treatment increased, rather than further decreased (post-hoc test on

LMER: *decrease* (A1 *vs* A2); t = -3.198, df = 410, p = 0.004, (B *vs* A2); t = -2.076, df = 410, p = 0.004, Table S3.18C).

b) Larval size

Larval size is affected by habitat manipulation, varying over time with different effects depending on treatment (LRT: interaction period*treatment; χ^2 = 11.039; p = 0.012; LMER: mR^2 = 0.136, cR^2 = 0.286, Table S3.19, Fig. 3.6b). Whilst we observed no difference in larval size before and after habitat manipulation at the *control* and *increase* sites (control: (B *vs* A1) t = -0.753; df = 335; p-value = 0.732; increase: (B *vs* A1) t = -0.848; df = 341; p-value = 0.673; Table S3.19C, Fig. 3.6b), anemonefish pairs living at sites where habitat size was *decreased* produced smaller larvae immediately after habitat manipulation (post-hoc test on LMER – (B *vs* A1) t = 2.370; df = 337; p-value = 0.048; Table S3.19C).

In terms of changes over a longer time period, we have no measures for the control sites but, anemonefish pairs living at sites where habitat size was *increased* produced larger larvae ((A1 *vs* A2) t = -3.310; df = 351; p-value = 0.003; Table S3.19C) yet at the sites whose habitat size was *decreased* we observed a similar response as with egg size, larval size increased on the longer time after manipulation, resulting in no difference in larval size compared to before (post-hoc test on LMER – (B *vs* A2) t = 0.065; df = 347; p-value = 0.998; (A1 *vs* A2) t = -2.071; df = 347; p-value = 0.097; Table S3.19C).

c) Larval swimming speed (U_{max})

Larval swimming performance (U_{max}) varies across treatments and over time (LRT: interaction period*treatment: χ^2 = 8.214, p = 0.042, LMER: mR^2 = 0.052, cR^2 = 0.154, Table S3.19C, Fig. 3.6c). Whilst we observed no difference in larval U_{max} before and after habitat manipulation at the *control* and *increase* sites (post-hoc on LMER: *control* (B vs A1); t-ratio = 0.803, df = 336, p = 0.701, *increase* (B vs A1); t-ratio = -0.334, df = 343, p = 0.940, Table S3.19C, Fig. 3.6c), we observed an increase in larval U_{max} after habitat manipulation in the *decrease* treatment (post-hoc on LMER: (B vs B1); t-ratio = -2.738, df = 338, p = 0.018, Table S3.19C, Fig. 3.6c).

In terms of changes over a longer time period, we have no measures for the control sites, but larval U_{max} is still higher in the *decrease* treatment (B vs A2: t-ratio = -2.612, df = 350, p = 0.025, Table S3.19C) and larval U_{max} also increases on the longer time after habitat manipulation in the *increase* treatment (A1 *vs* A2: t = -2.633; df = 353; p-value = 0.024; B *vs* A2: t-ratio = --2.870, df = 348, p = 0.012, Table S3.19C).

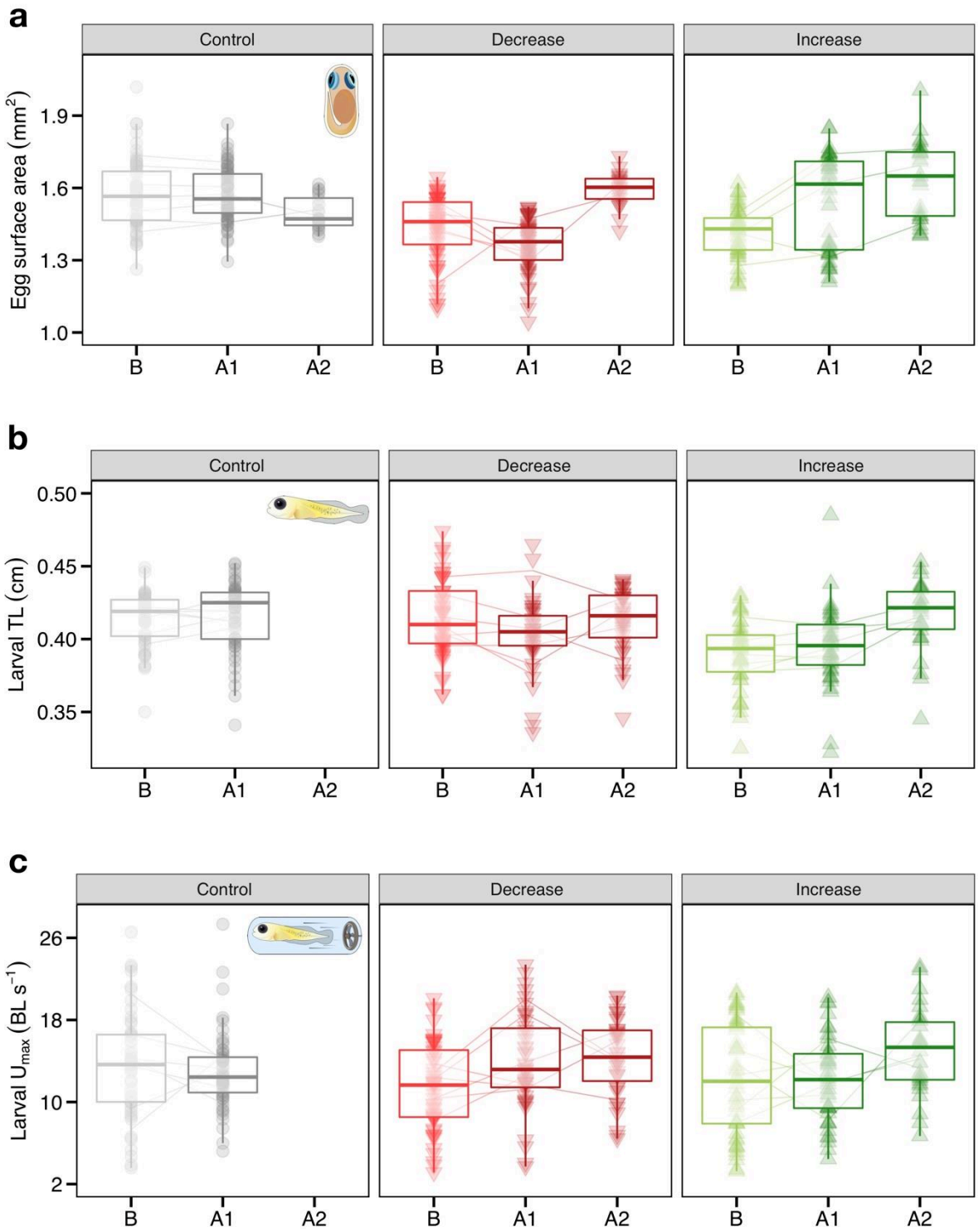

Figure 3. 6. Treatment effects (control, decrease and increase in habitat size) on offspring traits: (**a**) egg surface area of 10 eggs per clutch, (**b**) larval total length and (**c**) larval swimming performance (U_{max}). The treatment effect is represented by (B) before, (A1) immediately after and (A2) a longer period after habitat manipulation. Dots represent individual data points and lines connect each site before and after manipulation.

3.7. Discussion

Offspring phenotypes of free-living anemonefish showed plasticity when exposed to manipulated changes in habitat quality in the wild. Despite a positive relationship between reproductive success and natural anemone habitat size in the wild population of Moorea, anemonefish reproductive success was not significantly affected by habitat size manipulation. In terms of egg and larval phenotypes, no changes were observed in the control treatment, but when habitat quality decreased, anemonefish produced smaller eggs that hatched into smaller but faster larvae, and when habitat quality increased, anemonefish produced larger eggs, which did not immediately translate into any differences in larval traits. Three to five months after the increase in habitat quality, eggs further increased in size and now translated into increased larval size and swimming speed. When habitat quality was decreased, the immediate effects of habitat manipulation on larval swimming performance persisted over time, whereas larval and egg sizes recovered. Anemonefish fecundity correlated positively with maternal 17β-estradiol, and the conversion ratio of testosterone to 17β-estradiol increased when habitat size was artificially increased. Our habitat quality manipulation experiment demonstrates that parents dynamically adjust offspring phenotype in opposite ways depending on the direction of the manipulation and maternal reproductive hormones may be the proximate mechanism linking maternal phenotype with offspring phenotypes.

Parental effects

The observed plasticity in offspring phenotypes could be a direct effect of the manipulated environment on egg and larval phenotypes, however, this is highly unlikely as habitat quality does not directly impact egg and larval phenotypes, as eggs require no direct provisioning from anemones or the surrounding water. Parental effects are likely to be the mechanism underlying the observed offspring phenotypic plasticity in response to habitat change, and such effects may be non-adaptive or adaptive. First, non-adaptive maternal effects may occur when breeding females cannot maintain homeostasis in face of environmental change and offspring phenotypes are modified as a result. However, there is no evidence to suggest that parental homeostasis was not maintained, as parental baseline cortisol levels did not increase after manipulation and a parental stress response did not carry-over onto the offspring's phenotype. Secondly, non-adaptive parental effects may be due to parental allocation trade-offs which result in negative consequences on offspring fitness (Marshall and Uller 2007), in a similar way to how maladaptive developmental instability impacts variation in offspring size in stressful, novel or

less favourable environmental conditions (Debat and Patrice 2001; Olsson and Uller 2002; De Jong 2005; Pörtner and Farrell 2008). However, offspring phenotype was not only modified in the decreased, but also in the increased habitat quality treatment and in opposing ways, which rules out non-adaptive parental effects. Furthermore, in the decreased habitat quality manipulation, whilst smaller eggs hatched into smaller larvae, they swam faster which runs contrary to expectations if their fitness were compromised.

Our observed changes in offspring phenotype point towards adaptive parental effects representing an evolved parental adaptation to a particular environment. The environmental change that induced parental effects, habitat quality, is a biotic factor that anemonefish have depended on throughout their evolutionary history, and habitat size is a strong determinant of reproductive success in this species (Buston and Elith 2011, and Fig. 3.1). Moreover, this parental effect is likely adaptive as it enables parents to produce offspring with better swimming abilities when habitat quality declines. Such adaptive parental effects may be most important after local environmental perturbations such as cyclones or bleaching events, when habitats are rapidly degraded, and provide the larvae with the possibility of dispersing to better habitats, or in the case of climate change to expand their range.

Strength and direction of parental effects

Previous studies have demonstrated that the strength and direction of parental effects on offspring phenotype vary as a function of local environmental conditions. Maternal effects are considered to have stronger effects on offspring phenotype under severe perturbations and weaker effects under milder changes (Gliwicz and Guisande 1992; Fox et al. 1997; Mousseau and Fox 1998; Räsänen and Kruuk 2007). Immediately following an unfavourable habitat manipulation (decreased habitat quality) we observed strong parental effects on multiple offspring phenotypes including egg size, larval size and larval swimming performance, whereas the only observed phenotypic change when the manipulation was favourable (increased habitat quality) was on egg size. However, parental effects on offspring phenotypes were comparable between the opposing habitat manipulations over a longer-time period, after three to five months. Therefore, over a longer-time period not only is the strength of parental effects similar between treatments but parental effects are apparent regardless of the direction of perturbation of habitat quality. At the population level, there is natural variation in anemone habitat quality and therefore female anemonefish will be subjected to environmental heterogeneity in habitat quality. As such, we hypothesise that there will be selection for plasticity on offspring phenotype in anemonefish as habitat quality cannot be predicted until anemonefish have

changed sex into a breeding female and oogenesis has begun. At the individual level although breeding females do not change their anemone habitat during their lifetime and apart from a gradual but slow increase in total anemone surface area with time and with increasing group size (Holbrook and Schmitt 2005) the habitat size of breeding females will remain unaltered and selection on readjustment of offspring phenotype may be at most under very weak. However, environmental perturbations that impact anemone survival are common within and across populations such as outbreaks of Crown of Thorns sea stars (Vogler et al. 2008), predation (Holbrook and Schmitt 2005), cyclones and mass bleaching events (Hughes et al. 2018), and as such there should be selection on plasticity of offspring phenotype in response to habitat quality.

Proximate mechanisms

Habitat quality manipulation might have compromised parental care, impacting offspring phenotype and may be a possible proximate mechanism driving the observed changes. On the one hand, territorial defence, specifically heterospecific chasing and aggression (Mills et al. 2020), may increase when habitat size increases, but on the other hand, a reduction in habitat size and thus available shelter, may increase predation risk. Both scenarios will result in less time spent on parental care behaviours, which may impact offspring phenotype. A reduction in nest guarding might impact reproductive success, in particular number of eggs at hatching, but no differences were found between treatments. A reduction in egg fanning, which oxygenates eggs, is predicted to result in larger eggs (Einum et al. 2002), which indeed was found when habitat quality was increased, but the opposite was found when habitat quality was decreased, and thus cannot explain both results. Unfortunately, we did not record parental care behaviour, but if an increase in habitat quality increases territorial defence and aggression we would observe an accompanying elevation in male 11-ketotestosterone (11-KT) levels (Mills et al. 2020). 11-KT is a sex-specific steroid in many teleosts driving spermatogenesis, and involved in the development and maintenance of male reproductive traits, reproductive behavior, aggression and territory defense (Cochran 1987; Brantley et al. 1993; Borg 1994; Schulz and Miura 2002; Desjardins et al. 2008; Mills et al. 2018). However, we found no difference in the conversion ratio of T into 11-KT between males from the different habitat manipulation treatments. Therefore, our results suggest that paternal effects and parental care in anemonefish are unlikely to be the mechanisms driving plasticity in offspring phenotype.

The most parsimonious explanation for offspring phenotypic plasticity is via maternal effects, specifically maternal investment through cytoplasmic transfer of hormones. Changing

environmental conditions are known to influence the fluctuations in hormone levels that occur when females are transitioning between oogenesis and egg laying, leading to differential allocation of maternal resources to oocytes (Schwabl 1993; Groothuis and Schwabl 2002; Müller et al. 2002; Badyaev et al. 2006; Badyaev and Oh 2008; Rutkowska and Badyaev 2008). 17ß-estradiol (E_2) is the major oestrogen in fish reproduction, particularly in vitellogenesis and oocyte maturation (Fostier et al. 1983; Ng and Idler 1983; Lazier et al. 1987). Maternal levels of E_2 not only correlate with fecundity, but they also increased when habitat quality increased (Figure 3.3; unfortunately the sample size was too low to determine whether E_2 levels decreased when habitat quality decreased) and as such are the most plausible proximate mechanism driving differences in offspring phenotype. Exactly how a female's assessment of changes to anemone size is translated into elevated E_2 is less clear. Changes to habitat size will alter contact and possibly aggressive interactions with heterospecifics that share the host anemone, such as *Dascyllus trimaculatus* and may alter female endocrinology which are reflected in offspring phenotype as found for birds (Whittingham and Schwabl 2002). Future studies manipulating female hormones levels and determine the response on offspring phenotype would be ideal to test this hypothesis.

Fecundity

Habitat modification of anemonefish does not affect the number of eggs laid per clutch, despite the positive link between fecundity and habitat size in the natural environment. One explanation may be linked to the focal anemone under which anemonefish lay their eggs and receive protection during incubation. Anemonefish repeatedly lay on the same area of rock under an anemone throughout the year and in each territory we did not manipulate the preferred anemone to ensure continued reproduction. Our decision may have reduced the observed effects of a decline in habitat quality on fecundity and the effects of natural perturbations on offspring phenotype may not only be stronger than those we observed, but may also impact fecundity.

Potential implication for dispersal

Our observations that a decrease in habitat quality produces both smaller eggs and larvae, that nonetheless swim faster may represent a strategy for increasing dispersal under declining local conditions. Growing evidences show intra-specific variability in marine fish larvae (Nanninga and Berumen 2014) but linking their dispersive phenotypes with dispersal outcomes will likely remain a challenge for the near future, but much as been learnt from the interplay between environmental change and dispersal morphs in other animals (e.g. Zera and Denno 1997).

Certain marine invertebrates produce offspring with dimorphic phenotypes depending on the parental environment (Krug 2009; Krug et al. 2012), and avian mothers with few resources available produce offspring that are more likely to disperse, while those with access to plentiful resources produce offspring more likely to settle close to home (Fowler 2005; Duckworth 2009), however the opposite has also been found (Massot and Clobert 1995). Exposure to pollutants also impacts dispersal potential whereby the decreased condition of exposed bryozoans produce larger offspring that are not only more resistant to the pollutant but that also have a higher dispersal potential (Marshall 2008). However, these changes had negative impact on later stages, with larvae from pollutant-exposed mothers having lower survival and growth after metamorphosis (Marshall 2008). Therefore, it seems that positive maternal effects are context-dependent as maternal effects that increase performance during one offspring life-stage, decrease performance in others. In terms of our study, maternal effects in response to a decrease in habitat quality may trade-off their investment in egg size with increased larval swimming capacities. Indeed, reduced maternal investment in deteriorated environmental conditions has often been shown to increase offspring dispersal (Hughes et al. 2003a).

Theory states that larger offspring should be produced when conditions for juvenile growth and survivorship are poor (Sibly and Calow 1983; Parker and Begon 1986), however this was described for terrestrial species living in discrete habitats and may not take into account the vast area over which planktonic larvae of coral reef species disperse. Selection might therefore be expected to be higher on traits associated with choosing and reaching a suitable place to settle, such as larval swimming speed, than on other traits such as egg size. What is surprising is the lack of correlation between larval size and larval swimming ability and this link is considered to be strong (Fisher et al. 2000), unless this can be explained by the trade-off in performance discussed previously. The production of faster swimming larvae may be an investment in offspring that can potentially disperse further or swim more actively and thus be able to reach and better recognize a suitable habitat in which to settle (Nanninga and Berumen 2014). However, faster larvae may require more investment and thus may trade-off with egg and larval size (Marshall and Uller 2007).

Conclusions

A. chrysopterus fecundity (eggs laid and at hatching) is not affected by changes in parental habitat quality, however considerable changes in egg and larval traits support the evidence of plasticity in anemonefish parental investment. While it is well known that mothers can alter their offspring's phenotype when the environment is a good predictor of offspring environment

(Mousseau and Fox 1998; Agrawal 2002), the capacity of parents to modulate offspring phenotype is less well known in species with unpredictable offspring environments. Anemonefish are strongly attached to their habitat (Fautin 1991; Elliott et al. 1995; Buston 2003b) and are likely to have low adaptive potential to changes in their habitat due to low additive genetic variation (Salles et al. 2019). As such, plasticity in parental reproduction investment may be a key mechanism for coping to environmental changes in anemonefish (Barbasch et al. 2020). The production of larvae that swim faster when habitat size decreases may promote recruitment into new, better habitats suggesting that adaptive capacity to habitat change may arise from parental effects (Ghalambor et al. 2007). Adaptive parental effects that enable breeding pairs to influence their offspring dispersal phenotype in response to change in current environmental conditions may be particularly important on population dynamics and persistence to environmental change, especially in the current context of global climate change.

3.8. Acknowledgment

Financial support was provided by the Agence National de la Recherche to Glenn Almany, SCM, and RB (ANR-14-CE02-0005-01/Stay or Go) and to SCM (ANR-11-JSV7-012-01/Live and Let Die), and by LabEx "CORAIL" to RB and SCM ('Where do we go now?'). SSK was supported by Natural Environment Research Council Advanced Fellowship (NE/J019100/1) and European Research Council starting grant (640004). We particularly thank Till Deuss and Robin Mannion for their support and help in the CRIOBE anemonefish aquarium facility. We thank Sam Manning, Camille Vizon, Tara Cousins and Marc Besson for support in the field. We are also thankful to Gilles Siu, Pascal Ung, and all the technicians and CRIOBE community for support in the field.

3.9. Supplementary materials

Table S3. 1. List of sites including site ID, manipulation treatment, measured traits (fecundity, egg size and larval traits (larval size and larval swimming speed) and anemone surface area before and after habitat manipulation.

Site (pair ID)	Treatment	Response variables			Anemone surface (cm^2)	
		Fecundity	Egg size	Larval traits	Before	After
1	Control	X			8802	8802
2	Control	X			2125	2125
3	Control	X			6705	6705
4	Control	X	X	X	2716	2716
5	Control	X	X		2639	2639
6	Control	X		X	3017	3017
7	Control	X	X	X	1242	1242
8	Control	X	X	X	1342	1342
9	Control	X		X	11118	11118
10	Control	X		X	1341	1341
11	Control	X			16292	16292
12	Control		X		2970	2970
13	Control	X	X		5708	5708
14	Control	X	X	X	9748	9748
15	Decrease	X	X	X	4527	2157
16	Decrease	X	X	X	6010	3750
17	Decrease	X	X	X	11270	4723
18	Decrease	X		X	3141	1288
19	Decrease	X			8905	5955
20	Decrease	X		X	9512	5013
21	Decrease	X	X	X	5810	4065
22	Decrease	X	X	X	1980	1043
23	Decrease	X	X		6482	3095
24	Increase	X			15304	24804
25	Increase	X		X	1869	4169
26	Increase	X	X	X	4683	9418
27	Increase	X	X	X	8478	16045
28	Increase	X	X		956	2131
29	Increase	X	X		3532	6897
30	Increase	X		X	3155	6455
31	Increase	X		X	1506	2806
32	Increase	X	X	X	3054	6441
33	Increase	X			5364	9732
34	Increase	X		X	3001	5700
35	Increase	X			3593	6982
Sample size (n sites)		34	18	21		

Table S3. 2. Time between the manipulation and the periods of offspring measures (mean ± SD in days). Period (B) refers to the period prior to manipulation; Period (A1) refers to a shorter-term period, and Period (A2) refers to the longer-term period.

		Egg measurements		**Larval measurements**	
	Treaments	Mean	SD	Mean	SD
Period (B)	control	22.5	13.9	31.4	30.4
	decrease	29.8	23.8	23.6	16.2
	increase	36.4	19.2	31.8	18.7
Period (A1)	control	56.6	57.6	56.2	56.8
	decrease	14.0	3.2	23.9	13.7
	increase	49.6	18.6	54.9	50.7
Period (A2)	control	88.0	43.1	-	-
	decrease	165.0	30.8	66.6	41.7
	increase	111.7	38.2	83.0	23.8

Table S3. 3. Differences in anemone surface area among treatment groups after habitat manipulation.

Table S3. 3A. Results of the best-fit linear model using anemone surface area as a response variable and treatment as a predictor variable. R^2 is the multiple R-squared, which describe the proportion of the variance explained by the predictors variables included in the model. R^2_{adj} is the adjusted R-squared, which adjust the statistic based on the number of independent variable included in the model.

Fixed effects	**Estimate**	**SE**	**t-value**	**p-value**	R^2	R_{adj}^2
Intercept	5412	1278	4.234	<0.001	0.157	0.104
Treatment(*decrease*)	-1958	2044	-0.958	0.345		
Treatment(*increase*)	3053	1882	1.623	0.114		

Table S3. 3B. ANOVA of the best-fit model from Table S3.3A.

	Df	**SS**	**MS**	**F**	**p-value**
Treatment	2	136012346	68006173	2.9729	0.065
Residuals	32	732018272	22875571		

Table S3. 3C. Post-hoc test of the best fit model from table S3.3A.

Fixed effects	**Estimate**	**SE**	**df**	**t-value**	**p-value**
Control *vs* decrease	1957	2043	32	0.958	0.608
Control *vs* increase	-3053	1882	32	-1.623	0.251
Decrease *vs* increase	-5011	2109	32	-2.376	0.060

Supporting Information 1: Mortality during egg development and correction factor

To determine mortality during egg development, three photographs of the same egg clutch (N = 73) were taken across egg development over the whole experiment. The total number of eggs in each egg clutch was counted using ImageJ from a total of 219 photographs. Mortality over egg development was determined by fitting a generalized linear mixed model (GLMER) with a negative binomial distribution. The number of eggs was used as a count response variable and the developmental stage (from one to seven-days post fertilisation), the treatment (control, increase or decrease) and manipulation period (before or after manipulation) were used as fixed explanatory variables. Developmental stage was log-transformed due to scaling issues with the other model terms. In addition, site and nest ID (i.e. egg clutch) were used as random factors to account for non-independence of data. Interactions among model terms were initially included and subsequently dropped if not significant using the likelihood ratio test (LRT). The number of eggs decreased through development following the negative binomial distribution (GLMER: log(stage); $t = -0.352$, $p < 0.001$, $mR^2 = 192$, $cR^2 = 0.804$, Fig. S3.1, Fig. S3.2, Table S3.4B). Although the number eggs were higher in the period after manipulation (GLMER: $t = 2.772$, $p = 0.006$, Fig. S3.1, Table S3.4B), neither treatment nor manipulation period had an effect on mortality over developmental stage (the slope), as represented by the non-significant interaction between these variables (LRT: $\chi^2 = 9.34$, df = 15, $p = 0.096$, Table S3.4A).

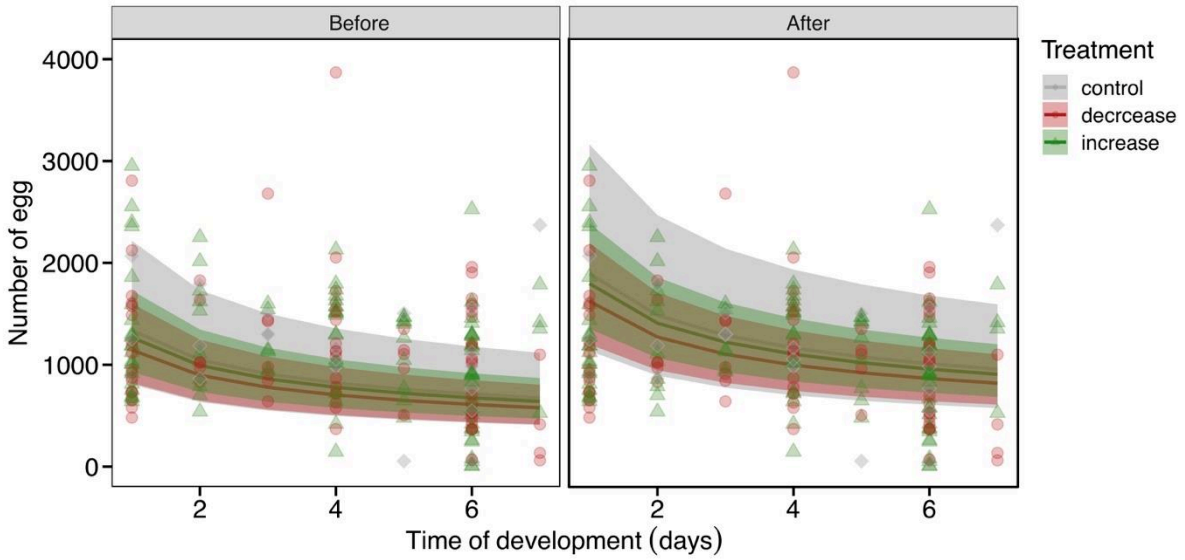

Figure S3. 1. Egg mortality over developmental stage represented as the decrease in number of eggs from one to seven-days post fertilization. The different colours refers to the three habitat

size treatments (control in grey, decrease in red and increase in green) and the boxed "B" and "A" refer to the period of habitat manipulation.

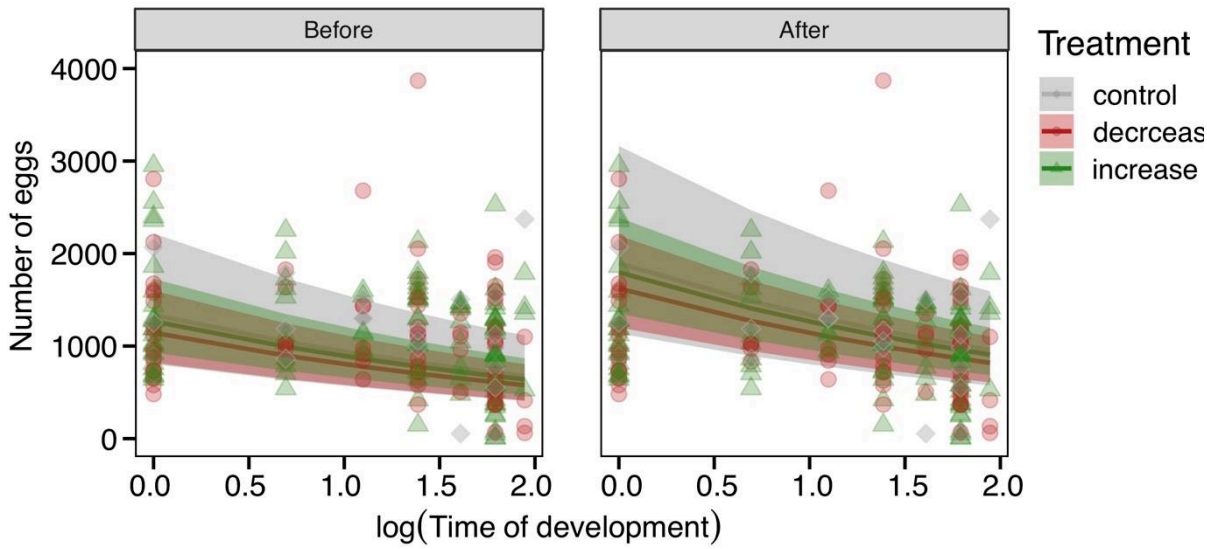

Figure S3. 2. Egg mortality over developmental stage represented as the decrease in number of log-transformed eggs from one to seven-days post fertilization. The different colours refers to the three habitat size treatments (control in grey, decrease in red and increase in green) and the boxed "B" and "A" refer to the period of habitat manipulation.

Table S3. 4. Models and model outputs for egg mortality through development analyses

Table S3. 4A. List of all negative binomial generalized linear mixed models (GLMM) performed with Y (number of eggs) as the response variable. Stage is the egg developmental stage from 1 to 7 days post fertilisation. Trtm is the habitat treatment (control, used as a reference, decrease and increase habitat size), Period is the manipulation period used as a categorical variable (before manipulation vs. after manipulation). The sites and egg clutch (nest) were used as random factors. Model selection was performed by sequentially dropping the non-significant interactions, and models were compared using the likelihood ratio test (LRT) in order to adopt the best-fit model (in bold) that included Period and treatment as predictor variables.

Model		df	LogLik	χ^2	p-value
1	$Y \sim log(stage) * Trtm * Period + (1\|site) + (1\|nest)$	15	-1644.1		
2	$Y \sim log(stage) + Trtm * Period + (1\|site) + (1\|nest)$	10	1648.8	9.344	0.096
3	$\boldsymbol{Y \sim log(stage) + Trtm + Period + (1\|site) + (1\|nest)}$	8	-1650.3	3.068	0.216

Table S3. 4B. Results of the best-fit model from Table S3.4A. mR^2 is the marginal R^2, which describes the proportion of variance explained by the fixed factors alone. cR^2 is the conditional R^2, which describes the proportion of variance explained by both fixed and random factors.

Fixed effects	Estimate	SE	z-value	p-value	mR^2	cR^2
Intercept	7.1978	0.258	27.863	<0.001	0.192	0.804

log(stage)	-0.3515	0.030	-11.593	<0.001
Treatment(*decrease*)	-0.1550	0.290	-0.535	0.593
Treatment(*increase*)	-0.0531	0.284	-0.187	0.851
Period(after)	0.3491	0.126	2.772	0.006

Number of photographs = 219 from 73 egg clutches (groups (NestID)) from 19 sites.

Table S3. 4C. ANOVA of the best-fit model from Table S3.4A

	χ	df	p-value
log(stage)	134.3861	1	<0.001
Treatment	0.4052	2	0.817
Period	7.6833	1	0.006

Percentage of daily mortality obtained from the results in Table S3B:

$\%\Delta y = \beta\ \%\Delta x$

Every day there is a decrease of 5.86% of eggs from the previous day.

Photographs of eggs

All sites used in the study were monitored every 2-3 days (monitoring was not possible on a daily basis). Consequently, we were not able to always photograph nests within one day of being laid (56% of the photographs were not taken on Day 1 of spawning), however photographs were taken as soon as a new clutch was observed. Similarly, but to a lesser extent, we did not always take a photograph on the day of hatching (44%). Due to the presence of egg mortality along development, in order to obtain accurate measurements of Fecundity and Number of eggs at hatching, egg counts from these photographs need to be corrected in order to account for mortality. In terms of Fecundity, eggs need to be added to the total number of eggs counted from photographs on Day 2, 3 and 4 to account for past mortality. In terms of Number of eggs at Hatching, eggs need to deducted from the number of eggs counted from photographs on Day 3, 4, and 5 to account for future mortality. A summary of the number of clutches that needed a correction is given in Table S2C.

Egg correction:

<u>To obtain Fecundity (on Day 1):</u>

<u>From day 2 photographs:</u>

$$Eggs\ D2 + \left(Eggs\ D2\ \times \frac{5.86}{100}\right)$$

<u>From day 3 photographs:</u>

$$Eggs\ D3 + \left(Eggs\ D3\ \times \frac{5.86}{100}\right) + \left(Eggs\ D3\ \times \frac{5.86}{100}\right) \times 1/(1 - \frac{5.86}{100})$$

<u>To obtain Number of eggs at Hatching (on Day 6):</u>

<u>From day 5 photographs:</u>

$$Eggs\ D5 - \left(Eggs\ D5 \times \frac{5.86}{100}\right)$$

<u>From day 4 photographs:</u>

$$Eggs\ D4 - \left(Eggs\ D4\ \times \frac{5.86}{100}\right) - \left(Eggs\ D4\ \times \frac{5.86}{100}\right) \times (1 - \frac{5.86}{100})$$

Etc…

Description of clutches that required egg correction:

For Fecundity analyses, we counted eggs from 172 photographs of nests, from which N = 75 were from photographs taken at one-dpf (days post fertilization), and N = 97 at later developmental stages (Table S3).

For Number of eggs at Hatching analyses, we counted eggs from 172 photographs of nests, from which N = 96 were from photographs taken on the day of hatching and N = 76 from earlier developmental stages (Table S3).

Table S3. 5. Number of counted and corrected nests.

Table S3. 5A. Fecundity (Day 1)

Treatment	**Counted from photographs taken on Day 1**	**Correction applied on photographs taken from Day 2-6**	**Total**
Control	23	31	54
Decrease	25	25	50
increase	27	41	68

Total	**75**	**97**	**172**

Table S3. 5B. Number of eggs at Hatching

Treatment	**Counted from photographs taken on Day of Hatching**	**Correction applied on photographs taken from Day 1-5**	**Total**
Control	26	28	54
Decrease	30	20	50
increase	40	28	68
Total	**96**	**76**	**172**

Table S3. 6. Repeatability analyses on the correction factor.

Table S3. 6. Repeatability tests of egg count for evaluating how consistent the number of eggs at day 1 and day 6 was among nests and across the real and corrected measures. Repeatability is calculated using the rptR package (Stoffel et al. 2017). Type is the type of measure (real or corrected), Trtm is the habitat quality treatment (control, decrease or increase) period is period before or after the habitat manipulation, and ID is the nest ID, which was included in the model as a random effect.

Model structure (number of eggs at day 1)	**R**	**95% CI**	**p-value**
1: $egg(day1) \sim type + Trtm + period + (1\|ID)$	0.71	0.562-0.828	<0.001

Model structure (number of eggs at day 6)	**R**	**95% CI**	**p-value**
1: $egg(day6) \sim type + Trtm + period + (1\|ID)$	0.878	0.83-0.919	<0.001

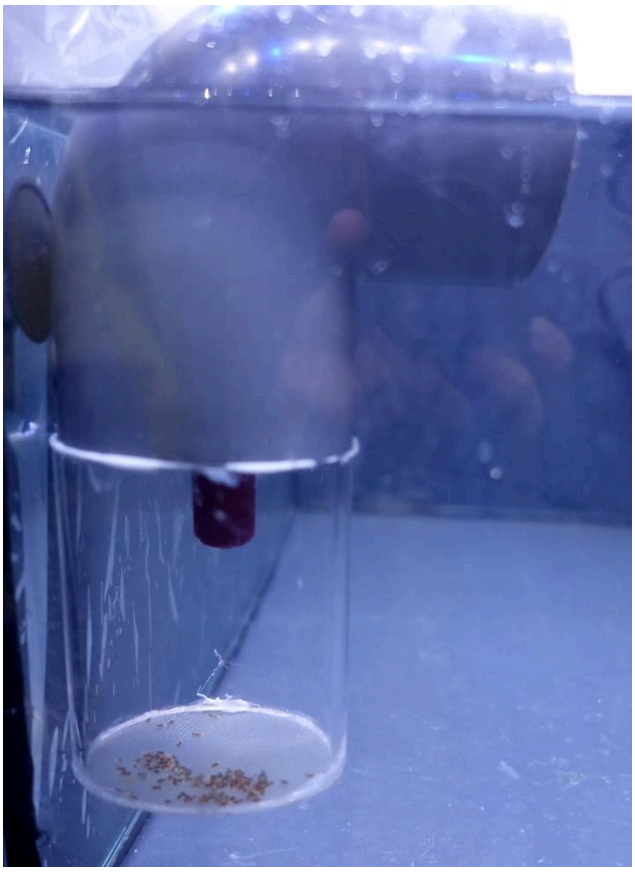

Figure S3. 3. Photo of laboratory set-up and egg rearing chambers. An air stone was introduced in the chamber to ensure water circulation.

Table S3. 7. Models and model outputs for fecundity (1dpf)/month analyses

Table S3. 7A. List of all linear mixed models (LMER) performed with Y [relative fecundity at day 1, i.e. number of eggs at 1 dpf (day post fertilization) spawned per lunar month] as the response variable. Anemone is the anemone surface area and is log-10 transform to solve model scaling problems; TL_F is adult female total length. The sites was used as random factors. Model selection was performed by sequentially dropping the non-significant interactions, and models were compared using the likelihood ratio test (LRT) in order to adopt the best-fit model (in bold) that included Anemone as predictor variables.

Model		df	LogLik	χ^2	p-value
1	$Y \sim log10(Anemone) * TL_F + (1\|site)$	6	-553.00		
2	$Y \sim log10(Anemone) + TL_F + (1\|site)$	5	-553.01	0.019	0.890
3	$\boldsymbol{Y \sim log10(Anemone) + (1\|site)}$	4	-553.28	0.526	0.468

Table S3. 7B. Results of the best-fit model from Table S3.7A. mR^2 is the marginal R^2, which describes the proportion of variance explained by the fixed factors alone. cR^2 is the conditional R^2, which describes the proportion of variance explained by both fixed and random factors.

Fixed effects	Estimate	SE	df	t-value	p-value	mR^2	cR^2
Intercept	-2077.950	2257.42	24.21	-0.920	0.366	0.097	0.692
Log10(Anemone)	1200.650	622.51	24.29	1.929	0.066		

Table S3. 8. Models and model outputs for number of eggs at hatching/month analyses

Table S3. 8A. List of all linear mixed models (LMER) performed with Y [relative fecundity at hatching, i.e. number of eggs at 6-7 dpf (day post fertilization) spawned per lunar month] as the response variable. Anemone is the anemone surface area and is log-10 transform to solve model scaling problems; TL_F is adult female total length. The sites was used as random factors. Model selection was performed by sequentially dropping the non-significant interactions, and models were compared using the likelihood ratio test (LRT) in order to adopt the best-fit model (in bold) that included Anemone as predictor variables.

Model		df	LogLik	χ^2	p-value
1	$Y \sim log10(Anemone) * TL_F + (1\|site)$	6	-532.15		
2	$Y \sim log10(Anemone) + TL_F + (1\|site)$	5	-532.18	0.059	0.808
3	$\boldsymbol{Y \sim log10(Anemone) + (1\|site)}$	4	-532.48	0.599	0.439

Table S3. 8B. Results of the best-fit model from Table S3.8A. mR^2 is the marginal R^2, which describes the proportion of variance explained by the fixed factors alone. cR^2 is the conditional R^2, which describes the proportion of variance explained by both fixed and random factors.

Fixed effects	Estimate	SE	df	t-value	p-value	mR^2	cR^2
Intercept	-2391.22	1649.44	23.50	-1.450	0.160	0.139	0.707
Log10(Anemone)	1072.74	454.85	23.58	2.358	0.027		

Table S3. 9. Models and model outputs egg size analyses

Table S3.9A. List of all linear mixed models (LMER) performed with Y [egg size (surface area)] as the response variable. Anemone is the anemone surface area and is log-10 transform to solve model scaling problems; TL_F is adult female total length. The sites was used as random factors. Model selection was performed by sequentially dropping the non-significant interactions, and models were compared using the likelihood ratio test (LRT) in order to adopt the best-fit model (in bold) that included Anemone as predictor variables.

Model		df	LogLik	χ^2	p-value
1	$Y \sim log10(Anemone) * TL_F + (1\|site/clutch)$	8	273.25		
2	$Y \sim log10(Anemone) + TL_F + (1\|site/clutch)$	7	272.66	1.183	0.267
3	$\boldsymbol{Y \sim log10(Anemone) + (1\|site/clutch)}$	6	272.05	1.219	0.270

Table S3. 9B. Results of the best-fit model from Table S3.9A. mR^2 is the marginal R^2, which describes the proportion of variance explained by the fixed factors alone. cR^2 is the conditional R^2, which describes the proportion of variance explained by both fixed and random factors.

Fixed effects	Estimate	SE	df	t-value	p-value	mR^2	cR^2
Intercept	1.3912	0.359	15.98	3.880	0.001	0.003	0.735
Log10(Anemone)	0.0256	0.100	16.01	0.255	0.802		

Table S3. 10. Models and model outputs for larval size analyses

Table S3. 10A. List of all linear mixed models (LMER) performed with Y [larva size (total length)] as the response variable. Anemone is the anemone surface area and is log-10 transform to solve model scaling problems; TL_F is adult female total length. The sites was used as random factors. Model selection was performed by sequentially dropping the non-significant interactions, and models were compared using the likelihood ratio test (LRT) in order to adopt the best-fit model (in bold) that included Anemone as predictor variables.

Model		df	LogLik	χ^2	p-value
1	$Y \sim log10(Anemone) * TL_F + (1\|site/clutch)$	7	486.39		
2	$Y \sim log10(Anemone) + TL_F + (1\|site/clutch)$	6	486.24	0.284	0.594
3	$\boldsymbol{Y \sim log10(Anemone) + (1\|site/clutch)}$	5	485.89	0.713	0.399

Table S3. 10B. Results of the best-fit model from Table S3.10A. mR^2 is the marginal R^2, which describes the proportion of variance explained by the fixed factors alone. cR^2 is the conditional R^2, which describes the proportion of variance explained by both fixed and random factors.

Fixed effects	Estimate	SE	df	t-value	p-value	mR^2	cR^2
Intercept	0.3783	0.042	17.38	8.953	<0.001	0.011	0.364
Log10(Anemone)	0.0081	0.012	17.43	0.684	0.503		

Table S3. 11. Models and model outputs for larval swimming speed analyses

Table S3. 11A. List of all linear mixed models (LMER) performed with Y [larval swimming speed (U_{max})] as the response variable. Anemone is the anemone surface area and is log-10 transform to solve model scaling problems; TL_F is adult female total length. The sites was used as random factors. Model selection was performed by sequentially dropping the non-significant interactions, and models were compared using the likelihood ratio test (LRT) in order to adopt the best-fit model (in bold) that included Anemone as predictor variables.

Model		df	LogLik	χ^2	p-value
1	$Y \sim log10(Anemone) * TL_F + (1\|site/clutch)$	7	-590.66		
2	$Y \sim log10(Anemone) + TL_F + (1\|site/clutch)$	6	-590.75	0.195	0.659
3	$\boldsymbol{Y \sim log10(Anemone) + (1\|site/clutch)}$	5	-591.10	0.691	0.406

Table S3. 11B. Results of the best-fit model from Table S3.11A. mR^2 is the marginal R^2, which describes the proportion of variance explained by the fixed factors alone. cR^2 is the conditional R^2, which describes the proportion of variance explained by both fixed and random factors.

Fixed effects	Estimate	SE	df	t-value	p-value	mR^2	cR^2
Intercept	12.1113	6.403	14.755	1.891	0.078	0.000	0.319
Log10(Anemone)	0.1284	1.799	15.045	0.071	0.944		

HORMONES

Table S3. 12. Models and model outputs for Conversion ratios of T to E_2 analyses (habitat manipulation)

Table S3. 12A. List of all generalized linear mixed model (LMER) performed with Y [conversion ratios of T to E_2] as response variable. Treatment is the habitat treatment (control, used as a reference, decrease and increase habitat size), Period is the manipulation period condition measure used as categorical variable (before manipulation, after manipulation). The sites was used as random factors to account for repeated measures on the same site. Model selection was performed by sequentially dropping the non-significant interaction, and models were compared using the likelihood ratio test (LRT) in order to adopt the best-fit model (in bold) that included Period and treatment as predictor variables.

Model		df	LogLik	χ^2	p-value
1	$Y \sim Treatment * Period + (1\|site)$	8	18.282		
2	$\boldsymbol{Y \sim Treatment + Period + (1\|site)}$	6	14.013	8.538	0.014

Table S3. 12B. Results of the best-fit model from Table S3.12A. mR^2 is the marginal R^2, which describes the proportion of variance explained by the fixed factors alone. cR^2 is the conditional R^2, which describes the proportion of variance explained by both fixed and random factors.

Fixed effects	Estimate	SE	df	t-value	p-value	mR^2	cR^2
Intercept	0.5979	0.069	10.0	8.661	<0.001	0.524	0.524
Period A	-0.0604	0.098	10.0	-0.618	0.550		
Treatment (decrease)	0.0443	0.098	10.0	0.453	0.660		
Treatment (increase)	-0.2303	0.085	10.0	-2.723	0.021		
Period A * Treatment (decrease)	0.0173	0.138	10.0	0.125	0.903		
Period A * Treatment (increase)	0.2676	0.120	10.0	2.238	0.049		

n = 8 sites

Table S3. 12C. Post-hoc test on the best-fit model form Table S3.12A.

	Fixed effects	Estimate	SE	df	t-value	p-value
Period (B)	control *vs* decrease	-0.0442	0.103	10.0	-0.430	0.904
	control *vs* increase	0.2302	0.091	8.0	2.541	0.080
	decrease *vs* increase	0.2745	0.085	10.0	3.247	0.022
Period (A)	control *vs* decrease	-0.0615	0.103	10.0	-0.598	0.824
	control *vs* increase	-0.0374	0.091	8.0	-0.412	0.912
	decrease *vs* increase	0.0242	0.085	10.0	0.286	0.956

	Fixed effects	Estimate	SE	df	t-value	p-value
Control	B *vs* A	0.0604	0.098	6.43	0.618	0.558
Decrease	B *vs* A	0.0431	0.098	6.43	0.441	0.674
Increase	B *vs* A	-0.2072	0.069	6.43	-3.002	0.022

Table S3. 13. Models and model outputs for 11-ketotestosterone (11-KT) analyses (habitat manipulation)

Table S3. 13A. List of all generalized linear mixed model (LMER) performed with Y [conversion ratios of T to 11-KT] as response variable. Treatment is the habitat treatment (control, used as a reference, decrease and increase habitat size), Period is the manipulation period condition measure used as categorical variable (before manipulation, after manipulation). The sites was used as random factors to account for repeated measures on the same site. Model selection was performed by sequentially dropping the non-significant interaction, and models were compared using the likelihood ratio test (LRT) in order to adopt the best-fit model (in bold) that included Period and treatment as predictor variables.

Model		df	LogLik	χ^2	p-value
1	$Y \sim Treatment * Period + (1\|site)$	8	19.759		
2	$\boldsymbol{Y \sim Treatment + Period + (1\|site)}$	6	18.209	3.101	0.212

Table S3. 13B. Results of the best-fit model from Table S3.13A. mR^2 is the marginal R^2, which describes the proportion of variance explained by the fixed factors alone. cR^2 is the conditional R^2, which describes the proportion of variance explained by both fixed and random factors.

Fixed effects	Estimate	SE	df	t-value	p-value	mR^2	cR^2
Intercept	0.5906	0.050	22.96	11.897	<0.001	0.020	0.135
Period A	0.0250	0.049	18.28	0.512	0.615		
Treatment (decrease)	0.0312	0.065	25.61	0.478	0.636		
Treatment (increase)	-0.0076	0.063	21.15	-0.120	0.905		

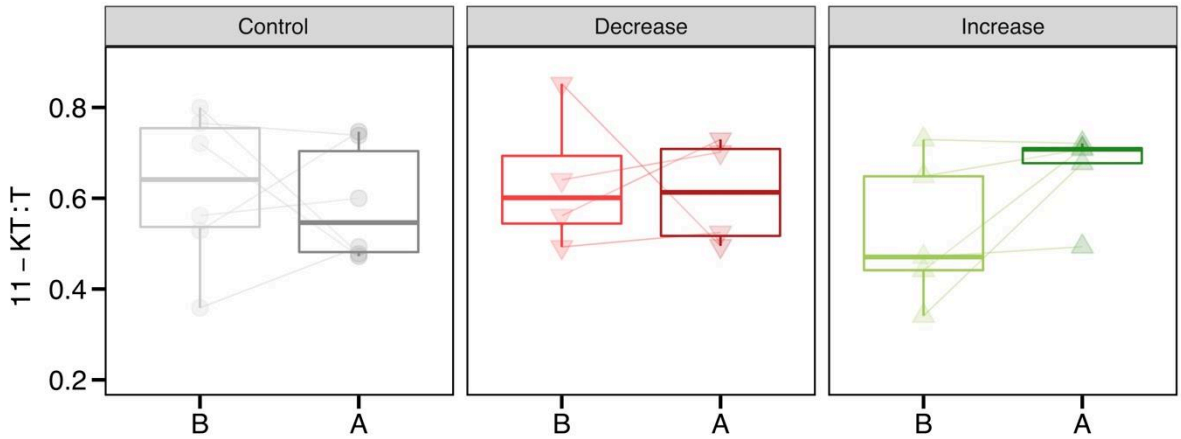

Figure S3. 4. T to 11-KT in each treatment condition (control decrease and increase habitat size), before (B) and before (B) and after (A) habitat manipulation. Dots and triangles represent individual data points and lines connect each site between two spawning events (n = 15 sites)

Table S3. 14. Models and model outputs for baseline cortisol concentration analyses (habitat manipulation)

Table S3. 14A. List of all linear mixed models (LMER) performed with Y [baseline cortisol concentration] as the response variable. Treatment is the habitat treatment (control, used as a reference, decrease and increase habitat size), Period is the manipulation period condition measure used as categorical variable (before manipulation, after manipulation). The sites was used as random factors to account for repeated measures on the same site. Model selection was performed by sequentially dropping the non-significant interaction, and models were compared using the likelihood ratio test (LRT) in order to adopt the best-fit model (in bold) that included Period and treatment as predictor variables.

Model		df	LogLik	χ^2	p-value
1	$Y \sim Treatment * Period + (1\|site)$	8	-316.38		
4	$\boldsymbol{Y \sim Treatment + Period + (1\|site)}$	6	-317.84	0.910	0.233

Table S3. 14B. Results of the best-fit model from Table S3. 14A. mR^2 is the marginal R^2, which describes the proportion of variance explained by the fixed factors alone. cR^2 is the conditional R^2, which describes the proportion of variance explained by both fixed and random factors.

Fixed effects	Estimate	SE	df	t-value	p-value	mR^2	cR^2
Intercept	25.1701	3.596	43.80	7.000	<0.001	0.035	0.345
Period A	0.3212	2.631	63.83	0.122	0.903		
Treatment (decrease)	-6.7260	4.477	65.87	-1.502	0.138		
Treatment (increase)	-0.9877	3.697	74.16	-0.267	0.790		

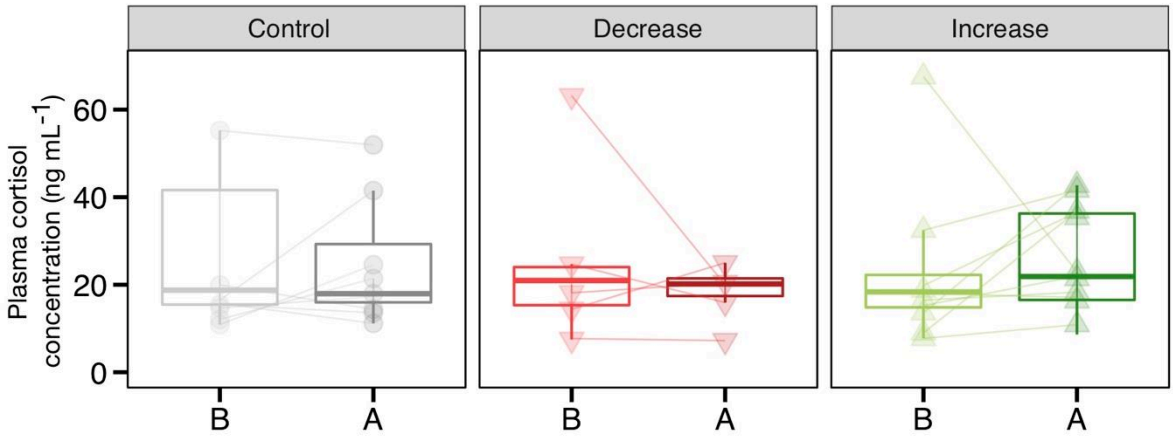

Figure S3. 5. Baseline cortisol concentration in each treatment condition (control decrease and increase habitat size), before (B) and after (A) habitat manipulation. Dots and triangles represent individual data points and lines connect each site between two spawning events (n = 17 sites)

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Table S3. 15. Models and model outputs for fecundity (1dpf)/month analyses (habitat manipulation)

Table S3. 15A. List of all generalized linear mixed model (LMER) performed with Y [Fecundity (1dpf)/month] as response variable. Treatment is the habitat treatment (control, used as a reference, decrease and increase habitat size), Period is the manipulation period condition measure used as categorical variable (before manipulation, after manipulation). The sites was used as random factors to account for repeated measures on the same site. Model selection was performed by sequentially dropping the non-significant interaction, and models were compared using the likelihood ratio test (LRT) in order to adopt the best-fit model (in bold) that included Period and treatment as predictor variables.

Model		df	LogLik	χ^2	p-value
1	$Y \sim Treatment * Period + (1\|site)$	14	-1077.2		
2	$\boldsymbol{Y \sim Treatment + Period + (1\|site)}$	8	-1080.5	6.599	0.360

Table S3. 15B. Results of the best-fit model from Table S3.15A. mR^2 is the marginal R^2, which describes the proportion of variance explained by the fixed factors alone. cR^2 is the conditional R^2, which describes the proportion of variance explained by both fixed and random factors.

Fixed effects	Estimate	SE	df	t-value	p-value	mR^2	cR^2
Intercept	1939.06	338.07	43.81	5.736	<0.001	0.056	0.574
Period A1	-223.00	222.08	91.40	-1.004	0.318		
Period A2	-197.88	226.78	92.04	-0.873	0.385		
Period A3	353.25	237.03	92.73	1.490	0.140		
Treatment (decrease)	565.35	483.96	31.22	1.168	0.252		
Treatment (increase)	387.66	448.18	31.59	0.865	0.394		

Table S3. 15C. Anova (LMER)

Fixed effects	SS	MS	Num df	Den df	F-value	p-value
Period	6157937	2052646	3	91.162	2.4481	0.069
Treatment	1265115	632557	2	31.178	0.7544	0.479

Table S3. 16. Linear model (LM) performed with fecundity (number of eggs at 1 dpf) as a response variable and 11β-estradiol as an explanatory variable.

Fixed effects	Estimate	SE	df	t-value	p-value	R^2	R^2_{adj}
Intercept	726.0	152.0	16	4.776	<0.001	0.519	0.489
Estradiol	598.6	144.1	16	4.155	<0.001		

Table S3. 17. Models and model outputs for number of eggs at hatching (6dpf)/month analyses (habitat manipulation)

Table S3. 17A. List of all generalized linear mixed model (LMER) performed with Y [Number of eggs at Hatching (6-7dpf)/month] as response variable. Treatment is the habitat treatment (control, used as a reference, decrease and increase habitat size), Period is the manipulation period condition measure used as categorical variable (before manipulation, after manipulation). The sites was used as random factors to account for repeated measures on the same site. Model selection was performed by sequentially dropping the non-significant interaction, and models were compared using the likelihood ratio test (LRT) in order to adopt the best-fit model (in bold) that included Period and treatment as predictor variables.

Model		df	LogLik	χ^2	p-value
1	$Y \sim Treatment * Period + (1\|site)$	14	-1040.5		
2	$\boldsymbol{Y \sim Treatment + Period + (1\|site)}$	8	-1044.2	6.5021	0.277

Table S3. 17B. Results of the best-fit model from Table S3.17A. mR^2 is the marginal R^2, which describes the proportion of variance explained by the fixed factors alone. cR^2 is the conditional R^2, which describes the proportion of variance explained by both fixed and random factors.

Fixed effects	Estimate	SE	df	t-value	p-value	mR^2	cR^2
Intercept	1309.01	250.27	44.88	5.230	<0.001	0.050	0.554
Period A1	-101.38	168.62	91.62	-0.601	0.549		
Period A2	-52.73	172.17	92.28	-0.306	0.760		
Period A3	373.41	179.93	93.01	2.075	0.041		
Treatment (decrease)	348.08	356.47	31.42	0.976	0.336		
Treatment (increase)	128.08	330.17	31.81	0.388	0.701		

Table S3. 17C. ANOVA(LMER) on the best-fit model from Table S3.16A.

Fixed effects	SS	MS	Num df	Den df	F-value	p-value
Period	3997923	1332641	3	92.416	2.7569	0.047
Treatment	492538	231269	2	31.382	0.4784	0.624

Table S3. 18. Models and model outputs for egg size analyses (habitat manipulation)

Table S3. 18A. List of all linear mixed model (LMER) performed with Y (egg size, surface) as response variable. Treatment is the habitat treatment (control, used as a reference, decrease and increase habitat size), Period is the manipulation period condition measure used as categorical variable (before manipulation, first spawn after manipulation, second spawn after manipulation). The sites was used as random factors to account for repeated measures on the same site. Model selection was performed by sequentially dropping the non-significant interaction, and models were compared using the likelihood ratio test (LRT) in order to adopt the best-fit model (in bold) that included Period and treatment as predictor variables.

Model		df	LogLik	χ^2	p-value
1	$\boldsymbol{Y \sim Treatment * Period + time + (1\|site)}$	12	377.28		
2	$Y \sim Treatment + Period + time + (1\|site)$	8	344.60	65.350	<0.001
3:	$Y \sim Treatment * Period + (1\|site)$	11	373.59	7.363	0.007

*model 3 is compared to model 1

Table S3. 18B. Results of the best-fit model from Table S3.18A. mR^2 is the marginal R^2, which describes the proportion of variance explained by the fixed factors alone. cR^2 is the conditional R^2, which describes the proportion of variance explained by both fixed and random factors.

Fixed effects	Estimate	SE	df	t-value	p-value	mR^2	cR^2
Intercept	1.5530	0.041	17.41	37.917	<0.001	0.294	0.676
Period(A1)	-0.0167	0.017	405.10	-0.958	0.339		
Period(A2)	-0.0499	0.031	413.60	-1.611	0.108		
Treatment(*decrease*)	-0.1380	0.059	16.37	-2.328	0.033		
Treatment(*increase*)	-0.1650	0.062	16.39	-2.644	0.017		
Time	0.0007	0.0002	496.80	2.716	0.007		
Period(A1) * Treatment(*decrease*)	-0.0043	0.026	406.70	-1.680	0.094		
Period(A2) * Treatment(*decrease*)	0.1420	0.043	413.30	3.322	<0.001		
Period(A1) * Treatment(*increase*)	0.1378	0.025	400.50	5.505	<0.001		
Period(A2) * Treatment(*increase*)	0.2014	0.036	405.60	5.672	<0.001		

n = 425, site = 18

Table S3. 18C. Post-hoc test on the results of the best-fit model from Table S3.18B.

	Fixed effects	Estimate	SE	df	t-value	p-value
Period (B)	control *vs* decrease	0.1380	0.059	16.3	2.328	0.080
	control *vs* increase	0.1650	0.062	16.3	2.644	0.044
	decrease *vs* increase	0.0270	0.064	16.2	0.419	0.908
Period (A1)	control *vs* decrease	0.1812	0.060	17.0	3.020	0.020
	control *vs* increase	0.0271	0.062	16.2	0.436	0.901
	decrease *vs* increase	-0.1540	0.065	16.7	-2.371	0.073
Period (A2)	control *vs* decrease	-0.0041	0.069	29.4	-0.059	0.998
	control *vs* increase	-0.0364	0.067	22.1	-0.540	0.852
	decrease *vs* increase	-0.0323	0.071	22.8	-0.459	0.891

	Fixed effects	Estimate	SE	df	t-value	p-value
Control	B *vs* A1	0.0167	0.017	405	0.957	0.605
	B *vs* A2	0.0499	0.031	414	1.605	0.245
	A1 *vs* A2	0.0332	0.028	411	1.167	0.474
Decrease	B *vs* A1	0.0599	0.018	403	3.406	0.002
	B *vs* A2	-0.0922	0.044	411	-2.076	0.096
	A1 *vs* A2	-0.1521	0.048	410	-3.198	0.004
Increase	B *vs* A1	-0.1211	0.019	402	-6.352	<0.001
	B *vs* A2	-0.1515	0.030	415	-5.075	<0.001
	A1 *vs* A2	-0.0304	0.028	414	-1.090	0.521

Table S3. 19. Models and model outputs for larval size analyses (habitat manipulation)

Table S3. 19A. List of all generalized linear mixed model (LMER) performed with Y (larval size) as response variable. Treatment is the habitat treatment (control, used as a reference, decrease and increase habitat size), Period is the manipulation period condition measure used as categorical variable (before manipulation, first spawn after manipulation, second spawn after manipulation). The sites was used as random factors to account for repeated measures on the same site. Model selection was performed by sequentially dropping the non-significant interaction, and models were compared using the likelihood ratio test (LRT) in order to adopt the best-fit model (in bold) that included Period and treatment as predictor variables.

Model		df	LogLik	χ^2	p-value
1	$Y \sim Treatment * Period + time + (1\|site)$	11	861.05		
2	$Y \sim Treatment + Period + time + (1\|site)$	8	855.53	11.039	0.012
3	$\boldsymbol{Y \sim Treatment * Period + (1\|site)}$	10	860.53	1.045	0.307

Table S3. 19B. Results of the best-fit model from Table S3.19A. mR^2 is the marginal R^2, which describes the proportion of variance explained by the fixed factors alone. cR^2 is the conditional R^2, which describes the proportion of variance explained by both fixed and random factors.

Fixed effects	Estimate	SE	df	t-value	p-value	mR^2	cR^2
Intercept	0.4134	0.005	29.36	86.613	<0.001	0.136	0.286
Period(A1)	0.0032	0.004	336.00	0.753	0.452		
Period(A2)	0.0218	0.005	352.90	3.973	<0.001		
Treatment(*decrease*)	0.0005	0.007	29.36	0.076	0.940		
Treatment(*increase*)	-0.0228	0.007	31.21	-3.327	0.002		
Period(A1) * Treatment(*decrease*)	-0.0137	0.006	336.90	-2.242	0.026		
Period(A2) * Treatment(*decrease*)	-0.0221	0.007	352.30	-3.055	0.002		
Period(A1) * Treatment(*increase*)	0.0008	0.006	338.90	0.120	0.904		

n = 361, site = 21

Table S3. 19C. Post-hoc test on the results of the best-fit model from Table S3.19B.

	Fixed effects	Estimate	SE	df	t-value	p-value
Period (B)	control *vs* decrease	-0.0005	0.007	28.5	-0.076	0.997
	control *vs* increase	0.0228	0.007	30.3	3.327	0.006
	decrease *vs* increase	0.0233	0.007	30.3	3.402	0.005
Period (A1)	control *vs* decrease	0.0132	0.007	31.2	1.911	0.153
	control *vs* increase	0.0221	0.007	31.0	3.198	0.009
	decrease *vs* increase	0.0089	0.007	33.8	1.254	0.430
Period (A2)	control *vs* decrease	-	-	-	-	-
	control *vs* increase	-	-	-	-	-
	decrease *vs* increase	0.0013	0.008	44.5	0.163	0.986

	Fixed effects	Estimate	SE	df	t-value	p-value
Control	B *vs* A1	-0.0032	0.004	335	-0.753	0.732
	B *vs* A2	-	-	-	-	-

	A1 *vs* A2	-	-	-	-	-
Decrease	B *vs* A1	0.0106	0.004	337	2.370	0.048
	B *vs* A2	0.0003	0.005	347	0.065	0.998
	A1 *vs* A2	-0.0103	0.005	347	-2.071	0.097
Increase	B *vs* A1	-0.0039	0.005	341	-0.848	0.673
	B *vs* A2	-0.0218	0.006	353	-3.949	<0.001
	A1 *vs* A2	-0.0179	0.005	351	-3.310	0.003

Table S3. 20. Models and model outputs for larval swimming speed analyses (habitat manipulation)

Table S3. 20A. List of all generalized linear mixed model (LMER) performed with Y (larval CAT) as response variable. Treatment is the habitat treatment (control, used as a reference, decrease and increase habitat size), Period is the manipulation period condition measure used as categorical variable (before manipulation, first spawn after manipulation, second spawn after manipulation). The sites was used as random factors to account for repeated measures on the same site. Model selection was performed by sequentially dropping the non-significant interaction, and models were compared using the likelihood ratio test (LRT) in order to adopt the best-fit model (in bold) that included Period and treatment as predictor variables.

Model		df	LogLik	χ^2	p-value
1	$Y \sim Treatment * Period + time + (1\|site)$	11	-1042.0		
2	$Y \sim Treatment + Period + time + (1\|site)$	8	-1046.2	8.418	0.038
3	$\boldsymbol{Y \sim Treatment * Period + (1\|site)}$	10	-1042.1	0.206	0.650

*model3 is compared to model1

Table S3. 20B. Results of the best-fit model from Table S3.20A. mR^2 is the marginal R^2, which describes the proportion of variance explained by the fixed factors alone. cR^2 is the conditional R^2, which describes the proportion of variance explained by both fixed and random factors.

Fixed effects	Estimate	SE	df	t-value	p-value	mR^2	cR^2
Intercept	13.6420	0.807	32.97	16.906	<0.001	0.052	0.154
Period(A1)	-0.6639	0.826	334.77	-0.803	0.422		
Period(A2)	3.0811	1.065	347.76	2.892	0.004		
Treatment(*decrease*)	-2.1420	1.141	32.97	-1.877	0.069		
Treatment(*increase*)	-1.5708	1.167	32.61	-1.347	0.187		
Period(A1) * Treatment(*decrease*)	3.0662	1.205	336.20	2.545	0.011		
Period(A2) * Treatment(*decrease*)	-0.6623	1.409	352.63	-0.470	0.639		
Period(A1) * Treatment(*increase*)	0.9665	1.226	338.91	0.788	0.431		

n = 361, site = 21

Table S3. 19C. Post-hoc test on the results of the best-fit model from Table S3.19B.

	Fixed effects	Estimate	SE	df	t-value	p-value
Period (B)	control *vs* decrease	2.1420	1.140	34.6	1.877	0.160
	control *vs* increase	1.5710	1.170	37.4	1.346	0.379
	decrease *vs* increase	-0.5710	1.170	37.4	-0.489	0.877
Period (A1)	control *vs* decrease	-0.9240	1.180	38.9	-0.784	0.715
	control *vs* increase	0.6040	1.180	38.4	0.514	0.865
	decrease *vs* increase	1.5280	1.210	42.8	1.260	0.425
Period (A2)	control *vs* decrease	-	-	-	-	-
	control *vs* increase	-	-	-	-	-
	decrease *vs* increase	-1.2330	1.360	55.8	-0.908	0.638

	Fixed effects	Estimate	SE	df	t-value	p-value
Control	B *vs* A1	0.6639	0.827	336	0.803	0.701
	B *vs* A2	-	-	-	-	-
	A1 *vs* A2	-	-	-	-	-
Decrease	B *vs* A1	-2.4023	0.877	338	-2.738	0.018
	B *vs* A2	-2.4188	0.926	350	-2.612	0.025
	A1 *vs* A2	-0.0165	0.971	350	-0.017	0.999
Increase	B *vs* A1	-0.3026	0.906	343	-0.334	0.940
	B *vs* A2	-3.0811	1.074	348	-2.870	0.012
	A1 *vs* A2	-2.7785	1.055	353	-2.633	0.024

Chapter 4: Parental and developmental water flow regime effects on anemonefish offspring phenotype and survival

4.1. Foreword

The aim of this chapter is to better understand the contribution of both the environment and the parental phenotype on offspring phenotype and survival. Specifically, I explored in this chapter whether the water flow can affect the phenotype of orange-fin anemonefish, *Amphiprion chrysopterus*, early life stages, on a wild population at Moorea island, French Polynesia; and whether these effects occur directly (developmental effects) or through parental determinants (transgenerational effects). Water flow was chosen as the environmental variable as coral reefs are catheterized by a high diversity of flow environments and anemones can live in both calm and high flow environments, which may affect the biology of their residents (e.g., *A. chrysopterus* adults).

This research was conceptualized and carried out within an international collaboration among myself, Dr. Suzanne C. Mills, Dr. Ricardo Beldade and Dr. Amelie Crespel and Dr. Shaun S. Killen from the University of Glasgow, and Dr. Tommy Norin from the University of Denmark. I performed most of the fieldwork with help from Dr. Suzanne C. Mills and Dr. Ricardo Beldade. I conducted all measures on larval stages, and morphological measures at all stages, while the physiological measurements on juveniles and adults where mainly performed by Dr. Amelie Crespel, Dr. Tommy Norin and Dr. Shaun S. Killen with contribution from myself. The data collection for this project lasted 4 months during the first year of my PhD. I then conducted all data analyses, and drafted the related manuscript under the supervision of Dr. Ricardo Beldade. Final writing was performed with inputs from of all co-authors.

4.2. Manuscript information

Title: Parental and developmental water flow regime effects on anemonefish offspring phenotype and survival

Authors: Daphne Cortese[1,], Amélie Crespel[2], Suzanne C. Mills[1,3], Tommy Norin[2,4], Shaun S. Killen[2], Ricardo Beldade[1,5]

Affiliations

[1] PSL Université Paris: EPHE-UPVD-CNRS, USR 3278 CRIOBE, BP 1013, 98729 Papetoai, Moorea, French Polynesia

[2] Institute of Biodiversity, Animal Health and Comparative Medicine, University of Glasgow, Graham Kerr Building, Glasgow G12 8QQ, UK

[3] Laboratoire d'Excellence "CORAIL", France

[4] DTU Aqua: National Institute of Aquatic Resources, Technical University of Denmark, 2800 Kgs. Lyngby, Denmark

[5] Estación Costera de Investigaciones Marinas and Center for Advanced Studies in Ecology and Biodiversity, Las Cruces, Pontificia Universidad Católica de Chile, Santiago de Chile

Keywords: water flow environment, parental influence, phenotypic plasticity, physiology, morphology, survival, coral reef fish, anemonefish

Status: manuscript in preparation; submission planned for March 2021.

4.3. Abstract

To maximize their fitness, it would be advantageous for wild organisms to locally adjust their phenotypes. The contribution of parental and developmental environment in determining individual phenotype is not yet fully understood, especially in species with a bipartite life cycle. We conducted a field experiment to explore the effects of high and low water flow experienced by the parents or the offspring on the survival, morphology, and physiology of a site-attached coral reef fish, the orange-fin anemonefish (*Amphiprion chrysopterus*). We measured early-life survival, fish growth, body shape, fins size and shape, and physiological performance (swimming speed and metabolic rate), at different life stages exposed to wild environments with differing amounts of water flow. Results show that offspring born from parents living at high flow sites, independently from where they developed, had slower growth and lower survival in the wild compared to offspring from parents inhabiting low flow sites. Parents living in high flow also produce larvae with higher caudal fin aspect ratio, but larval caudal fin length was determined by parental caudal fin length. At the juvenile stage, we did not observed differences in morphology nor physiological performance associated with the developmental or parental flow and they survived equally well in distinct developmental environments. These results give important insights into the influences of the parental contribution, both genetic or non-genetic, or by the direct effect of their environment on embryos, in determining larval phenotype and post-settlement growth and survival. However, our findings also show that there is some flexibility to cope with a wide range of flow environments. Such flexibility could minimize the loss of individuals when larvae settle into environments relatively different from their parental origin.

4.4. Introduction

Specific phenotypic traits may be advantageous in certain environments while being disadvantageous in others. As such, the role of the environment in shaping an organism's phenotype may have important fitness consequences (Rodrigues and Beldade 2020). Morphological, physiological and behavioral variation can be attributed to phenotypic plasticity, the development of alternative phenotypes without any change in the genotype (West-Eberhard 1989). For example, sticklebacks are known to exhibit different morphologies depending on the complexity of the environment they are living in, likely associated with foraging, competition and predation pressure (Day and McPhail 1996; Wund et al. 2008, 2012; Garduño-Paz et al. 2010). Phenotypic variation can also be attributed to parental contributions, which may include a genetic component, parental effects, or epigenetic effects (Riddell et al. 1981; Taylor and McPhail 1985; Uller 2008; Feil and Fraga 2012). Parents may produce alternative offspring phenotypes depending on the environmental conditions that they themselves have experienced, which may maximize maternal (Mousseau and Fox 1998; Uller 2008) and offspring fitness, under predictable or unpredictable environments (Crean and Marshall 2009; Krug 2009). Many marine species have evolved with a bipartite life cycle where a dispersal phase may implicate a final offspring location in an unpredictable and relatively different environment than their parents. The extent to which developmental adjustments and parental effects, either separately or combined, determine phenotype and survival in such species is still not well understood.

Natural environments are structurally complex and, in aquatic environments, water flow is a key physical factor that contributes to this complexity (Monismith et al. 2006). In tropical coral reefs, for example, interactions among topographic complexity, water motion, and currents can be highly variable over time (e.g. diurnal), and space (Lenhardt 1991; Hearn 2011; Leichter et al. 2013). Fish, living in hydrodynamically complex environments are good candidates to study environmentally-induced polymorphisms. Fish have specific morphological traits correlated with their swimming modes and life-history traits, which are likely associated with the specific water flow environment which they inhabit (Webb 1984; Webb and Weihs 1986; Fulton et al. 2001; Langerhans et al. 2003; Langerhans and Reznick 2010). Across species, elongated bodies, a high proportion of red muscle, and a high caudal fin aspect ratio (i.e. the squared height of the caudal fin divided by the surface area of the fin) should enhance steady swimming, while deeper bodies, with a high proportion of white muscle and smaller caudal fin aspect ratio, but larger and more elongated caudal fins, enhances unsteady swimming, i.e. irregular movements involving changes in velocity or direction, such as burst swimming speed,

fast-starts and maneuverability (Videler 1993; Domenici and Blake 1997; Webb 1998; Blake 2004; but see Kasapi et al. 1993). Empirical and modelling studies have also observed fins and body morphological differentiations among individuals of the same species living in different habitats or water flow regimes, likely to enhance the swimming capacity in that specific environment. These include elongated bodies and high caudal fin aspect ratios that enhance steady swimming, but also allow fish to maintain position in high water flow while minimizing drag (Langerhans 2008; Páez et al. 2008; Haas et al. 2010; Cureton II and Broughton 2014). The pectoral fins are main generator of movement in labriform fish (Breder Jr 1926; Webb 1994; Fulton et al. 2005) and are used for maneuvering (Domenici and Blake 1997). Most labriforms (including anemonefish such as *Amphiprion* sp.) will switch from using their pectoral fins at high speeds to include the caudal fin (pectoral-caudal swimming) (Drucker 1996; Drucker and Jensen 1996; Johansen et al. 2007). These two swimming modes have distinct purposes and energetic costs, and each of them can be advantageous under different situations encountered in the complex surrounding environment. It is unclear whether in specific water flow environments, the swimming performance or morphological traits associated with each swimming mode are transmitted by parents, via genetic or epigenetic effects, or whether the water flow experienced during development, via phenotypic plasticity, may also contribute to the variability in traits.

Living in different water flow environments may also require different bioenergetic traits to support more or less metabolically expensive swimming modes (Boisclair and Tang 1993). For example, tropical reef fish living in high flow areas have faster critical swimming speeds, higher aerobic scopes and fast-start escape response (Binning et al. 2014; Nadler et al. 2018), while in lower flow areas fish have lower metabolic rates, aerobic scopes and swimming speeds (Binning et al. 2014, 2015). Such differences in physiological capacities have been rarely shown in juveniles during ontogeny, making it difficult to know if observed differences come from the present or past environment. Despite some studies that have explored the effects of water flow on developmental morphology in freshwater species and salmonids (Pakkasmaa and Piironen 2001; Imre et al. 2002; Fischer-Rousseau et al. 2010), almost nothing is known for coral reef fishes, which live in a highly complex environment, generally have a pelagic larval phase, and ultimately settle in environments that are unpredictable for their parents.

Many fish on coral reefs are territorial, including anemonefish (*Amphiprion sp.*). Anemonefish live in a close symbiosis with sea anemones (Fautin 1991), which they depend on for shelter, and are thus site-attached (Elliott et al. 1995; Buston 2003b). Anemones can live in a strikingly diverse array of environments, from very shallow to very deep water (Bridge et

al. 2012), in very calm waters or under high flow environments (Fautin 1992; Brolund et al. 2004; Holbrook and Schmitt 2005), as well as over broad geographic ranges (Emms et al. 2020). Anemonefish, hosted by sea anemones, may be locally adjusted to the environment in which they and their host live and phenotypic differences among flow regimes may be expected. Like many other coral reef fishes (Levin 2006; Simpson et al. 2014; Almany et al. 2017), anemonefish larvae develop in open water, and may settle in anemones located in different water flow environments than experienced by their parents (Beldade et al. 2016). It is unclear whether parents would invest in their offspring's phenotype to enhance the offspring's performance in the same environment experienced by the parents themselves, whether the offspring modulate their phenotype through plasticity to match the specific water flow they end up in after settlement, or finally, the degree to which offspring survival is affected by both the water flow environment in which their parents lived and/or where they themselves end up settling.

We used a transgenerational cross-transplant field-based experiment to understand the morphological and physiological effects of water flow environment on adult and early life stages of the orange-fin anemonefish *Amphiprion chrysopterus*. We aimed to understand whether parental contribution or developmental plasticity could explain variation in offspring phenotypes across water flow environments, and whether offspring or parental water flow environments affect post-settlement larval survival.

4.5. Materials and methods

4.5.1. Parental water flow sites, adult and larval traits at the end of the larval stage

Twenty-three breeding pairs of orange-fin anemonefish, *Amphiprion chrysopterus,* from sites known to encompass a range of water flows, were monitored in the lagoon surrounding the island of Moorea, French Polynesia, in 2017 (Fig. 4.1A, Fig. S4.1, Table S4.1). The twenty-three sites were categorized into high and low parental water flow sites according to water flow velocity (see Environmental Measurements section) and morphological and physiological traits were measured from the adult anemonefish pairs at these same sites (Fig. 4.1A, Table S4.2). At each site anemonefish were captured, placed against a scaled underwater slate, photographed using a Sony DSC-RX100 III camera, and immediately released back onto their anemones. Morphological traits were measured from these photographs using ImageJ software. Physiological traits were measured in eight of the anemonefish pairs after bringing the fish into the the laboratory at the CRIOBE research centre (see Physiological Measure section below).

A subset of eleven breeding pairs from sites with the most extreme parental water flows (seven low and four high) were selected (Table S4.2) and monitored every 2-3 days over 2 months for spawning. When egg clutches were found (Fig. 4.1B), egg development stage was determined based on egg colour and visual developmental cues (Beldade *et al.*, 2017, Supplementary Fig. 3) and used to predict hatching date. On the day of predicted hatching (7 days after fertilization during the spring) one fifth of the egg clutch (~200-300 eggs; Table S4.2) was collected while SCUBA diving. This was done using a scalpel blade to detach the eggs from the substrate while manually and gently pumping the egss into a plankton bag. Eggs were then transferred under water into a zip-lock plastic bag filled with fresh sea water from there moved to a cooler onboard the boat. After a short boat-ride to CRIOBE, eggs were transferred into hatching chambers inside glass aquaria (30 L volume) containing fresh seawater from the lagoon, in which the eggs hatched overnight. All newly hatched larvae were reared under the same laboratory conditions, including water flow through the aquaria, water temperature (27.5 ± 1°C, mean ± SD), algae and food (see details in section 1 of Supporting information). At the end of the larval stage, i.e. 18 days post-hatching (dph) spent entirely in aquaria, physiological (larval swimming speed; see below) and morphological (body and fin sizes; see below) traits were determined for six larvae from each clutch. Larval development in the aquaria tend to be slower than in the wild, therefore the end of the larval stage was determined by the appearance of the body white stripe (i.e. at 18 dph).

4.5.2. Developmental water flow sites, exposure and juvenile traits

Over a further three days, larvae from each egg clutch were kept in individual 5 L aquaria (at 28 ± 1°C, mean ± SD) containing a small anemone (~5 cm diameter), in order to facilitate settlement prior to release into the lagoon. Six developmental water flow sites (two low and four high flow) were selected on sandy flats in the northern lagoon of Moorea (Fig. 4.1A, S4.2, Table S4.3) and eight cages each containing a healthy anemone of approximately equal size (224 ± 83 cm^2). Cages were used to prevent anemone predation and were placed 10 m apart at each site. The juvenile anemonefish were able to swim in and out of the cage mesh (3 x 3 cm squares).

After 21 dph in aquaria, the six full-sibling post-settled larvae previously measured from the same cohort were randomly cross-transplanted in the six developmental water flow sites (one at each site) in the caged anemones (Fig. 4.1). If a post-settled larva died within a week after release, we replaced it with a new post-settled larva from the same cohort, but for which

we did not measure the physiological or morphological trait at 18dph measures (21 out of 67 larvae were replaced).

After six weeks exposed in the developmental water flow treatments, surviving juveniles were captured, brought back to the laboratory where physiological traits (swimming speed, growth and metabolism: see below) were measured. Juveniles were then released back again onto their respective anemones. After an additional 2 to 5 weeks, surviving juveniles were again caught and morphological traits measured (i.e. after ~8-11 weeks total exposure with no difference in exposure time between the developmental flow treatments; ANOVA, all $p > 0.05$, Table S4.4; Table S4.5). Morphological traits were measured after this longer exposure to the water flow treatments (8-11 weeks) compared to physiological traits (6 weeks) due to the longer time required for morphological plasticity (months in response to water flow; Kelley *et al.*, 2017) compared to physiological plasticity (weeks in response to abiotic factors; Fangue *et al.*, 2014; Sandblom *et al.*, 2014) to occur in fish (Kingsolver and Huey 1998).

Ethical approval for the study was granted from The Animal Ethics Committee, Centre National de la Recherche Scientifique (permit number 006725).

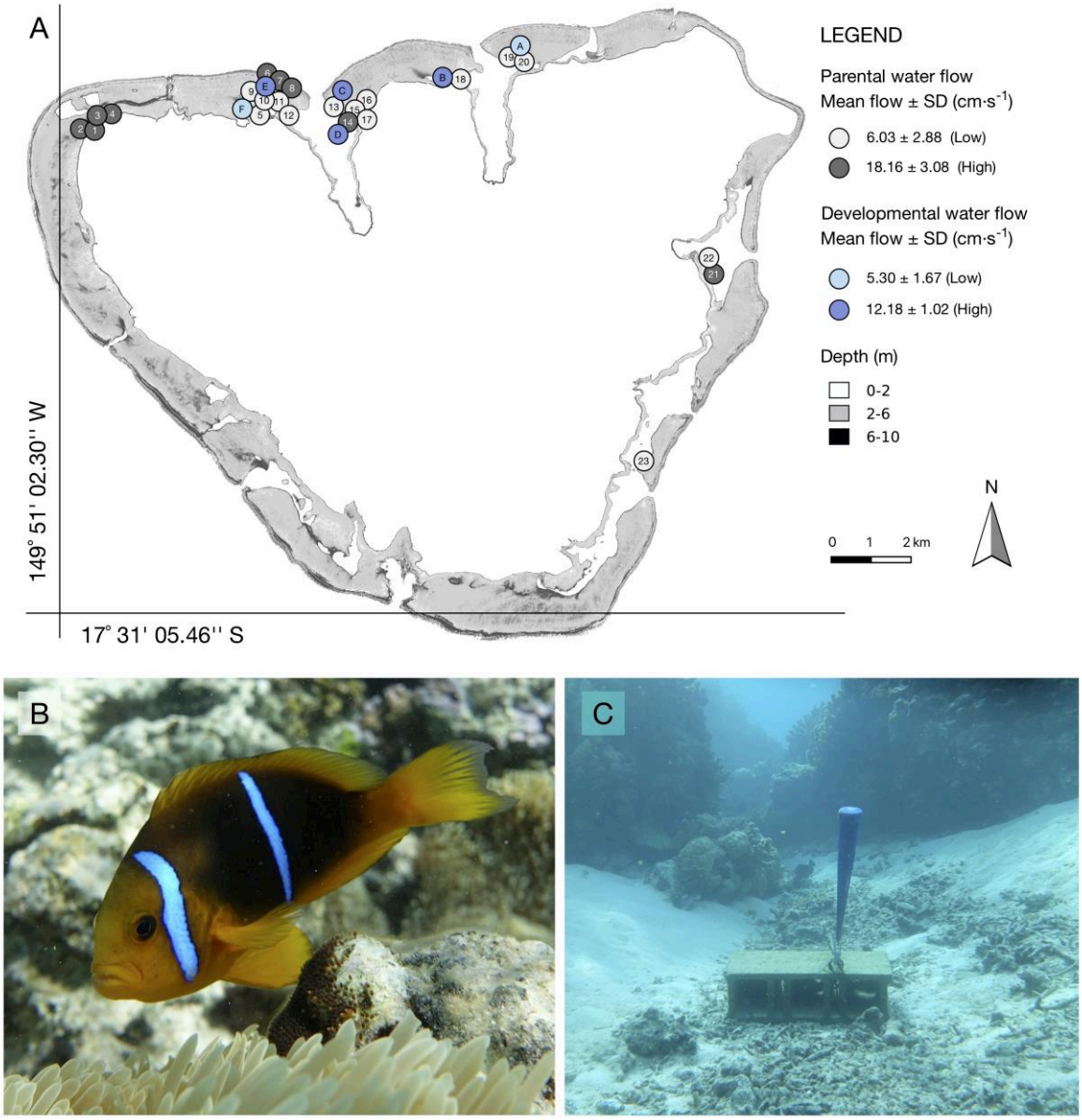

Figure 4. 1. Study sites and experimental photographs in situ. (A) Map of the lagoon around Moorea with highlighted adult sites (1-23, Table S4.1), flow regime category (light grey = low flow and dark grey = high flow, Table S4.1) and developmental flow sites (A-F, Table S4.3) with their respective flow regime category (light blue = low flow and dark blue = high flow, Table S4.3). (B) Male *Amphiprion chrysopterus* guarding a nest of 3 day old eggs. (C) Marotte HS current meter deployed at one of the sites.

4.5.3. Environmental measurements (water flow, temperature and depth)

Water flow velocities were measured over twelve days prior to egg collection at each parental site and for ~11 weeks (the approximate duration of the offspring exposure in October-December 2017) at the developmental sites using Marotte HS (High Sampling Rate) drag-tilt current meters (Marine Geophysics laboratory, James Cook University; Fig. 4.1C). The current meters were placed ~1-2 m away from either the focal anemone at each parental site, or from

each cage at the developmental sites. The drag-tilt was recorded at a fixed point every 10 minutes and converted to water flow velocity by post-processing with a tilt-to-speed calibration curve using the MarotteHSConfig Software (Version 3.0, Marine Geophysics laboratory, James Cook University). Water flow velocity (mean, maximum and variation) were combined in a Principal Component Analysis (PCA, supplementary materials Fig. S4.1A-E, Table S4.1, Fig. S4.1A-E, Table S4.3) to identify two categories of water flow based on their PCA scores: high and low flow (Fig. S4.1E, S4.3E, Table S4.1, S4.3).

Water temperature was recorded using a temperature sensor glued at the bottom of the Marotte HS current meter (measured once every 10 minutes with ± 0.3 ˚C error), and the depth of each site was recorded using an underwater depth meter (Table S4.1B). At each parental site the territory size, in terms of surface area of anemones was also determined (details on anemone size measure in Supporting information section 2 and Table S4.1B).

4.5.4. Survival

Larval survival (absence/presence) at each developmental water flow treatment was regularly monitored over the 11-week experiment (every 1-2 days for the first week and every 4-5 days thereafter). Given that juvenile anemonefish are unlikely to voluntarily leave their anemone due to risk of predation (Buston, 2003a; Elliott et al., 1995), the absence of a fish from its anemone was equated to mortality. Despite the use of cages, three anemones disappeared during the experiment, together with their anemonefish, which were not accounted for in the survival analyses. Nine additional fish were excluded from survival analyses due to non-natural mortality (they died during transport or during holding in the laboratory). Total sample size for survival was 55 fish.

4.5.5. Constant acceleration test (U_{max})

Fish swimming performance was measured on 18 dph larvae and juveniles using the constant acceleration test (Farrell 2008), which gives an indication of the maximum swimming speed (U_{max}) of a fish. Measurements were carried out in a 170 mL Blaza-style swim tunnel (Loligo System, Viborg, Danmark) immersed in a larger aquarium where temperature was maintained at 27.5 ± 1°C. The swim tunnel system consists of a two glass cylinders inside each other, one larger than the other. The inner cylinder (the swimming chamber) contains the fish and a impeller at one end (separated from the fish by a honeycomb screen), which circulates water through the inner cylinder and return is through the space between the inner and outer cylinders.

This ensures a one-way flow through the inner cylinder against which the fish swims (Brett 1964). The impeller velocity is regulated by a voltmeter. A fish was introduced into the swim tunnel and kept at a water velocity of ~0.5-1 body length (BL) s^{-1} for an acclimation period (10 min for 18 dph larvae and 30 min for ~2 months old juveniles) before the trials begun (Plaut 2001). Water flow was then gradually increased at a rate equivalent to approximately half a body length every 30 sec until the fish was fatigued, could no longer maintain its position in the flow, and was swept towards a honeycomb screen located on the downstream end of the water tunnel (as described in Killen, Nati and Suski, 2015). The equation used to calculate U_{max} of larvae is (Brett 1964): $U_{max} = U_i + [U_j \times (T_i/T_j)]$; where U_i is the penultimate speed (the highest velocity maintained for the whole interval measured in cm s^{-1}), U_j is the velocity increment (0.5 BL, 0.5 cm s^{-1} in larvae of 18 dph and 1.0 cm s^{-1} in juvenile fish), T_i is the time swum in the final velocity increment, and T_j is the prescribed interval time (30 sec). Swimming speed was then calculated in body length per seconds to account for fish size, which is likely to affect swimming speed (Fisher and Hogan 2007).

4.5.6. Metabolic rate measurements (SMR, MMR, Aerobic scope)

Measurements of fish metabolic rates [standard metabolic rate (SMR) and maximum metabolic rate (MMR)], were carried out in respirometry chambers where fish oxygen uptake rate is recorded as a proxy for metabolic rate (Norin & Clark, 2016; Svendsen, Bushnell, & Steffensen, 2016). Adult MMR, adult SMR, and juvenile SMR were obtained using a static intermittent-closed respirometry, while juvenile MMR was measured in the swim tunnel respirometer (Clark et al. 2013). Fish aerobic scope (AS) was calculated as the difference between MMR and SMR.

The static intermittent-closed respirometry setup was composed of a set of rectangular plastic (food-grade polypropylene) chambers of 2000 mL volume for adults, while glass cylindrical chambers of 35 or 110 mL volume (depending on fish size) were used for juveniles. The respirometry chambers were submersed in a 40 L tank filled with normoxic seawater from the lagoon (29.4 ± 0.05˚C, mean ± SE). For the adults, an Eheim Compact 300 pump (EHEIM GmbH & Co., Deizisau, Germany) was inserted into a loop of PVC tubing connected to each end of each respirometry chamber. The pump was modified to be in-line by removing the detachable plastic cover in front of the impellor and replacing it with a hose-connector glued onto the front of the pump to create a full seal. This pump continuously recirculated water at a rate of approximately one chamber volume per minute through the respirometry chamber, past the fish, and past a fibre-optic oxygen probe inserted into the PVC tubing in the recirculation

loop and connected to an oxygen meter (FireStingO_2; Pyro Science GmbH, Aachen, Germany). The respirometry chambers used for the juveniles were set up similarly, except that water was recirculated using a peristaltic pump (Masterflex L/S; Cole-Parmer, Vernon Hills, IL, USA), also at a rate of approximately one chamber volume per minute. For all respirometers, an automated flush pump (EHEIM Compact 300) was connected to one end of each chamber (one pump per chamber for adults; one pump per four chambers for juveniles) and intermittently flushed the respirometry chamber with fresh and fully aerated seawater from the 40 L ambient tank at a rate of approximately one chamber volume per minute. These automated pumps created alternating closed (sealed) phases (5 min for adults; 6 min for juveniles), during which fish oxygen consumption was measured every 2 s with the oxygen probes, and flush phases (5 min for all fish), during which the water was refreshed. The flush water exited through an overflow tube connected to the other end of the respirometry chamber, with the tip of the flush tube extending above the water surface.

Eight of the 11 wild breeding anemonefish pairs were brought to CRIOBE and transferred into 30 L individual tanks. After 36 h of fasting, metabolic rate measurements were taken. For maximum metabolic rate measurements (MMR), fish were manually chased in a circular tub containing seawater for 2 min until exhaustion, then immediately transferred into the static intermittent-closed respirometry setup during a closed phase to measure the oxygen consumption rate immediately post-chase, which is a proxy of fish maximum metabolic rate (Norin and Clark 2016). The standard (resting) metabolic rate (SMR) of the fish was then obtained by leaving them in the respirometry chambers overnight (for ~20 h), resulting in ~109 to 120 separate oxygen uptake rate recordings (depending on flush duration). We also recorded background microbial oxygen consumption rates in a empty chamber overnight. Slopes of the decline in oxygen concentration over time during the closed phases of the intermittent-respirometry cycle were calculated using labchart (Macknight 1988). Fish oxygen uptake rates were then calculated by multiplying these slopes by the volume of the respirometry chamber after subtracting fish volume and background microbial respiration. SMR was calculated as the lowest 10% of these data. After completion of respirometry measurements, we recorded fish body mass to the nearest 0.1 g.

Juveniles in the caged anemones from all sites were caught after six weeks and brought to CRIOBE. After fasting for ~24 h, juveniles were transferred into the static intermittent-closed respirometry chambers and their SMR was measured overnight following the same protocol as adult fish. The following day, juvenile MMR was measured in the same swim tunnel used for the U_{max} measurement, containing an oxygen probe which recorded fish oxygen consumption

immediately after swimming performance measurements (post-exercise respirometry). After fish exhaustion, the velocity in the swim tunnel respirometer was reduced to 1 BL s^{-1} and three cycles of 10 minutes (6 minutes close and 4 minutes flush) were used to estimate the maximum slope of oxygen consumption. Microbial oxygen consumption was also recorded in an empty chamber before and after swimming the fish swim. After all metabolic measurements, juvenile body mass was measured (to the nearest 0.001 g).

After laboratory measurements, all fish were fed and released back into their original site in the field.

4.5.7. Specific growth rate

The total length of 18 dph larval anemonefish (initial time) was measured to the nearest 0.001 cm (photographs were taken with a binocular microscope, LEICA EX4W), and was measured a second time on juveniles aged approx. 70-96 dph (i.e. after 8-11 weeks in the field – final time). Specific growth rate (SGR) was determined as the percentage increase in individual body size per day (Hopkins 1992) as: $\mathrm{SGR} = (\ln(TL_{t2}) - \ln(TL_{t1}))/n * 100$; where TL is the body total length at t_2 (final time) and t_1 (initial time), and n is the number of days between the two consecutive measures.

4.5.8. Morphological measures

After physiological measurements, adults were temporally immobilized within hand nets and photographed. 18 dph larvae were anesthetised (0.1 g of MS222 diluted in 1L of water) and on losing equilibrium, were transferred to a petri dish containing clean filtered water where lateral photographs were taken with a binocular microscope (LEICA EX4W) before regaining normal activity. Juveniles were photographed after being scarified with an overdose of MS222. The following morphological traits were measured from photographs using ImageJ software for all fish life-stages, from larvae to adults (Fig. 4.2, sample size in Table S4.5): body elongation as a ratio of the total or fork length (TL in larvae and juveniles: distance from head to end of tail; and FL in adults: distance from head to the fork of the tail) to body height (BH: distance from the pelvic fin and the start of the dorsal fin); pectoral fin length ratio, which is the pectoral fin length (PL: length of the pectoral fin from insertion to the longest tip of the leading edge) divided by fish body length; caudal fin length ratio measured as the ratio of the caudal fin (CL: length of the caudal fin from the end of the standard length to the longest tip) and fish body length. The caudal fin shape, i.e. caudal fin aspect ratio (CAR), which is the squared length of

the caudal fin height (CH) divided by the surface of the caudal fin (CS), was also measured in larvae and juveniles.

Female and male sizes are generally correlated within each anemonefish breeding pair due to social status growth regulation (Buston, 2003b). We also observed a similar correlation in size for the orange-fin anemonefish around Moorea (R = 0.56, t = 2.92, df = 19, p = 0.009, n = 21 pairs), but within the subset of eleven selected breeding pairs used for the analyses, there was no significant correlation in size (R = 0.16, t = 0.45, df = 8, p = 0.67, n = 10 pairs).

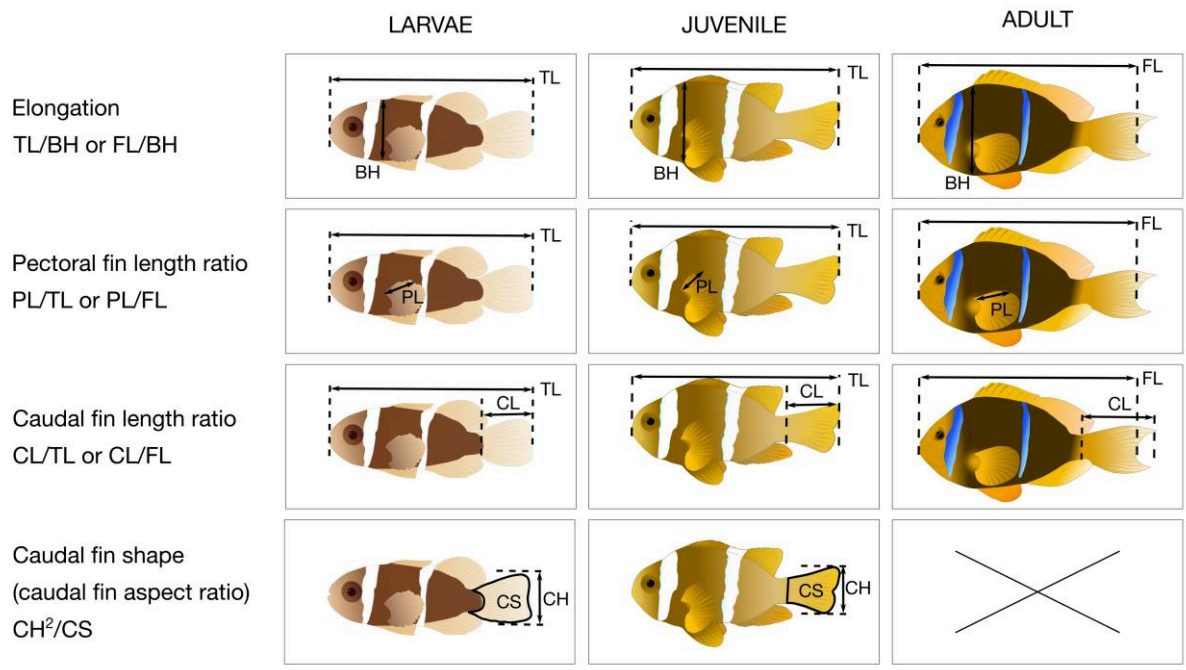

Figure 4. 2. Illustration of morphological traits measured at each developmental stage of A. chrysopterus: larvae at settlement stage (18 dph), juveniles (8-11 weeks) and adults. Pectoral and caudal fin length ratio were then reported as percentages. TL = total length; FL = fork length; BH = body height; PL = pectoral fin length; CL = caudal fin length; CS = caudal fin surface area; CH = caudal fin height.

4.5.9. Statistical analyses

All statistical analyses were carried out in R software, version 3.6.1 (R Core Team 2019).

First, we obtained mass-adjusted metabolic rate values using a linear model (LM) of $\log_{10}$-transformed metabolic rate (MR) as the response variable and $\log_{10}$-transformed body-mass as the explanatory variable. Then, using model residuals we adjusted each individual MR to the overall mean body-mass of fish used in this study (adult mean body-mass = 59.5 g; juvenile mean body-mass = 0.304 g). Adjusted-MR (SMR_{adj}, MMR_{adj}, and AS_{adj}) were then used as explanatory and response variables in the following models.

To explore the effect of water flow regime on adult fish morphology and physiology we used a linear model (LM) with adult elongation, TL ratio, CL ratio, MMR_{adj}, SMR_{adj} and AS_{adj} as response variables. Explanatory variables were fish sex, parental water flow, and anemone surface area. All co-variates were initially included with interactions, and subsequently removed if non-significant using the likelihood ratio test (LRT) *via* the *lmtest* package (Zeileis and Hothorn 2002) to determine the best-fit model (Table S4.6). To reveal differences among groups on model results we carried out a Tuckey post-hoc test via *emmeans* package (Lenth 2020).

Individual offspring survival was also determined using a mixed cox proportional hazard model *via* the 'coxme' function in the *coxme* R package (Therneau 2020a). Parental water flow and developmental water flow treatments were used as explanatory variables, the hazard function (risk of death at time *t*, which refers to the end of the experiment) used as a response variable and parental ID was used as a random effect (Table S4.7A). The effect of parental and developmental water flow was also tested on offspring SGR by fitting a LMER with SGR as a response variable and developmental and parental water flow as explanatory variables. Initial offspring length was also included as a co-variate in the model to account for their asymptotic growth. Parental ID and offspring site were used as random factors (Table S4.8A).

We determined the effect of parental water flow on larval phenotype using a separate mixed-effect linear models (LMERs). Larval elongation, PL ratio, CL ratio, CAR and U_{max} as response variables. Larval CAR was log10-transformed to alleviate non-normality. Parental water flow was used as an explanatory variable and parental ID as a random factor to account for non-independence of full-siblings (Table S4.9).

To determine the influence of parental phenotype on offspring phenotype, we fitted a LM that included larval morphology response variables (elongation, PL ratio, CL ratio, TL) and the same maternal and paternal morphological trait as explanatory variables. The interaction among parental traits was included to explore synergetic effects of both parents (Table S4.10).

To explore the effect of water flow on juvenile morphology after 8-11 weeks we fitted a LMER with the parental and developmental water flow treatment as a predictor variable and parental ID and offspring sites as random effects. Elongation, PL ratio, CL ratio and CAR were used as response variables. Interactions among parental and developmental water flows, as well as larval total length in the CAR model were initially included and subsequently removed if no significant (LRT, Table S4.11A). We also determined the effect of water flow on juvenile

physiology after 6 weeks by fitting a similar LMER with the parental and developmental water flow treatment as a predictor variable and parental ID and offspring sites as random effects. Juvenile SMR_{adj}, MMR_{adj}, and AS_{adj} and U_{max} were used as response variables (Table S4.12A).

Finally, we also explored the effect of developmental water flow treatment on each individual over time we fitted a LMER with developmental water flow treatment and fish stage (larvae *vs.* juvenile) as explanatory variables, initially with all interactions that were dropped if not significant (likelihood ratio test). Model response variables were: elongation, PL ratio, CL ratio, CAR and U_{max} as response variables. Fish ID was also included as a random variable in order to account for repeated measures. Parental ID and offspring site were also included as random factors to account for non-independence of full-siblings and juveniles that grew at the same site (Table S4.13).

4.6. Results

4.6.1. Effect of parental water flow on adult phenotype

We observed no differences in body elongation among adults living in low and high flow (LM: flow(H); t = 0.688, p = 0.496, R^2 = 193, R^2_{adj} = 0.150, table S4.6A, Fig. 4.3A). Sex had an effect on body elongation, with males being more elongate than females (LM: sex(male); t = 2.928, p = 0.006, table S4.6A, Fig. 4.3A). We observed no differences in pectoral fin length ratio across parental water flow sites and across sex (LM (sex removed from the model according to LRT: p > 0.05): Flow (H); t = -1.817, p = 0.077, R^2 = 0.080, R^2_{adj} = 0.056, Fig. 4.3B).

Parental water flow impacted adult caudal length ratio but differently for the two sexes (LM: parental flow*sex; t = -2.209, p = 0.034, R^2 = 227, R^2_{adj} = 0.141, Table S4.6A, Fig. 4.3C). Males at low parental water flow sites tend to have a longer caudal fin in relation to body length compared to those living in high flow (post-hoc test: t = 1.957, p = 0.058, Table S4.6B, Fig. 4.3C), while no difference was observed for the females. Within low parental water flow sites, females had a shorter caudal fin length in relation to body length compared to males (post-hoc test: t = -2.585, p = 0.014, Table S4.6B).

Fish adjusted standard metabolic rate (SMR_{adj}) was also affected by the interaction between paternal water flow and fish sex (LM: parental flow*sex; t = -2.254, p = 0.044, R^2 = 346, R^2_{adj} = 0.182, Table 4.6A, Fig. 4.3D). Females tend to have a lower SMR_{adj} in low water flow compared to females in high water flow (post-hoc test: t = -1.988, p = 0.070, Table S4.6B). Males do not show differences in SMR among paternal water flow sites (post-hoc test: t =1.201,

p = 0.2531, Table S4.6B). Within high water flow, females have a higher SMR_{adj} compared to males (post-hoc test: t = 2.285, p = 0.041, Table S4.6B). Neither parental water flow nor sex had any effect on fish MMR or AS (LM(MMR): Flow (H); t = -1.444, p = 0.171, R^2 = 0.130, R^2_{adj} = 0.067; LM(AS): Flow (H); t = -1.738, p = 0.104, R^2 = 0.178, R^2_{adj} = 0.119, Table 4.6A, Fig. 4.3E and 4.3F respectively).

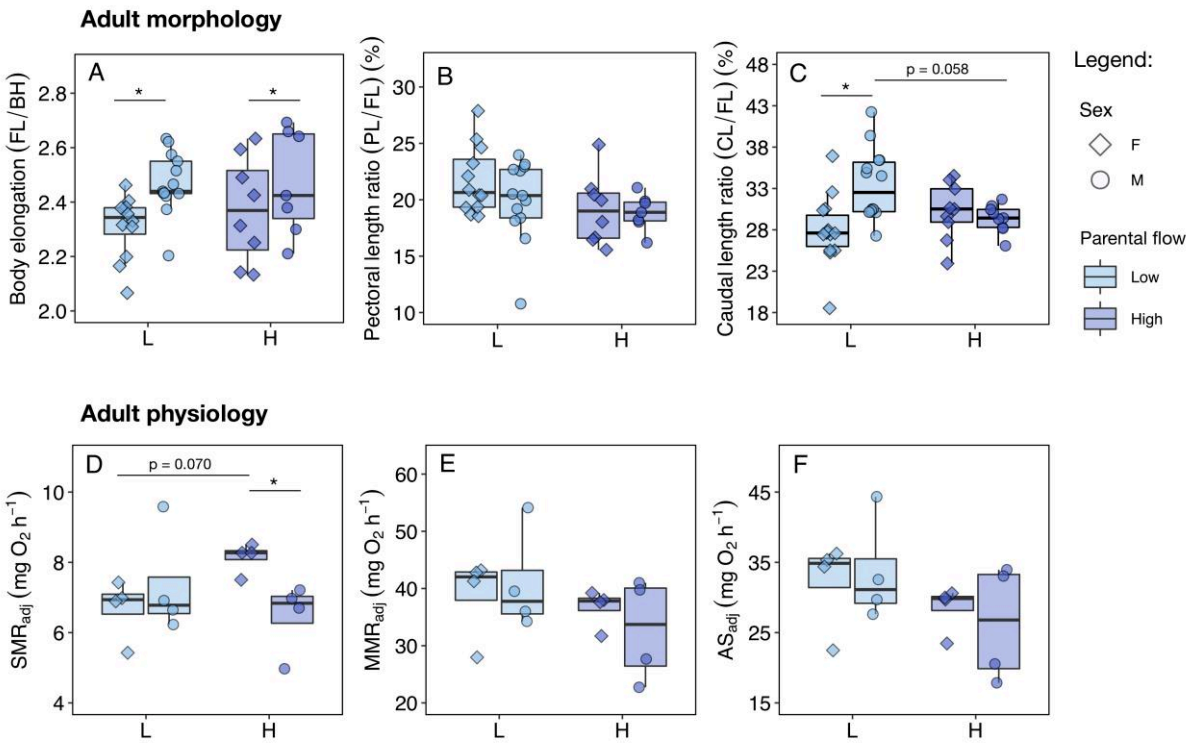

Figure 4. 3. Phenotypes of adult fish (females and males) living in different parental water flows (low L and high H). Adult morphological traits included body elongation (A), pectoral length ratio (B) and caudal length ratio (C). Adult physiological traits included standard metabolic rate (SMR, D), maximum metabolic rate (MMR, E) and aerobic scope (AS, F). Metabolic data have been adjusted (using model residuals) to the overall mean body mass of measured adult fish (59.5 g). Symbols represent individual raw data points of morphological traits and body-mass adjusted values of physiological traits. In each figure, the boxplot with the median, the inter-quartile range (IQR) and whiskers are shown. The upper whisker extends to the largest observation less than or equal to upper hinge +1.5 IQR while the lower whisker extends up to the smallest observation greater than or equal to lower hinge -1.5 IQR. P-value of significant differences across water flow regimes or across sex are indicated in the figure (* refers to p < 0.05).

4.6.2. Effect of parental and developmental water flow on offspring survival and growth

Offspring from parents living in high parental water flow sites had a lower survival probability throughout the experiment (coxme: coef = 1.150, z = 2.350, p = 0.019, Table S4.7, Fig. 4.4A) regardless of developmental water flow treatment (LRT: $flow_P$*$flow_D$; p > 0.05, Table S4.7A)

with about 81% of their mortality happening within three days after release, i.e. at settlement. Even if lower, the majority of mortality of offspring from parents living in low flow also occurred within four days after release, resulting in about 82% of the overall mortality occurring at settlement. The survival of juvenile anemonefish was not affected by the developmental water flow treatment (coxme: coef = 0.077, z = 1.220, p = 0.220, Table S4.7). The specific growth rate (SGR) was lower in offprisng from parents living under high compared to low flow (LMER: flow(H); t = -2.392, p = 0.036, mR^2 = 0.728, cR^2 = 0.728, Table S4.8, Fig. 4.4B) while it was not affected by developmental flow (LRT: $flow_D$; p > 0.05, Table S4.8A).

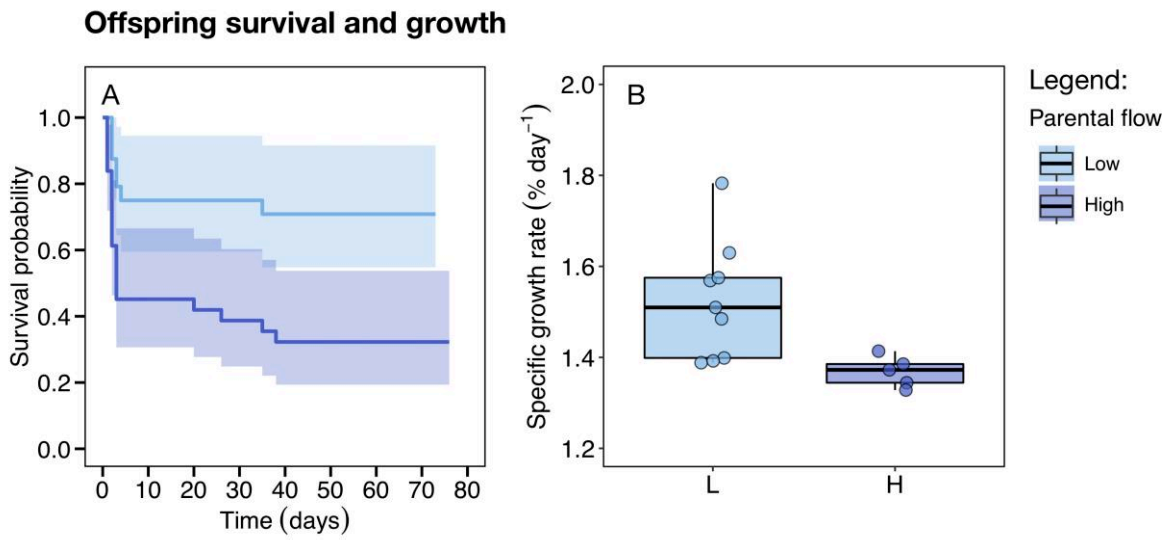

Figure 4. 4. Survival curve over 11 weeks (~75 days) (A). Time 0 corresponds to the settlement stage, the time at which fish were released in the lagoon. Solid grey lines are survival probabilities curves, surrounded by 95 % confidence bands. Specific growth rate (B) measured from settlement (18 dph) to 8-11 weeks of flow exposure. Symbols represent individual data points adjusted by the initial body size (1.08 cm, size at settlement). The boxplot with the median, the inter-quartile range (IQR) and whiskers are shown. The upper whisker extends to the largest observation less than or equal to upper hinge +1.5 IQR while the lower whisker extends up to the smallest observation greater than or equal to lower hinge -1.5 IQR. Colours refer to parental water flow sites (low, L, and high, H, in light and dark grey respectively)

4.6.3. Effect of parental water flow on larval phenotype

Anemonefish pairs living at high parental water flow sites produced larvae with a different caudal fin shape than those from low parental water flow sites. In high water flow the height of the fins was greater in relation to their surface area (LMER: flow(H); t = 3.289, p = 0.001, mR^2 = 0.196, cR^2 = 0.196, Table S4.9, Fig. 4.5A). No other larval morphological traits varied according to parental water flow (LMER: flow(H); all p > 0.005) nor larval swimming capacity (LMER: flow(H); t = -0.355, p = 0.746, mR^2 = 0.006, cR^2 = 0.423).

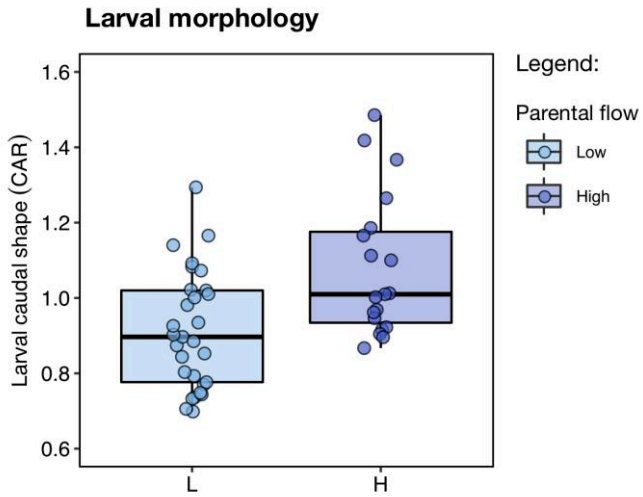

Figure 4. 5. (A) Larval caudal length aspect ratio (CAR) of 18 dph larvae produced by parents living in different flow regimes (low and high flow). Symbols represent individual raw data points. In each figure, the boxplot with the median, the inter-quartile range (IQR) and whiskers are shown. The upper whisker extends to the largest observation less than or equal to upper hinge +1.5 IQR while the lower whisker extends up to the smallest observation greater than or equal to lower hinge -1.5 IQR.

4.6.4. Effect of parental morphological phenotype on larval morphological phenotype

There is an interaction among maternal and paternal caudal length ratio on offspring caudal length ratio (LMER: flow(H); $t = 2.866$, $p = 0.007$, $mR^2 = 0.180$, $cR^2 = 0.115$; Table S4.10, Fig. 4.6). Offspring caudal fin ratio increases with maternal caudal fin ratio, but only if mothers are paired with fathers that also have a high caudal fin ratio. An opposite trend is observed if mothers are paired with a male that has a low caudal fin ratio (Fig. 4.6).

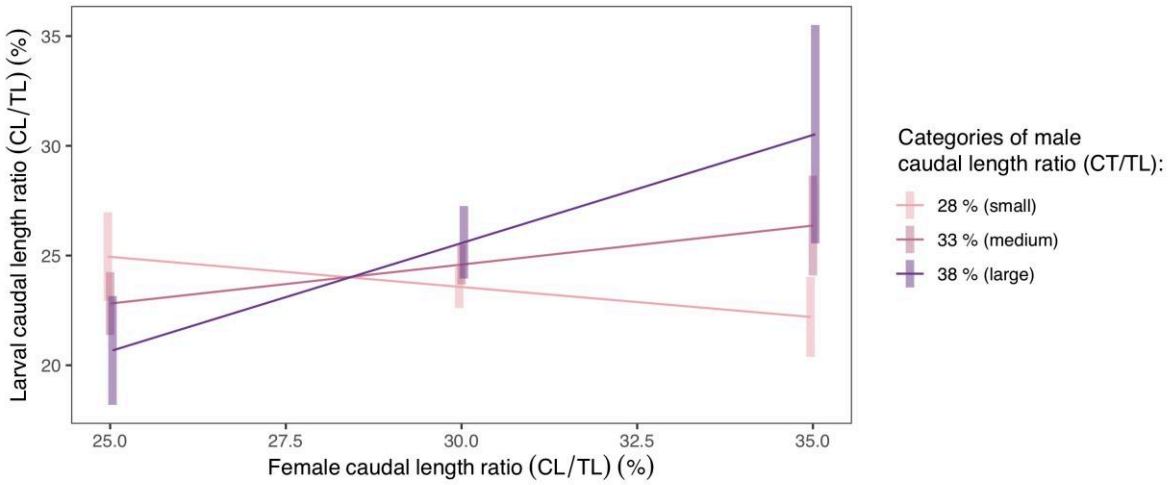

Figure 4. 6. Interaction of maternal and paternal caudal length ratio effect on offspring caudal length. Predicted lines from the model show the effect of maternal caudal length ratio while holding constant male caudal fin length ratio (three categories are represented). The graph was plotted via the 'emmip' function in the emmeans package in R (Lenth 2020).

4.6.5. Effects of parental and developmental water flow on juvenile morphology and physiology

We found no effect of parental nor developmental flow on any morphological trait of juvenile anemonefish after 8-11 weeks of developmental flow exposure (LRT: all $p > 0.05$, removed parental factor); LMER: developmental flow; all $p > 0.05$, $mR^2 = 0.023\text{-}0.090$; $cR^2 = 0.090\text{-}0.365$; Table S4.11, Fig. 4.7). Similarly, parental flow had no effect on fish MMR, AS or U_{max} (LRT: all $p > 0.05$, removed parental factor) and there was no effect of parental flow on juvenile SMR (LMER, parental flow; $t = 0.515$, $p = 0.610$, $mR^2 = 0.011$; $cR^2 = 0.011$). Finally, we found no change in SMR, MMR, AS or U_{max} of juvenile anemonefish living in different developmental water flow treatments after 6 weeks of flow exposure (LMER: all $p > 0.05$, $mR^2 = 0.011\text{-}0.038$; $cR^2 = 0.011\text{-}0.314$; Table S4.12, Fig. 4.8).

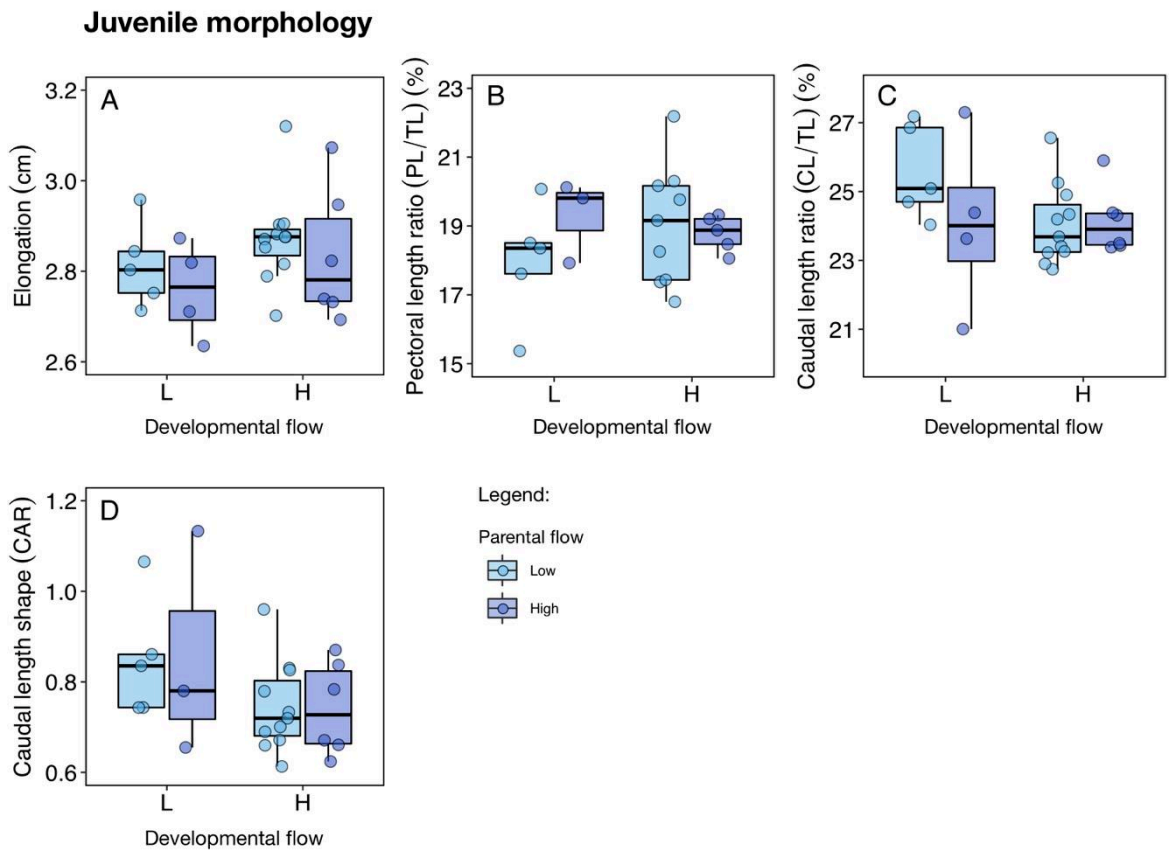

Figure 4. 7. Morphological traits of juveniles after experiencing different water flow regimes during development (low L and high H). Elongation (A), pectoral fin length ratio (B), caudal fin length ratio (C) and caudal fin shape (D), were performed on juveniles after 8-11 weeks in the field under high and low water flow regimes. Symbols represent individual raw values. The upper whisker extends to the largest observation less than or equal to upper hinge +1.5 IQR while the lower whisker extends up to the smallest observation greater than or equal to lower hinge -1.5 IQR.

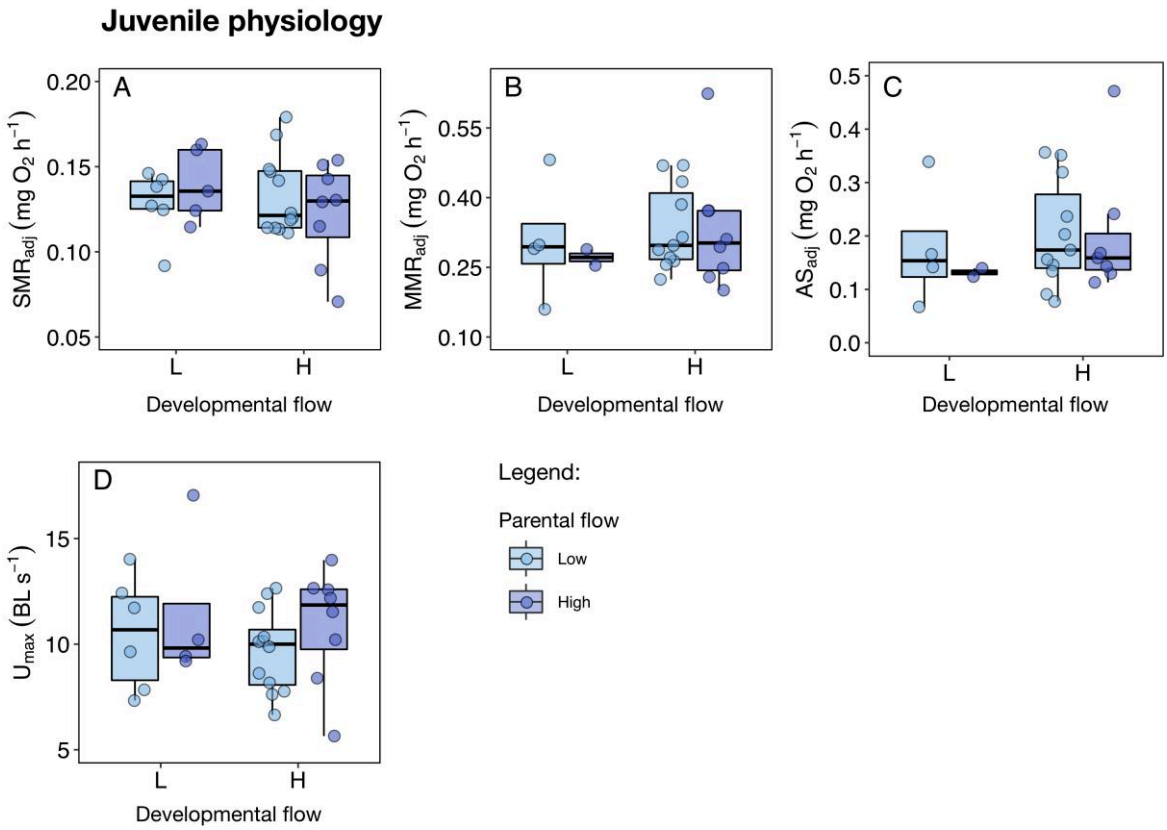

Figure 4. 8. Physiological traits of juveniles after experiencing different water flow regimes during development (low L and high H). Standard metabolic rate (SMR, A), maximum metabolic rate (MMR, B), aerobic scope (AS, C) and constant acceleration test ($\boldsymbol{U_{max}}$, D), were performed on juveniles after 6 weeks in the field under high and low water flow regimes. Symbols represent individual values adjusted by mean body-mass (0.304 g). The upper whisker extends to the largest observation less than or equal to upper hinge +1.5 IQR while the lower whisker extends up to the smallest observation greater than or equal to lower hinge -1.5 IQR.

4.6.6. Developmental plasticity: Effects of developmental water flow on change in offspring traits

Variation in individual phenotype across early life stages (from larval to juvenile stage) shows that across development the caudal length ratio increases in individuals living in low developmental water flow (LMER: flow(H)*stage(juvenile); t = -2.790, p = 0.016, mR^2 = 0.269, cR^2 = 0.396; post-hoc: flow(L): stage(larvae) *vs* stage(juvenile); t = -3.397, p = 0.005, Table S4.13A and S4.13B, Fig. 4.9), but does not under high developmental water flow (post-hoc: t = 0.110, p = 0.914, Table S4.13B, Fig. 4.9) regardless of the parental flow origin. It has to be noted that at the juveniles stage the caudal length ratio among developmental flow treatments didn't show significant differences (post-hoc: stage(juvenile): flow(L) *vs* flow(H); t = 1.098, p

= 0.328). For caudal fin aspect ratio there is also a significant interaction between flow and developmental stage, but due to only 2 individuals at low flow sites being measured twice, results must be interpreted with caution (Table S4.13A). Developmental flow category had no effect on any other measured morphological traits (Table S4.13A).

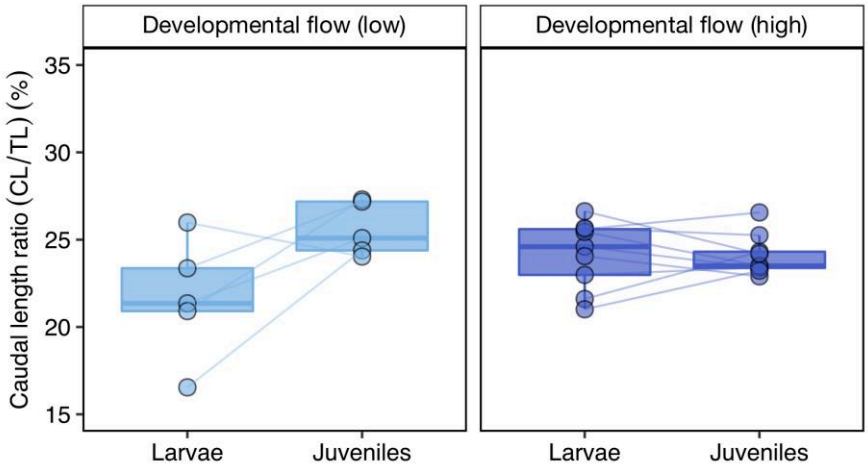

Figure 4. 9. Caudal fin length ratio changes during development, from larvae to juveniles, in low (left) and high developmental flow (right) treatments. Symbols represent individual raw data points and lines connect the same individual at different developmental stages. The upper whisker extends to the largest observation less than or equal to upper hinge +1.5 IQR while the lower whisker extends up to the smallest observation greater than or equal to lower hinge -1.5 IQR.

4.7. Discussion

Both parental phenotype and environment (flow) influenced offspring morphology and survival. Parents living in high water flow produce offspring that have higher mortality and slower growth, regardless of the developmental flow conditions they were in. Furthermore, offspring from parents in higher flow also have a higher caudal fin aspect ratio (shape) at settlement. When both parents have long caudal fins, their larvae also have long caudal fins, but the same is not true for parents with short caudal fins. In contrast, neither parental nor developmental flow regimes had a consistent effect on the morphology, energetics, or performance of juveniles (i.e. post-settlement stage). Our study reveals the importance of the parental contribution and parental environment in determining offspring phenotype and survival in the wild, even in species with unpredictable offspring environment. Our results could have important repercussions on the adult fish fitness as differences in anemonefish growth at early stages

(from settlement to juvenile stage) may translate into reduced size-at-age with implication in competition, ability to escape predators and sexual maturation (Arendt 1997; Sogard 1997).

Parents living in high flow environments produce larvae with higher caudal fin aspect ratio. This type of shape is expected to be associated to steady swimming in high flow environments (Langerhans, 2008; Webb, 1998). The effect of parental flow on larval caudal fin shape suggests that environmental mediated parental effects, genetic inheritance or plasticity of embryos exposed to parental flow (during egg development) may be involved in the determination of this trait. While we cannot separate the effects of these mechanisms, offspring from parents living in high flow had higher mortality rate compared to those from low flow parents, regardless of the developmental flow conditions. The inheritance, genetic or epigenetic, or plasticity of caudal fin shape did not seem to provide any survival advantage. Having a higher caudal fin shape may be more decisive at a different developmental stage. For example, during the dispersal phase having a higher caudal fin aspect ratio may help maintaining sustained swimming against the water current, which is a relatively common behaviour of the pelagic larval phase (Leis 2006). Alternatively, traits that enhance swimming abilities at high flow environments may give an advantage at older ages (>2-3 months post settlement), i.e. when anemonefish reduce the contact with anemone tentacles and swim out of the anemones into the water column (Ross 1978a). Our results also show that maternal and paternal caudal length have a synergetic effect on larval caudal length suggesting that this morphological trait possess some genetic inheritance. However, the lack of differences in the caudal length according to parental flow suggests that there may be no local adaptation to water flow for this trait. Indeed, long caudal fin may not be particularly advantageous/disadvantageous in coping with a specific flow. In high flow long caudal fins are disadvantageous because the increase in drag (Langerhans 2008), however long caudal fins can be advantageous in capturing prey and evade predators, for example by enabling fast-start movements that require a high thrust (Videler 1993; Domenici and Blake 1997; Blake 2004).

Parental flow can also have repercussions on larval development and juvenile survival. This environment effect can cascade into the offspring's phenotype via parental effects, though a variety of mechanisms, including maternal provisioning, hormonal transfer, RNA transfer, DNA methylation or parental care. It is well established that fish may invest less into reproductive output in order to maximize their own fitness during unfavorable environmental conditions (Stearns 1989; Reznick et al. 2000; Donelson et al. 2008; Green 2008). Anemonefish largely feed on plankton swimming in the water column (Allen, 1972; Fautin, 1991; Mariscal, 1970) and despite abundant resource availability in high flow environments, routine activity is

likely to be more energetically demanding (Boisclair and Tang 1993). If fish living in high flow have a reduced energy budget to use for reproduction, this could affect offspring swimming performance and explain the decreased growth and survival. While we did not find evidence of differences in adult metabolic traits in response to water flow, there could still be differences in energy expenditure while swimming and performing routine daily activities in areas of high and low flow.

After settlement, neither the parental nor the developmental flow influenced individual physiology or morphology. Offspring were able to cope equally in both developmental flow environments (no differences in survival according to developmental flow). These results suggest that anemonefish produce offspring with phenotypes suited for heterogeneous environments, which is consistent with the various flow environment, anemones are found at (Fautin 1992; Brolund et al. 2004; Holbrook and Schmitt 2005). While some tropical reef fish larvae are able to delay metamorphoses until they reach a suitable environment to settle (Victor 1986; McCormick 1999; Parmentier et al. 2004) *via* environmental modulation of the thyroid-hormones (Holzer et al. 2017), the probability of encountering anemones on reefs is relatively low (Fautin 1991; Chadwick and Arvedlund 2005). Therefore, the water flow environment may not be a key determinant of settlement. Anemonefish may have alternative ways of coping with the flow environment, for example via behavioural adjustments. Early post-settlement anemonefish mostly dwell among anemone tentacles (Ross 1978a) thus avoiding high flow regimes in the same way, other coral reef fish use holes and the space among coral branches as shelter from strong currents (Fulton et al. 2001; Johansen et al. 2008). Overall, it appears that other environmental factors than water flow may be involved in determining individual's morphology and physiology after settlement.

The caudal fin length ratio increased through development in low flow regimes, while no length change was found among early life stages living in high flow. These results suggest that, to some extent, anemonefish caudal fins may be plastic to developmental environmental flow, as observed in other fish species, such as salmonids (Fischer-Rousseau et al. 2010). In environments with low (mean) but highly variable flow, unsteady swimming is likely to be the main mode adopted by fish and this may elicit the higher growth of the caudal fin.

While water flow regime does not seem to affect adult physiology and morphology, it does accentuate differences between sexes, with males having longer caudal fin at low flow compared to females, and females having higher standard metabolic rate at high flow compared to males. Sex specific behavioural differences may explain these results. Both male and female anemonefish are territorial, but the intensity of aggression, and the dimension and spatial

distribution of territory defence may differ between sexes. *Amphiprion xanthurus* males defend nests and peripheric areas of the territory, while females defend larger areas within the territory (Moyer & Sawyers, 1973). Male behaviours entailing fast-starts, rapid turns, and maneuvers could benefit from longer caudal fins (Videler 1993; Domenici and Blake 1997). Females may need to stay higher in the water column to control the larger territory, which means that in high flow regimes this would translate in a higher energetic demand and increased standard metabolic rate (Weiner 1992), especially if they had longer caudal fins. Anemonefish are protandrous species and once the female dies the second largest fish, i.e. the breeding male, changes sex to become a female. However, the marginal differences in caudal length ratio between flow regimes are not observed in females (that were once males), which can be explained by the regulation of body growth and its dependence on social rank (Buston 2003a). Once the highest social rank (female) die, the growth suppression disappears (Hattori and Yanagisawa 1991) and the same mechanism may be involved in caudal shape adjustment.

Overall, our study reveals the importance of parental contribution and their environment in determining offspring morphology and survival across variable and complex natural environments. The fitness implications of living in high and low water flow sites may cascade across life-stages, for example though reduction of size-at-age and could have consequences in competition, foraging, reproduction and survival (Arendt 1997; Sogard 1997). However, the capacity to equally survive after settlement in different developmental flow environments shows how anemonefish may adjust their phenotypic traits in order to cope with a wide range of flow environments. Finally, in our study both genetic and epigenetic effects and plasticity during embryonic development can have been involved in determining offspring phenotype. Such functional mechanisms modulating the parental contribution and the influence of environment at embryonic stages still need to be dissociated in order to have a better picture of how species cope with their local environment and how this may have carry-over effects across generations.

4.8. Acknowledgment

We particularly thank Till Deuss for his support and help in the CRIOBE anemonefish aquarium facility. We are also thankful to Marguerite Taiarui, Nao Nakamura, Gilles Siu, Pascal Ung, Anne Haguenauer, Frederic Zuberer, Franck Lerouvreur and all the CRIOBE student community for support in the field. We would like to express our gratitude to Glenn Almany. Financial support was provided by the Agence National de la Recherche to Glenn Almany, SCM, and RB (ANR-14-CE02-0005-01/Stay or Go) and to SCM (ANR-11-JSV7-012-01/Live and Let Die), by the Pacific Funds to SCM and by LabEx "CORAIL" to RB and SCM ('Where do we go now?'). TN was supported by the Danish Council for Independent Research (DFF-4181-00297) and the European Union's Horizon 2020 research and innovation programme under the Marie Skłodowska-Curie grant agreement No. 713683. SSK was supported by Natural Environment Research Council Advanced Fellowship (NE/J019100/1) and European Research Council starting grant (640004).

4.9. Supplementary materials

1. *Larval rearing protocol*

All newly hatched larvae were reared under the same conditions, including water flow, temperature (T) of 27.5 ± 1°C, algae and food. All larvae were fed daily with rotifers (*Brachionus plicatilis*) enriched with live *Isochrysis galbana* (phytoflagellate) and SELCO (Self Emulsified Lipid Concentrate) during the first 6-8 days, after which they were fed with enriched brine shrimps (*Artemia salina*).

2. *Environmental measurements: territory size*

Anemone surface area correlates with anemonefish size (Barbasch et al. 2020) and is related to anemonefish fitness (Buston and Elith 2011), therefore we determined territory size at each parental site. Anemone surface area was measured in Image J from photographs taken with a SONY camera (DSC-RX100M3) and anemones were considered as two-dimensional ellipses when viewed from above (Holbrook and Schmitt 2005). Adult territory size varied from ~615 cm^2 (1 anemone) to ~3500 cm^2 (24 anemones) per anemonefish pair (Table S4.1B).

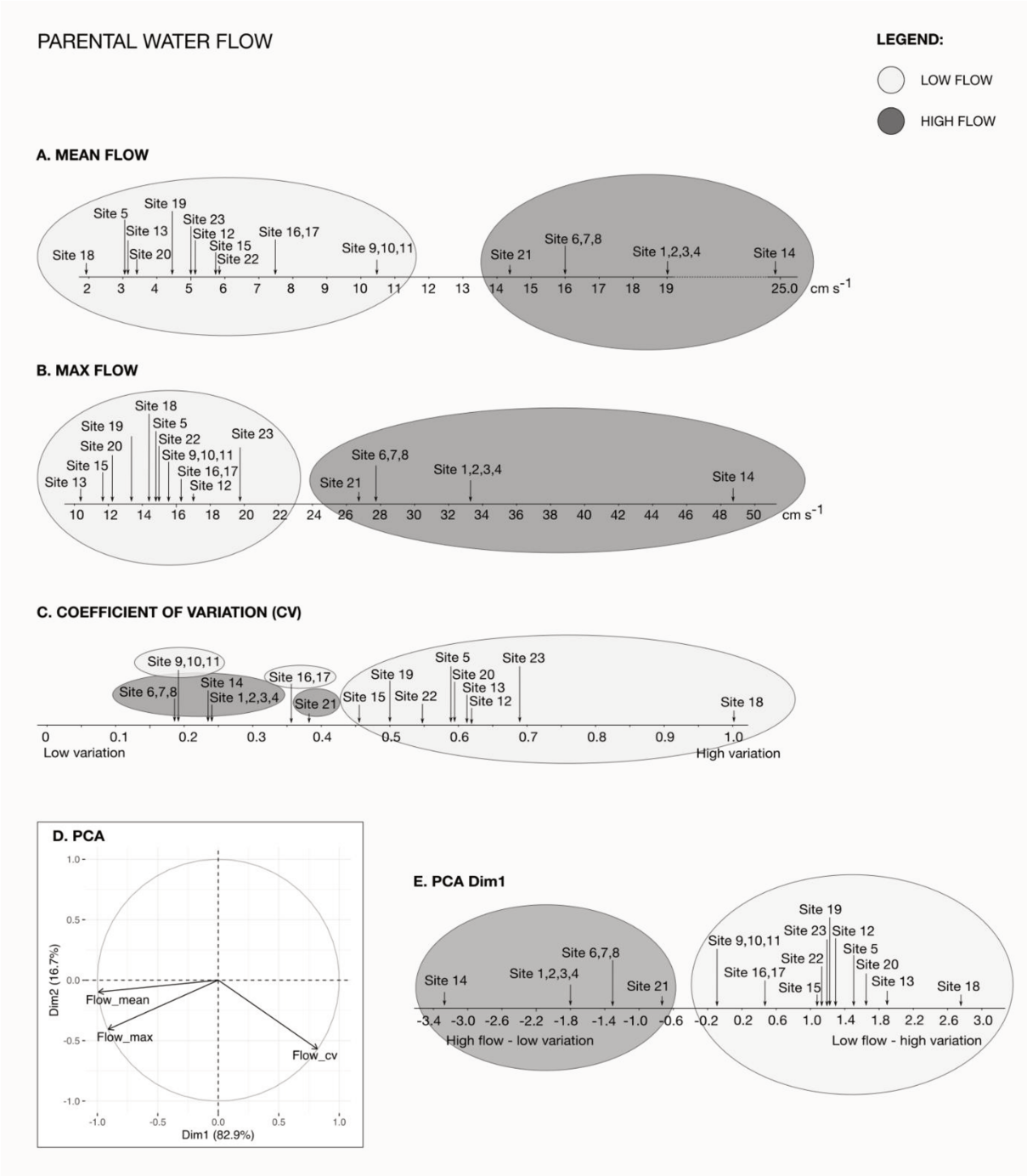

Figure S4. 1. Water flow regime at the 23 parental sites. (A) Mean flow, (B) maximum flow and (C) coefficient of variation (i.e. standard deviation/mean) are shown for each site on a horizontal scale (from left to right: low to high flow in A and B and low to high variation in C). (D) Principal Component Analyses showing the relationship among water flow variables (results are shown in Table S4.1), and (E) distribution of each site on the first PCA axis, which explain the 82.9% of variance, and identification of two categories of flow: low flow and high variation (light grey) vs. high flow and low variation (dark grey).

Table S4. 1. Environmental measures at adult sites.

Table S4. 1A. Principal Component Analyses (PCA) determined on the mean, the maximum and the coefficient of variation (CV) of water flow regimes in adults' sites.

Principal Component Analyses	**Adult sites**	
	PCA1 (82.9%; eigenvalue = 2.5)	
Flow	**Correlation (R)**	**loading**
Mean	-0.99	-0.63
Max	-0.91	-0.58
CV	0.82	0.52

Table S4. 1B. Summary of environmental measures at adult sites and PCA results from Table S4.1A.

Site (pair ID)	Temperature (˚C) (mean ± SD)	Anemone surface area (cm^2)	Number of anemones	Depth (m)	Flow ($cm\ s^{-1}$) (mean ± SD)	Flow categories (high, low)	PCA score
1	29.23 ± 0.77	11270	13	2.0-2.1	19.0 ± 4.7	H	-1.80
2	29.23 ± 0.77	6010	5	2.0-2.2	19.0 ± 4.7	H	-1.80
3	29.23 ± 0.77	956	2	2.3-2.4	19.0 ± 4.7	H	-1.80
4	29.23 ± 0.77	8802	16	2.0	19.0 ± 4.7	H	-1.80
5	29.27 ± 0.37	615	1	4.0	3.1 ± 1.8	L	1.57
6	29.34 ± 0.28	1271	2	2.0	16.0 ± 3.0	H	-1.33
7	29.34 ± 0.28	4527	13	2.0-2.2	16.0 ± 3.0	H	-1.33
8	29.34 ± 0.28	3532	6	1.9-2.0	16.0 ± 3.0	H	-1.33
9	29.29 ± 0.19	3229	7	2.0	10.5 ± 2.0	L	-0.10
10	29.29 ± 0.19	5810	7	2.3-2.5	10.5 ± 2.0	L	-0.10
11	29.29 ± 0.19	3155	4	2.0-2.5	10.5 ± 2.0	L	-0.10
12	28.79 ± 0.33	3054	10	6.0-6.2	5.2 ± 3.2	L	1.36
13	27.13 ± 0.20	5364	2	4.0-4.3	3.1 ± 1.9	L	1.89
14	29.36 ± 0.29	6730	8	9.8-10.0	24.9 ± 6.2	H	-3.25
15	28.89 ± 0.34	7938	6	4.2-4.5	5.7 ± 2.6	L	1.19
16	29.36 ± 0.40	2499	2	3.5	7.5 ± 2.6	L	0.53
17	29.36 ± 0.40	3385	2	1.9-2.0	7.5 ± 2.6	L	0.53
18	29.33 ± 0.40	2797	10	3.0-3.5	1.9 ± 2.0	L	2.77
19	29.40 ± 0.29	6507	13	1.8-2.0	4.5 ± 2.3	L	1.33
20	29.23 ± 0.19	1559	3	6.0	3.5 ± 2.1	L	1.70
21	29.44 ± 0.30	34957	24	15.0	14.4 ± 5.4	H	-0.69
22	28.80 ± 0.20	25295	12	18.0	5.9 ± 3.2	L	1.22
23	29.00 ± 0.17	16292	13	3.0	5.1 ± 3.5	L	1.32

Table S4. 2. Summary of selected pairs used for adult and offspring measures.

Pairs ID	Flow categories (high, low)	Adult morphology	Adult physiology	Egg collection	Larval measures	Cross-transplant experiment
1	H	Yes	Yes	Yes	Yes	Yes
2	H	Yes				
3	H	Yes				
4	H	Yes				
5	L	Yes				
6	H	Yes				
7	H	Yes	Yes	Yes	Yes	Yes
8	H	Yes	Yes	Yes	Yes	Yes
9	L	Yes				
10	L	Yes	-	Yes	Yes	-
11	L	Yes	Yes	Yes	Yes	Yes
12	L	Yes	Yes	Yes	Yes	Yes
13	L	Yes	Yes	Yes	Yes	Yes
14	H	Yes	Yes	Yes	-	Yes
15	L	Yes	-	Yes	Yes	-
16	L	Yes	-	Yes	Yes	-
17	L	Yes	Yes	Yes	Yes	Yes
18	L	Yes				
19	L	Yes				
20	L	Yes				
21	H	Yes				
22	L	Yes				
23	L	Yes				

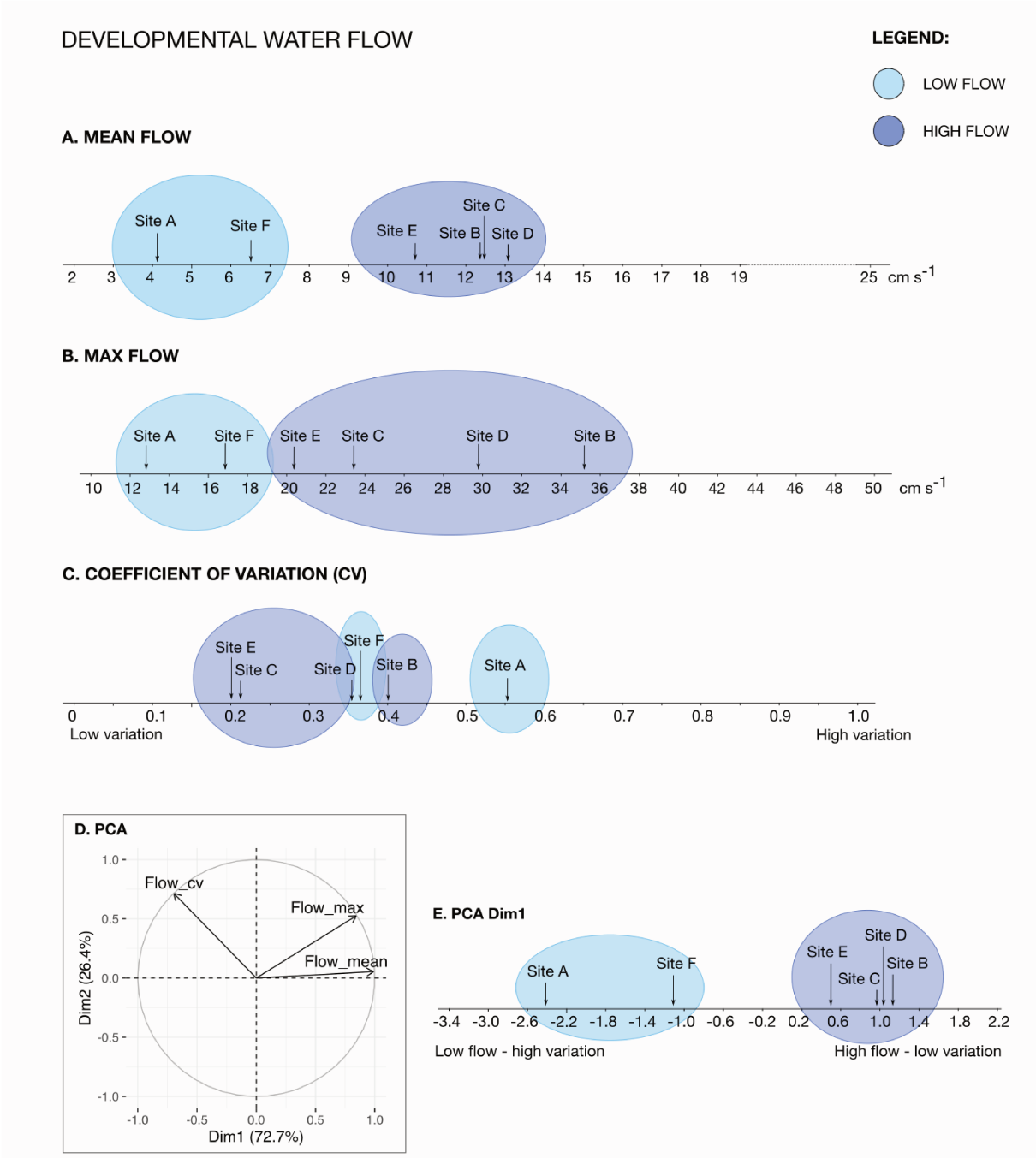

Figure S4. 2. Water flow regime at the six sites of offspring development. (A) Mean flow, (B) maximum flow and (C) coefficient of variation (i.e. standard deviation/mean) are shown for each site on a horizontal scale (from left to right: low to high flow in A and B and low to high variation in C). (D) Principal Component Analyses showing the relationship among water flow variables (results are shown in Table S4.3), and (E) distribution of each site on the first PCA axis, which explain the 72.7% of variance, and identification of two categories of flow: low flow and high variation (light blue) vs. high flow and low variation (dark blue).

Table S4. 3. Environmental measures at developmental sites

Table S4. 3A. Principal Component Analyses (PCA) determined on the mean, the maximum and the coefficient of variation (CV) of water flow regimes on sites of offspring development.

Principal Component Analyses	**Developmental site**	
	PCA1 (72.7%; eigenvalue = 2.2)	
Flow	**Correlation (R)**	**loading**
Mean	0.99	0.67
Max	0.84	0.57
CV	-0.70	-0.47

Table S4. 3B. Summary of environmental measures at developmental sites and PCA results from Table S4.3A.

Offspring locations	Temperature (˚C) (mean ± SD)	Depth (m)	Flow (cm s^{-1}) (mean ± SD)	Flow categories (high, low)	PCA score
A	27.19 ± 0.58	2.1	4.1 ± 2.3	L	-2.49
B	27.65 ± 0.62	4.2	12.4 ± 5.0	H	1.11
C	-	2.4	12.5 ± 2.7	H	0.97
D	27.64 ± 0.62	10.5	13.1 ± 4.7	H	1.02
E	27.64 ± 0.58	2.2	10.7 ± 2.1	H	0.49
F	27.72 ± 0.64	2.0	6.5 ± 2.4	L	-1.11

Table S4. 4. ANOVA to test for differences in the time that offspring spent in the field between flow regimes for each morphological trait.

		df	Sum Sq	Mean Sq	F-value	p-value
elongation	$Flow_O$	1	6	6.0	0.149	0.703
	Residuals	25	1010	40.4		
PL ratio	$Flow_O$	1	14.2	14.20	0.371	0.549
	Residuals	21	805.1	38.34		
CL ratio	$Flow_O$	1	6	6.0	0.149	0.703
	Residuals	25	1010	40.4		
CAR	$Flow_O$	1	0	0.00	0	1
	Residuals	24	938	39.083		

Table S4. 5. Sample size (n) of morphological and physiological measures carried out at each fish stage (larvae, juveniles, development and adults).

	Traits measured	larvae	juvenile	development*	Adults
morphology	elongation	55	26	14	40
	CL/TL	58	26	14	40
	PL/TL	57	22	11	40
	CAR	48	25	10	-
physiology	SMR	-	31	-	16
	MMR	-	25	-	16
	AS	-	25	-	16
	U_{max}	56	30	16	-
	Growth	-	14	-	-
survival		55		-	-

*from larvae to juveniles, repeated measures in same individuals

Table S4. 6. Models and model outputs for adult phenotype analyses

Table S4. 6A. Summary of the best-fit linear models (LM) of flow effect on adult phenotype (elongation, pectoral fin length, caudal fin length, standard metabolic rate, maximum metabolic rate and aerobic scope). References of categorical variables used in the model are female (sex) and low flow (L, flow). H indicates high flow. R^2 is the multiple R-squared, which describe the proportion of the variance explained by the predictors variables included in the model. R^2_{adj} is the adjusted R-squared, which adjust the statistic based on the number of independent variable included in the model.

Best fit model		Estimate	SE	df	t-value	p-value	R^2	R^2_{adj}
1. Elongation	**Intercept**	2.3232	0.038	37	61.384	<0.001	0.193	0.150
	Sex (male)	0.1355	0.046	37	2.928	0.006		
	Flow (H)	0.0329	0.048	37	0.688	0.496		
2. PL ratio	**Intercept**	20.7964	0.607	38	34.252	<0.001	0.080	0.056
	Flow (H)	-1.8013	0.992	38	-1.817	0.077		
3. CL ratio	**Intercept**	41.093	5.835	36	6.997	<0.001	0.227	0.141
	Sex (male)	4.312	1.668	36	2.585	0.014		
	Flow (H)	2.048	1.765	36	1.160	0.234		
	Log10(Anemone)	-3.431	1.604	36	-2.139	0.040		
	Sex (male) * Flow (H)	-5.674	2.569	36	-2.209	0.034		
4. SMR	**Intercept**	6.6820	0.518	12	12.903	<0.001	0.346	0.182
	Sex (male)	0.6615	0.732	12	0.903	0.384		
	Flow (H)	1.4557	0.732	12	1.988	0.070		
	Sex (male) * Flow (H)	-2.3351	1.036	12	-2.254	0.044		
5. MMR	**Intercept**	39.889	2.536	14	15.731	<0.001	0.130	0.067
	Flow (H)	-5.177	3.586	14	-1.444	0.171		
6. AS	**Intercept**	32.830	2.211	14	14.848	<0.001	0.178	0.119
	Flow (H)	-5.435	3.127	14	-1.738	0.104		

Table S4. 6B. Post-hoc test on the best-fit model from table S4.6A.

Model	Fixed effects	Estimate	SE	df	t-value	p-value
3. CL ratio	Flow (L): sex (female) *vs* sex (male)	-4.31	1.67	35	-2.585	0.014
	Flow (H): sex (female) *vs* sex (male)	1.36	1.94	35	0.702	0.487
	Sex (female): flow (L) *vs* flow (H)	-2.05	1.77	35	-1.160	0.254
	Sex (male): flow (L) *vs* flow (H)	3.63	1.85	35	1.957	0.058
4. SMR	Flow (L): sex (female) *vs* sex (male)	-0.661	0.732	12	-0.903	0.384
	Flow (H): sex (female) *vs* sex (male)	1.674	0.732	12	2.285	0.041
	Sex (female): flow (L) *vs* flow (H)	-1.456	0.732	12	-1.988	0.070
	Sex (male): flow (L) *vs* flow (H)	0.879	0.732	12	1.201	0.253

Table S4. 7. Models and model outputs for larval survival analyses

Table S4. 7A. Cox proportional hazard mixed model with the hazard function (defined as the risk of death over time) used as a response variable. *Time* is the survival time in weeks, *Status* (presence/absence) is used for censoring, $Flow_P$ is the parental water flow regime (low vs high) and $Flow_D$ is the water flow regime at developmental sites (low vs high). Parental (P) and developmental (D) *site* were used as random factors to account for non-independence of data. Model selection was performed by comparing models using the anova function in R, in order to identify the best-fit model (in bold) that included parental and offspring flow as predictor variables.

Model	LogLik	χ^2	p-value
1: $(Time, Status) \sim Flow_P * Flow_D + (1\|site_P) + (1\|site_D)$	-98.464		
2: $(\boldsymbol{Time}, \boldsymbol{Status}) \sim \boldsymbol{Flow_P} + \boldsymbol{Flow_D} + (\mathbf{1}\|\boldsymbol{site_P}) + (\mathbf{1}\|\boldsymbol{site_D})$	-98.382	0.164	0.685

Table S4. 7B. Results of the best-fit model from Table S4.7A.

	coef	Exp(coef)	Se(coef)	z-value	p-value
$Flow_O$ (H)	0.5909	1.8057	0.463	1.280	0.200
$Flow_P$ (H)	1.1550	3.1741	0.495	2.330	0.020

Table S4. 8. Models and model outputs for specific growth rate analyses

Table S4. 8A. Linear midex models (LMER) of parental flow and developmental flow effect on offspring specific growth rate in length (SGR) used as a response variable. The log-transformed initial fish length at the start of the experiment was included as a covariate to account for the non-linear relationship of fish growth over time. Parental (P) and developmental (D) site were used as random factors to account for non-independence of data. Parental flow and developmental flow are used as a predictor variables. Model selection was performed by sequentially dropping non-significant variables, starting with the interaction, and models were compared using the likelihood ratio test in R, in order to identify the best-fit model (in bold).

Model	Df	LogLik	χ^2	p-value
1: $y \sim log(TL_i) + Flow_P * Flow_D + (1\|site_P) + (1\|site_D)$	8	12.517		
2: $y \sim log(TL_i) + Flow_P + Flow_D + (1\|site_P) + (1\|site_D)$	7	12.483	0.069	0.793
3: $y \sim log(TL_i) + Flow_P + (1\|site_P) + (1\|site_D)$	6	12.348	0.270	0.603

Table S4. 8B. Results of the best-fit model from Table S4.8A. mR^2 is the marginal R^2, which describes the proportion of variance explained by the fixed factors alone. cR^2 is the conditional R^2, which describes the proportion of variance explained by both fixed and random factors.

Best fit model			Estimate	SE	df	t-value	p-value	mR^2	cR^2
1	SGR	Intercept	1.6611	0.047	11.00	35.04	<0.001	0.728	0.728
		$log(TL_i)$	-1.7617	0.302	11.00	-5.833	<0.001		
		$Flow_P$ (H)	-0.1567	0.066	11.00	-2.392	0.036		

Table S4. 9. Summary of the mixed-effect linear model (LMER) of parental flow ($Flow_P$) effect on larval phenotype. H indicates high flow. mR^2 is the marginal R^2, which describes the proportion of variance explained by the fixed factors alone. cR^2 is the conditional R^2, which describes the proportion of variance explained by both fixed and random factors. Parental Site is used as random factors to account for non-independence of data.

Best fit model			Estimate	SE	df	t-value	p-value	mR^2	cR^2
1	**elongation**	**Intercept**	3.4554	0.067	6.62	51.757	<0.001	0.069	0.399
		$Flow_P$ (H)	-0.1472	0.115	7.20	-1.275	0.242		
2	**PL ratio**	**Intercept**	20.502	0.542	8.68	37.848	<0.001	0.028	0.074
		$Flow_P$ (H)	-1.0771	0.949	7.39	-1.135	0.292		
3	**CL ratio**	**Intercept**	22.9640	0.530	8.90	43.303	<0.001	0.020	0.210
		$Flow_P$ (H)	0.7065	0.915	8.95	0.772	0.460		
4	**Log10(CAR)**	**Intercept**	-0.0450	0.013	46.00	-3.352	0.002	0.196	0.196
		$Flow_P$ (H)	0.0723	0.021	46.00	3.289	0.001		
5	**U_{max}**	**Intercept**	22.6971	1.29	8.16	17.608	<0.001	0.006	0.423
		$Flow_P$ (H)	-0.7822	2.34	7.93	-0.335	0.746		

Table S4. 10. Summary of the LMs of parental phenotype influence on larval phenotype. The interaction among maternal and paternal phenotype was included to account for potential synergetic effects of parents on offspring phenotype. R^2 is the multiple R-squared, which describe the proportion of the variance explained by the predictors variables included in the model. R^2_{adj} is the adjusted R-squared, which adjust the statistic based on the number of independent variable included in the model.

Best fit model			Estimate	SE	df	t-value	p-value	R^2	R^2_{adj}
1	**elongation**	**Intercept**	17.9030	15.489	46	1.156	0.254	0.038	-0.024
		elongation (mother)	-6.6780	6.888	46	-0.969	0.337		
		elongation (father)	-5.9030	6.362	46	-0.928	0.358		
		elongation (mother)* elongation ratio (father)	2.7210	2.819	46	0.965	0.340		
2	**PL ratio**	**Intercept**	-9.3104	84.933	48	-0.110	0.913	0.045	-0.015
		PL ratio (mother)	1.5031	4.104	48	0.366	0.716		
		PL ratio (father)	2.0274	4.395	48	0.461	0.647		
		PL ratio (mother)* PL ratio (father)	-0.1052	0.212	48	-0.496	0.622		
3	**CL ratio**	**Intercept**	132.0110	38.724	38	3.409	0.002	0.180	0.115
		CL ratio (mother)	-3.8036	1.346	38	-2.825	0.007		
		CL ratio (father)	-3.5783	1.257	38	-2.847	0.007		
		CL ratio (mother)* CL ratio (father)	0.1260	0.044	38	2.866	0.007		
4	**TL**	**Intercept**	3.5814	5.377	49	0.666	0.509	0.081	0.025
		FL (mother)	-0.1573	0.367	49	-0.429	0.670		
		FL (father)	-0.2483	0.446	49	-0.556	0.581		
		FL (mother)* FL (father)	0.0160	0.030	49	0.525	0.602		

Table S4. 11. Models and model outputs for juvenile morphology analyses

Table S4. 11A. Linear mixed model (LMER) of parental flow and developmental flow effect on offspring morphological traits. Juvenile elongation, pectoral fin length (PL ratio), caudal fin length (CL ratio) and caudal fin shape (CAR) used as a response variable. Parental (P) and developmental (D) site were used as random factors to account for non-independence of data. Parental flow and developmental flow are used as predictor variables and their interaction tested via the likelihood ratio test (LRT).

1. Elongation model	**Df**	**LogLik**	**χ^2**	**p-value**
1: $y \sim Flow_P * Flow_D + (1\|site_P) + (1\|site_D)$	7	21.902		
2: $\boldsymbol{y \sim Flow_P + Flow_D + (1\|site_P) + (1\|site_D)}$	6	21.883	0.037	0.847

2. PL ratio model	Df	LogLik	χ^2	p-value
1: $y \sim Flow_P * Flow_D + (1\|site_P) + (1\|site_D)$	7	-36.341		
2: $\boldsymbol{y \sim Flow_P + Flow_D + (1\|site_P) + (1\|site_D)}$	6	-37.574	2.467	0.116

3. CL ratio model	Df	LogLik	χ^2	p-value
1: $y \sim Flow_P * Flow_D + (1\|site_P) + (1\|site_D)$	7	-44.113		
2: $\boldsymbol{y \sim Flow_P + Flow_D + (1\|site_P) + (1\|site_D)}$	6	-45.073	1.920	0.166

4. CAR model	Df	LogLik	χ^2	p-value
1: $y \sim TL + Flow_P * Flow_D + (1\|site_P) + (1\|site_D)$	8	19.213		
2: $y \sim TL + Flow_P + Flow_D + (1\|site_P) + (1\|site_D)$	7	18.163	0.025	0.875
3: $\boldsymbol{y \sim Flow_P + Flow_D + (1\|site_P) + (1\|site_D)}$	6	18.163	2.100	0.147

Table S4. 11B. Summary of the best-fit model from Table S4.11A. mR^2 is the marginal R^2, which describes the proportion of variance explained by the fixed factors alone. cR^2 is the conditional R^2, which describes the proportion of variance explained by both fixed and random factors.

Best fit model			**Estimate**	**SE**	**df**	**t-value**	**p-value**	**mR^2**	**cR^2**
1	**elongation**	**Intercept**	2.8092	0.040	26.00	71.117	<0.001	0.128	0.128
		$Flow_P$ (H)	-0.0438	0.042	26.00	-1.036	0.310		
		$Flow_D$ (H)	0.0650	0.043	26.00	1.507	0.144		
2	**PL ratio**	**Intercept**	18.5828	0.676	7.236	27.474	<0.001	0.025	0.363
		$Flow_P$ (H)	0.1384	0.792	6.265	0.175	0.867		
		$Flow_D$ (H)	0.4419	0.604	4.590	0.732	0.500		
3	**CL ratio**	**Intercept**	25.0748	0.584	7.87	42.952	<0.001	0.095	0.183
		$Flow_P$ (H)	-0.4655	0.532	20.38	-0.876	0.391		
		$Flow_D$ (H)	-0.8304	0.660	5.75	-1.258	0.257		
4	**CAR**	**Intercept**	0.8520	0.045	9.25	18.760	<0.001	0.164	0.167
		$Flow_P$ (H)	0.0001	0.049	20.40	0.002	0.999		
		$Flow_D$ (H)	-0.1090	0.050	6.33	-2.161	0.071		

Table S4. 12. Models and model outputs for juvenile physiology analyses

Table S4. 12A. Linear mixed model (LMER) of parental flow and developmental flow effect on offspring physiological traits. Juvenile body mass adjusted SMR, MMR, AS, and U_{max} are used as a response variable. Parental (P) and developmental (D) site were used as random factors to account for non-independence of data. Parental flow and developmental flow are used as predictor variables and their interaction tested via the likelihood ratio test (LRT).

1. SMR_{adj} model	**Df**	**LogLik**	**χ^2**	**p-value**
1: $y \sim Flow_P * Flow_D + (1\|site_P) + (1\|site_D)$	7	73.392		
2: $y \sim \boldsymbol{Flow_P + Flow_D + (1\|site_P) + (1\|site_D)}$	6	72.622	1.539	0.215

2. MMR_{adj} model	Df	LogLik	χ^2	p-value
1: $y \sim Flow_P * Flow_D + (1\|site_P) + (1\|site_D)$	7	15.826		
2: $y \sim \boldsymbol{Flow_P + Flow_D + (1\|site_P) + (1\|site_D)}$	6	15.824	0.003	0.953

3. AS_{adj} model	Df	LogLik	χ^2	p-value
1: $y \sim Flow_P * Flow_D + (1\|site_P) + (1\|site_D)$	7	3.852		
2: $y \sim \boldsymbol{Flow_P + Flow_D + (1\|site_P) + (1\|site_D)}$	6	3.807	0.091	0.764

4. U_{max} model	Df	LogLik	χ^2	p-value
1: $y \sim Flow_P * Flow_D + (1\|site_P) + (1\|site_D)$	7	-68.298		
2: $y \sim \boldsymbol{Flow_P + Flow_D + (1\|site_P) + (1\|site_D)}$	6	-68.299	0.002	0.967

Table S4. 12B. Summary of the best-fit model from Table S4.12A. mR^2 is the marginal R^2, which describes the proportion of variance explained by the fixed factors alone. cR^2 is the conditional R^2, which describes the proportion of variance explained by both fixed and random factors.

Best fit model			**Estimate**	**SE**	**df**	**t-value**	**p-value**	**mR^2**	**cR^2**
1	**SMR_{adj}**	**Intercept**	0.1346	0.084	28.0	16.000	<0.001	0.010	0.010
		$Flow_P$ (H)	-0.0027	0.009	28.0	-0.298	0.768		
		$Flow_D$ (H)	-0.0045	0.009	28.0	-0.490	0.628		
2	**Log10(MMR_{adj})**	**Intercept**	-0.5463	0.059	22.0	-9.265	<0.001	0.034	0.034
		$Flow_P$ (H)	-0.0173	0.056	22.0	-0.308	0.761		
		$Flow_D$ (H)	0.0568	0.064	22.0	0.884	0.387		
3	**Log10(AS_{adj})**	**Intercept**	-0.8273	0.097	11.2	-8.497	<0.001	0.036	0.066
		$Flow_P$ (H)	-0.0197	0.097	5.7	-0.203	0.846		
		$Flow_D$ (H)	0.0953	0.104	20.6	0.918	0.369		
4	**U_{max}**	**Intercept**	10.5542	1.173	6.84	8.998	<0.001	0.058	0.325
		$Flow_P$ (H)	1.0024	1.086	4.26	0.923	0.405		
		$Flow_D$ (H)	-0.8303	1.230	4.05	-0.675	0.536		

Table S4. 13. Models and model outputs for developmental plasticity analyses

Table S4. 13A. Summary of the best-fit LMER on developmental plasticity of offspring phenotype (elongation, pectoral fin length caudal fin length, caudal fin shape and swimming sped). $Flow_D$ is the flow at developmental sites (low vs high) and Stage is the developmental stage (larvae vs juvenile). Parental and offspring Site are used as random factors to account for non-independence of data. In addition, fish ID is used as random factor to account for repeated measures on the same individual.

Best fit model			Estimate	SE	df	t-value	p-value	R^2	R^2_{adj}
1	**elongation**	**Intercept**	3.4002	0.088	5.32	38.597	<0.001	0.707	0.758
		$Flow_D$ (H)	0.1100	0.098	3.96	1.118	0.327		
		Stage (j)	-0.5825	0.067	19.48	-8.741	<0.001		
2	**PL ratio**	**Intercept**	20.5240	1.158	10.03	17.718	<0.001	0.076	0.402
		$Flow_D$ (H)	-1.1141	1.286	8.03	-0.867	0.411		
		Stage (j)	-1.1219	0.956	10.00	-1.174	0.268		
3	**CL ratio**	**Intercept**	21.6307	0.909	23.30	23.809	<0.001	0.269	0.396
		$Flow_D$ (H)	2.5461	1.133	23.30	2.247	0.034		
		Stage (j)	3.9675	1.168	12.00	3.397	0.005		
		$Flow_D$ (H)*Stage (j)	-4.0634	1.457	12.00	-2.790	0.016		
4	**CAR**	**Intercept**	1.9809	0.196	13.61	10.081	<0.001	0.569	0.871
		TL	-0.8220	0.154	9.07	-5.328	<0.001		
		$Flow_D$ (H)	-0.0860	0.113	3.52	-0.761	0.494		
		Stage (j)	1.3710	0.307	9.04	4.465	0.002		
5	**U_{max}**	**Intercept**	22.2786	1.137	29.00	19.593	<0.001	0.717	0.717
		$Flow_D$ (H)	-0.1126	1.227	29.00	-0.092	0.927		
		Stage (j)	-10.5219	1.188	29.00	-8.860	<0.001		

Table S4. 13B. Post-hoc test on the best-fit model from table S4.13A. mR^2 is the marginal R^2, which describes the proportion of variance explained by the fixed factors alone. cR^2 is the conditional R^2, which describes the proportion of variance explained by both fixed and random factors.

Model		Fixed effects	Estimate	SE	df	t-value	p-value
3.	**CL ratio**	$Flow_D$ (L): stage (larvae) *vs* stage (juvenile)	-3.967	1.17	12	-3.397	0.005
		$Flow_D$ (H): stage (larvae) *vs* stage (juvenile)	0.096	0.87	12	0.110	0.914
		Stage (larvae): $flow_D$ (L) *vs* $flow_D$ (H)	-2.550	1.38	4.44	-1.843	0.132
		Stage (juvenile): $flow_D$ (L) *vs* $flow_D$ (H)	1.520	1.38	4.44	1.098	0.328

Chapter 5: Physiological and behavioural effects of anemone bleaching on symbiont anemonefish in the wild

5.1. Foreword

I conceptualised this research project with Dr. Tommy Norin, University of Denmark, within an international collaboration among Dr. Suzanne C. Mills, Dr. Ricardo Beldade, and Dr. Shaun S. Killen and Dr. Amélie Crespel from the University of Glasgow, based on our previous study (Norin et al. 2018, in which I am co-author, attached in Appendix 1) where we observed that short-term exposure (2 weeks) to anemone bleaching affects juvenile anemonefish metabolism by increasing their oxygen demand. The following up question was therefore to understand the effects of anemone bleaching over a longer period (from 1 to 9 months), to provide a more comprehensive and realistic understanding of these effects, as anemone bleaching can last for several months. In addition, this chapter aims at understanding whether juvenile anemonefish can acclimate to bleaching.

To achieve this, I have planned and conducted intensive fieldwork, and received help from Dr. Suzanne C. Mills, Dr. Ricardo Beldade and several volunteering students that accompanied me on the field. The laboratory-based experiments and subsequent analyses were performed in close collaboration with Dr. Tommy Norin. I drafted the related manuscript under the supervision of Dr. Suzanne C. Mills with important contribution from Dr. Tommy Norin. Final writing was performed with inputs from of all co-authors. This project was conducted during the two first years of my PhD.

5.2. Manuscript information

Title: Physiological and behavioural effects of anemone bleaching on symbiont anemonefish in the wild

Authors: Daphne Cortese[1,*], Tommy Norin[2,3,*], Ricardo Beldade[1,4], Amélie Crespel[2], Shaun S. Killen[2], Suzanne C. Mills[1,5]

*These authors contributed equally

Affiliations

[1] PSL Université Paris: EPHE-UPVD-CNRS, USR 3278 CRIOBE, BP 1013, 98729 Papetoai, Moorea, French Polynesia

[2] Institute of Biodiversity, Animal Health and Comparative Medicine, University of Glasgow, Graham Kerr Building, Glasgow G12 8QQ, UK

[3] DTU Aqua: National Institute of Aquatic Resources, Technical University of Denmark, 2800 Kgs. Lyngby, Denmark

[4] Estación Costera de Investigaciones Marinas and Center for Advanced Studies in Ecology and Biodiversity, Las Cruces, Pontificia Universidad Católica de Chile, Santiago de Chile

[5] Laboratoire d'Excellence "CORAIL", France

Keywords: climate change; standard metabolic rate; growth rate; survival; environmental stressor; coral reef fish; temperature; acclimation

Status: Published in *Functional Ecology* (in Appendix 2):

Cortese D, Norin T, Beldade R, Crespel A, Killen SS, Mills SC. Physiological and behavioural effects of anemone bleaching on symbiont anemonefish in the wild. Funct Ecol. 2021;00:1–11. https://doi.org/10.1111/1365-2435.13729

5.3. Abstract

Climate change causes extreme heat waves that have induced worldwide mass coral bleaching. The impacts of temperature-induced bleaching events on the loss of algal endosymbionts in both corals and anemones are well documented. However, the cascading impacts of bleaching on animals that live in association with corals and anemones are understudied. We performed a field-based experiment to investigate how host anemone bleaching affected the metabolic rate, growth, behaviour, and survival of wild juvenile orange-fin anemonefish *Amphiprion chrysopterus* over 1, 2, and (for survival) 9 months. We found that the standard metabolic rate of anemonefish residing in bleached anemones decreased over time but was unaffected in fish from healthy anemones. Despite the reduced metabolic cost, the growth rate of fish from bleached anemones was significantly lower compared to fish from healthy anemones, suggesting that animals residing in bleached hosts are at an energetic disadvantage. This was corroborated by our finding that fish from bleached anemones spent more time out of their anemones, suggestive of a greater need to forage in the water column. However, fish from bleached anemones were overall less active and used less space around the anemone, resulting in a negative correlation between space use and survival after 4 weeks. Our results provide insight into the physiological and behavioural effects of host bleaching on juvenile fish in the wild, and highlight how relatively short-term thermal anomalies can have long-lasting impacts beyond the bleached anemones or corals themselves.

5.4. Introduction

Anthropogenic climate change and extreme weather events are global phenomena that impact terrestrial and aquatic organisms. The increasing frequency and duration of global episodes of coral reef bleaching (Hughes et al. 2017) are testament to the severity of climate-driven impacts on coral reef ecosystems. Corals are not alone, as other marine invertebrates, including sea anemones, also bleach in response to environmental stressors (McClanahan et al. 2009). If these organisms survive, the temporary loss of endosymbiotic dinoflagellates can last several months before recovery (Lang et al. 1992; Jones 1997; Saenz-Agudelo et al. 2011; Beldade et al. 2017). As such, short-term climatic stressors such as transient heat waves lasting from days to weeks (Glynn 1996; Oliver et al. 2018) are outlived by the ensuing longer-term bleaching episodes. The impacts of these bleaching events may cascade onto other animals associated with corals and anemones for either shelter, foraging, or recruitment (Jones and Syms 1998; Wilson et al. 2006; Graham et al. 2007). However, the physiological and behavioural impacts of bleaching on associated species, such as coral reef fish, have rarely been tested.

Fishes are essential components of coral reef ecosystems, due to their role in reef health, diversity, resilience, and local economy (Moberg and Folke 1999; Bellwood et al. 2004; Graham et al. 2006). Bleaching-induced coral mortality can drive fish mortality due to the degradation and loss of suitable habitats (Bonin et al. 2009), but recent evidence has shown that temporary bleaching of anemones also impacts the physiology and reproduction of associated anemonefish (Beldade et al. 2017; Norin et al. 2018). To cope with prolonged bleaching, fish may need to adjust their physiology and behaviour through phenotypic plasticity (e.g. acclimation) to enhance fitness while living in a bleached environment. For example, bleaching-associated decreases in anemonefish food sources (e.g. plankton in the water column, anemone waste products, and symbiont algae; Tada et al. 2003; Piontkovski and Castellani 2009; Verde et al. 2015) may require anemonefish to alter their behaviour to maximise foraging, or to adjust their energy expenditure to compensate for reduced food intake (Dill 1983; Höjesjö et al. 1999). As fish associated with bleached (white) hosts are visually more conspicuous (Coker et al. 2009), the risk of predation potentially increases too, which may reduce foraging and other behaviours outside of the anemone (Lima and Dill 1990). The previously-observed increased metabolic demands of anemonefish after two weeks of bleaching in the laboratory (Norin et al. 2018) may also trade-off with growth due to competition for a finite energy budget (energy allocation trade-off; Weiner, 1992). However, to what extent these behavioural and physiological adjustments occur in nature remains unknown.

Here, we quantify the effects of varying bleaching exposure durations on a suite of life-history traits of anemonefish living in the wild. We conducted an extensive field-based study with wild site-attached juvenile orange-fin anemonefish *Amphiprion chrysopterus* transplanted onto healthy (unbleached) or bleached anemones in the lagoonal reef system of Moorea, French Polynesia. Anemonefishes live in a permanent obligatory mutualistic symbiosis with sea anemones (Fautin 1991) and remain with the host anemone on which they settled as juveniles (Hattori 1994; Buston 2003b), even if it bleaches (Saenz-Agudelo et al. 2011), making them especially vulnerable to bleaching and an ideal model species (Beldade et al. 2017; Mills et al. 2018, 2020). We compared standard (resting) metabolic rate (SMR), behaviour (activity and space use) and growth rate of anemonefish after 4 and 8 weeks of living with unbleached or bleached anemones, and tracked the fish's survival for 9 months. These exposure durations are ecologically relevant both to bleaching and anemone recovery (Lang et al. 1992; Jones 1997), and sufficient to observe the potential for acclimation (Fangue et al. 2014; Sandblom et al. 2014). We also explored correlations among the measured traits, as environmental disturbances are known to alter the relationship between physiological and behavioural traits (Killen et al. 2013).

5.5. Materials and methods

5.5.1. Experimental overview

Magnificent sea anemones *Heteractis magnifica* (n = 53) and juvenile orange-fin anemonefish *Amphiprion chrysopterus* (n = 47) were collected in the northern lagoon of Moorea, French Polynesia (17°32'19.8"S, 149°49'46.3"W) from September to November 2017.

Unbleached anemones were collected 2 to 3 weeks before the fish, during which time about half of the anemones were thermally-induced to bleach in the laboratory at the CRIOBE research centre (see 'Host anemones' below). Two days before placing fish onto them, unbleached and bleached anemones of similar size were individually placed back into the field in 47 cylindrical cages (60 × 40 cm) randomly distributed across four sites in Moorea's northern lagoon (Fig. 5.1). Environmental conditions (water flow, water temperature and depth) were measured at the sites (Table S5.1) consisting of a sandy flat, such that all cages were 5 m away in any direction from any larger coral structures ('bommies'). Cages were used to prevent anemone predation from, for example, turtles, and were placed at least 10 m apart.

Anemonefish were caught from unbleached anemones in the lagoon (there was no bleaching at this time) and placed individually into the 47 cages with either an unbleached (n = 26) or a bleached (n = 21) anemone.

The metabolic rate, growth rate, and behaviour (see individual sections below) of the anemonefish were measured approximately four weeks (26-29 days; Table S5.2A) after residing in unbleached or bleached anemones, and a random subset of individuals (n = 23) were measured a second time after approximately 8 weeks (46-65 days; Table S5.2A), with no difference in exposure time between treatments for either exposure period (Table S5.3). While the experimental exposures to unbleached or bleached anemones varied around the target 4 and 8 weeks, we will, for simplicity, refer to the two measurement points as 'Week 4' and 'Week 8'. The first day of the experiment, when fish were placed onto anemones, is referred to as 'Week 0'.

Anemonefish survival and anemone bleaching status were monitored weekly over the first 8 weeks and then every 2 weeks over a further 7 months. At the end of the ninth month of monitoring, all anemonefish were returned to the un-caged host anemone from which they were originally collected, and anemones were returned to anemone patches in the lagoon.

Ethical approval for the study was granted from The Animal Ethics Committee, Centre National de la Recherche Scientifique (permit number 006725).

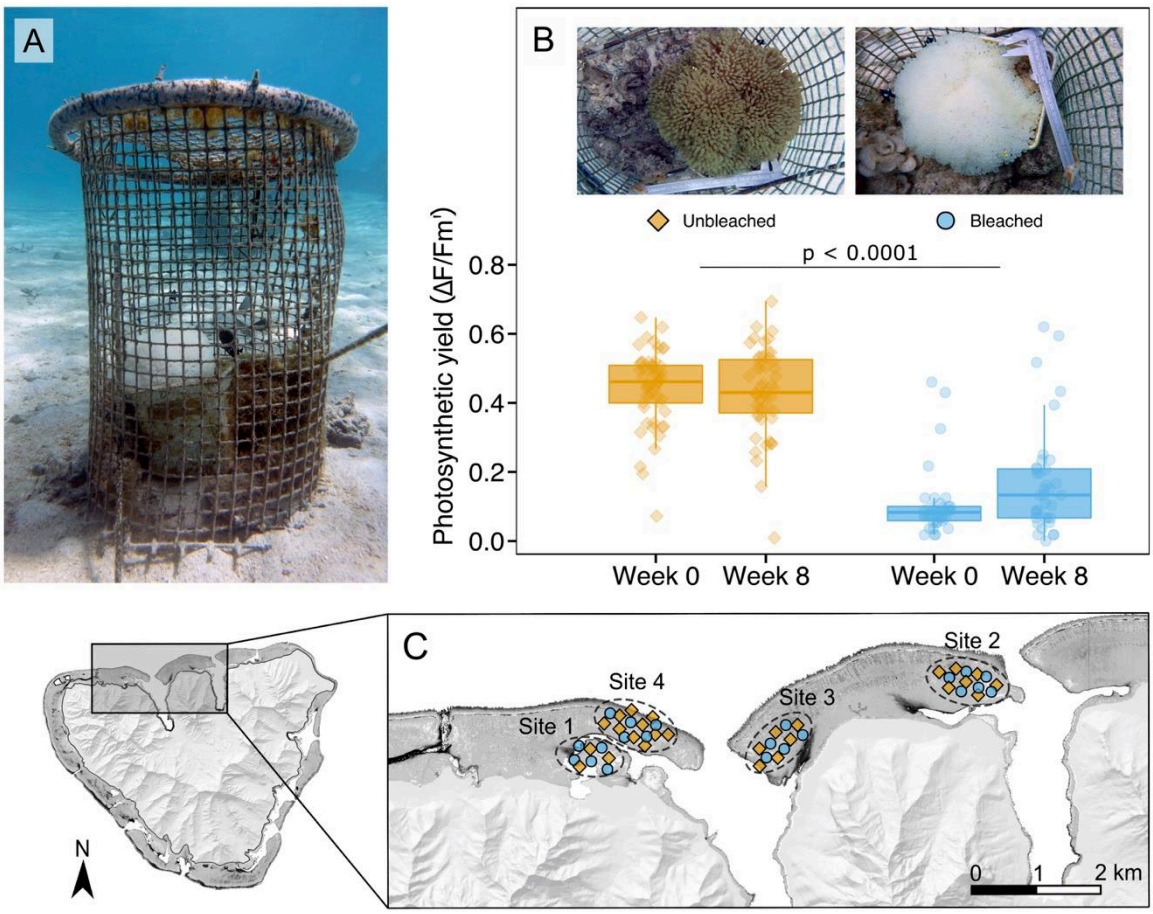

Figure 5.1. In situ experimental photographs and study sites. (A) Experimental set-up with a bleached anemone and a juvenile orange-fin anemonefish *Amphiprion chrysopterus* inside a cage (3 × 3 cm mesh size). The anemonefish could freely swim in and out of the cage through the mesh. The cage was used to prevent anemone predation and secured in place with steel rods. (B) Photosynthetic yield in unbleached and bleached anemones at the start (Week 0) and after 8 weeks (Week 8). ΔF is the difference between Fm′ (maximum fluorescence yield) and F (fluorescence yield of a dark reference). The photos show the top-view from within a cage containing an unbleached (left) and a bleached (right) anemone, each with a juvenile anemonefish and 20-cm long callipers included for scale. (C) Map of the island of Moorea with an insert of the northern lagoon (17°32'19.8"S, 149°49'46.3"W) showing the location of the two treatments (unbleached and bleached anemones) distributed across four sites.

5.5.2. Host anemones

Anemones (containing no anemonefish) were collected by hand while SCUBA diving at depths of 3-6 m and brought to the CRIOBE laboratory in coolers filled with water from the collection sites. In the laboratory, the anemones were kept in aquaria receiving flow-through water from the lagoon and fed brine shrimp daily. About half were maintained at 28°C for two weeks and remained unbleached. The others were bleached by heating the aquaria water to 31°C for 2 weeks.

Once positioned in the field, anemone bleaching status was ascertained visually on a weekly basis and confirmed by measuring photosynthetic activity at Week 0 and Week 8 of the experiment. Photosynthetic activity in the anemones was measured in triplicate using an underwater diving pulse amplitude modulated fluorometer (DIVING-PAM; Heinz Walz GmbH, Effeltrich, Germany). All the anemones from one site were measured on the same night, and measures were taken from all sites between 17:12 and 17:37. Photosynthetic activity was significantly higher in unbleached than bleached anemones both at the beginning of the experiment and after 8 weeks (linear mixed-effects models, LME: Treatment; $t = 11.793$, $p < 0.0001$; Time; $t = 1.000$, $p = 0.324$, $mR^2 = 0.583$, $cR^2 = 0.712$, Fig. 5.1B; Table S5.4B). Anemone size (surface area) was measured at Weeks 0, 4, and 8 (details in Supporting Information).

Three out of the 21 bleached anemones started to show signs of recovery after 4 weeks and were replaced with other bleached anemones of approximately the same size. As replacing a recovering bleached anemone may disturb associated anemonefish, to ensure equal disturbance between treatments, we correspondingly replaced a healthy anemone at the same site with another healthy anemone of approximately the same size.

5.5.3. Anemonefish

Anemonefish were caught by hand-netting while free or SCUBA diving between September and November 2017 (Table S5.2), transferred in water-filled coolers to CRIOBE's aquarium facilities, weighed and, on the same day, returned to the lagoon onto one of the caged anemones, with no significant difference in start date between treatments (Table S5.3). Fish were released within ~10 cm of the caged anemone and always swam straight to the anemone and hid within its tentacles.

Natural recruitment of *A. chrysopterus* is limited in Moorea (Schmitt and Holbrook 2000; Beldade et al. 2012, 2016) and post-settlement mortality is high due to intraspecific aggression (Buston 2003b). We therefore maintained the number of *A. chrysopterus* juveniles at one individual per anemone throughout the experiment, and any natural recruits (smaller in size than our focal fish) were noted and removed. *Dascyllus trimaculatus* also use anemones during a part of their life cycle (O'Donnell et al. 2017), however, they are subjected to heterospecific aggression from *A. chrysopterus* (Mills et al. 2020), which depresses their density and survival (Schmitt and Holbrook 1996; Holbrook and Schmitt 2004), but their presence has little to no effect on anemonefish (Schmitt and Holbrook 2000). Nevertheless, to control for any aggression, growth, or density-dependent effects on focal experimental *A.*

chrysopterus, all natural recruits of *D. trimaculatus* were removed.

Focal *A. chrysopterus* were monitored weekly. Even though the anemonefish were able to move through the netting of their cages, the distance between cages (>10 m) and high predation risk outside the anemone tentacles (Mariscal 1970; Elliott et al. 1995; Buston 2003b), especially for juveniles (Buston and Garcia 2007), made the chance of fish exchanging anemone host unlikely. Nevertheless, to confirm individual identification during the experiment, focal fish were photographed and identified by colour patterns based on the second and third vertical white stripes (example photos in Figure S5.1).

5.5.4. Metabolic rate

At the end of Week 4 and 8, the anemonefishes were caught and transferred by boat in a water-filled cooler to CRIOBE's aquarium facilities. The fish were initially held with unfed unbleached or bleached anemones (as per their respective treatments in the field) for ~20 h in aquaria receiving filtered, flow-through water from the lagoon. This was done to ensure that the fish were not digesting during subsequent metabolic rate measurements. Each individual fish was then transferred to an intermittent-closed respirometry setup where its oxygen uptake rate could be recorded as a proxy for its metabolic rate (Svendsen et al. 2016).

The respirometry setup was shielded from surrounding disturbances and comprised a 40 L (water volume) tank receiving flow-through normoxic seawater from the lagoon at 29.16 ± 0.04°C (mean ± SE temperature in the respirometry setup across all experiments), glass respirometry chambers (35 or 110 mL volume, depending on fish size) in which the oxygen uptake rate of the fish could be measured by use of fibre-optic oxygen meters with probes (FireStingO_2; Pyro Science GmbH) and accompanying software (Pyro Oxygen Logger; Pyro Science GmbH), a peristaltic pump (Masterflex L/S; Cole-Parmer) with gas-tight tubing that recirculated water through the respirometry chambers and past the oxygen probes, and a set of flush pumps (EHEIM Compact; EHEIM GmbH & Co) which intermittently flushed fresh and fully aerated seawater through the respirometry chambers for 5 min in every 9 to 10 min intermittent-closed respirometry cycle (flush and close durations were adjusted based on chamber volumes and fish sizes). The respirometry chambers were supported by two plastic pipes in between which anemones from the respective treatment were placed such that their tentacles touched the bottom and sides of the respirometry chambers (photos in figure 1 in Norin et al., 2018). This allowed the fish to be surrounded by and see (but not touch) the anemones, and also receive olfactory cues from the anemones in the flush water. Fish were introduced to respirometry chambers a few minutes before the first automated oxygen uptake

rate recordings were started in the afternoon and remained there for ~17 hr until the following morning. The fish were then removed from the respirometry chambers and their body mass recorded.

Fish oxygen uptake rates were calculated by multiplying the slopes (over 3 to 4 min) for the decline in oxygen inside the respirometry chambers during the closed phases of the respirometry cycles by the volume of the respirometry chamber after subtracting fish volume and background bacterial respiration (calculated from a respirometry chamber without fish). The SMR of each individual fish was then estimated by first calculating the mean of the lowest 10% of its oxygen uptake rate measurements from the ~17 h respirometry trial, then excluding any outliers (data points outside the mean ± 2 SDs, or data points with r^2 values for the linear regressions of the decline in oxygen over time lower than the mean r^2 – 2 SDs), and finally re-calculating the mean of the remaining data points.

Due to electrical problems at the research station, some of the scheduled respirometry trials could not be completed, resulting in a reduced sample size for metabolic rate measurements (n = 15 and n = 14 fish from unbleached and bleached anemones, respectively, at Week 4, and n = 12 and n = 9 fish, respectively, at Week 8; Table S5.2A).

5.5.5. Behaviour

A GoPro camera was placed on the top of each cage in the lagoon (~50 cm from the anemone) in the afternoon of an experimental day (between approximately 14:00 and 15:00), and 20 min videos were recorded. The first 10 min of these videos were discarded as the acclimation period (Nanninga et al. 2017), while the following 10 min were used to quantify: (a) *Time spent out of the anemone* – defined as the percentage difference between the total observation time and the time that at least 50% of the fish's body was within the anemone tentacles from observations of a fish's location every 3 s (n = 200 observations); (b) *Activity* – the total number of times a gridline was crossed per minute, calculated by digitally separating the video frame into a grid of 10 × 6 sections of equal size (Fig. S5.2) and counting each time the fish crossed a gridline within three haphazardly selected periods of 30 s; and (c) *Space use* – the total number of unique squares a fish occupied per minute, calculated from the number of unique squares on the digital grid (Fig. S5.2) that the fish occupied within three haphazardly selected periods of 30 s.

Due to time restrictions in the field, only a subset of the fish was filmed at Week 4 (n = 11 and n = 12 in unbleached and bleached treatments, respectively; Table S5.2A). At Week 8, 23 fish were filmed but three videos were unusable due to poor camera placement on the cage, resulting in n = 12 and n = 8 in unbleached and bleached anemones, respectively (Table S5.2A).

5.5.6. Growth rate

Anemonefish were weighed to the nearest 0.001 g at the start of the experiment (Table S5.2A), at which point body masses were not significantly different between treatment groups (Wilcoxon-test; W = 209, p = 0.81, n = 40). Fish were re-weighed at Week 4 (n = 20 and n = 20 in unbleached and bleached treatments, respectively; Table S5.2A) and Week 8 (n = 13 and n = 9 in unbleached and bleached treatments, respectively; Table S5.2A).

Specific growth rate (SGR) was determined as the percentage increase in individual body mass per day as SGR = $\ln(BM_{t_2}) - \ln(BM_{t_1}) \times t^{-1} \times 100$, where BM is body mass at t_2 (final time) and t_1 (initial time), and t is the time (days) between the two consecutive measures (Hopkins 1992).

5.5.7. Survival

Anemonefish survival (absence/presence) was recorded weekly over the first 8 weeks and every 2 weeks over the following 7 months. Given that juvenile anemonefish are unlikely to voluntarily leave their anemone due to risk of predation (Elliott et al. 1995; Buston 2003b), the absence of a fish from its anemone was equated to mortality. Despite the use of cages, seven anemones (and thus also the anemonefish) disappeared during the nine months survival study, but these absent fish were not included in the survival analyses. In addition, non-natural mortality linked to electrical problems at the research station was also excluded from survival analyses (final n = 29, Table S5.2B).

5.5.8. Statistical analysis

Statistical analyses were performed in R version 3.6.1 (R Core Team, 2019).

Linear mixed-effects models (LMEs) were used to explore the effect of the explanatory variables – anemone treatment (categorical), exposure time (continuous), fish body mass (continuous), and site (categorical) – on fish SMR, growth rate, and behaviour. Fish ID (categorical) was included as a random effect to account for non-independence of data (i.e. when fish were measured twice over time). Time was included as a continuous variable since not all measurements were taken at exactly four or eight weeks but spread around these target time points for logistical reasons. All LMEs were fitted using the *lmerTest* package (Kuznetsova et al. 2017), while marginal (mR^2) and conditional (cR^2) R^2 where obtained with the package *piecewiseSEM* (Lefcheck 2016). The selection of the best-fit model was determined

using likelihood ratio tests (LRTs), starting from the most complex model and subsequently removing non-significant interactions and explanatory variables (Tables S5.6A, S7A, S8A, S9A, and S10A) *via* the *lmtest* package (Zeileis and Hothorn 2002). In addition, SMR and fish body mass were $\log_{10}$-transformed to account for their nonlinear (power) relationship. The 'ns' function in the *splines* package was used in the growth rate model to account for non-linear body mass effects on fish growth. Space use was $\log_{10}$-transformed to alleviate non-normality. In each model, residuals were visually inspected to ensure that all assumptions were met. For graphical representation, regressions were fitted on predicted values obtained using the 'ggemmens' function in the *ggeffect* package (Lüdecke 2018).

The consistency of each individual fish's behaviour across the three haphazardly chosen sections of 30 s video was evaluated by calculating the adjusted repeatability (R_{adj}) of activity and space use using the *rptR* package (Stoffel et al. 2017). We calculated overall R_{adj} using the same model structure as for the LMEs (Table S5.5A), with chronological order of the three 30 s sections added as an additional explanatory variable. We also calculated R_{adj} for each anemone treatment separately by sub-setting the data for each treatment and removing treatment as a fixed effect in these models. Behaviour was significantly repeatable in all cases (R_{adj} = 0.318 to 0.464, $p \leq 0.0072$; Table S5.5B).

We tested for differences in survival between treatments using a Cox proportional hazard model *via* the 'coxph' function in the *survival* R package (Therneau and Grambsch 2000; Therneau 2020b). Site was used as a covariate. Cox proportional hazard models calculate survival as the probability that an individual survives from the time origin (start of experiment) to a specified future time (end of experiment). To do this, the hazard function (risk of death over time) is used as a response variable.

Correlations among all traits were performed using Pearson's correlation coefficient or Spearman's rank correlation (parametric and non-parametric data, respectively). For each trait where fish body mass had an effect, body-mass-adjusted values were used for correlation analyses. Body-mass-adjusted values were obtained by calculating model partial residuals (i.e. fixing body mass and removing its partial effect) using the 'remef' function from the *remef* package in R (Hohenstein and Kliegl 2020).

5.6. Results

5.6.1. Physiology

The SMR of anemonefish varied over time depending on anemone treatment (bleached or unbleached, Fig. 5.2), as indicated by the significant interaction between treatment and exposure time (LME: Time*Treatment; t = 2.024, df = 45, p = 0.049; $mR^2 = 0.904$, $cR^2 = 0.904$; Table S5.6B). SMR was the same between treatment groups after approximately 4 weeks of anemone exposure, but decreased in anemonefish from bleached anemones between 4 and 8 weeks of exposure (Fig. 5.2).

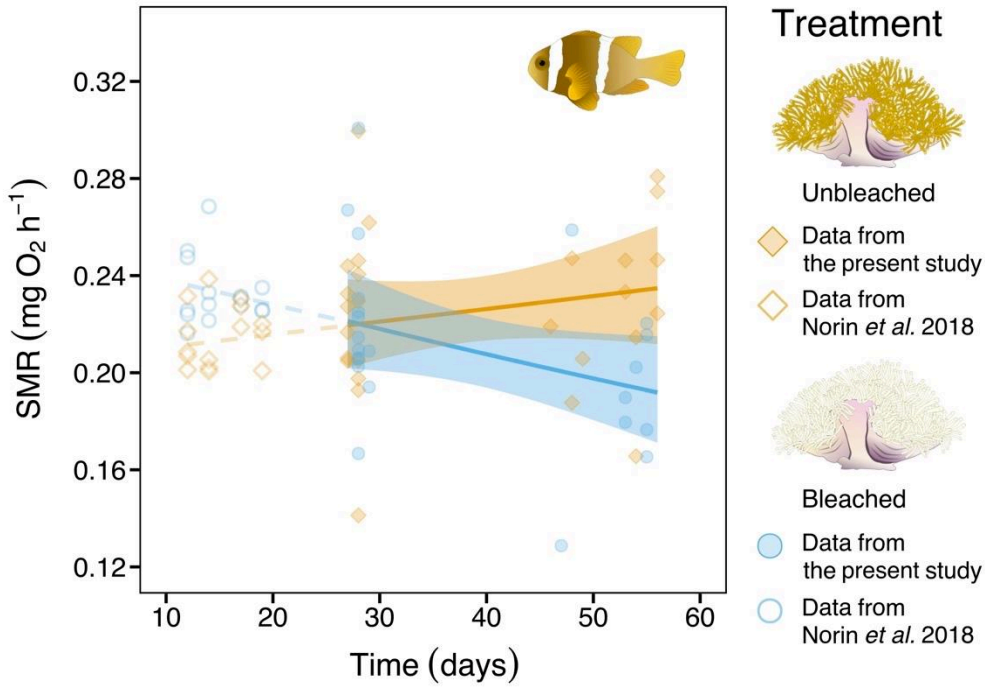

Figure 5.2. Standard metabolic rate (SMR) of *Amphiprion chrysopterus* juveniles residing in unbleached or bleached anemones over time. Solid orange and blue lines are model predicted regression lines fitted to the data from the present study, surrounded by 95% confidence bands. Data shown in open symbols at 12-19 days are from a previous laboratory study by Norin et al. (2018) and the regression lines have been extended to these data (dashed lines) for visual comparison only [i.e. the data from Norin et al. (2018) are not part of the fit]. The presented SMR data, including those from Norin et al. (2018), have all been adjusted (using model residuals) to the overall mean body mass of the fish from the present study (0.59 g), to allow direct comparisons between data [note that the SMR data from Norin et al. (2018) were adjusted to a body mass of 1 g in their Figure 3].

5.6.2. Behaviour

Time spent out of the anemone

Anemone treatment had a significant effect on the time fish spent out of the anemone (LME: Treatment; t = -2.946, df = 29.69, p = 0.006; $mR^2 = 0.331$, $cR^2 = 0.592$; Table S5.7B), with fish from bleached anemones spending 22.7% more time out of the anemone compared to fish from unbleached anemones (Fig. 5.3A). In addition, the amount of time fish spent out of the anemone increased over time by 0.77% each day (LME: Time; t = -3.893, df = 23.61, $p < 0.001$; Fig. 5.3A; Table S5.7B).

Activity

Anemone treatment had a significant effect on fish activity (LME: Treatment; t = 2.276, df = 38, p = 0.029; $mR^2 = 0.339$, $cR^2 = 0.339$; Table S5.8B), with fish from unbleached anemones being more active than fish from bleached anemones (Fig. 5.3B). Exposure duration did not have a significant effect on fish activity (LME: Time; t = 1.795, df = 38, p = 0.081; Table S5.8B), but larger fish tended to be more active than smaller fish (LME: Body mass; t = 1.928, df = 38, p = 0.061; Table S5.8B).

Space use

Anemone treatment also had a significant effect on fish space use (LME: Treatment; t = 2.495, df = 38, p = 0.017; $mR^2 = 0.492$; $cR^2 = 0.492$; Table S5.9B), with fish from unbleached anemones using more space over and around the anemone than fish from bleached anemones (Fig. 5.3C). Fish also increased space use over time by ~1.2% each day (LME: Time; t = 3.027, df = 38, p = 0.004; Table S5.9B). As expected, larger fish used more space around the anemone (LME: Body mass; t = 2.627, df = 38, p = 0.012; Table S5.9B).

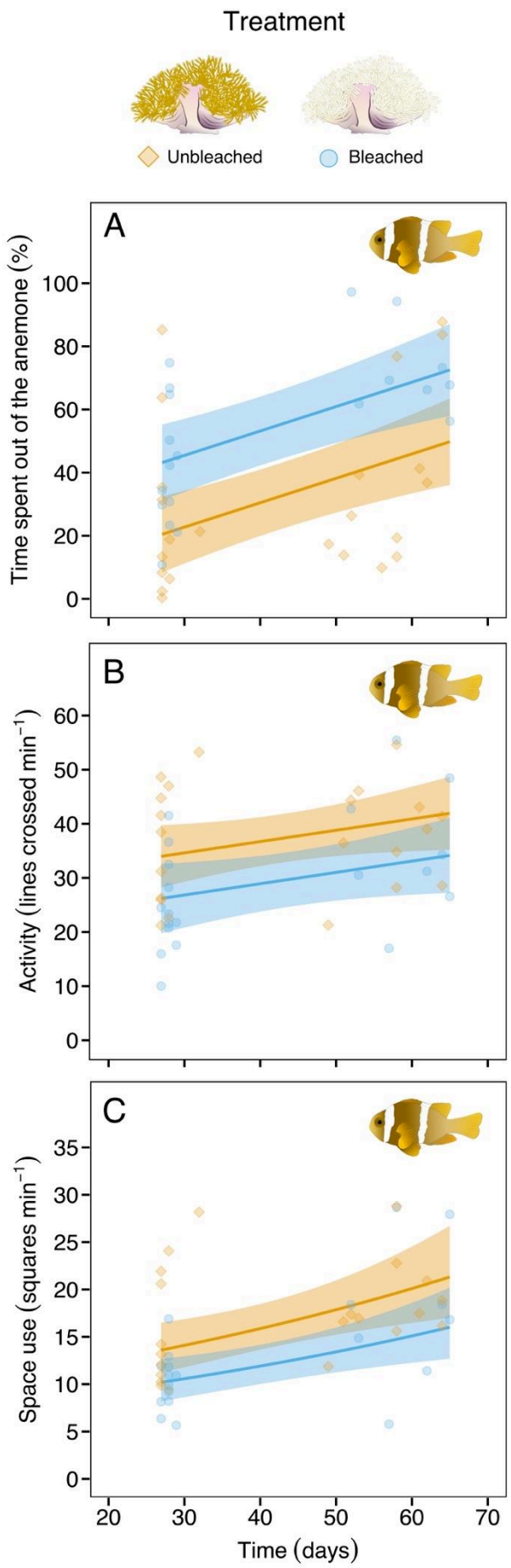
Treatment
Unbleached
Bleached
A
Time spent out of the anemone (%)
0
20
40
60
80
100
B
Activity (lines crossed min^{-1})
0
10
20
30
40
50
60
C
Space use (squares min^{-1})
0
5
10
15
20
25
30
35
20
30
40
50
60
70
Time (days)

Figure 5.3. Behaviours of *Amphiprion chrysopterus* juveniles residing in unbleached or bleached anemones over time. (A) time spent out of the host anemone, (B) movement activity, and (C) horizontal space use above the anemone. Symbols represent individual raw data points in (A) while in (B) and (C) data points are adjusted (using model residuals) to the overall mean body mass of the fish from the present study (0.59 g), as body mass significantly affected activity and space use but not time spent out of the anemone. Lines are model predicted regression lines surrounded by 95% confidence bands.

5.6.3. Growth rate

Anemone treatment had a significant effect on fish growth rate (LME: Treatment; t = 4.047, df = 57, $p < 0.001$; $mR^2 = 0.707$, $cR^2 = 0.707$; Table S5.10B), with fish of a given mass growing 0.85% per day faster when residing in unbleached than bleached anemones across the entire experiment (Fig. 5.4A). Treatment exposure time had no significant effect on fish growth rate (LME: Time; t = 1.217, df = 57, p = 0.229; Fig. 5.4A; Table S5.10B).

5.6.4. Survival

Although survival appeared to diverge between treatments after 10 weeks (Fig. 5.4B), there was no significant difference in survival between fish from unbleached and bleached anemones throughout the 9 months of treatment exposure (coxph model: Treatment; coef = -0.56, z = -1.11, p = 0.268, Table S5.11B). However, study site had an impact on fish survival with Site 3 showing higher survival probability compared to Sites 2 and 4 (coxph model: Site 3; coef = -1.66, z = -2.31, p = 0.021; Table S5.11B).

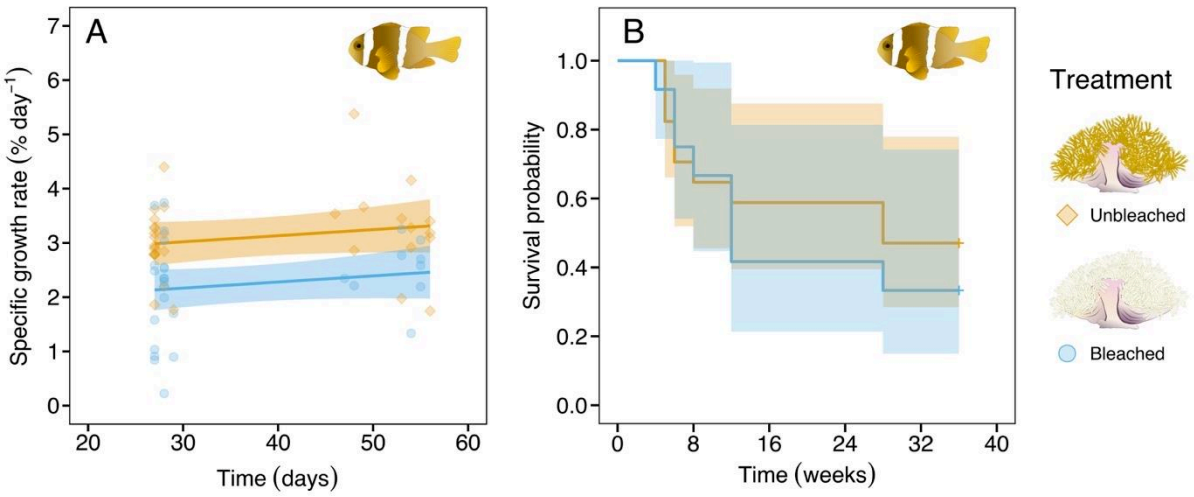

Figure 5.4. Growth and survival of *Amphiprion chrysopterus* juveniles residing in unbleached or bleached anemones over time. (A) Specific growth rate. Symbols represent individual data

points adjusted (using model residuals) to the overall mean body mass of the fish used in the growth analyses (t_1, 0.23 g). Solid orange and blue lines are model predicted regression lines fitted to the data, surrounded by 95% confidence bands. (B) Survival over 9 months (initial 8 weeks of the main experiment plus an additional 28 weeks of monitoring the fish in the same treatments). Solid orange and blue lines are survival probability curves, surrounded by 95% confidence bands.

5.6.5. Correlations among traits

Standard metabolic rate correlated negatively with growth rate after 4 weeks in bleached anemones (Fig. 5.5). Behaviours correlated positively with each other: time spent out of the anemone was positively correlated with fish activity and space use after 4 weeks in bleached anemones, and activity and space use were highly positively correlated in both treatments after both 4 and 8 weeks (Fig. 5.5). Activity was also positively correlated with survival in fish inhabiting unbleached anemones after 4 weeks, but did not correlate in fish from bleached anemones at either 4 or 8 weeks (Fig. 5.5). Space use was negatively correlated with survival in bleached anemones after 4 weeks (Fig. 5.5). Growth rate was negatively correlated with activity and space use in unbleached anemones after 4 weeks, but there were no significant correlations among these traits in fish from bleached anemones (Fig. 5.5). All test statistics are summarised in Table S5.12.

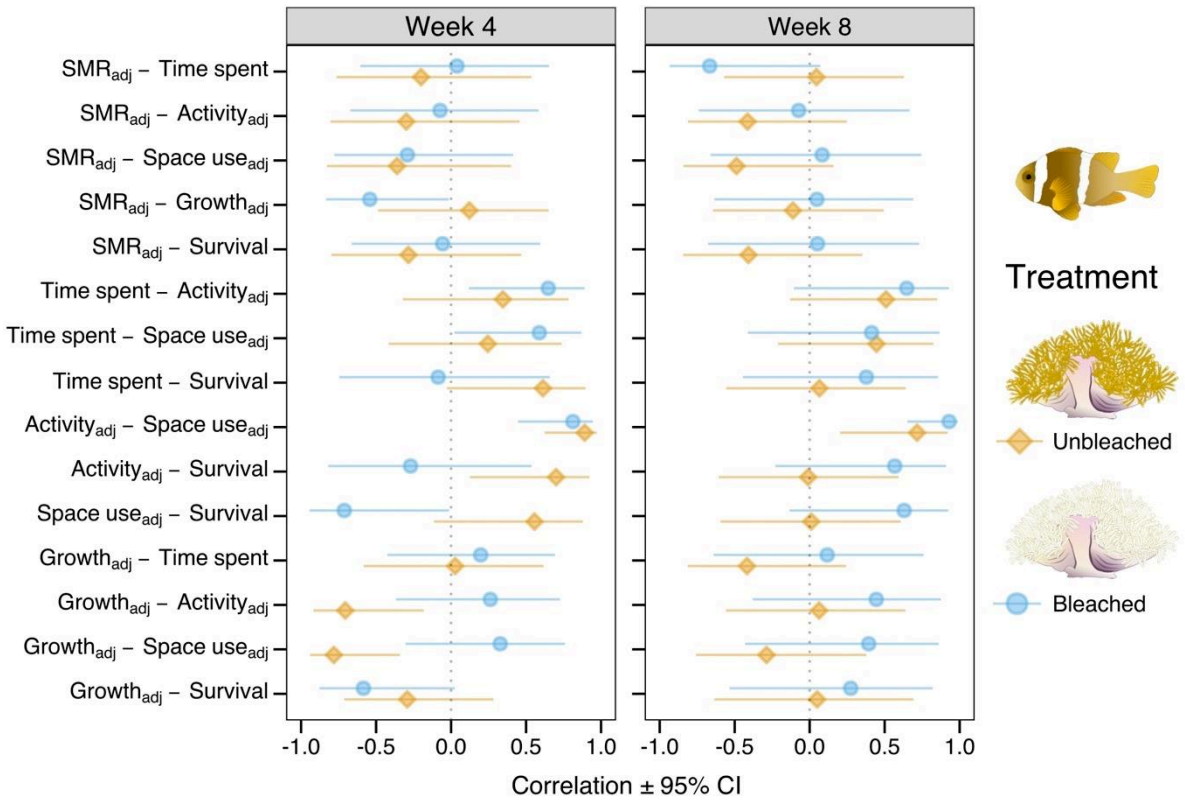

Figure 5.5. Correlations between all measured traits after 4 and 8 weeks of residing in unbleached or bleached anemones. Correlations (circles and diamonds) where the 95% confidence interval (horizontal error bars) does not cross zero (dashed vertical lines) are significant. Body mass significantly affected standard metabolic rate (SMR), activity, space use and growth rate, so body-mass-adjusted ($_{adj}$) values were used in correlations. For time spent (out of the anemone), raw values were used as fish body mass had no effect on this trait (Fig. 5.3A). All test statistics are summarised in Table S5.12.

5.7. Discussion

Our field-based experiment allowed us to test the indirect effects of climate change and warming-induced bleaching on wild coral reef fish in the absence of elevated temperature, whilst also exposing fish and anemones to natural variability arising from selection and environmental variables, including water current, food availability, solar radiation, and inter- and intra-specific interactions (all small organisms < 4 cm body depth could traverse the mesh cages). We found that bleaching induced a reduction in fish's activity, a reduction in hiding as fish spent more time out of bleached anemones, a decrease in standard metabolic rate (SMR) over time, and lower growth rates. However, despite these differences in fish behaviour and physiology, after nine months there was no impact of host anemone bleaching on fish survival.

At first glance, our results for SMR appear to contradict a previous laboratory study showing that bleaching increased the metabolic demands of juvenile anemonefish after two weeks (Norin et al., 2018a). However, the increased SMR observed by Norin et al. (2018) fits well with our SMR results when extrapolated back to a 2-week exposure period (Fig. 5.2), despite the different experimental conditions (laboratory *vs*. field). Combined, these results suggest that anemonefish initially experience increased SMR in response to the short-term stressor of bleaching, but that metabolic rates decrease over a longer, and more ecologically relevant, exposure to bleaching. Reduced food intake is a likely contributing factor to the observed decrease in SMR, and is known to cause a reduction in SMR in other fish species (O'Connor et al. 2000; Van Leeuwen et al. 2012; Auer et al. 2015). The lower growth rate of anemonefish from bleached anemones further corroborates that reduced food intake is likely causing the decrease in SMR, which is also supported by the negative correlation between SMR and growth in fish from bleached, but not unbleached, anemones after 4 weeks.

The decrease in SMR over time in fish from bleached anemones suggests that individuals with a relatively high SMR were energetically disadvantaged, had little excess capacity for growth, and were forced to down-regulate their SMR. While a down-regulated SMR can be advantageous in food-limited environments (Metcalfe et al. 1995; Metcalfe 1998; O'Connor et al. 2000; Auer et al. 2015), there is a limit to how much SMR can be reduced without affecting basic physiological functioning, and reduced tissue-level metabolic rates, as a consequence of reduced food intake, carry an oxidative cost in the form of harmful reactive oxygen species (Salin et al. 2018). The indication of a decreasing SMR up to 8 weeks of exposure to bleached anemones provides no evidence for acclimation (stabilisation) in SMR, unlike other studies on coral reef fish that have reported physiological and behavioural acclimation to the thermal stressor itself (Donelson et al. 2011). Therefore, our observed decrease in SMR between 4 to 8 weeks of bleaching is more likely to reflect a deteriorating condition rather than acclimation. An interesting next step would be to investigate if fish are able to back-regulate their metabolic physiology after anemones have recovered from bleaching. Moreover, we conducted our study on bleaching-naïve wild juveniles about 1 month old and, as such, phenotypically plastic responses over multiple bleaching events remain to be explored.

The negative impact of bleaching is further supported by our behavioural data, as survival correlates positively with space use and activity in unbleached anemones (Fig. 5.5), but negatively in bleached anemones. High activity and space use are likely important for foraging on planktonic prey in the water column and for territorial behaviour and competition with both con- and hetero-specifics (e.g. *D. trimaculatus*), behaviours that are all energetically costly

(Koteja 2000; Schmitz 2005; Yeates et al. 2007; Biro and Stamps 2010; Barry 2014). Anemonefish receive their nutrients from plankton, whose density declines under thermally-induced bleaching (Tada et al. 2003; Piontkovski and Castellani 2009), and from anemone waste products and symbiont algae (Verde et al. 2015); both of these food sources may be reduced or lost entirely when anemones bleach. Under normal, unbleached conditions, the positive correlation between survival and behaviour (space use and activity) indicates that foraging and territoriality are balanced with food intake from the territory. However, during bleaching episodes, even though fish from bleached anemones spent more time out of their anemone (Fig. 5.3A), they were less active and used less space around the anemone compared to fish from unbleached anemones (Fig. 5.3B,C), with no indication that they maximised foraging or compensated energetically for the diminished food availability (Dill 1983; Höjesjö et al. 1999). The absence of energetic compensation can have detrimental outcomes (Werner and Anholt 1993; Brown and Kotler 2004), emphasised here by the negative correlation between survival and space use in bleached anemones. The energetic cost of spending more time outside the anemones might be balanced with other benefits, such as finding a better, unbleached habitat. However, the anemones were placed 10 m apart in our study and, as anemonefishes are sedentary species (Hattori 1994), we did not observe any movement between cages.

The loss of symbiont algae and the likely decreased availability of waste products in bleached anemones, coupled with an absence of compensatory foraging, are two likely causes of the lower growth rate observed for fish from bleached anemones (Fig. 5.4A). A third cause may be initially higher metabolic rates, which can occur two weeks after the onset of bleaching (Norin et al. 2018; Figure 2). Although fish with a higher SMR can digest and grow faster if enough food is available to cover their increased maintenance costs (Millidine et al. 2009; Reid et al. 2012), in the absence of any compensatory foraging (as our behavioural data suggest), the initially higher SMR may have impacted growth for the remaining six-week exposure period through competition for a finite energy budget and an inability to catch up on growth later (Metcalfe and Monaghan 2001). This is supported by the observed negative correlation between SMR and growth after 4 weeks in bleached anemones. In addition, the subsequent decrease in SMR of fish from bleached anemones may have further reduced their growth rate. Growth is an especially important trait in anemonefishes, as they live in size-dependent hierarchies, which determine the timing of sex change and reproductive status (Fricke 1979; Buston 2003a). The finding that bleached anemone hosts lower the growth of associated fish is therefore likely to have cascading and life-long consequences for individual anemonefish (in addition to reduced

reproduction; Beldade et al. 2017), but also for other fish species associated with hosts that bleach. Indeed, a poor start in life during bleaching episodes, with lower growth and reduced size-at-age, results in smaller fish more likely to lose in competition for space and more vulnerable to predation (Arendt 1997; Sogard 1997).

Despite all the indications that host anemone bleaching affects the resident anemonefish negatively, bleaching did not significantly reduce anemonefish survival over 9 months. The use of cages (to reduce anemone predation) could also have reduced natural predation on anemonefish by predators larger than the cage mesh size, especially considering that juveniles from bleached anemones spent less time in their anemone, which should render them more vulnerable to predation (Mariscal 1970; Elliott et al. 1995; Buston 2003a). Moreover, the bleached, white coloration of anemones enhances the visual contrast between anemone and fish, rendering them more visible to predators (Coker et al. 2009). However, our results corroborate those of previous natural field observations in which neither anemonefish densities nor adult survival were affected by bleached anemones (Saenz-Agudelo et al. 2011; Beldade et al. 2017).

Overall, the evidence of detrimental effects, together with the strong habitat dependency of coral reef species (~12% of coral reef fishes live in symbiosis with hosts that bleach; Beldade et al. 2017) emphasises the importance of mitigating and regulating human actions that contribute to climate-induced bleaching events.

5.8. Acknowledgment

Financial support was provided by the Agence National de la Recherche to Glenn Almany, SCM, and RB (ANR-14-CE02-0005-01/Stay or Go) and to SCM (ANR-11-JSV7-012-01/Live and Let Die), by the Pacific Funds to SCM and by LabEx "CORAIL" to RB and SCM ('Where do we go now?'). TN was supported by the Danish Council for Independent Research (DFF-4181-00297) and the European Union's Horizon 2020 research and innovation programme under the Marie Skłodowska-Curie grant agreement No. 713683. SSK was supported by Natural Environment Research Council Advanced Fellowship (NE/J019100/1) and European Research Council starting grant (640004). We particularly thank Till Deuss for his support and help in the CRIOBE anemonefish aquarium facility. We thank Laetitia Hédouin for the use of the diving pulse amplitude modulated fluorometer. We are thankful to Paul Johnson for his suggestions on statistics. We are also thankful to Gilles Siu, Pascal Ung, Anne Haguenauer, Frederic Zuberer, Franck Lerouvreur and all the CRIOBE community for support in the field. We would like to express our gratitude to Glenn Almany (RIP) for his support and unwavering enthusiasm for this project.

5.9. Supplementary materials

Anemone size

The size of each anemone was calculated as their surface area, considering them as a two-dimensional ellipse when viewed from above (Holbrook and Schmitt 2005). There were no significant differences in anemone size between treatments (unbleached *vs*. bleached) at the beginning (Week 0; independent samples t-test, t = -1.846, df = 36.994, p = 0.073) and middle (Week 4; independent samples t-test, t = -1.917, df = 14.236, p = 0.076) of the experiment, but anemone size tended to decrease for bleached anemones over the course of the experiment (Week 8; Wilcoxon-test size, W = 25, p = 0.053), which was unsurprising as anemones generally shrink due to the loss of photosynthesis when they bleach (Jones et al. 2008; Hobbs et al. 2013).

Table S5. 1. Summary of the environmental variability at the four field sites. Daily water flow and water temperature were measured with a drag-tilt current meter (Marotte HS, Marine Geophysics Laboratory, James Cook University, Australia) over the first eight weeks of the experiment (October-December 2017) and are shown here as means ± SDs. The depth range of cages at each site are also shown for each reef location (also see map in Fig. 5.1 in the main manuscript). In addition, the four sites were chosen due to the presence of a sandy flat, such that all cages were 5 m away in any direction from any large coral structures ("bommies").

	Water flow (m s^{-1})	**Water temperature (°C)**	**Depth range (m)**	**Reef location**
Site 1	0.065 ± 0.02	27.72 ± 0.52	2.5 – 3.0	Fringing reef
Site 2	0.041 ± 0.04	27.65 ± 0.53	3.0 – 3.2	Barrier reef
Site 3	0.124 ± 0.02	NA	2.8 – 3.2	Barrier reef
Site 4	0.107 ± 0.02	27.64 ± 0.52	3.5 – 4.0	Barrier reef

Table S5. 2. Experimental start dates and treatment durations for the 47 fish used in the study

Table S5. 2A. The fish ID, treatment, field site, experimental start date (Week 0) on which fish were placed onto a bleached or an unbleached anemone, and exact treatment exposure durations (in days) prior to recording growth rate, standard metabolic rate (SMR), and behaviour at Week 4 and Week 8 of the experiment. Site refers to the four sites shown in Fig. 5.1 in the main manuscript. A dash indicates the absence of a measurement.

ID	Treatment	Site	Week 0	Week 4 (mean: 28 days)			Week 8 (mean: 56 days)		
			Date	Growth rate	SMR	Behaviour	Growth rate	SMR	Behaviour
1	Bleached	1	26/09/17	27	-	-	-	-	-
2	Unbleached	1	26/09/17	27	-	-	-	-	-
3	Bleached	1	26/09/17	27	-	27	-	-	-
4	Bleached	1	26/09/17	27	-	27	-	-	-
5	Unbleached	1	26/09/17	27	-	-	-	-	-
6	Bleached	1	26/09/17	27	-	-	-	-	-
7	Bleached	1	26/09/17	27	-	-	-	-	-
8	Unbleached	1	26/09/17	27	-	27	-	-	-
9	Bleached	2	28/09/17	28	28	28	-	-	-
10	Unbleached	3	28/09/17	27	-	-	-	-	-
11	Bleached	3	28/09/17	28	28	-	-	-	-
12	Bleached	2	28/09/17	28	28	28	-	-	-
13	Unbleached	4	28/09/17	27	-	-	-	-	-
14	Bleached	4	28/09/17	28	28	28	-	-	-
15	Unbleached	4	28/09/17	27	-	27	-	-	-
16	Bleached	4	28/09/17	28	28	-	-	-	-
17	Unbleached	4	28/09/17	27	-	-	-	-	-
18	Unbleached	2	28/09/17	27	27	-	-	-	-
19	Bleached	2	28/09/17	27	-	-	-	-	-
20	Unbleached	4	28/09/17	27	27	27	-	-	-
21	Bleached	4	28/09/17	28	28	28	-	-	-
22	Bleached	2	28/09/17	28	28	-	55	55	64
23	Unbleached	2	28/09/17	27	27	27	56	56	64
24	Unbleached	2	28/09/17	27	27	27	56	56	64
25	Bleached	4	28/09/17	28	28	28	55	55	65
26	Unbleached	4	28/09/17	27	27	27	56	56	-
27	Bleached	4	28/09/17	28	28	-	55	55	65
28	Unbleached	2	28/09/17	27	27	27	56	56	62
29	Bleached	2	28/09/17	28	28	28	55	55	62
30	Unbleached	4	01/10/17	-	28	-	-	-	-
31	Unbleached	4	02/10/17	-	28	-	53	53	61
32	Bleached	2	03/10/17	-	-	-	54	54	57
33	Unbleached	3	04/10/17	28	28	-	54	54	58
34	Bleached	3	04/10/17	29	29	29	53	53	-
35	Unbleached	4	04/10/17	-	28	-	54	-	-
36	Unbleached	3	04/10/17	28	28	28	54	54	58
37	Bleached	3	04/10/17	29	29	29	53	53	58
38	Unbleached	4	05/10/17	-	-	-	53	53	58
39	Unbleached	3	09/10/17	29	29	28	49	49	53
40	Bleached	3	09/10/17	28	28	28	48	48	53
41	Unbleached	2	10/10/17	28	28	-	48	48	51
42	Unbleached	3	10/10/17	28	28	27	48	48	52
43	Bleached	3	10/10/17	27	27	27	47	47	52
44	Unbleached	4	11/10/17	-	-	-	-	-	56
45	Unbleached	2	12/10/17	-	-	-	46	46	49
46	Unbleached	3	01/11/17	28	28	32	-	-	-
47	Unbleached	2	01/11/17	27	-	-	-	-	-

Table S5. 2B. The fish ID, treatment, field site, survival time, and survival status (absent vs. present) of all 29 fish used in the survival analyses. Status was used for censoring in the statistical model (Table S5.11), as the exact date of death (absence) is unknown due to weekly monitoring.

ID	Treatment	Site	Survival time (weeks)	Status
10	Unbleached	3	5	Absent
11	Bleached	3	6	Absent
13	Unbleached	4	5	Absent
14	Bleached	4	4	Absent
15	Unbleached	4	5	Absent
17	Unbleached	4	6	Absent
18	Unbleached	2	6	Absent
19	Bleached	2	6	Absent
21	Bleached	4	8	Absent
22	Bleached	2	36	Present
23	Unbleached	2	36	Present
24	Unbleached	2	28	Absent
25	Bleached	4	12	Absent
26	Unbleached	4	12	Absent
29	Bleached	2	12	Absent
32	Bleached	2	12	Absent
33	Unbleached	3	36	Present
34	Bleached	3	36	Present
37	Bleached	3	36	Present
38	Unbleached	4	36	Present
39	Unbleached	3	36	Present
40	Bleached	3	36	Present
41	Unbleached	2	36	Present
42	Unbleached	3	28	Absent
43	Bleached	3	28	Absent
44	Unbleached	4	36	Present
45	Unbleached	2	36	Present
46	Unbleached	3	36	Present
47	Unbleached	2	8	Absent

Table S5. 3. Summary statistics for differences among anemone treatments

Summary statistics for differences among anemone treatments (bleached *vs*. unbleached) analysed with Wilcoxon-Mann Whitney U tests on a) the experimental start date (Week 0; date on which fish were placed onto their anemone) and b) the treatment exposure duration. Treatment exposure duration for growth rate measurements at Week 4 came out as significantly different between treatments (p = 0.036), however, the exposure durations were very similar between treatments, ranging from 27 to 29 days (Table S5.2A), with means of 27.4 and 27.8 days for unbleached and bleached anemones, respectively. Similarly, exposure duration was borderline significantly different between treatments for behaviour (p = 0.054) but, again, means were 27.6 and 27.9 days for unbleached and bleached anemones, respectively. These differences of 0.3-0.4 days between treatments are not biologically meaningful and were driven by the low variation in exposure duration within each treatment.

Trait measured	**Exposure time group**	**a) Experimental start date**		**b) Treatment exposure duration**	
		W	**p-value**	**W**	**p-value**
Growth rate	Week 4	156.0	0.201	269.5	0.036
	Week 8	46.5	0.428	56.5	0.920
Standard metabolic rate	Week 4	73.5	0.137	142.0	0.058
	Week 8	43.5	0.463	52.0	0.914
Behaviour (time spent out of the anemone)	Week 4	58.0	0.617	95.0	0.054
	Week 8	31.5	0.206	62.0	0.290
Behaviour (activity)	Week 4	58.0	0.617	95.0	0.054
	Week 8	31.5	0.206	62.0	0.290
Behaviour (space use)	Week 4	58.0	0.617	95.0	0.054
	Week 8	31.5	0.206	62.0	0.290

Table S5. 4. Models and model outputs for analyses of photosynthesis of anemones (for assessment of bleaching status).

Table S5. 4A. List of all linear mixed-effects models (LMEs) performed with Y (relative measure of the number of reaction centres actively involved in photosynthesis) as response variable. Y is calculated as $(\boldsymbol{Fm'} - \boldsymbol{F})/\boldsymbol{Fm'}$, where $\boldsymbol{Fm'}$ is the maximum fluorescence yield and F is the fluorescence yield of a dark reference. Time is the time of measurement (Week 0 or Week 8; used as categorical variable), Trtm is anemone treatment (bleached vs. unbleached), and Site is field site. Three replicate measurements of Y were taken for each anemone, so individual anemone (anemoneID) and replicate number (Replicate) were included as random factors. Model selection was performed by sequentially dropping non-significant variables, starting with the interaction, and models were compared using likelihood ratio tests in order to identify the best-fit model (in bold) that included time and treatment as predictor variables.

Model	df	LogLik	χ^2	p-value
1: $Y \sim Time * Trtm + Site + (1\|anemoneID) + (1\|Replicate)$	10	129.01		
2: $Y \sim Time + Trtm + Site + (1\|anemoneID) + (1\|Replicate)$	9	128.06	1.907	0.167
3: $\boldsymbol{Y \sim Time + Trtm + (1\|anemoneID) + (1\|Replicate)}$	**6**	**125.73**	**4.647**	**0.200**

Table S5. 4B. Results of the best-fit model from Table S5.4A. mR^2 is the marginal R^2, which describes the proportion of variance explained by the fixed factors alone. cR^2 is the conditional R^2, which describes the proportion of variance explained by both fixed and random factors.

Fixed effects	Estimate	SE	df	t-value	p-value	mR^2	cR^2
Intercept	0.1270	0.022	46.38	5.807	<0.0001	0.583	0.712
***Time* (Week 8)**	0.0236	0.024	34.15	1.000	0.324		
***Trtm* (Unbleached)**	0.3015	0.026	28.13	11.793	<0.0001		

Figure S5. 1. Example photographs of fish at the start of the experiment (Week 0) and after eight weeks in the field (Week 8), used to confirm individual identification of fish. The fish ID is indicated on the left side of pictures.

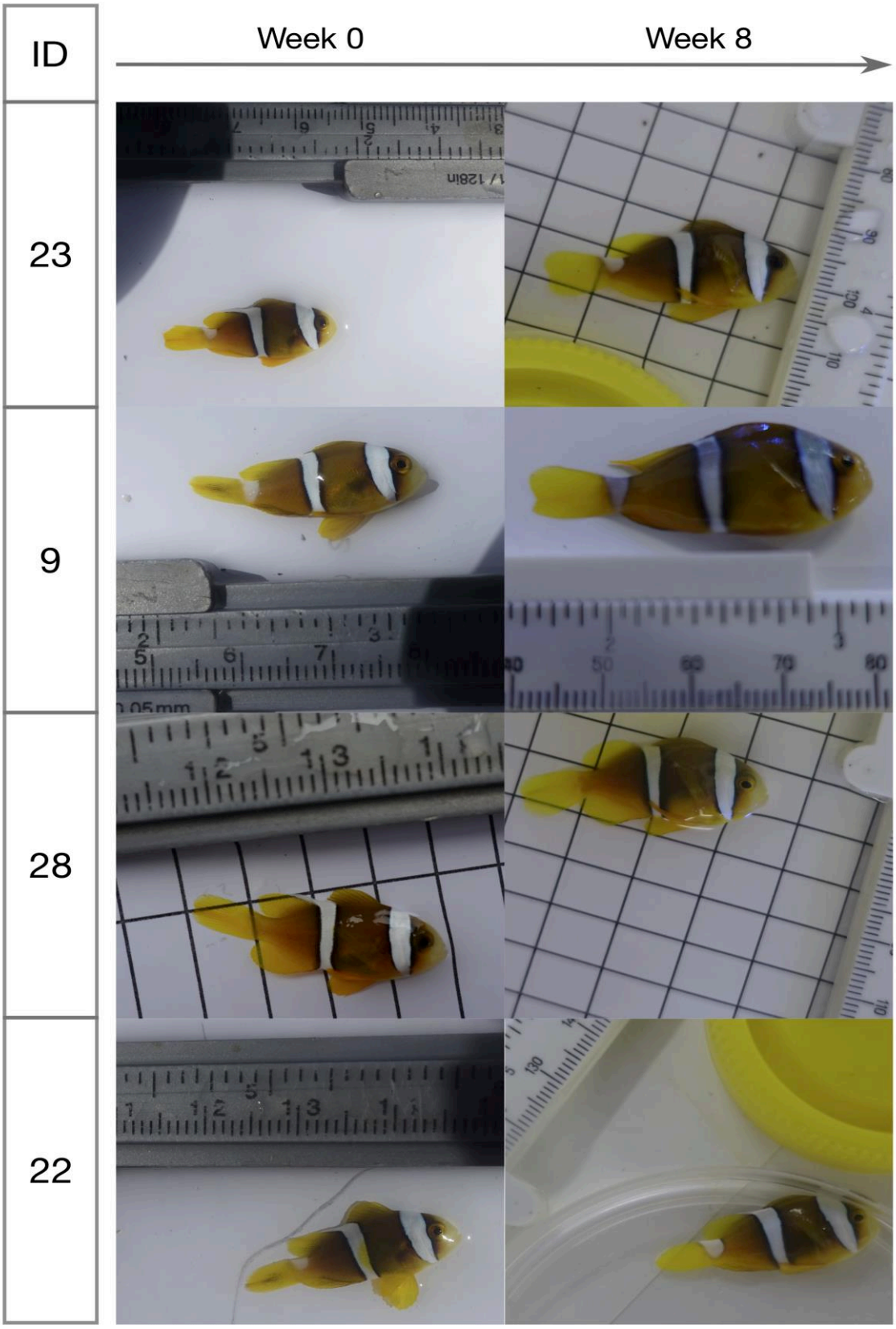

Figure S5. 2. Graphical representation of the grid used to digitally separate video frames of the 10 min video recordings into 10 × 6 sections of equal size in order to quantify fish activity. The fish is highlighted by the red circle. Activity was quantified as the total number of times a gridline was crossed per minute, calculated by counting each time the fish crossed a gridline within three haphazardly selected periods of 30 s. Fish space use was quantified as the total number of unique squares a fish occupied per minute, calculated by counting the number of unique squares on the grid that the fish occupied within the three 30 s periods.

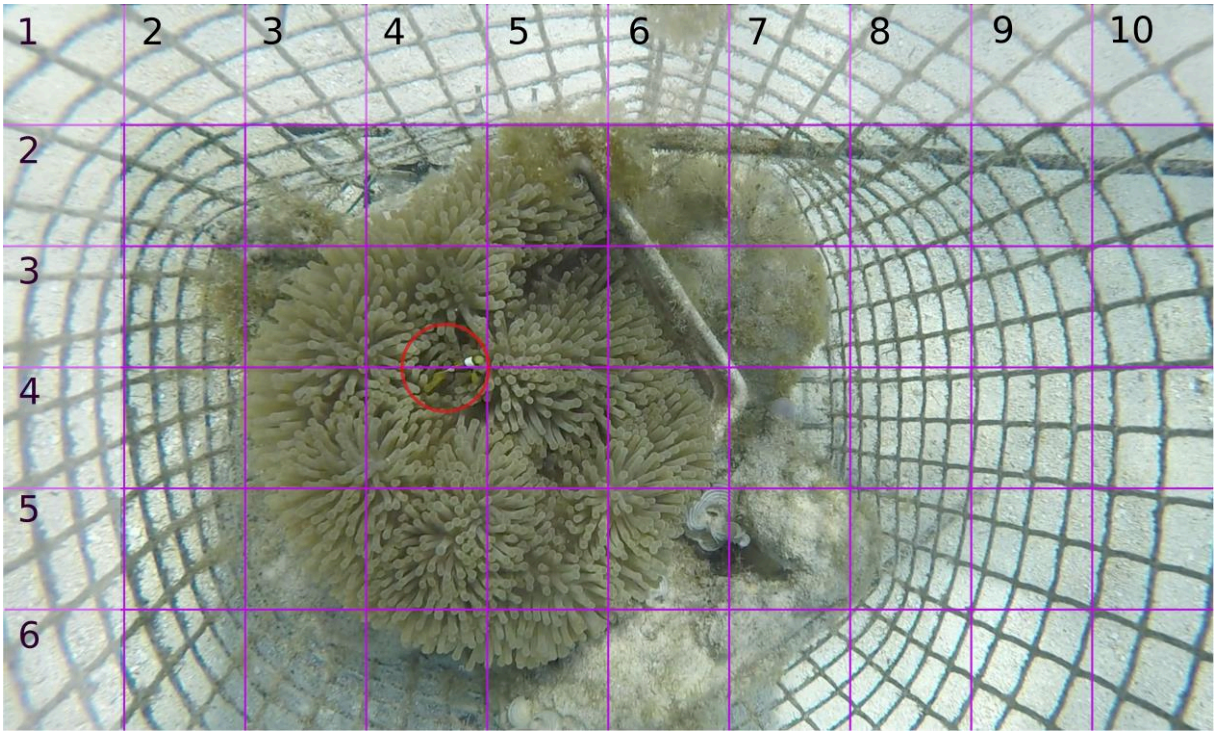

Table S5. 5. Repeatability tests of behaviour for evaluating how consistent activity and space use was among individual fish across the three sections of 30 s video (cf. Fig. S5.2).

Table S5. 5A. Adjusted repeatability (R_{adj}) of activity and space use was calculated using the rptR package (Stoffel et al. 2017). Order is the chronological order of the three 30 s video sections, Mass is fish body mass at the time of the behavioural measurement, Time is duration of treatment exposure, and Trtm is anemone treatment (bleached vs. unbleached). ID is fish ID, which was included in the model as a random effect.

Model structure (activity)	**R_{adj}**	**95% CI**	**p-value**
1: $Activity \sim Order + log10(Mass) + Time + Trtm + (1\|ID)$	0.318	0.109-0.506	0.0011

Model structure (space use)	**R_{adj}**	**95% CI**	**p-value**
1: $log10(Space\ use) \sim Order + log10(Mass) + Time + Trtm + (1\|ID)$	0.361	0.164-0.561	0.0003

Table S5. 5B. R_{adj} calculated for each treatment (bleached *vs*. unbleached anemones) separately, by sub-setting the data for each treatment and removing *Trtm* as a fixed effect in the models.

Behaviour	**Treatment**	**R_{adj}**	**95% CI**	**p-value**
Activity	Bleached	0.426	0.093-0.682	0.0067
	Unbleached	0.400	0.112-0.664	0.0030
Space use	Bleached	0.464	0.148-0.722	0.0020
	Unbleached	0.336	0.039-0.593	0.0072

Table S5. 6. Models and model outputs for standard metabolic rate (SMR) analyses

Table S5. 6A. List of all linear mixed-effects models (LMEs) performed with SMR as response variable. Mass is fish body mass at the time of SMR measurement, Trtm is anemone treatment (bleached vs. unbleached), Time is duration of treatment exposure, and Site is field site. ID is fish ID, which was included in the model as a random effect. Model selection was performed by sequentially dropping non-significant variables, starting with the interaction, and models were compared using likelihood ratio tests (LRTs) in order to identify the best-fit model (in bold) that included time and treatment as predictor variables.

Model	df	LogLik	χ^2	p-value
1: $log10(SMR) \sim log10(Mass) * Trtm + Time * Trtm + Site + (1\|ID)$	10	68.553		
2: $log10(SMR) \sim log10(Mass) + Time * Trtm + Site + (1\|ID)$	9	68.315	0.437	0.509
3: $log10(SMR) \sim log10(Mass) + Time + Trtm + Site + (1\|ID)$	8	66.157	4.315	0.038
4: $\boldsymbol{log10(SMR) \sim log10(Mass) + Time * Trtm + (1\|ID)}$	**7**	**66.624**	**3.382**	**0.184†**

†Result of the LRT between model 2 and model 4.

Table S5. 6B. Results of the best-fit model from Table S5.6A. mR^2 is the marginal R^2, which describes the proportion of variance explained by the fixed factors alone. cR^2 is the conditional R^2, which describes the proportion of variance explained by both fixed and random factors.

Fixed effects	Estimate	SE	df	t-value	p-value	mR^2	cR^2
Intercept	-0.4421	0.051	45	-8.654	<0.0001	0.904	0.904
log10(*Mass*)	0.6721	0.036	45	18.739	<0.0001		
Time	-0.0021	0.001	45	-1.818	0.076		
***Trtm* (Unbleached)**	-0.0883	0.062	45	-1.424	0.161		
***Time**Trtm* (Unbleached)**	0.0031	0.002	45	2.024	0.049		

Table S5. 7. Models and model outputs for percentage of time spent out of the anemone analyses

Table S5. 7A. List of all linear mixed-effects models (LMEs) performed with percentage time spent out of the anemone (Time spent) as response variable. Mass is fish body mass at the time of behavioural measurement, Trtm is anemone treatment (bleached vs. unbleached), Time is duration of treatment exposure, and Site is field site. ID is fish ID, which was included in the model as a random effect. Model selection was performed by sequentially dropping non-significant variables, starting with the interaction, and models were compared using likelihood ratio tests in order to identify the best-fit model (in bold) that included time and treatment as predictor variables.

Model	df	LogLik	χ^2	p-value
1: $Time\ spent \sim log10(Mass) * Trtm + Time * Trtm + Site + (1\|ID)$	11	-182.96		
2: $Time\ spent \sim log10(Mass) + Time * Trtm + Site + (1\|ID)$	10	-182.97	0.015	0.903
3: $Time\ spent \sim log10(Mass) + Time + Trtm + Site + (1\|ID)$	9	-183.76	1.588	0.208
4: $Time\ spent \sim log10(Mass) + Time + Trtm + (1\|ID)$	6	-187.22	6.915	0.075
5: $\boldsymbol{Time\ spent \sim Time + Trtm + (1\|ID)}$	**5**	**-188.95**	**3.460**	**0.063**

Table S5. 7B. Results of the best-fit model from Table S5.7A. mR^2 is the marginal R^2, which describes the proportion of variance explained by the fixed factors alone. cR^2 is the conditional R^2, which describes the proportion of variance explained by both fixed and random factors.

Fixed effects	Estimate	SE	df	t-value	p-value	mR^2	cR^2
Intercept	22.4989	9.724	33.79	2.314	0.0269	0.331	0.592
Time	0.7733	0.199	23.61	3.893	0.0007		
***Trtm* (Unbleached)**	-22.7568	7.725	29.69	-2.946	0.0062		

Table S5. 8. Models and model outputs for activity analyses

Table S5. 8A. List of all linear mixed-effects models (LMEs) performed with activity as response variable. Mass is fish body mass at the time of behavioural measurement, Trtm is anemone treatment (bleached vs. unbleached), Time is duration of treatment exposure, and Site is field site. ID is fish ID, which was included in the model as a random effect. Model selection was performed by sequentially dropping non-significant variables, starting with the interaction, and models were compared using likelihood ratio tests in order to identify the best-fit model (in bold) that included time and treatment as predictor variables.

Model	df	LogLik	χ^2	p-value
1: $Activity \sim log10(Mass) * Trtm + Time * Trtm + Site + (1\|ID)$	11	-152.94		
2: $Activity \sim log10(Mass) + Time * Trtm + Site + (1\|ID)$	10	-153.07	0.247	0.619
3: $Activity \sim log10(Mass) + Time + Trtm + Site + (1ID)$	9	-154.07	1.999	0.157
4: $\boldsymbol{Activity \sim log10(Mass) + Time + Trtm + (1\|ID)}$	**6**	**-156.94**	**5.750**	**0.124**
5: $Activity \sim Time + Trtm + (1\|ID)$	5	-158.90	3.918	0.048

Table S5. 8B. Results of the best-fit model from Table S5.8A. mR^2 is the marginal R^2, which describes the proportion of variance explained by the fixed factors alone. cR^2 is the conditional R^2, which describes the proportion of variance explained by both fixed and random factors.

Fixed effects	Estimate	SE	df	t-value	p-value	mR^2	cR^2
Intercept	23.6914	6.710	38	3.531	0.001	0.339	0.339
log10(*Mass*)	13.0031	6.746	38	1.928	0.061		
Time	0.2089	0.116	38	1.795	0.081		
***Trtm* (Unbleached)**	7.7763	3.416	38	2.276	0.029		

Table S5. 9. Models and model outputs for space use analyses

Table S5. 9A. List of all linear mixed-effect models (LME) performed with space use as response variable. Mass is fish body mass at the time of behavioural measurement, Trtm is anemone treatment (bleached vs. unbleached), Time is duration of treatment exposure, and Site is field site. ID is fish ID, which was included in the model as a random effect. Model selection was performed by sequentially dropping non-significant variables, starting with the interaction, and models were compared using likelihood ratio tests in order to identify the best-fit model (in bold) that included time and treatment as predictor variables.

Model	df	LogLik	χ^2	p-value
1: $log10(Space\ use) \sim log10(Mass) * Trtm + Time * Trtm + Site + (1\|ID)$	11	23.453		
2: $log10(Space\ use) \sim log10(Mass) + Time * Trtm + Site + (1\|ID)$	10	22.307	2.292	0.130
3: $log10(Space\ use) \sim log10(Mass) + Time + Trtm + Site + (1\|ID)$	9	21.703	1.208	0.272
4: $\boldsymbol{log10(Space\ use) \sim log10(Mass) + Time + Trtm + (1\|ID)}$	**6**	**21.080**	**1.246**	**0.742**
5: $log10(Space\ use) \sim Time + Trtm + (1\|ID)$	5	17.576	7.008	0.008

Table S5. 9B. Results of the best-fit model from Table S5.9A. mR^2 is the marginal R^2, which describes the proportion of variance explained by the fixed factors alone. cR^2 is the conditional R^2, which describes the proportion of variance explained by both fixed and random factors.

Fixed effects	Estimate	SE	df	t-value	p-value	mR^2	cR^2
Intercept	0.9377	0.097	38	9.686	<0.0001	0.492	0.492
log10(*Mass*)	0.2556	0.097	38	2.627	0.012		
Time	0.0051	0.002	38	3.027	0.004		
***Trtm* (Unbleached)**	0.1230	0.049	38	2.495	0.017		

Table S5. 10. Models and model output for growth rate analyses

Table S5. 10A. List of all linear mixed-effects models (LMEs) performed with growth rate (Growth) as response variable. Mass is fish body mass at the experimental start date (Week 0), Trtm is anemone treatment (bleached vs. unbleached), Time is duration of treatment exposure, and Site is field site. ID is fish ID, which was included in the model as a random effect. 'ns' is a function in the spline package in R (R Core Team 2019) for non-linear effects of continuous covariates. Two spline curves were used in order to account for quadratic mass effects on fish growth. Model selection was performed by sequentially dropping non-significant variables, starting with the interaction, and models were compared using likelihood ratio tests in order to identify the best-fit model (in bold) that included time and treatment as predictor variables.

Model	df	LogLik	χ^2	p-value
1: $Growth \sim ns(log10(Mass), 2) * Trtm + Time * Trtm + Site + (1\|ID)$	13	-69.195		
2: $Growth \sim ns(log10(Mass), 2) + Time * Trtm + Site + (1\|ID)$	11	-70.567	2.745	0.254
3: $Growth \sim ns(log10(Mass), 2) + Time + Trtm + Site + (1\|ID)$	10	-70.663	0.191	0.662
4: $\boldsymbol{Growth \sim ns(log10(Mass), 2) + Time + Trtm + (1\|ID)}$	**7**	**-72.419**	**3.512**	**0.319**
5: $Growth \sim Time + Trtm + (1\|ID)$	5	-106.19	67.540	<0.0001

Table S5. 10B. Results of the best-fit model from Table S5.10A. mR^2 is the marginal R^2, which describes the proportion of variance explained by the fixed factors alone. cR^2 is the conditional R^2, which describes the proportion of variance explained by both fixed and random factors.

Fixed effects	Estimate	SE	df	t-value	p-value	mR^2	cR^2
Intercept	5.0160	0.445	57	11.265	<0.0001	0.707	0.707
ns(log(*Mass*), 2) 1	-7.0722	0.786	57	-8.999	<0.0001		
ns(log(*Mass*), 2) 2	-3.7221	0.473	57	-7.866	<0.0001		
Time	0.0112	0.009	57	1.217	0.229		
***Trtm* (Unbleached)**	0.8531	0.211	57	4.047	0.0002		

Table S5. 11. Models and model output for survival analyses

Table S5. 11A. Cox proportional hazard model with the hazard function (defined as the risk of death over time) used as a response variable. Time is the survival time in weeks, Status (presence/absence; cf. Table S5.2B) is used for censoring as the exact date of death (absence) is unknown due to weekly monitoring, Trtm is anemone treatment (bleached vs. unbleached), and Site is field site. Model selection was performed by sequentially dropping non-significant variables, starting with the interaction, and models were compared using likelihood ratio tests in order to identify the best-fit model (in bold) that included time and treatment as predictor variables.

Model	df	LogLik	χ^2	p-value
1: $(Time, Status) \sim Trtm * Site$	5	-47.393		
2: $(\boldsymbol{Time}, \boldsymbol{Status}) \sim \boldsymbol{Trtm} + \boldsymbol{Site}$	**3**	**-48.060**	**1.3347**	**0.513**
3: $(Time, Status) \sim Trtm$	1	-51.095	6.0697	0.048

Table S5. 11B. Results of the best-fit model from Table S5.11A.

Fixed effects	Coef.	Exp. (coef.)	SE (coef.)	z-value	p-value	R^2
***Site* 2**	-1.6504	0.5218	0.5426	-1.199	0.231	0.31
***Site* 3**	-1.6628	0.1896	0.7201	-2.309	0.021	
***Trtm* (Unbleached)**	-0.5608	0.5707	0.5064	-1.107	0.268	

Table S5. 12. Correlation test statistics for all trait correlations

Correlation test statistics for all trait correlations, as shown graphically in Fig. 5.5 in the main manuscript. For standard metabolic rate (SMR), activity, space use, and growth rate, body-mass-adjusted ($_{adj}$) values were used in correlations, as body mass significantly affected these traits. Body-mass-adjusted values were obtained by calculating partial effects (i.e. fixing body mass) using the remef function from the remef package in R (Hohenstein and Kliegl 2020). For time spent (out of the anemone), raw values were used as fish body mass had no significant effect on this trait (Table S5.7A). Significant correlations ($p < 0.05$) are highlighted in bold, while near-significant correlations ($p < 0.10$) are indicated with an asterisk.

	Treatment	**Week**	**Test**	**cor/rho**	**t/S**	**df**	**p-value**
SMR_{adj} *vs.* Time spent	Unbleached	4	Spearman	-0.200	144		0.61
		8	Spearman	0.045	210		0.90
	Bleached	4	Pearson	0.040	0.11	8	0.91
		8	Pearson	-0.667	-2.19	6	0.07*
SMR_{adj} *vs.* $Activity_{adj}$	Unbleached	4	Pearson	-0.298	-0.83	7	0.44
		8	Pearson	-0.414	-1.36	9	0.21
	Bleached	4	Pearson	-0.073	-0.21	8	0.84
		8	Pearson	-0.073	-0.18	6	0.86
SMR_{adj} *vs.* Space use_{adj}	Unbleached	4	Pearson	-0.360	-1.02	7	0.34
		8	Pearson	-0.488	-1.68	9	0.13
	Bleached	4	Pearson	-0.291	-0.86	8	0.41
		8	Pearson	0.083	0.20	6	0.84
SMR_{adj} *vs.* $Growth_{adj}$	Unbleached	4	Pearson	0.122	0.39	10	0.71
		8	Pearson	-0.112	-0.36	10	0.73
	Bleached	4	Pearson	-0.542	-2.23	12	**0.045**
		8	Pearson	0.048	0.13	7	0.90
SMR_{adj} *vs.* Survival	Unbleached	4	Spearman	-0.284	154.13		0.46
		8	Spearman	-0.408	169		0.28
	Bleached	4	Spearman	-0.056	174.32		0.88
		8	Spearman	0.052	79.62		0.90
Time spent *vs.* $Activity_{adj}$	Unbleached	4	Spearman	0.345	144		0.30
		8	Spearman	0.509	108		0.11
	Bleached	4	Pearson	0.648	2.69	10	**0.02**
		8	Pearson	0.647	2.08	6	0.08*
Time spent *vs.* Space use_{adj}	Unbleached	4	Spearman	0.245	166		0.47
		8	Spearman	0.445	122		0.17
	Bleached	4	Pearson	0.589	2.30	10	**0.044**
		8	Pearson	0.413	1.11	6	0.31
Time spent *vs.* Survival	Unbleached	4	Spearman	0.613	63.89		0.06*
		8	Spearman	0.065	205.67		0.85
	Bleached	4	Spearman	-0.086	91.22		0.84
		8	Spearman	0.378	52.25		0.36
$Activity_{adj}$ *vs.* Space use_{adj}	Unbleached	4	Pearson	0.891	5.89	9	**0.0002**
		8	Pearson	0.716	3.08	9	**0.01**
	Bleached	4	Pearson	0.812	4.41	10	**0.001**
		8	Pearson	0.930	6.18	6	**0.0008**
$Activity_{adj}$ *vs.* Survival	Unbleached	4	Spearman	0.700	49.44		**0.02**
		8	Spearman	-0.010	222.2		0.98
	Bleached	4	Spearman	-0.270	106.69		0.52
		8	Spearman	0.567	36.38		0.14
Space use_{adj} *vs.* Survival	Unbleached	4	Spearman	0.557	73.17		0.09*
		8	Spearman	0.010	217.8		0.98
	Bleached	4	Spearman	-0.712	143.81		**0.048**
		8	Spearman	0.630	31.09		0.09*

Growth$_{adj}$ *vs.* Time spent	Unbleached	4	Spearman	0.027	214		0.95
		8	Spearman	-0.418	312		0.20
	Bleached	4	Pearson	0.198	0.64	10	0.54
		8	Pearson	0.117	0.29	6	0.79
Growth$_{adj}$ *vs.* Activity$_{adj}$	Unbleached	4	Pearson	-0.705	-2.98	9	**0.01**
		8	Pearson	0.063	0.19	9	0.85
	Bleached	4	Pearson	0.262	0.86	10	0.41
		8	Pearson	0.444	1.21	6	0.27
Growth$_{adj}$ *vs.* Space use$_{adj}$	Unbleached	4	Pearson	-0.781	-3.76	9	**0.005**
		8	Pearson	-0.288	-0.90	9	0.39
	Bleached	4	Pearson	0.328	1.09	10	0.30
		8	Pearson	0.394	1.05	6	0.33
Growth$_{adj}$ *vs.* Survival	Unbleached	4	Spearman	-0.292	587.85		0.31
		8	Spearman	0.050	114.02		0.90
	Bleached	4	Spearman	-0.584	348.56		0.06*
		8	Spearman	0.274	61		0.51

Chapter 6: Long-term exposure to artificial light at night (ALAN) in the wild decreases growth and survival of a coral reef fish

6.1. Foreword

Artificial Light At Night (ALAN) is an increasing global stressor. While there are a few studies examining the effects of ALAN on terrestrial animals biology and ecology, very little is known about their effects on aquatic organisms, in particular on wild populations. The aim of this chapter is to examine how early life stages can cope with human-induced environmental stressors, such as ALAN, in the wild. Specifically, I investigated the long-term (~2 years) effects of ALAN on growth and survival of *Amphiprion chrysopterus* in their natural environment.

I conceptualised this research project with my supervisors Dr. Suzanne C. Mills and Dr. Ricardo Beldade, in collaboration with Dr. Stephen Swearer, from the University of Melbourne. Due to the long-term experiment (2 years) I focused on the first year of the project (my second year of PhD) and Jules Schligler (Master student) focused on the second year. I performed the fieldwork with help from volunteering students (especially Camille Vizon, Marie-Louise Elian and Ignacio Pita Vaca) and the analyses were carried out in close collaboration with Jules Schligler. The manuscript was drafted by Jules Schligler under the contribution and supervision of myself and Dr. Suzanne C. Mills.

6.2. Manuscript information

Title: Long-term exposure to artificial light at night (ALAN) in the wild decreases growth and survival of a coral reef fish

Authors: Jules Schligler[1,*], Daphne Cortese[1,*], Ricardo Beldade[1, 4], Stephen E. Swearer[2], Suzanne C. Mills[1,3]

*co-first author

Affiliations:

[1] PSL Université Paris: EPHE-UPVD-CNRS, USR 3278 CRIOBE, BP 1013, 98729 Papetoai, Moorea, French Polynesia.

[2] National Centre for Coasts and Climate and School of BioSciences, University of Melbourne, Parkville, Victoria 3010 Australia

[3] Laboratoire d'Excellence "CORAIL"

[4] Estación Costera de Investigaciones Marinas and Center for Advanced Studies in Ecology and Biodiversity, Las Cruces, Pontificia Universidad Católica de Chile, Santiago de Chile.

Keywords: Ecological light pollution, artificial lightscapes, anthropogenic stressor, mortality, pollutant

Status: Submitted to Proceeding B. ID: RSPB-2021-0454.

6.3. Abstract

Artificial light at night (ALAN) is an increasing anthropogenic pollutant, closely associated with human population density, and now well recognised in both terrestrial and aquatic environments. However, we have a relatively poor understanding of the effects of ALAN in the marine realm. Here, we carried out a field experiment in the coral reef lagoon of Moorea, French Polynesia, to investigate the effects of long-term exposure (18-23 months) to chronic light pollution at night on the growth and survival of wild juvenile orange-fin anemonefish, *Amphiprion chrysopterus*. Long-term exposure to environmentally relevant underwater illuminance (mean: 4.3 lx), reduced growth (mean: 44%) and survival (mean: 36%) of juvenile anemonefish compared to that of juveniles exposed to natural moonlight underwater (mean: 0.03 lx). Our study carried out in an ecologically realistic situation in which the direct effects of artificial lighting on juvenile anemonefish are combined with the indirect consequences of artificial lighting on other species, such as their competitors, predators and prey, revealed the negative impacts of ALAN on life-history traits. Not only are there immediate impacts of ALAN on mortality, but the decreased growth of surviving individuals may also have considerable fitness consequences later in life. Future studies examining the mechanisms behind these findings are vital to understand how organisms can cope and survive in nature under this globally increasing pollutant.

6.4. Introduction

Artificial light at night (ALAN) is a globally widespread environmental pollutant with direct ecological impacts on multiple terrestrial and aquatic ecosystems (Davies and Smyth 2018; Dominoni and Nelson 2018; Sanders and Gaston 2018) including ecosystem functioning (Sanders et al. 2020). ALAN has been identified as a new major pollutant in the context of global environmental change (Gaston et al. 2015; Davies and Smyth 2018). Levels of light pollution are closely associated with human population density and economic activity (Gallaway et al. 2010). Approximately one tenth of the world's population (600 million people) live in coastal areas that are less than 10 m above sea level, resulting in considerable anthropogenic light pollution (Davies et al. 2014), which is expected to increase in parallel with global human population increases along the world coastline (Grimm et al. 2008). Light pollution is a recognised threat for wildlife and biodiversity worldwide (Hölker et al. 2010; Gaston et al. 2013), directly affecting biological and ecological processes across taxa, including changes in key life-history traits, such as immune function (Becker et al. 2020; Durrant et al. 2020), survival (McLay et al. 2017), aging (Vinogradova et al. 2010) and fecundity (Willmott et al. 2018), however, the impacts of ALAN have rarely been assessed for marine species in the wild.

Organisms have evolved biological rhythms, and light cycles are the strongest and most predictable of environmental cues, such that cues from circadian, circalunar, seasonal and annual rhythms are vital for biological processes such as reproduction, migration, dispersal, etc (Hölker et al. 2010; Tan et al. 2010; Kronfeld-Schor et al. 2013). Natural light regimes may be compromised by anthropogenic night-time light pollution, directly disrupting physiology, behaviour and reproduction in many organisms (Gaston et al. 2014). Artificial light at night may also alter interactions with other species, on the one hand by facilitating foraging and energy acquisition (Rydell 1992; Dwyer et al. 2013), but also by increasing vulnerability to predators (Rodríguez et al. 2017). It is therefore unsurprising that there is little consensus as to ALAN's effects on an organism's survival and growth. The studies comparing continuous exposure to light have produced conflicting results, ranging from increased growth (Malek and Haim 2019; Thawley and Kolbe 2020), no difference in growth (Hallaråker et al. 1995), variation in growth responses across species (Brüning et al. 2011) and decreased growth (Dananay and Benard 2018; Durrant et al. 2018). Furthermore, exposure to artificial light at night has been shown to increase animal mortality in some species (McLay et al. 2017; Fobert et al. 2019), but not in others (Willmott et al. 2018). The majority of these studies were conducted under laboratory conditions, which may limit our understanding of ALAN's effects

on growth and survival in natural populations (Gaston et al. 2015) where animals interact with predators and prey, and where habitat complexity and coping behaviours may hamper detrimental effects of ALAN.

We investigated the impact of long-term exposure of ALAN on the growth and survival of juvenile site-attached orange-fin anemonefish, *Amphiprion chrysopterus* (Cuvier, 1830) in the lagoon of Moorea, French Polynesia. Anemonefish live in an obligate mutualism with sea anemones, mainly with the magnificent sea anemone, *Heteractis magnifica*, making them an ideal model species for long-term *in situ* studies (Beldade et al. 2017; Emms et al. 2020; Mills et al. 2020). Anemonefish actively feed on plankton in the water column (Allen 1970; Mariscal, 1970) and on anemone waste products (Verde et al. 2015). Sea anemones also provide anemonefish and their eggs shelter from predators, especially at night when anemonefish reduce their activity and rest among anemone tentacles (Mariscal 1970). Anemonefish are restricted to living near their host anemone, and thus are unable to move away from the direct effects of a potential stressor such as light pollution. Light pollution may also indirectly impact anemonefish via its direct impacts on their planktonic invertebrate prey (Watson et al. 2001), anemones (Wijgerde et al. 2012), or predators (Becker et al. 2013).

In a field-based experiment we aimed to determine the impacts of artificial light at night on survival and growth of juvenile orange-fin anemonefish over, on average 20.5 months. We predicted that the long-term exposure to light pollution would (1) lower survival due to the potential of light to attract natural predators (Becker et al. 2013) and (2) increase anemonefish growth due to the increased availability of planktonic prey that are attracted to light (Watson et al. 2001; Navarro-Barranco and Hughes 2015), coupled with the potential for extended daily foraging (Russ et al. 2015) and increasing foraging rates (Utne 1997) by these diurnal zooplanktivores (Allen 1970; Mariscal 1970). However, if such advantages trade-off with any direct physiological costs of ALAN and sleep deprivation (Raap et al. 2016), (3) light pollution would have no impact on growth. We specifically tested our predictions on juvenile anemonefish given the high mortality (McGurk 1986), and greater potential for developmental acclimation e.g. (Angilletta 2009) during this life stage.

6.5. Materials and methods

6.5.1. Experimental sites

Three subtidal fringing reef sites exposed to long-term terrestrial lighting (ALAN) were surveyed along the shores of Moorea, French Polynesia (17°32'19.8"S, 149°49'46.3"W; Fig.

6.1). Each of the three ALAN sites was paired with a nearby control site (within 100 m) of comparable depth and reef structure, but with no artificial illumination and only moonlight (and potential skyglow). Six anemonefish territories were chosen at each control and ALAN site, except one control site where 12 anemonefish territories were chosen (42 territories in total). Each territory consisted of one healthy magnificent sea anemone, *Heteractis magnifica*, which had been collected in the northern lagoon of Moorea and randomly distributed across the 42 territories. Light intensities at control and ALAN sites were measured underwater as illuminance (in Lux) and photosynthetic active radiation (PAR in W m^{-2}) at the same depth as anemones (60 cm) using a SpectroSens2+ (Skye Instruments Ltd). Measurements were taken in triplicate at one location in each of the six sites at night after moonrise, weekly (corresponding to the four lunar phases) on 12 different nights over four months (216 measurements in total, 36 per site).

6.5.2. Anemonefish monitoring

Wild-spawned, laboratory-reared, juvenile orange-fin anemonefish, *Amphiprion chrysopterus*, of one to three months of age from ~ 50 wild breeding pairs were used in the experiment. Eggs were collected from natural nests in Moorea lagoon over three months and hatched and reared in aquaria at CRIOBE (Supp Mat 6.1). After ~ 1-3 months, 42 juveniles were photographed and weighed (± 0.01g) using an Ohaus Scout Pro Portable Electronic Balance. Total length (TL: distance from tip of lower jaw to end of tail) and body height (distance from the pelvic fin and the start of the dorsal fin) were measured (± 0.001 cm) from photographs using ImageJ software. Individual juvenile anemonefish were randomly placed into each experimental anemone, either control (n = 24) or ALAN (n = 18) with no difference in length, height or weight between treatments (Supp Mat 6.2). Juveniles were released within ~10 cm of the anemone and always swam straight into the anemone (Fig. 6.1B).

Juvenile anemonefish were exposed to their respective treatments for a mean duration of 20.5 months [mean exposure (± SE): Control = 20 months and 8 days (± 13 days); ALAN = 20 months and 20 days (± 13 days)]. The developmental life-stages in anemonefish are determined by gonadal development and encompass juveniles, immature subadults and functional males and females. The timing of developmental stages is not known for *A. chrysopterus*, but juvenile saddleback anemonefish, *A. polymnus*, become immature subadults after 2-4 months and sex inversion into a functional female occurs between 12-14 months (Rattanayuvakorn et al. 2006). To determine whether exposure to ALAN was more sensitive

across each transition period we aimed to monitor survival prior to and after these developmental stages. Juvenile *A. chrysopterus* were therefore monitored for survival on four occasions: after 35 days (~1 month as juveniles), 70 days (~2 months as immature sub-adults), 12-17 months (mean: 15 months prior to or during sex inversion) and 18-23 months (mean: 20.5 months as functional adults).

6.5.3. Survival

Anemonefish survival (absence/presence), as well as their host anemone survival (absence/presence), were determined during the four monitoring periods. Even though the anemonefish were able to move, the distance between anemones within treatment and especially between treatments, and the high predation risk outside the anemone tentacles (Mariscal 1970; Elliott et al. 1995; Buston 2003b), especially for juveniles (Buston and Garcia 2007), made the chance of fish exchanging anemone host unlikely. Nevertheless, to confirm individual identification, focal fish were photographed at each monitoring period and identified by colour patterns based on the shape and length of the second and third vertical white stripes. The absence of a fish from its anemone was equated to mortality, which is particularly high upon settlement (Elliott et al. 1995; Buston 2003b). The exact date of death (absence) is unknown, therefore, individual survival times were determined as the average survival (in months) between the maximum survival time (i.e. date of most recent monitoring) and the minimum survival (i.e. date of the preceding monitoring). If both the anemonefish and anemone were absent, individual anemonefish were removed from the survival analysis (3 Controls and 4 ALAN) since the cause of fish absence is not known (either a direct treatment effect or indirectly from the disappearance of the host anemone, Cortese et al. 2021).

6.5.4. Growth rate

At the end of the last two exposure periods (15 and 20.5 months), surviving individuals were re-captured, re-weighed and re-photographed. Specific growth rate (SGR) was determined as the percentage increase in individual size (body mass, height or total length) per day as SGR = $\ln(S_{t_2}) - \ln(S_{t_1}) \times t^{-1} \times 100$, where S is the fish size (body mass, height or total length) at t_2 (final time) and t_1 (initial time), and t is the time (days) between the two consecutive measures (Hopkins 1992).

Ethical approval for the study was granted from The Animal Ethics Committee, Centre National de la Recherche Scientifique (permit number 006725).

6.5.5. Statistical analyses

Statistical analyses were performed in R version 3.6.1 (R Core Team 2019). Linear mixed-effect models (LMMs) determined differences in illuminance (lx) and PAR among sites and treatments (both categorical variables). All measures were taken in triplicate within the same night, therefore the variable replicate was included in the model as a random effect to account for non-independence of data. In addition, linear models (LM) determined differences in maximum illuminance (lx) and PAR among sites and treatments. Both illuminance (lx) and PAR were log10(x+1)-transformed to alleviate non-normality of model residuals. LMMs were fitted using the *lmerTest* package (Kuznetsova et al. 2017), while marginal (mR^2) and conditional (cR^2) R^2 were obtained with the package *piecewiseSEM* (Lefcheck 2016).

A general linear model (GLM) with a binomial distribution was used to explore the effect of treatment and site on the probability of fish survival over time. Survival curves were fitted using the *survminer* package (Kassambara et al. 2019).

LMERs were fitted to analyze the effect of treatment, site, period of treatment exposure (0-15 vs. 15-20.5 months) and initial fish height, length and weight (log-transformed) on fish SGR (height, length and log-transformed weight), including fish ID as a random factor to account for repeated measures on the same individuals at the two different periods.

Best-fit models were determined using likelihood ratio tests (LRTs), starting from the most complex model and subsequently removing non-significant interactions and explanatory variables via the *lmtest* package (Zeileis and Hothorn 2002), and Tukey post-hoc tests were used to determine differences among groups via the 'emmeans' function in the *emmeans* package (Lenth 2020).

6.6. Results

6.6.1. Light intensities

Mean light intensities (in Lux) at ALAN territories were, on average, 143 times higher than intensities at control territories at all sites (Table S6.1A-C; Post-hoc test on LMER – Site 1 (control vs ALAN): $t = -4.865$, $p < 0.001$; Site 2: $t = -6.140$, $p < 0.001$; Site 3: $t = -10.164$; $p < 0.001$; Fig. 6.1C) and similarly for PAR (Table S6.2A-C; Site 1: $t = -3.690$, $p = 0.001$; Site 2: $t = -6.128$, $p < 0.001$; Site 3: $t = -8.218$; $p < 0.001$; Fig. 6.1D). The light intensities at each of the

three ALAN sites (in Lux and PAR) were significantly different from each other, with Site 3 > Site 2 > Site 1 (all $p < 0.01$; Tables S6.1D and S6.2D; Fig. 6.1C, D). However, there were no differences in light intensities among control sites (all $p > 0.92$; Table S6.1D and S6.2D; Fig. 6.1C, D). The variability observed within each site was due to differences among triplicate measures (Table S6.2A and S6.2B), rather than lunar phase or month (removed from the final best-fit model, LRTs: $p > 0.05$, Table S6.1A and S6.2A). The same results were found if we only used the maximum of the triplicate light intensity measures (in Lux and in PAR)(Tables S6.3A-D and S6.4A-D).

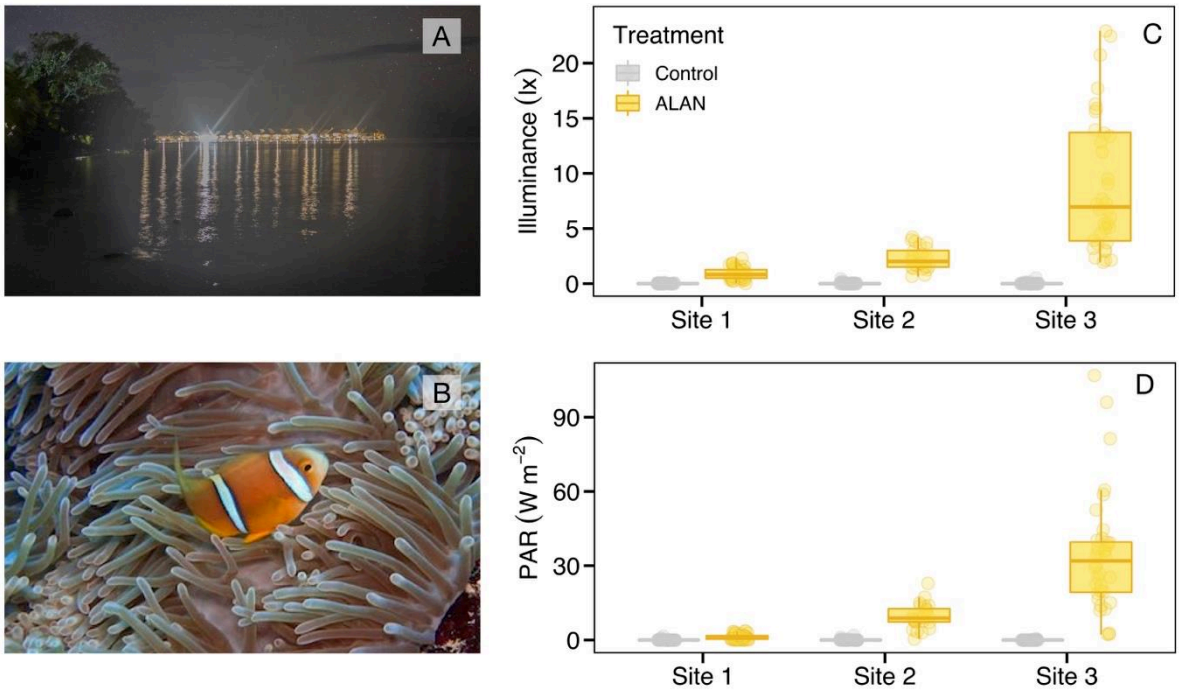

Figure 6. 1. Photographs of (A) artificial light at night (ALAN) in Moorea, French Polynesia (Photo credit: Jules Schligler), and (B) an orange-fin anemonefish, *Amphiprion chrysopterus*, in its host anemone *Heteractis magnifica* (Photo credit: Anne Haguenauer), and underwater light intensity measurements in (C) illuminance (Lux) and (D) photosynthetic active radiation (PAR) in both treatments at each of the three sites.

6.6.2. Survival

Only approximately half of the anemonefish were present in their host anemones 12-17 months later (15 Controls, 6 ALAN and one new recruit on an ALAN territory) and only one third 18-23 months later (12 Controls and 3 ALAN). The survival probability of fish exposed to artificial light at night was on average 36% lower than that of control individuals exposed to natural light at night (GLM: treatment (ALAN); $n = 35$; z-value = 1.921; $p = 0.055$; Table S6.5B, Fig. 6.2). The survival probability of anemonefish decreased over time in a similar manner between the

treatments except during two periods where anemonefish exposed to ALAN had lower survival: prior to the first monitoring period (juvenile stage) and after approx. 20 months of exposure (as functional adults) (Fig. 6.2). In addition, differences were observed among sites, with fish from site 2 having 51% and 55% higher survival resp. compared to fish from site 1 (z-value = 0.416; p = 0.047; Table S6.5C) and site 3 (z-value = 0.303; p = 0.045; Table S6.5C).

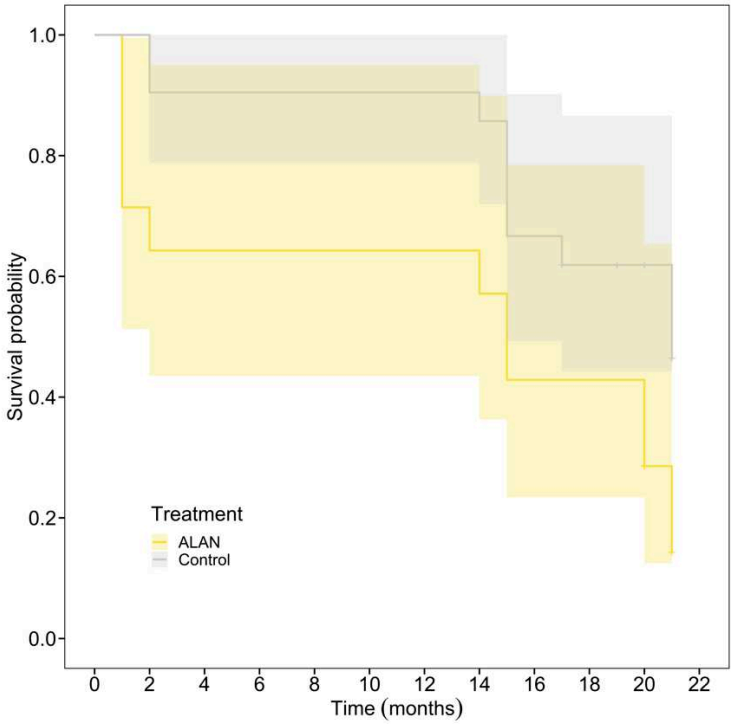

Figure 6. 2. Survival rate with 95% confidence bands of juvenile orange-fin anemonefish, *Amphiprion chrysopterus*, exposed to either Control (natural light cycle) or ALAN over a maximum of 23 months.

6.6.3. Specific growth rate

Growth was measured for 21 individuals (15 Controls and 6 ALAN) after 12-17 months, and 15 individuals (12 controls and 3 ALAN) after 18-23 months. The specific growth rate of juvenile anemonefish height (adjusted to initial size) was on average 15% lower in the ALAN treatment compared to the control (LMER: treatment (ALAN); t = -2.260, p = 0.036, mR^2 = 0.906, cR^2 = 0.963, Table S6.6B, Fig. 6.3A), and they tended to have a 51% lower specific growth rate in terms of weight (LMER: t = -1.850, p = 0.074, mR^2 = 0.397, cR^2 = 0.397, Table S6.8B, Fig. 6.3C). Despite a 21% lower specific growth rate in terms of length, we found no statistical difference (LMER: t = -1.184, p = 0.251, mR^2 = 0.846, cR^2 = 0.888, Table S6.7B, Fig. 6.3B). We did not observe any interaction among treatment and time of exposure (Table S6.6A, S6.7A and S6.8A). Overall, daily growth rate was higher during the second period of

exposure than in the first (LMER: height (2nd exposure period); $t = 3.752$, $p < 0.001$, length (2nd exposure period); $t = 3.796$, $p < 0.001$, weight (2nd exposure period); $t = 1.914$, $p = 0.065$).

We did not observe a difference in juvenile growth among sites (all $p > 0.05$: Tables S6.6B, S6.7B, S6.8B), however, we did observe an interaction between exposure duration and site on growth in height with fish from site 2 growing more than those from site 3, but only in the first exposure period (Table S6.6C; post-hoc test on LMER – 1st exposure period (Site 2 vs Site 3); $t = 3.241$, $p = 0.009$). As expected, given that daily growth declines with size (Morais and Bellwood 2018), initial fish height, length and body mass had a negative effect on growth rate (LMER: initial height; $t = -16.490$, $p < 0.001$, Table S6.6B; LMER: initial length; $t = -11.728$, $p < 0.001$, Table S6.7B; LMER: initial weight; $t = -3.723$, $p < 0.001$, Table S6.8B).

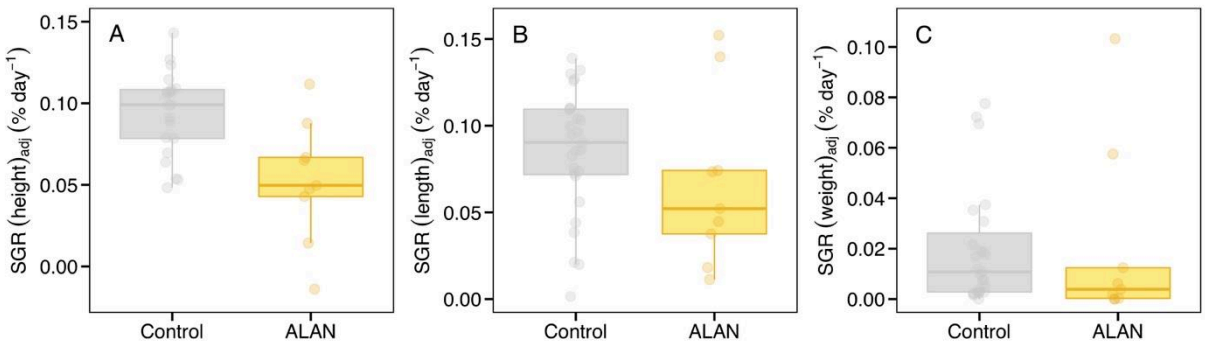

Figure 6. 3. Specific growth rate (SGR) of individual orange-fin anemonefish, *Amphiprion chrysopterus* (each data point), over both monitoring periods if alive (but repeated measures were accounted for the in the model) for (A) height, (B) total length and (C) weight. Individual data points were adjusted (using model residuals) to the overall mean measure of the fish size used in the growth analyses: (A) height (2.7 cm), (B) length (7.1 cm) and (C) body mass (11.0 g).

6.7. Discussion

Our study provides the first evidence that long-term exposure to artificial light at night pollution (ALAN), over 18-23 months, negatively impacts the survival and growth of a wild coral reef fish. Our results present a comprehensive understanding of the impact of ALAN on survival and growth in the natural environment simultaneously considering the focal fish, their predators and prey. The levels of illuminance measured underwater at our three ALAN sites, 0.9, 2.3 and 8.9 lx, are representative of light pollution that shallow fringing reefs are exposed to under street lighting and hotel lights. As these levels are considerably lower than those produced by LED lights used at ports (Bolton et al. 2017), the impacts of ALAN in coastal marine ecosystems could be greater than observed in the present study.

Our results agree with our prediction of lower survival in the ALAN treatment due to its likely negative effects on physiology and/or the potential for light to attract natural predators (Fig. 6.2). Over the total monitoring period of 23 months, the survival probability of fish exposed to ALAN decreased by 36% compared to the control group. A previous laboratory study on *Drosophila melanogaster* found that ALAN caused a 12% reduction in lifespan, and acknowledged that the biological impact may be greater in the wild (McLay et al. 2017). The juvenile life stage of anemonefish was most affected by ALAN with high mortality during the first month of exposure. Juvenile survival in the wild was low, but this is comparable to levels previously observed for wild juvenile anemonefish (Cortese et al. 2021). Lower survival may be due to physiological stress as ALAN decreased the condition of wild bird nestlings (Grunst et al. 2019). However, our results do not allow us to differentiate between physiological stress and predation to determine the cause of higher mortality under ALAN. Previous studies have demonstrated that the abundance of large fish increases when exposed to artificial light pollution, which remain close to light (Becker et al. 2013). As such, visual piscivorous predators may increase their activity under artificial light at night and enhance their foraging ability (Becker et al. 2013), therefore the higher observed mortality may have been due to increased predation under ALAN. Sea anemone hosts provide a natural refuge from predation (Mariscal 1970) and they may have partially mitigated the impact of increased predation and hence mortality. As such ALAN may have greater impacts on fish species, other than anemonefish, that are not associated with refuges from predation. Furthermore, hiding in anemones for long periods to avoid the increased presence of predators near lit sites, may have hindered anemonefish feeding rates. If feeding was decreased under continuous light exposure, then anemonefish growth may have traded-off against survival and may explain the observed decrease in growth under ALAN. Future studies should measure physiological traits and monitor the nocturnal behaviour of anemonefish, and other less site-attached species, as well as their predators, under ALAN to determine the cause of mortality.

Contrary to our predictions, we observed a negative, rather than a positive, impact of ALAN on growth, especially on fish height. Growth is a complex process influenced by many interacting physiological and ecological factors and their trade-offs (Morais and Bellwood 2018) and the observed lower growth may be due to negative effects on growth-related physiology. One such effect may be the requirement of a period of inactivity for optimal growth. *Amphiprion melanopus*, when exposed to continuous lighting, slowed their growth (Arvedlund et al. 2000). Similarly, in our study, the lack of darkness may have compromised growth. Additionally, several hormonal mechanisms, for example a decrease in the production of

melatonin, a hormone responsive to changes in photoperiod and involved in growth regulation (Falcón et al. 2007; Brüning et al. 2017), or thyroid hormones (Ouyang et al. 2018) may potentially contribute to reduced growth. Finally, the lack of sleep may result in increased metabolism, with a subsequent higher energetic demand (Stephenson et al. 2007), which despite the higher availability of planktonic prey attracted to light (Watson et al. 2001; Navarro-Barranco and Hughes 2015) coupled with the potential for extended daily foraging (Russ et al. 2015), might not be fully realised, especially if anemonefish hid in their anemones and actually fed less, resulting in less energy available for growth. The initial size of individual fish impacted growth, this is likely due to the fact that growth in fish is nonlinear and asymptotic, so larger fish will grow less than smaller fish over the same time period of time (Morais and Bellwood 2018).

The physiological responses of juveniles could have carry-over effects on fitness-related traits later in life. Exposure to ALAN during early life stages that lower growth could result in a smaller size at maturity rendering these small fish more likely lose in competition for space and be more vulnerable to predation (Arendt 1997; Sogard 1997). In addition, a poor start in life might further compromise adult reproduction that may already be negatively impacted by ALAN, as demonstrated by reduced egg hatching in a short-term laboratory study (Fobert et al. 2019) with potential repercussions for population dynamics (Rickman et al. 2000; Beldade et al. 2012; Saenz-Agudelo et al. 2014). If the decrease in growth is sustained throughout life, it is likely that fish will be smaller at breeding, which, given the known positive size/fecundity relationship (Barneche et al. 2018), fish impacted by light will be less fecund in adulthood (Beldade et al. 2012).

Alternatively, the differences in growth may be due to phenotypic plasticity in morphology in response to ALAN and may be adaptive. ALAN may have induced a phenotypic change in resource allocation, giving priority to growth in length (and weight) over body height. The morphology of crucian carp has evolved differently in the presence/absence of predators to adaptively avoid predation (Brönmark and Miner 1992) and anemonefish are able to adjust their growth plastically throughout life in response to social and ecological contexts (Buston 2003a; Reed et al. 2019). A lower growth rate, resulting in smaller height, may therefore be the optimal strategy for anemonefish exposed to ALAN, and may facilitate escaping predation by either decreasing drag or hiding inside anemones.

Pelagic fish larvae before settlement are not site-attached and are less expected to experience chronic exposure to ALAN (O'Connor et al. 2019), however ALAN might impact settlement. During our study we observed a new anemonefish recruit at one of the ALAN

exposed sites which may reflect a well-known effect of light on larval attraction. Light is a well-known tool to capture fish larvae (Doherty 1987) and Pomacentridae, the family in which anemonefish belong, are one of the most abundant families captured by light traps (Nakamura et al. 2009), representing up to 93% of the fish families attracted by light (Choat et al. 1993). Furthermore, this recruit survived at the ALAN site reaching 11.3 cm in length, likely a functional adult. Even though larval recruitment of orange-fin anemonefish in Moorea is very low (Beldade et al. 2012, 2016), one recruit is not sufficient to draw any solid conclusions concerning the impacts of ALAN on recruitment. Nevertheless, this is the first study to report that ALAN does not prevent the settlement and successful recruitment of larval fish in the wild.

In conclusion, combining the growing number of studies showing the negative impacts of ALAN in marine ecosystems with the projections of global population increases, especially along coastlines and the close association with levels of light pollution and population density, ALAN is already a risk to our marine ecosystems and will only exacerbate in the future. Marine protected areas (MPAs) are not excluded from ALAN and due to the current lack of legislation, 20% of MPAs are already exposed to artificial light at night and 14.7% are exposed to increasing levels of light pollution (Davies et al. 2016), therefore mitigation measures should be of paramount importance. Mitigation measures and policy changes are urgently needed including maintaining and creating dark areas, only lighting part of the night and improving lighting technology in terms of directing light where it is needed, reducing light intensities and changing spectra (Gaston et al. 2017). There is also a rising concern regarding the combined interactions of multiple anthropogenic stressors (Perkin et al. 2011) including boat noise (Mills et al. 2020) and the worldwide impact of these cumulative stressors needs to be better understood to help future management strategies (Halpern et al. 2015).

6.8. Acknowledgment

We are enormously grateful to: Camille Vizon, Marie-Louise Elian and Ignacio Pita for their considerable help at night with light intensity measures; Anne Haguenauer, Frederic Zuberer, Guillaume Iwankow and Frank Lerouvreur for their help with monitoring in the field and to Mathieu Kerneur for his help in the field. We thank Till Deuss and Robin Mannion for their support in anemonefish larval rearing and in the aquaria facility. Financial support was provided by the Agence National de la Recherche (ANR-14-CE02-0005-01/Stay or Go) to Glenn Almany, SCM and RB, from Appel à projets exceptionnel 2020 par le Haut-commissariat de la République en Polynésie française, RESIPOL (BLEACH & ALAN) to SCM and Pacific Funds (BLEACH & ALAN) to SCM. The Underwater LUX Sensor and SpectroSens2+ handheld logging meter was kindly donated to SCM for coral reef research by Skye Instruments Ltd (http://www.skyeinstruments.com/coral-research-and-skye-instruments/). We wish to thank Sofitel Moorea Ia Ora Beach Resort, Manava Beach Resort & Spa Moorea and Intercontinental Moorea Resort & Spa for their help and support in the field.

6.9. Supplementary materials

Suppl. Mat. 1: Anemonefish rearing

All newly hatched larvae were reared at a temperature of 27.5 ± 1°C and fed daily with rotifers (Brachionus plicatilis) enriched with live Isochrysis galbana (phytoflagellate) and SELCO (Self Emulsified Lipid Concentrate) for the first 6-8 days, after which they were fed with brine shrimp (Artemia salina). At 18 days post hatching (dph), one larva per nest was transferred to outdoor aquaria on a natural light regime (12L:12D) with anemones into which they settled and were fed twice-daily Otohime (Marubeni Nisshin Feed Co., Ltd) at ~ 10% of their body mass.

Suppl. Mat. 2: Initial anemonefish size at release

Length in cm: Control = 5.14 ± 0.40 and ALAN = 5.41 ± 0.33; n = 42, W = 227, p = 0.792; Height in cm: Control = 1.90 ± 0.17 and ALAN = 2.03 ± 0.14; n = 42, W = 236, p = 0.624; Weight in g: Control = 6.30 ± 1.05 and ALAN = 4.91 ± 0.97; n = 42, W = 189, p = 0.505.

Table S6. 1. Models and model outputs for LUX in triplicate analyses

Table S6. 1A. List of all linear mixed-effects models (LMEs) performed with illuminance (LUX) as a response variable. *Treatment* is the light treatment (Control vs. ALAN) and *Site* is field site. *Moon* refers to the lunar phase (full, new, first quarter and third quarter). *Month* is the month of measure (from February to May). Three repeated measures of illuminance were taken for each replicate, so *Replicate* was included as a random factor. Model selection was performed by using likelihood ratio tests in order to test the significance of the interaction and identify the best-fit model (in bold).

Model	df	LogLik	χ^2	p-value
1: $log_{10}(LUX+1) \sim Treatment * Site + moon * month + (1\|Replicate)$	21	171.60		
2: $log_{10}(LUX+1) \sim Treatment * Site + moon + month + (1\|Replicate)$	14	167.25	8.691	0.276
3 $log_{10}(LUX+1) \sim Treatment + Site + moon + month + (1\|Replicate)$	12	137.55	59.416	<0.001
4 $log_{10}(LUX+1) \sim Treatment * Site + moon + (1\|Replicate)$	11	165.86	2.782	0.427
5 $\boldsymbol{log_{10}(LUX+1) \sim Treatment * Site + (1\|Replicate)}$	8	165.50	0.726	0.867

†Model 4 is compared to model 2

Table S6. 1B. Results of the best-fit model from Table S6.1A. References of categorical variables used in the model are Site1 and treatment (control). mR^2 is the marginal R^2, which describes the proportion of variance explained by the fixed factors alone. cR^2 is the conditional R^2, which describes the proportion of variance explained by both fixed and random factors.

Fixed effects	Estimate	SE	df	t-value	p-value	mR^2	cR^2
Intercept	0.007892	0.037	64	0.211	0.833	0.847	0.954
Treatment (ALAN)	**0.259760**	**0.051**	**64**	**5.108**	**<0.001**		
Site 2	0.005916	0.053	64	0.112	0.911		
Site 3	0.003466	0.053	64	0.066	0.948		
Treatment (ALAN) * Site 2	**0.220815**	**0.078**	**64**	**2.835**	**0.006**		
Treatment (ALAN) * Site 3	**0.659951**	**0.073**	**64**	**9.004**	**<0.001**		

Random effect	Variance	SD
Replicate (intercept)	0.0146	0.121
Residual	0.0062	0.079

Table S6. 1C. Post-hoc tests on the transformed estimates from the best-fit model from Table S6.1A.

Fixed effects	Estimate	SE	df	t-value	p-value
Site 1: treatment (control) *vs* treatment (ALAN)	**-0.834**	**0.171**	**64**	**-4.865**	**<0.001**
Site 2: treatment (control) *vs* treatment (ALAN)	**-2.089**	**0.340**	**64**	**-6.140**	**<0.001**
Site 3: treatment (control) *vs* treatment (ALAN)	**-7.506**	**0.738**	**64**	**-10.164**	**<0.001**

Table S6. 1D. Post-hoc tests on the transformed estimates from the best-fit model from Table S6.1A.

Fixed effects	Estimate	SE	df	t-value	p-value
Treatment (control):					

Site 1 *vs* Site 2	-0.0140	0.125	64	-0.112	0.993
Site 1 *vs* Site 3	-0.0082	0.124	64	-0.066	0.998
Site 2 *vs* Site 3	0.0058	0.125	64	0.046	0.999
Treatment (ALAN):					
Site 1 *vs* Site 2	**-1.2696**	**0.360**	**64**	**-3.526**	**0.002**
Site 1 *vs* Site 3	**-6.6803**	**0.748**	**64**	**-8.933**	**<0.001**
Site 2 *vs* Site 3	**-5.4107**	**0.803**	**64**	**-6.734**	**<0.001**

Table S6. 2. Models and model outputs for PAR in triplicate analyses

Table S6. 2A. List of all linear mixed-effects models (LMEs) performed with illuminance (PAR) as a response variable. Treatment is the light treatment (control vs. ALAN) and Site is field site. Moon refers to the lunar phase (full, new, first quarter and third quarter). Month is the month of measure (from February to May). Three repeated measures of illuminance were taken for each replicate, so repeated measure of replicate (Replicate) were included as random factors. Model selection was performed by using likelihood ratio tests in order to test the significance of the interaction and identify the best-fit model (in bold).

Model	df	LogLik	χ^2	p-value
1: $log_{10}(PAR+1) \sim Treatment * Site + moon * month + (1\|Replicate)$	21	80.075		
2: $log_{10}(PAR+1) \sim Treatment * Site + moon + month + (1\|Replicate)$	14	74.543	11.064	0.136
3: $log_{10}(PAR+1) \sim Treatment + Site + moon + month + (1\|Replicate)$	12	33.411	82.265	<0.001
4: $log_{10}(PAR+1) \sim Treatment * Site + moon + (1\|Replicate)$	11	74.266	0.554	0.907
5: $\boldsymbol{log_{10}(PAR+1) \sim Treatment * Site + (1\|Replicate)}$	8	71.891	4.751	0.191

†Model 4 is compared to model 2

Table S6. 2B. Results of the best-fit model from Table S6.2A. References of categorical variables used in the model are Site1 and treatment (control). mR^2 is the marginal R^2, which describes the proportion of variance explained by the fixed factors alone. cR^2 is the conditional R^2, which describes the proportion of variance explained by both fixed and random factors.

Fixed effects	Estimate	SE	df	t-value	p-value	mR^2	cR^2
Intercept	0.0249	0.051	64	0.490	0.626	0.874	0.949
Treatment (ALAN)	**0.2695**	**0.069**	**64**	**3.890**	**<0.001**		
Site 2	0.0118	0.072	64	0.164	0.870		
Site 3	-0.0153	0.072	64	-0.213	0.832		
Treatment (ALAN) * Site 2	**0.6604**	**0.106**	**64**	**6.224**	**<0.001**		
Treatment (ALAN) * Site 3	**1.1567**	**0.100**	**64**	**11.587**	**<0.001**		

Random effect	Variance	SD
Replicate (intercept)	0.0252	0.159
Residual	0.0174	0.132

Table S6. 2C. Post-hoc test on the transformed estimates from the best-fit model from Table S6.2A

Fixed effects	Estimate	SE	df	t-value	p-value
Site 1: treatment (control) *vs* treatment (ALAN)	**-0.911**	**0.247**	**64**	**-3.690**	**0.001**
Site 2: treatment (control) *vs* treatment (ALAN)	**-8.170**	**1.333**	**64**	**-6.128**	**<0.001**
Site 3: treatment (control) *vs* treatment (ALAN)	**-26.255**	**3.195**	**64**	**-8.218**	**<0.001**

Table S6. 2D. Post-hoc test on the transformed estimates from the best-fit model from Table S6.2A

Fixed effects	Estimate	SE	df	t-value	p-value
Treatment (control):					
Site 1 *vs* Site 2	-0.0291	0.178	64	-0.164	0.985
Site 1 *vs* Site 3	0.0366	0.172	64	0.213	0.975
Site 2 *vs* Site 3	0.0657	0.175	64	0.376	0.925
Treatment (ALAN):					
Site 1 *vs* Site 2	**-7.2885**	**1.344**	**64**	**-5.422**	**<0.001**
Site 1 *vs* Site 3	**-25.3077**	**3.200**	**64**	**-7.909**	**<0.001**
Site 2 *vs* Site 3	**-18.0192**	**3.457**	**64**	**-5.212**	**<0.001**

Table S6. 3. Models and model outputs for maximum values of LUX analyses

Table S6. 3A. List of all linear models (LM) performed with the maximum illuminance (LUX) recorded at each measurement as a response variable. Treatment is the light treatment (control vs. ALAN) and Site is field site. Moon refers to the lunar phase (full, new, first quarter and third quarter). Month is the month of measure (from February to May). Model selection was performed by using likelihood ratio tests in order to test the significance of the interaction and identify the best-fit model (in bold).

Model	df	LogLik	χ^2	p-value
1: $log_{10}(LUXmax + 1) \sim Treatment * Site + moon * month$	20	45.073		
2: $log_{10}(LUXmax + 1) \sim Treatment * Site + moon + month$	13	41.266	7.614	0.368
3: $log_{10}(LUXmax + 1) \sim Treatment + Site + moon + month$	11	13.406	55.72	<0.001
4: $log_{10}(LUXmax + 1) \sim Treatment * Site + moon$	10	39.327	3.878	0.275
5: $\boldsymbol{log_{10}(LUXmax + 1) \sim Treatment * Site}$	7	39.148	0.359	0.949

†Model 4 is compared to model 2

Table S6. 3B. Results of the best-fit model from Table S6.3A. References of categorical variables used in the model are Site1 and treatment (control). R^2 is the multiple R-squared, which describe the proportion of the variance explained by the predictors variables included in the model. R^2_{adj} is the adjusted R-squared, which adjust the statistic based on the number of independent variable included in the model.

Fixed effects	Estimate	SE	df	t-value	p-value	R^2	R^2_{adj}
Intercept	0.0177	0.042	64	0.423	0.674	0.880	0.871
Treatment (ALAN)	**0.3102**	**0.057**	**64**	**5.451**	**<0.001**		
Site 2	0.0203	0.059	64	0.344	0.732		
Site 3	0.0134	0.059	64	0.227	0.821		
Treatment (ALAN) * Site 2	**0.2283**	**0.087**	**64**	**2.620**	**0.011**		
Treatment (ALAN) * Site 3	**0.7008**	**0.082**	**64**	**8.544**	**<0.001**		

Table S6. 3C. Post-hoc test on the transformed estimates from the best-fit model from Table S6.3A

Fixed effects	Estimate	SE	df	t-value	p-value
Site 1: treatment (control) *vs* treatment (ALAN)	**-1.09**	**0.214**	**64**	**-5.069**	**<0.001**
Site 2: treatment (control) *vs* treatment (ALAN)	**-2.68**	**0.456**	**64**	**-5.873**	**<0.001**
Site 3: treatment (control) *vs* treatment (ALAN)	**-9.94**	**1.064**	**64**	**-9.342**	**<0.001**

Table S6. 3D. Post-hoc test on the transformed estimates from the best-fit model from Table S6.3A

Fixed effects	Estimate	SE	df	t-value	p-value
Treatment (control):					
Site 1 *vs* Site 2	-0.0499	0.145	64	-0.344	0.937
Site 1 *vs* Site 3	-0.0326	0.144	64	-0.227	0.972
Site 2 *vs* Site 3	0.0172	0.147	64	0.117	0.993
Treatment (ALAN):					
Site 1 *vs* Site 2	**-1.6441**	**0.483**	**64**	**-3.405**	**0.003**
Site 1 *vs* Site 3	**-8.8885**	**1.076**	**64**	**-8.260**	**<0.001**
Site 2 *vs* Site 3	**-7.2444**	**1.149**	**64**	**-6.307**	**<0.001**

Table S6. 4. Models and model outputs for maximum values of PAR analyses

Table S6. 4A. List of all linear models (LM) performed with the maximum PAR value recorded at each measurement as a response variable. Treatment is the light treatment (control vs. ALAN) and Site is field site. Moon refers to the lunar phase (full, new, first quarter and third quarter). Month is the month of measure (from February to May). Model selection was performed by using likelihood ratio tests in order to test the significance of the interaction and identify the best-fit model (in bold).

Model	df	LogLik	χ^2	p-value
1: $log_{10}(PARmax + 1) \sim Treatment * Site + moon * month$	20	18.267		
2: $log_{10}(PARmax + 1) \sim Treatment * Site + moon + month$	13	13.027	10.481	0.163
3: $log_{10}(PARmax + 1) \sim Treatment + Site + moon + month$	11	-19.518	65.091	<0.001
4: $log_{10}(PARmax + 1) \sim Treatment * Site + moon$	10	12.913	0.227	0.973
5: $\boldsymbol{log_{10}(PARmax + 1) \sim Treatment * Site}$	7	11.527	2.773	0.428

†Model 4 is compared to model 2

Table S6. 4B. Results of the best-fit model from Table S6.4A. References of categorical variables used in the model are Site1 and treatment (control). R^2 is the multiple R-squared, which describe the proportion of the variance explained by the predictors variables included in the model. R^2_{adj} is the adjusted R-squared, which adjust the statistic based on the number of independent variable included in the model.

Fixed effects	Estimate	SE	df	t-value	p-value	R^2	R^2_{adj}
Intercept	0.0747	0.062	64	1.206	0.232	0.887	0.879
Treatment (ALAN)	**0.3472**	**0.084**	**64**	**4.111**	**<0.001**		
Site 2	0.0250	0.088	64	0.285	0.777		
Site 3	-0.0600	0.088	64	-0.684	0.496		
Treatment (ALAN) * Site 2	**0.6611**	**0.129**	**64**	**5.112**	**<0.001**		
Treatment (ALAN) * Site 3	**1.1895**	**0.122**	**64**	**9.775**	**<0.001**		

Table S6. 4C. Post-hoc test on the transformed estimates from the best-fit model from Table S6.4A

Fixed effects	Estimate	SE	df	t-value	p-value
Site 1: treatment (control) *vs* treatment (ALAN)	**-1.45**	**0.388**	**64**	**-3.748**	**<0.001**
Site 2: treatment (control) *vs* treatment (ALAN)	**-11.56**	**2.248**	**64**	**-5.145**	**<0.001**
Site 3: treatment (control) *vs* treatment (ALAN)	**-34.56**	**5.080**	**64**	**-6.803**	**<0.001**

Table S6. 4D. Post-hoc test on the transformed estimates from the best-fit model from Table S6.4A

Fixed effects	Estimate	SE	df	t-value	p-value
Treatment (control):					
Site 1 *vs* Site 2	-0.0703	0.247	64	-0.285	0.956
Site 1 *vs* Site 3	0.1531	0.225	64	0.681	0.775
Site 2 *vs* Site 3	0.2234	0.232	64	0.961	0.604
Treatment (ALAN):					
Site 1 *vs* Site 2	**-10.1806**	**2.267**	**64**	**-4.490**	**<0.001**
Site 1 *vs* Site 3	**-32.9521**	**5.090**	**64**	**-6.474**	**<0.001**
Site 2 *vs* Site 3	**-22.7715**	**5.550**	**64**	**-4.103**	**<0.001**

Table S6. 5. Models and model outputs for survival analyses

Table S6. 5A. List of all general linear models (GLM) performed with survival as response variable and using a binomial distribution and a logit link. Status (presence/absence) is used for censoring as the exact date of death (absence) is unknown due to monitoring in three occasions throughout the study. Treatment is the light treatment (control vs. ALAN) and Site is the field site. Model selection was performed by sequentially dropping non-significant variables, starting with the interaction, and models were compared using likelihood ratio tests in order to identify the best-fit model (in bold) that included treatment as predictor variables.

Model	df	LogLik	χ^2	p-value
1: $(Status) \sim Treatment * Site$	6	-14.599		
2: $(\boldsymbol{Status}) \sim \boldsymbol{Treatment} + \boldsymbol{Site}$	**4**	**-16.516**	**3.844**	**0.146**
3: $(Status) \sim Treatment$	2	-21.615	10.198	0.006

Table S6. 5B. Results of the best-fit model from Table S6.5A. Null deviance (D_0), Residual deviance (D) and their respective degree of freedom (df).

Fixed effects	Estimate	SE	z-value	p-value	D_0	D_0 df	D	D df
Intercept	-0.071	0.799	-0.089	0.013	47.804	34	33.032	31
Treatment (ALAN)	**1.877**	**0.977**	**1.921**	**0.055**				
Site 2	**2.643**	**1.115**	**2.371**	**0.018**				
Site 3	0.081	1.181	0.068	0.946				

Table S6. 5C. Post-hoc test on the best-fit model from Table S6.5A**.**

Fixed effects	Estimate	SE	df	z-value	p-value
Site 1 *vs* Site 2	-2.6426	1.11	Inf	0.416	0.047
Site 1 *vs* Site 3	-0.0807	1.18	Inf	0.572	0.997
Site 2 *vs* Site 3	2.5619	1.07	Inf	0.303	0.045

Table S6. 6. Models and model outputs for growth rate in height analyses

Table S6. 6A. List of all linear mixed effect models (LMER) performed with SGR_H (specific growth rate in height) as response variable. H_i is the initial fish height at the start of the experiment, Treatment is the light treatment (control vs. ALAN). Period refer to the 2 periods of treatment exposure (i.e. 0-15 months and 15-20 months), and Site is field site. Individual ID was included as a random factor to account for repeated measures. Model selection was performed by sequentially dropping non-significant variables, starting with the interaction, and models were compared using likelihood ratio tests in order to identify the best-fit model (in bold) that included Treatment, Period and Site as predictor variables.

Model	df	LogLik	χ^2	p-value
1: $SGR_H \sim log(H_i) + Treatment * Period * Site + (1\|ID)$	14	86.194		
2: $SGR_H \sim log(H_i) + Treatment * Period + Site + (1\|ID)$	9	80.624	11.141	0.049
3: $SGR_H \sim log(H_i) + Treatment + Period * Site + (1\|ID)$	10	83.314	5.760	0.218

†Model 3 is compared to model 1

Table S6. 6B. Results of the best-fit model from Table S6A. mR^2 is the marginal R^2, which describes the proportion of variance explained by the fixed factors alone. cR^2 is the conditional R^2, which describes the proportion of variance explained by both fixed and random factors.

Fixed effects	Estimate	SE	Df	t-value	p-value	mR^2	cR^2
Intercept	**0.2641**	**0.020**	**24.52**	**13.472**	**<0.001**	0.906	0.963
log(H_i)	**-0.2172**	**0.013**	**25.22**	**-16.490**	**<0.001**		
Treatment (ALAN)	**-0.0309**	**0.014**	**18.21**	**-2.260**	**0.036**		
2nd exposure period	**0.0758**	**0.020**	**17.09**	**3.752**	**<0.001**		
Site 2	0.0325	0.019	22.52	1.691	0.105		
Site 3	-0.0263	0.023	23.31	-1.162	0.257		
2nd exposure period : Site 2	**-0.0433**	**0.020**	**13.92**	**-2.169**	**0.048**		
2nd exposure period : Site 3	-0.0168	0.025	14.03	-0.661	0.519		

Table S6. 6C. Post-hoc test on the best-fit model from Table S6.6A

Fixed effects	Estimate	SE	df	t-value	p-value
1st exposure period					
Site 1 *vs* Site 2	-0.0325	0.019	21.9	-1.690	0.232
Site 1 *vs* Site 3	0.0263	0.023	22.7	1.156	0.491
Site 2 *vs* Site 3	**0.0589**	**0.018**	**25.0**	**3.241**	**0.009**
2nd exposure period					
Site 1 *vs* Site 2	0.0107	0.022	26.6	0.490	0.877
Site 1 *vs* Site 3	0.0431	0.028	27.7	1.522	0.296
Site 2 *vs* Site 3	0.0324	0.022	27.9	1.490	0.311

Table S6. 7. Models and model outputs for growth rate in length analyses

Table S6. 7A. List of all LMER performed with SGR_L (specific growth rate in length) as response variable. L_i is the initial fish length at the start of the experiment, Treatment is the light treatment (control vs. ALAN). Period refer to the 2 periods of treatment exposure (i.e. 0-15 months and 15-20 months), and Site is field site. Individual ID was included as a random factor to account for repeated measures. Model selection was performed by sequentially dropping non-significant variables, starting with the interaction, and models were compared using likelihood ratio tests in order to identify the best-fit model (in bold) that included Treatment, Period and Site as predictor variables.

Model	df	LogLik	χ^2	p-value
1: $SGR_L \sim log(L_i) + Treatment * Period * Site + (1\|ID)$	14	83.450		
2: $SGR_L \sim log(L_i) + Treatment * Period + Site + (1\|ID)$	9	78.471	9.957	0.076
3: $SGR_L \sim log(L_i) + Treatment + Period + Site + (1\|ID)$	8	76.931	3.081	0.079

Table S6. 7B. Results of the best-fit model from Table S7A. mR^2 is the marginal R^2, which describes the proportion of variance explained by the fixed factors alone. cR^2 is the conditional R^2, which describes the proportion of variance explained by both fixed and random factors.

Fixed effects	Estimate	SE	Df	t-value	p-value	mR^2	cR^2
Intercept	**0.4671**	**0.033**	**25.90**	**14.093**	**<0.001**	0.846	0.888
log(L_i)	**-0.2050**	**0.017**	**29.08**	**-11.728**	**<0.001**		
Treatment (ALAN)	-0.0161	0.014	19.23	-1.184	0.251		
2nd exposure period	**0.0515**	**0.014**	**26.66**	**3.796**	**<0.001**		
Site 2	0.0079	0.017	18.20	0.455	0.655		
Site 3	-0.0333	0.021	18.78	-1.569	0.133		

Table S6. 8. Models and model outputs for growth rate in weight analyses

Table S6. 8A. List of all LMER performed with SGR_W (specific growth rate in weight) as response variable. W_i is the initial fish weight at the start of the experiment, Treatment is the light treatment (control vs. ALAN). Period refer to the 2 periods of treatment exposure (i.e. 0-15 months and 15-20 months), and Site is field site. Individual ID was included as a random factor to account for repeated measures. Model selection was performed by sequentially dropping non-significant variables, starting with the interaction, and models were compared using likelihood ratio tests in order to identify the best-fit model (in bold) that included Treatment, Period and Site as predictor variables.

Model	df	LogLik	χ^2	p-value
1: $\log(SGR_W) \sim log(W_i) + Treatment * Period * Site + (1\|ID)$	14	-45.947		
2: $\log(SGR_{W)} \sim log(W_i) + Treatment * Period + Site + (1\|ID)$	9	-49.413	6.933	0.226
3: $\boldsymbol{log(SGR_W) \sim log(W_i) + Treatment + Period + Site + (1\|ID)}$	8	-50.626	2.426	0.119

Table S6. 8B. Results of the best-fit model from Table S8A. mR^2 is the marginal R^2, which describes the proportion of variance explained by the fixed factors alone. cR^2 is the conditional R^2, which describes the proportion of variance explained by both fixed and random factors.

Fixed effects	Estimate	SE	Df	t-value	p-value	mR^2	cR^2
Intercept	-0.3961	0.597	30.0	-0.664	**0.512**	0.397	0.397
log(W_i)	**-0.7070**	**0.190**	**30.0**	**-3.723**	**<0.001**		
Treatment (ALAN)	**-0.7874**	**0.426**	**30.0**	**-1.850**	**0.074**		
2nd exposure period	0.8427	0.440	30.0	1.914	0.065		
Site 2	-0.3848	0.541	30.0	-0.712	0.482		
Site 3	-0.2458	0.668	30.0	-0.368	0.716		

Chapter 7: General discussion

This thesis provides new knowledge into the determinants of larval dispersal associated traits and the sensitivity of post-settlement juveniles to environmental changes, using the orange-fin anemonefish *Amphiprion chrysopterus* as a model species.

I have shown that maternal size plays an important role in larval traits associated with dispersal, with larger mothers producing more offspring, which swim faster. The parental environment is also a major determinant of these larval associated traits with variation in parental habitat characteristics eliciting a modification of larval phenotype and post-settlement survival. Particularly, I observed that variation in parental habitat quality (anemone surface area) can affect offspring morphology and larval swimming abilities via parental effects. Moreover, water flow conditions around parental sites modulate offspring morphology and survival. Finally, I showed that habitat variation caused by human-induced global change, such as anemone bleaching and exposure to artificial light at night (ALAN), can modify juvenile fish phenotype, with impacts in survival when exposed to ALAN. Such results provide new insights into the importance of parental phenotypes and environmental conditions in determining larval dispersal traits through to post-settlement.

In the light of these results, I discuss in the sections below: the importance of the maternal phenotype in determining larval traits associated to dispersal (7.1.); the role of environmental variability and stressors in determining offspring phenotype associated to dispersal or post-settlement survival through parental effects (7.2.) and through direct effects (7.3.); the importance and limitations of carrying out experiments in the wild using natural populations (7.4.); and, lastly, I present the main conclusions and implications of this thesis in the final subsection (7.5.)

7.1. Maternal phenotype (size) determines larval traits associated to dispersal

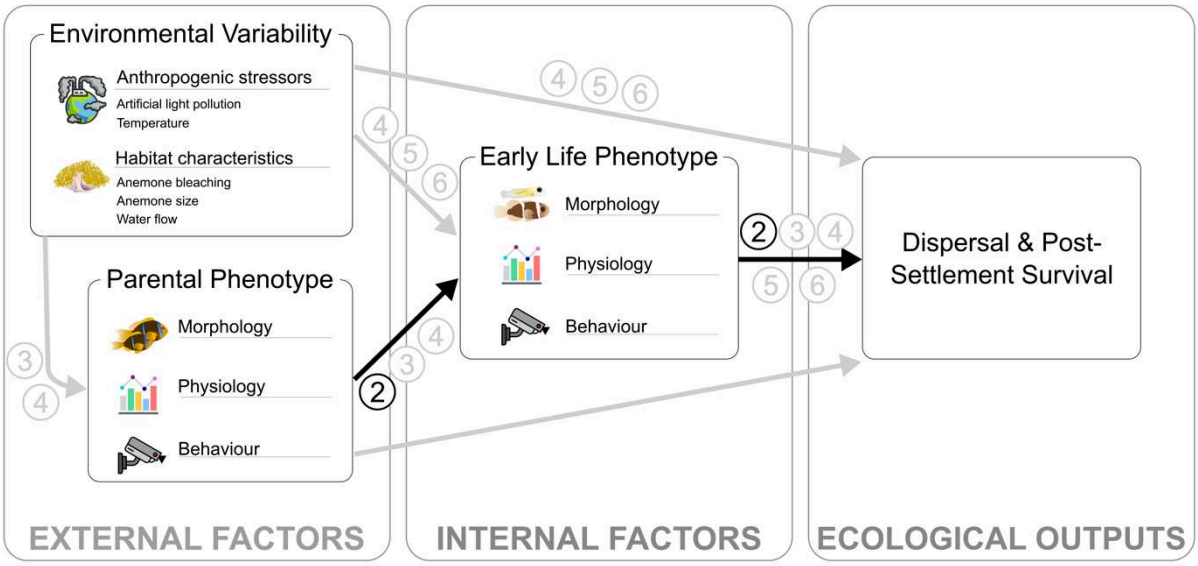

Figure 7. 1. Schematic representation of the relationships between the multiple determinants of dispersal associated traits and post-settlement survival. Arrows indicate the links between biological and ecological processes. Black arrows indicate the effects that are discussed in this subsection, while grey arrows indicate the effects that are discussed in other subsections. Circled numbers indicate the thesis chapters that examine these effects.

In *A. chrysopterus*, the parental phenotype (for example maternal size) can affect larval traits such as larval swimming speed and is thus one of the main determinants of dispersal associated traits. First, I demonstrated that maternal size did not affect spawning frequency but that larger mothers have higher fecundity: i.e. higher number of eggs produced and higher number of eggs hatching, which confirms previous findings in other fish species (Hislop 1988; Jennings et al. 2009). A higher number of larvae produced over time can lead to a higher probability of recruitment (e.g. Rickman et al. 2000). However, empirical and theoretical evidence has shown that recruitment fluctuation does not only depend on fecundity suggesting that other factors linked to larval survival, such as larval size or food availability, can also affect dispersal and recruitment success (Hjort 1914; Mertz and Myers 1996; Beldade et al. 2012). In this thesis, I found that offspring from larger mothers are superior swimmers during a large part of their dispersal phase. Indeed, I observed that larval swimming speed was positively and significantly influenced by maternal size at different developmental stages: from hatching to up to nine days post hatching. In contrast, maternal size did not influence the dispersal patterns of the larvae that were released and tracked in their natural environment. However, larvae were only tracked for a limited period of time: 7 minutes, which is short in comparison to their overall period of dispersal in the wild (~11-14 days; Schligler et al. *in prep;* Beldade et al. 2016). Thus, the

maternal effects observed on larval swimming speed may impact their dispersal abilities (i) at other time scales, for example over longer periods and during the late larval stage when swimming abilities increase significantly, and (ii) in other aspects, such as reducing the cost of dispersal (e.g. reducing mortality). As swimming abilities can impact smaller scale movements and activities, increased swimming speeds could mitigate predator avoidance and capture of moving planktonic prey (Downie et al. 2020), ultimately enhancing survival. In addition to producing larvae that swim faster, in several fish species maternal size or age is associated with egg size (Hixon et al. 2014), energetic content (Sogard et al. 2008) and growth rate (Berkeley et al. 2004). A recent metanalysis reported that in many fish species larger mothers produce disproportionally more reproductive output (i.e. fecundity, egg size and absolute egg energetic content) (Barneche et al. 2018). All this evidence highlights the role of maternal size or age in the dynamics and persistence of populations and beg a review of current fisheries political and conservation plans. Indeed, nowadays the politics on fisheries catches are focused on the preservation of smaller individuals, in order to leave them the chance to reach the reproductive stage and contribute to population replenishment. Such a strategy will result in the removal of all larger fish from a population with deleterious effects on fishery productivity and the size structure of populations (Hixon et al. 2014). In addition, marine protected areas that include "no take" areas have rapidly shown the positive effects on the size structure of fish populations (Watson et al. 2009). Management of marine fisheries and conservation plans should consider fish size as a target phenotype to protect.

The underlying mechanism that drive this relationship among larger mothers and their offspring may involve different processes. It may involve changes in maternal anatomy or physiology (greater energy resources) with increasing maternal size (Roff 1992), or adaptive response linked to the relationship between maternal phenotype and offspring environment (Marshall and Keough 2004; Green 2008; Marshall et al. 2010; Hixon et al. 2014). For example, in rockfishes larger mothers produce better quality larvae but that are released earlier in the season when environmental conditions are more adverse (Sogard et al. 2008). In those species, maternal phenotype provides a temporal spread of spawning effort to maximise offspring survival in diverse environments (diversified bet-hedging strategy).

7.2. Environmental variability determines offspring dispersal associated phenotype and post-settlement survival via parental effects

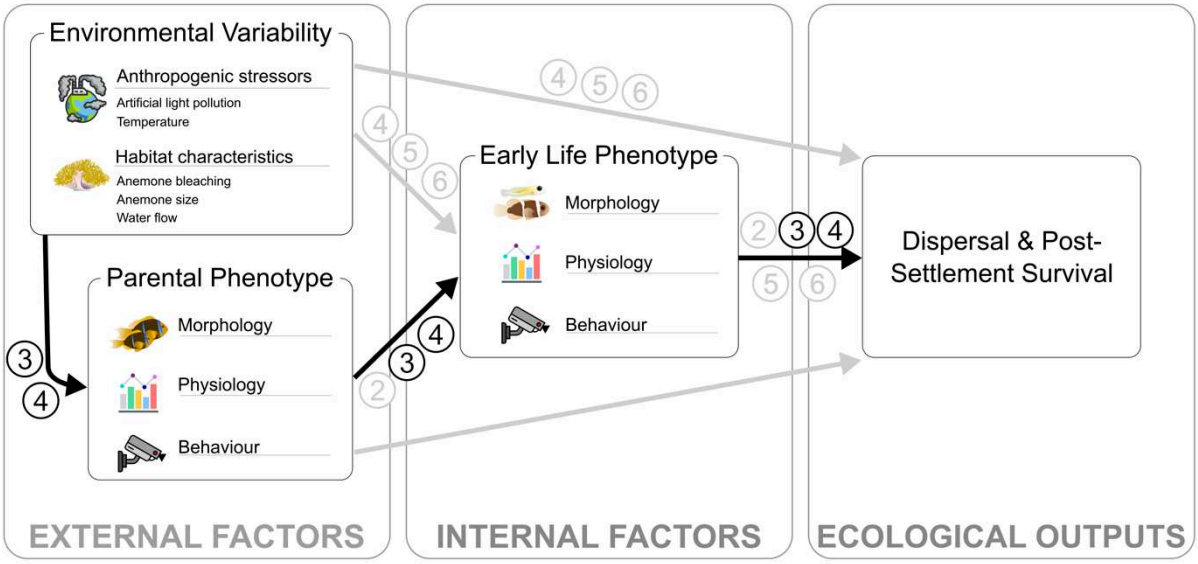

Figure 7. 2. Schematic representation of the relationships between the multiple determinants of traits associated with dispersal and post-settlement survival processes. Arrows indicate the effects of biological and ecological processes on one another. Black arrows indicate the effects that are discussed in this subsection, while gray arrows indicate the effects that are discussed in other subsections. Circled numbers indicate the thesis chapters that examine these effects.

Parental effects may be an important mechanism through which the environment mediates offspring traits and their survival (Bernardo 1996b). Indeed, environmental perturbations can affect parental condition (Roff 1992; Mousseau and Fox 1998), and therefore maternal provisioning to eggs (hormonal transfer and RNA transfer, DNA methylation) and parental care, ultimately affecting offspring quality, phenotype, and survival (Bernardo 1996a; Green 2008; Uller 2012). Here, I found that a degradation of the parental habitat (i.e. a decrease in anemone surface area) led to decrease in the size of the eggs and larvae, but an increase in larval swimming speed. Following habitat degradation, parents may have to maximize investment towards their own fitness rather than offspring fitness (Stearns 1989; Reznick et al. 2000; Donelson et al. 2008; Green 2008), which could explain the decrease in egg and larval sizes. The production of faster larvae, on the other hand, may indicate an investment in offspring that could disperse further away from the degraded habitat, to reach a more suitable habitat in which to settle (Nanninga and Berumen 2014). Swimming faster remains, however, energetically costly, and can result in smaller larvae (Marshall & Uller, 2007). In contrast, increased habitat size enhances the production of larger offspring, which may be associated with a higher amount of resources available to parents in larger anemones (Fautin and Allen 1992; Buston and Elith

2011; Verde et al. 2015). Larger offspring may be advantageous during the dispersal phase and at settlement, via avoidance of predators, access to a wider range of food resources and thus increased growth and survival ('bigger is better hypotheses'; Anderson, 1988; Bailey & Houde, 1989; Meekan & Fortier, 1996; Miller, Crowder, Rice, & Marschall, 1988; Vigliola & Meekan, 2002; but see Litvak & Leggett, 1992). These results highlight the importance of the silver spoon effect (Stamps 2006): individuals born from parents living in a good habitat have the chance to be larger and to have enhanced capacities to survive during the dispersal phase. Despite strong parental plasticity on egg and larval phenotypes in response to habitat changes, in this thesis I found that reproductive success (number of eggs laid and at hatching) was not affected by habitat changes. In a recent study on a different anemonefish species, *Amphiprion percula,* authors found that local resource availability and habitat size had a positive effect on the number of eggs laid and parental care (Barbasch et al. 2020). At first glance, the results from this thesis on *A. chrysopterus* and those on *A. percula* from Barbasch *et al.* (2020) seem contradictory. However, both species respond plastically to changes in their habitat structure or resource availability by modulating the number and/or quality of their offspring, underlining the importance of habitat characteristics in site-attached species such as anemonefishes.

In this thesis, I also explored whether the water flow conditions at parental anemones can modulate offspring morphology, physiology and survival. I found that parents living in high flow produce larvae that exhibit a larger caudal fin aspect ratio, but with lower growth and reduced survival after settlement, in comparison to offspring produced by parents living in low flow and independently on the water flow they experienced as after settlement. Caudal fins with a higher aspect ratio are advantageous in high flow environments as they can enhance steady swimming and allow fish to maintain their position while minimizing drag (Videler 1993; Blake 2004; Langerhans 2008) and possibly during the dispersal phase as well (Leis 2006). However, offspring from parents in high flow do not show a survival advantage when after settlement they settle onto high flow *vs* low flow sites. The negative parental effects on post-settlement growth and survival may be explained by reduced parental investment in order to maximize fitness in perhaps unfavorable environmental conditions, such as high water flow (Stearns 1989; Reznick et al. 2000; Donelson et al. 2008; Green 2008). While resource availability may not be a limiting factor in high flow, as plankton is part of anemonefish diet (Allen, 1972; Fautin, 1991; Mariscal, 1970), routine activity is likely to be more energetically demanding (Boisclair and Tang 1993). If fish living in high flow have a reduced energy budget to use for reproduction, this could cascade into lower investment in offspring and explain their decreased growth and survival.

While mothers are known to be able to modulate their offspring phenotype when they are able to predict the environment their offspring will be exposed to (Mousseau and Fox 1998; Agrawal 2002), the capacity of parents to modulate offspring phenotype in species with unpredictable offspring environments is less well known. In this context, this thesis brings new insights into parental plasticity in response of environmental changes. Anemonefish are strongly attached to their habitat (Buston, 2003a; Elliott et al., 1995; Fautin, 1991) and are likely to have low adaptive potential to habitat changes due to low additive genetic variation (Salles et al. 2019). Plasticity in reproduction has been suggested to be critical for coping with habitat changes (Barbasch et al. 2020). However, here I found that parents modulated their offspring phenotype when their habitat structure changed and when living in high flow environments, with potential implications for dispersal and repercussions for post-settlement survival. The different direction of plasticity depending on the nature of the habitat change suggests that maternal effects are not a passive consequence of a maternal response to local environmental conditions but an active maternal adjustment of resource allocation, highlighting the critical role of parental effects for coping with global changes. The production of larvae that swim faster when habitat size decreases may allow larvae to reach novel/better habitats, suggesting that parental plasticity to habitat change may be adaptive (Ghalambor et al. 2007). Further research should focus on the adaptive potential of parental reproductive plasticity in species that, like anemonefishes, are strongly attached to their habitat and with low genetic variation. It would also be valuable to determine if the results found here in anemonefish can be translated into other species with different life cycle. The adaptive potential of parental effects may have important implications on population dynamics and persistence, especially in the current context of global change and it is therefore critical to understand how species in different ecological context respond to such changes.

7.3. Environmental change (natural or anthropogenically-induced) effects on post-settlement phenotype and survival

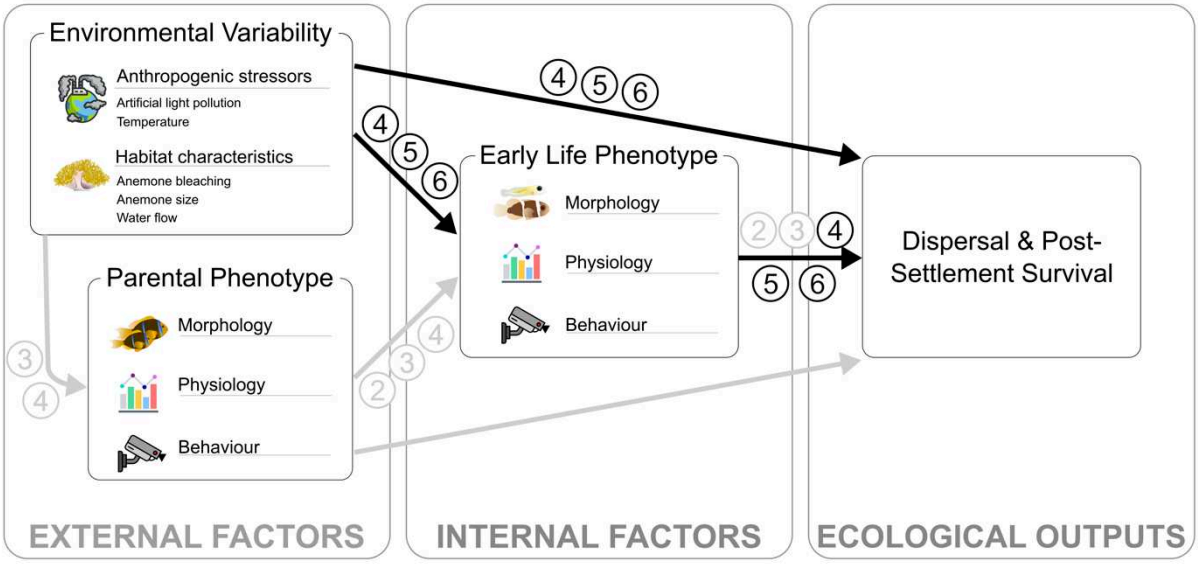

Figure 7. 3. Schematic representation of the relationships between the multiple determinants of traits associated with dispersal and post-settlement survival processes. Arrows indicate the effects of biological and ecological processes on one another. Black arrows indicate the effects that are discussed in this subsection, while gray arrows indicate the effects that are discussed in other subsections. Circled numbers indicate the thesis chapters that examine these effects.

Here I explored the effect of a natural environmental stressor (water flow regime) on the morphology, physiology and survival of post-settlement anemonefish. In addition, I studied the effect of two anthropogenic-induced environmental stressors (anemone bleaching and light pollution) on the physiology and survival of juvenile *A. chrysopterus*. I found stronger effects on the phenotype of post-settlement fish stages for anthropogenically-induced stressors, especially anemone bleaching, rather than water flow environment and light pollution impact on juvenile survival while anemone bleaching and water flow did not affect fish survival. The water flow experienced by juveniles after settlement had no consistent effects on the morphological and physiological traits that I measured, nor on their post-settlement survival. These results suggest that other environmental factors may be involved in determining an individual's morphology and physiology after settlement, or that anemonefish have alternative ways to cope with varying water flow regimes. For example, as in other coral reef fish species that use holes, crevices, branching corals and rubble to shelter from strong currents (Fulton et al. 2001; Johansen et al. 2008), juvenile anemonefish mostly dwell among anemone tentacles (Ross 1978a), avoiding high flow regimes.

Global change is causing increasing and widespread bleaching events (Hughes et al. 2017), which is a major threat for coral reef associated animals (Graham et al. 2007). However, the cascading effects of bleaching on animals that live in association with corals or anemones for shelter, foraging, or recruitment (Graham et al., 2007; Jones & Syms, 1998; Wilson, Graham, Pratchett, Jones, & Polunin, 2006) have rarely been tested. Here I provide the first evidence of the effects of anemone bleaching on fish physiology and behaviour over time (Cortese et al., 2021; Norin et al., 2018b). Short-term exposure to anemone bleaching (two weeks) causes an initial increase of anemonefish oxygen demand, which suggests that they are stressed (Norin et al., 2018b). However, the longer-term experiment presented in this thesis revealed that over time (two months) the standard metabolic rate (SMR) of anemonefish residing in bleached anemones decreases, while remaining unaffected in fish from healthy anemones (Cortese et al. 2021). Despite the reduced metabolic cost, fish from bleached anemones also experienced reduced growth compare to fish from healthy anemones, suggesting that while in bleached hosts anemonefish have an energetic disadvantage. Anemonefish nutrition is based mostly on plankton, whose density declines under thermally-induced bleaching (Tada et al. 2003; Piontkovski and Castellani 2009), but anemonefish also ingest anemone waste products and symbiont algae (Verde et al. 2015), which may also change during bleaching (Beldade et al., 2017). A reduced food intake in fish residing in bleached anemones may explain their lower growth and progressive decrease in metabolic rate. Fish from bleached anemones also spent more time out of their anemone compared to fish from unbleached anemones. The time spent out of the anemone, and the space they use around it is important for clownfish foraging and territorial defense. However, fish in unbleached anemones were also less active and used less space around the anemone, resulting in a negative correlation between space use and survival after four weeks of exposure suggesting neither increased foraging nor territoriality. This result together with the lack of evidence of stabilisation in fish standard metabolic rate suggests that anemonefish are negatively impacted by the bleaching of their host rather than acclimating to this condition.

Fish exposed to light pollution, i.e. artificial light at night (ALAN), showed lower growth, especially in body height, over ~20 months of exposure. The observed impact on growth may emerge from multiple stimuli. Growth is a complex process influenced by many interacting physiological and ecological factors (Parker and Larkin 2011). While ALAN may increase the availability of planktonic prey (which are usually attracted by light) (Watson et al. 2001; Navarro-Barranco and Hughes 2015) and extend the foraging period of diurnal zooplanktivors (Russ et al. 2015), it may also affect the organism's physiology and cause sleep

deprivation (Raap et al. 2016). The lack of a period of inactivity (Arvedlund et al. 2000), can cause an hormonal imbalance, such as for melatonin (Brüning et al. 2017) and thyroid hormones (Ouyang et al. 2018), with consequences for growth. Potential ALAN impacts on fish metabolism (Gaston et al. 2015) may have also led to a lower fish growth. The increased mortality of fish living in territories exposed to ALAN may also be explained by physiological effects, or by increased predation under ALAN due to the light attraction of predators and increase of their piscivorous activity (Becker et al. 2013).

While ALAN directly impacted anemonefish survival, anemone bleaching did not directly affect their survival, suggesting that their impacts may not be important enough to directly affect individual survival or that anemonefish had alternative ways to cope with such stressors. The adjustment of organism phenotype via plasticity is an important mechanism that allow species to cope with changes in their local environment (West-Eberhard 1989; Beldade et al. 2011) and may partially explain the lack of effects of bleaching on fish survival and the effect of ALAN only on fish height rather than length over body height. Indeed, despite bleaching effects suggest negative impacts over long-term exposure, the observed modifications of fish behaviour may allow for short-term survival. In addition, the lower growth rate of anemonefish exposed to ALAN and resulting in smaller height, may represent a phenotype more able to escape predators, e.g. by decreasing drag or facilitating hiding inside anemones. Studies on the modification of fish behaviour under ALAN would help to shed light into the mechanisms involved into the observed effects. It would also be interesting to explore whether individuals that already experienced a bleaching event are more able to cope with this stressor compared to naïve fish. However, despite the limited mortality following the exposure to some stressors (e.g. bleaching), the behavioural and physiological effects may have repercussions on later life stages. With a reduced size-at-age, individuals are more likely to lose in competition and may be more vulnerable to predation (Arendt 1997; Sogard 1997). In anemonefish there are strong size-dependent hierarchies, which drive the timing of sex-change and reproductive status (Fricke 1979; Buston 2003a), thus smaller individuals may only reach the breeding status later. More generally, all life stage are connected and a bad experience in early life may have repercussions in future stages (Burton and Metcalfe 2014).

7.4. Advantages and limitations of studies in the natural environment

Field-based experiments necessarily encompass natural variability arising from natural biotic and abiotic factors, and provide a more realistic picture of ecological processes than more classical approaches based solely on laboratory experiments. This may be especially important when studying variation in offspring phenotype under environmental changes. Indeed, trait correlations may change under different environments and laboratory conditions can themselves weaken or alter the correlation among traits (Killen et al. 2013), leading to a simplification and misinterpretation of the ecological consequences in the natural world. Despite the outstanding advantage of carrying out studies in natural populations, in nature multiple variables may correlate with themselves, which may confound results and limit the perception of key variables. In addition, carrying out fieldwork may have important sampling logistic restrictions, in terms of facilities, access to the field, time involved and unpredictable events that require continuous adjustments in the work plan.

In chapter 5 of this thesis for example, we cannot dissociate among physiological changes and increased predation pressure to explain the increased mortality under artificial light at night. Further studies on anemonefish behaviour, physiology and predator density would be necessary to understand better the causes behind the observed effects. In addition, the multiple variables to which organisms are exposed in the natural world may lead to a large variability in organisms' phenotypes, which may require a high sample size to be able to observe specific effects (e.g. chapter 2). On the other hand, studies in the laboratory cannot take into consideration all this natural variability. Field-based studies are therefore essential to be able to explore, understand and predict the realistic interactions of species with their environment and their capacity to cope with it.

7.5. Conclusion

Overall, this thesis brings new insights into the role of the environment and parents in determining offspring morphology, physiology, and survival, in a coral reef fish species. Such effects can translate into differences in the dispersal potential and post-settlement survival with important implications for population connectivity and persistence (Bernardo 1996a; Hixon et al. 2014). The parental ability to adjust offspring phenotype may be a key factor involved in response to environmental changes. Further studies on the adaptive potential of parental and environmental effects in other species both with similar and different ecology (habitat, life cycle) than anemonefish would be valuable for a better understanding of their capacity to cope with

global change and for conservation plans. In addition, future studies on parental effects on offspring phenotype could deeply focus on the mechanisms involved, such as hormonal transfer but also RNA transfer, DNA methylation and parental care. Furthermore, under the current conditions, organisms are rarely exposed to single stressors therefore the impacts of multiple stressors in the natural environment should also be taken into account. The new knowledge provided in my PhD thesis may provide additional information to better model larval dispersal and simulate global change impacts. Together with the findings of direct effects of anemone bleaching and ALAN on early life stages, this PhD helps to better predict how fish populations will respond to climate change. Such predictions are key to mitigating the impacts and improving conservations strategies, for example by targeting the most important marine areas and fish phenotypes to protect.

Traduction générale en français (French translation)

Chapitre 1 : Introduction

Les changements environnementaux induits par les activités humaines ont un impact sur les écosystèmes marins et terrestres du monde entier (Chapin III et al. 2000; Halpern et al. 2008; Jenny et al. 2016; Hughes et al. 2017). Face à ces perturbations, les espèces peuvent s'adapter, par exemple par sélection de génotypes adaptés aux nouvelles conditions environnementales (Hofmann and Todgham 2010), se déplacer vers des habitats plus adaptés, par exemple via la dispersion (Hill et al. 2002), ou encore s'acclimater à ces écosystèmes changeants, par exemple via la plasticité phénotypique, un processus qui permet le développement de phénotypes alternatifs sans changement de génotype (West-Eberhard 1989; Charmantier et al. 2008; Uller 2008).

La dispersion joue un rôle central dans la dynamique et l'évolution des populations spatialement structurées, et dans la persistance globale des espèces face aux extinctions locales (Ronce 2007). Au cours de leur cycle de vie, de nombreuses espèces marines comme les poissons des récifs coralliens produisent des larves pélagiques. Cette phase larvaire constitue souvent l'unique opportunité de dispersion de ces espèces qui sont sédentaires au stade adulte (Thorson 1949; Cowen and Sponaugle 2009). En effet, la grande majorité des stades larvaires des espèces de poissons coralliens se développent et dispersent en milieu océanique, avant de revenir s'installer dans les habitats récifaux. Cette dispersion larvaire dépend de facteurs physiques, tels que les courants océaniques, mais est également largement déterminée par les traits biologiques des larves, tels que leur comportement, leur morphologie, et leur physiologie (Cowen 2002; Leis and McCormick 2002; Fiksen et al. 2007; Paris et al. 2007; Cowen and Sponaugle 2009; Nanninga and Berumen 2014; Faillettaz et al. 2018). Par exemple, la taille et la vitesse de nage des larves déterminent leur capacité à échapper aux prédateurs et à se nourrir, et influencent donc directement la dispersion, en augmentant la probabilité de survie (Sogard 1997; Fisher et al. 2000; Leis and McCormick 2002; Fuiman and Cowan 2003; Fisher 2005; Leis et al. 2007; Nanninga and Berumen 2014). Le phénotype des larves peut par la suite également influencer leur retour vers le récif et leur survie dans ce nouvel habitat. En effet, les capacités natatoires et sensorielles des larves sont primordiales durant cette phase de colonisation récifale, par exemple afin de sélectionner et de rejoindre un habitat propice à leur croissance et leur survie, qui leur permettra in fine de recruter dans les populations adultes (Kingsford et al. 2002; Simpson et al. 2004).

Le phénotype des larves peut être déterminé par le phénotype parental, des facteurs

environnementaux, ou les deux (Figure 1). Cependant, l'influence de ces déterminants les traits larvaires associés à la dispersion reste peu étudiée, en particulier dans le contexte des changements environnementaux actuels. De plus, étant donné que les conditions environnementales peuvent affecter à la fois les traits parentaux et ceux de la progéniture, il est souvent difficile de faire la distinction entre les réponses phénotypiques induites par des facteurs environnementaux, ou parentaux, ou les deux. Enfin, les jeunes poissons peuvent aussi être affectés par les stress environnementaux après leur phase larvaire, une fois installés dans les récifs. Les effets environnementaux sur les phénotypes des jeunes stades de vie peuvent avoir des effets cascades sur d'autres stades de vie et sur les générations suivantes (Burton and Metcalfe 2014). Par conséquent, il est essentiel de comprendre comment le phénotype des juvéniles sont affectés par l'hétérogénéité environnementale, qu'elle soit d'origine naturelle ou anthropique, y compris s'ils sont capables de s'acclimater à une telle variabilité, pour une meilleure compréhension des effets des changements climatiques sur la dynamique des populations de poissons (Pechenik et al. 1998; Burton and Metcalfe 2014).

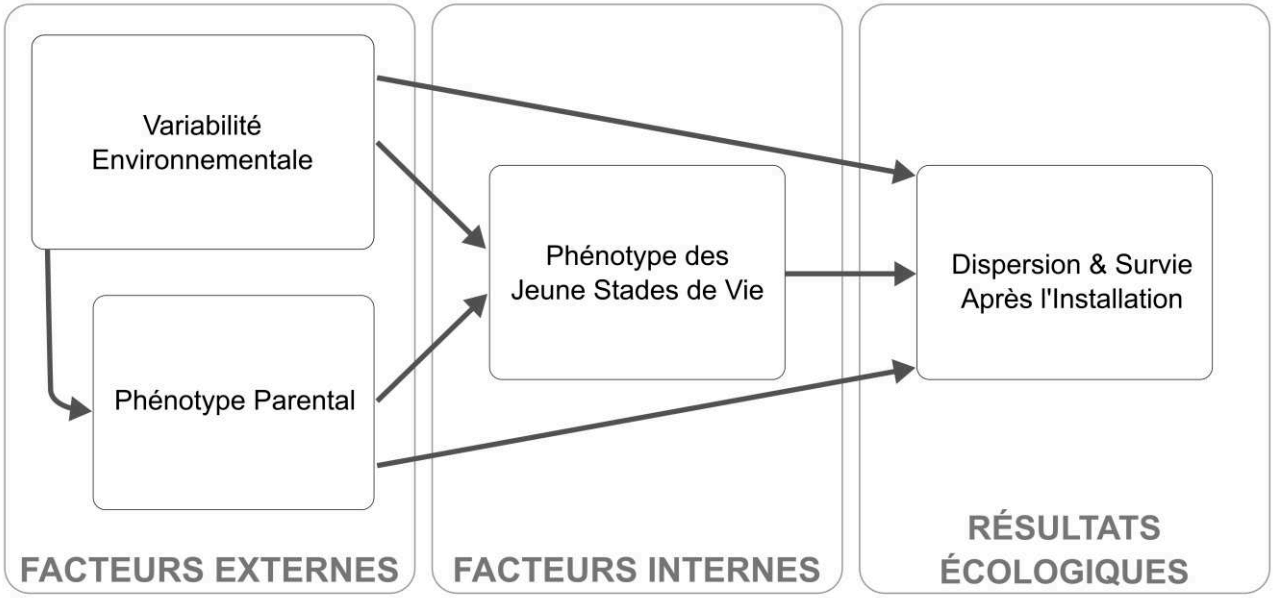

Figure 1. Représentation schématique des relations entre les multiples déterminants des traits associés aux processus de dispersion et de survie après l'installation. Les flèches indiquent les effets des processus biologiques et écologiques les uns sur les autres.

Plan de recherche

Ma thèse vise à comprendre comment les stress environnementaux actuels et les phénotypes parentaux affectent les phénotypes de la progéniture chez les poissons, plus particulièrement

sur les traits de dispersion des larves du poisson-clown à nageoires orange, *Amphiprion chrysopterus*. Des individus de poissons-clowns de la population naturelle de l'île de Moorea, en Polynésie française, ont été utilisé comme organisme modèle dans cette thèse (Figure 2). Le poisson-clown est une espèce emblématique des récifs coralliens, qui sont parmi les écosystèmes les plus menacés de la planète (Hughes et al. 2003b; Pandolfi et al. 2003). Les poissons-clowns vivent en symbiose avec les anémones de mer, ces dernières fournissant un abri aux poissons-clowns contre leurs prédateurs (les poissons-clowns étant immunisés contre la toxicité des tentacules des anémones), et en échange les poissons-clowns permettent une bonne ventilation des anémones en nageant entre les tentacules des anémones, et leur fournissent des nutriments essentiels via leur déjections (Fautin 1991). Le risque de prédation étant extrêmement élevé dans les récifs coralliens, les poissons-clowns, juvéniles et adultes, sont donc très sédentaires et restent dans les anémones après leur stade larvaire de dispersion. Comme les emplacements des anémones sont durables et le plus souvent fixes, ces espèces sont de bons modèles pour conduire des suivis *in situ* au long terme, et sur différents stades de vie : adultes, juvéniles, mais aussi œufs, qui sont pondus sur un substrat (par exemple une roche) à côté des anémones.

Figure 2. Couple (en haut de l'anémone) et juvénile (sur la partie droite de l'anémone) de poissons-clowns à nageoire orange *Amphiprion chrysopterus* dans leur anémone *Heteractis magnifica* dans le lagon de Moorea, en Polynésie française. Photo réalisée par Marc Besson.

Dans l'objective de mieux comprendre comment les populations peuvent faire face aux perturbations environnementales, naturelles ou induites par l'homme, il est nécessaire d'étudier l'influence des parents et de l'environnement sur les traits associés à la dispersion des larves dans le milieu naturel. Par conséquent, ma thèse s'articule autour de cinq chapitres qui cherchent à répondre aux questions suivantes (figure 3) :

1) Le phénotype maternel (taille) peut-il affecter les caractères larvaires associés à la dispersion ? (Chapitre 2)
2) Les perturbations environnementales, via les effets parentaux, peuvent-elles moduler le phénotype associé à la dispersion des larves ? (Chapitre 3)
3) Y a-t-il des effets développementaux et/ou intergénérationnels de la courantologie sur les premiers stades de vie d'*Amphiprion chrysopterus* ? (Chapitre 4)

4) Comment les premiers stades de la vie font-ils face aux facteurs de stress environnementaux d'origine humaine, tels que le blanchissement des anémones et la lumière artificielle la nuit, dans le milieu naturel ? (Chapitres 5 et 6)

Pour répondre à ces problématiques, j'ai utilisé une combinaison de suivi *in situ*, d'expériences sur le terrain, et d'expériences en laboratoire. J'ai ainsi pu suivre des couples reproducteurs de poissons-clowns pendant une période de 3 ans, grâce auxquels j'ai pu prélever et faire éclore des œufs, pour ensuite élever des larves. L'approche que j'ai utilisée, impliquant des expériences sur le terrain et des individus appartenant à une population naturelle, a permis d'intégrer la variabilité environnementale naturelle et offre ainsi une image plus réaliste des processus régissant le déterminisme des traits larvaires associés à la dispersion dans le milieu naturel. Plus précisément, dans les chapitres 2, 3, 4 et 6, j'ai prélevé des œufs produits par un sous-ensemble de couples reproducteurs appartenant à la population de poissons-clowns autour de Moorea, et j'ai élevé les larves en laboratoire. Dans le chapitre 5, des juvéniles directement échantillonnés dans le milieu ont été utilisés. Ma thèse a également impliqué des manipulations *in situ* des habitats parentaux (chapitre 3) et des habitats de développement (chapitre 5), ainsi que des expériences sur le terrain profitant de gradients environnementaux naturels non manipulés (chapitres 3 et 4) ou d'origine anthropique (chapitre 6). Enfin, dans chaque chapitre des mesures de laboratoire ont également été réalisées au CRIOBE sur des larves, des juvéniles ou des adultes. Différentes catégories de données évaluant la morphologie, la physiologie et le comportement m'ont permis d'apprendre des méthodologies de différentes disciplines pour répondre à des questions liées à l'écologie et à l'évolution.

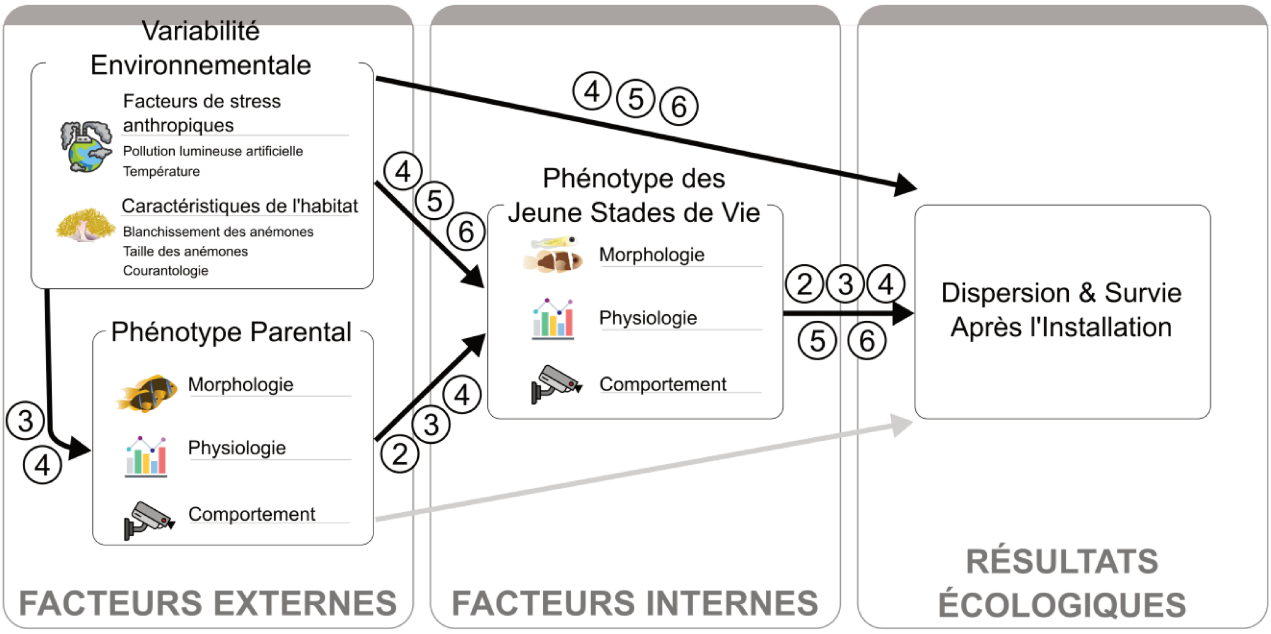

Figure 3. Représentation schématique des relations entre les multiples déterminants des traits associés aux processus de dispersion larvaire et de survie après l'installation. Les flèches indiquent les effets des processus biologiques et écologiques les uns sur les autres. Les flèches noires indiquent les effets étudiés dans cette thèse. Les nombres encerclés indiquent les chapitres de cette thèse qui examinent ces effets.

Chapitres 2 : Chez les poissons les grandes femelles produisent plus de descendants et qui nagent plus rapidement, que les petites femelles

Chez les poissons, la taille des reproducteurs est un facteur qui détermine leur importance dans le repeuplement des populations, avec notamment une relation hyper-allométrique qui lie taille et fécondité chez les femelles. En dehors de cette relation bien démontrée dans la littérature, on ne sait que peu de chose sur l'importance des grands individus reproducteurs dans le repeuplement des populations de poissons. Au-delà des différences de fécondité, des différences en termes de fréquence de ponte et de qualité des larves produites pourraient être présentes entre reproducteurs de différentes tailles. Dans une population sauvage de poissons-clowns à nageoire orange, *Amphiprion chrysopterus*, espèce emblématique des récifs coralliens, j'ai exploré le lien entre la taille maternelle et la fécondité, ainsi qu'entre cette même taille et les traits larvaires importants pour la dispersion : taille et vitesse de nage des larves. Enfin j'ai également observé comment la taille maternelle pouvait influencer la dispersion larvaire en relâchant et suivant des larves dans le milieu naturel et en comparant leur mouvement avec le mouvement de larves dérivantes passives (larves mortes). Ce chapitre de ma thèse montre que (i) les mères plus grandes produisent plus d'œufs et plus de larves à l'éclosion ; (ii) les descendants de mères plus grandes possèdent de meilleures capacités de nage pendant la majeure partie de leur phase de dispersion larvaire ; (iii) le mouvement *in situ* des larves à éclosion (moins de 24h après éclosion) était significativement différent de celui des larves dérivantes passives. Ces différences en termes de fécondité, de capacités natatoires et de mouvement dans le milieu naturel apportent de nouveaux éléments quant à l'importance des poissons de grandes tailles dans les populations sauvages. Ces résultats sont importants pour la gestion des pêches et les politiques de conservation.

Chapitres 3 : Effets parentaux sur le phénotype de la progéniture : dispersion larvaire dans un monde en changement

Les populations de poissons font face à de nombreuses variations, en particulier diminution et fragmentation, de leurs habitats à cause des activités humaines. Les effets parentaux non génétiques peuvent être des déterminants importants dans les processus de relocalisation par dispersion dans un habitat convenable ou d'acclimatation via plasticité phénotypique, mais ils n'ont été que rarement étudiés chez les poissons marins. Durant ma thèse, j'ai mené une expérience sur le terrain afin de déterminer si des changements dans la qualité de l'habitat peuvent affecter la physiologie des reproducteurs (taux de cortisol, 17β-estradiol, testostérone et de 11-cétotestostérone), leur fécondité, mais également de tester si ces changements d'habitat peuvent avoir des effets intergénérationnels en affectant le phénotype des larves, notamment leurs traits associés à la dispersion. En manipulant la taille du territoire (surface des anémones) des poissons-clowns à nageoires orange, *Amphiprion chrysopterus*, j'ai constaté que la diminution de la qualité de l'habitat (c'est-à-dire une diminution du nombre d'anémones et donc un territoire plus petit) conduit les parents à produire des larves plus petites mais plus rapides juste après cette manipulation de l'habitat. A l'inverse, une augmentation de la qualité de l'habitat (c'est-à-dire une augmentation du nombre d'anémones et donc un territoire plus grand) conduit les femelles à produire des œufs plus gros, et augmente leur concentration en hormone maternelle 17β-estradiol. Des effets à plus long terme suggèrent une récupération du phénotype des jeune stades de vie à des niveau pré-manipulation quand l'habitat décroit et des effets positifs retardés sur le phénotype larvaire quand la qualité de l'habitat augmente. Ces résultats mettent en évidence les effets du changement d'habitat des adultes sur leur progéniture et suggèrent la potentielle capacité des populations à se déplacer après un changement d'habitat, avec des répercussions potentielles sur l'abondance, la répartition et la diversité des écosystèmes.

Chapitres 4 : Effets de l'environnement parental et de l'environnement de développement sur le phénotype et la survie des jeunes stades de vie des poissons-clowns

La capacité de certains organismes à ajuster localement leur phénotype leur permet de maximiser leur fitness dans un environnement variable. La contribution de l'environnement où vivent les parents et de l'environnement de développement dans la détermination du phénotype des individus n'est pas encore entièrement comprise, en particulier chez les espèces avec un cycle de vie biphasique comme la grande majorité des poissons coralliens. Dans ce contexte, j'ai mené une expérience sur le terrain afin explorer les effets de la courantologie (courant fort ou faible) de l'environnement parental et de l'environnement développement sur la survie, la morphologie et la physiologie des poissons-clowns à nageoires orangées (*Amphiprion chrysopterus*). J'ai observé chez les juvéniles issus de parents vivant dans des sites à courant fort une croissance plus lente et une survie plus faible en comparaison avec les juvéniles issus de parents résidant dans des sites avec un courant faible. Les parents vivant dans un environnement avec courant fort produisent également des larves avec un rapport hauteur / largeur de la nageoire caudale plus élevé. J'ai également observé une corrélation positive entre la longueur de la nageoire caudale des larves et la longueur de la nageoire caudale des parents. Chez les juvéniles en revanche, aucune différence significative de survie et de phénotype n'a été observé entre sites de différentes courantologies. De plus, au stade juvénile, à l'exception de la croissance, aucun autre trait morphologique ou physiologique n'était associé aux conditions de courantologie parental. Ces résultats donnent des informations importantes sur la contribution des parents, à la fois génétique et non génétique, à la détermination du phénotype larvaire et à la croissance et à la survie des juvéniles de poissons-clowns. Ces résultats montrent également qu'il existe une certaine flexibilité chez cette espèce, lui permettant de faire face à des différentes conditions de courantologie, ce qui pourrait minimiser la perte d'individus lorsque les larves s'installent dans des environnements relativement différents de leur origine parentale.

Chapitres 5 : Effets physiologiques et comportementaux du blanchiment des anémones sur les poissons-clowns

Le changement climatique est à l'origine de vagues de chaleur extrêmes qui provoquent des évènements massifs de blanchissement des coraux dans le monde entier. Les impacts de ces événements sur la perte d'algues symbiotiques chez les coraux et les anémones sont bien documentés. Cependant, les effets cascade du blanchissement sur les animaux qui vivent en association avec les coraux et les anémones ne sont pas encore bien connus. Nous avons effectué une expérience sur le terrain afin d'étudier comment le blanchissement des anémones hôtes peut affecter le taux métabolique, la croissance, le comportement et la survie des juvéniles de poissons-clowns à nageoires orange (*Amphiprion chrysopterus*) après 1, 2 et (pour la survie) 9 mois d'exposition. J'ai constaté que le taux métabolique standard de poissons-clowns résidant dans les anémones blanchies diminuait avec le temps, mais n'était pas affecté chez les poissons vivant dans des anémones saines. De plus, le taux de croissance des poissons provenant d'hôtes blanchis était significativement plus faible que celui des poissons d'anémones saines, ce qui suggère que les poissons résidants dans les hôtes blanchis sont désavantagés sur le plan énergétique. Ce résultat est corroboré par le fait que les juvéniles résidant dans des anémones blanchies passent plus de temps hors de leurs anémones que ceux résidant des anémones saines, suggérant un plus grand besoin de se nourrir dans la colonne d'eau. Cependant, les juvéniles dans les anémones blanchies étaient globalement moins actifs et utilisaient moins d'espace autour de l'anémone, entraînant une corrélation négative entre l'utilisation de l'espace et la survie après quatre semaines. Nos résultats donnent un aperçu des effets du blanchissement des hôtes sur la physiologie et le comportement des juvéniles de poissons dans le milieu naturel, et soulignent comment des anomalies thermiques à relativement court terme peuvent avoir des impacts à long terme allant au-delà des effets sur les anémones ou les coraux eux-mêmes, en affectant les populations de poissons qui vivent en associations avec ces organismes blanchis.

Chapitres 6 : L'exposition à long terme à la lumière artificielle nocturne (ALAN) dans le milieu naturel diminue la croissance et la survie d'un poisson de récif corallien.

La lumière artificielle nocturne (ALAN) est un polluant anthropique en croissance, strictement associé à la densité de la population humaine, et aujourd'hui présent dans de nombreux environnements terrestres et aquatiques. Cependant, nous avons aujourd'hui qu'une compréhension relativement faible des effets d'ALAN sur les organismes marins. Dans ce contexte, j'ai mené une expérience de terrain dans le lagon de Moorea, en Polynésie française, pour étudier les effets d'une exposition à long terme (18-23 mois) à ALAN sur la croissance et la survie de juvéniles de poissons-clowns à nageoire orange (*Amphiprion chrysopterus*) d'une population dans le milieu naturel. L'exposition au long terme à une lumière artificielle nocturne (de 4,3 lx en moyenne, équivalente aux éclairages sous-marins côtiers) a causé une diminution de 44% de la croissance et de 36% la survie des juvéniles de poissons-clowns vivant dans les zones artificiellement éclairées par rapport à ceux simplement exposés au clair de lune (luminosité moyenne de 0,03 lx). Cette étude réalisée dans des conditions écologiquement réalistes, où les effets directs de l'éclairage artificiel sur les juvéniles de poissons-clown se combinent aux conséquences indirectes de l'éclairage artificiel sur d'autres espèces, telles que leurs prédateurs et proies, a révélé les impacts négatifs de ALAN sur la vie et les traits d'histoire de vie d'un poisson de récif corallien. Non seulement ALAN a des effets immédiats sur la mortalité, mais la diminution de la croissance des individus survivants peut également avoir des conséquences considérables sur leur fitness au long terme, par exemple pour des raisons de compétition et de succès de reproduction qui dépendent fortement de la taille des individus chez cette espèce. Des études futures examinant les mécanismes qui peuvent expliquer ces découvertes sont essentielles pour comprendre comment dans le milieu naturel les organismes peuvent faire face et survivre quand exposés à ce polluant qui est en augmentation dans le monde entier.

Chapitres 7 : Discussion générale

Cette thèse, en utilisant le poisson-clown à nageoire orange *Amphiprion chrysopterus* comme espèce modèle, apporte de nouvelles connaissances sur les déterminants des traits associés à la dispersion larvaire et la sensibilité aux changements environnementaux des juvéniles après installation.

J'ai montré que la taille maternelle joue un rôle important dans les traits larvaires associés à la dispersion. En effet, les mères plus grandes produisent plus de descendants, et qui nagent plus vite. L'environnement parental est également un déterminant majeur des traits associés à la dispersion larvaire. Les variations des caractéristiques de l'habitat parental causent des modifications du phénotype larvaire et peuvent affecter la survie après l'installation. En particulier, j'ai observé que la variation de la qualité de l'habitat des parents (surface des anémones) peut affecter la morphologie des larves ainsi que leurs capacités de nage via des effets parentaux. De plus, les conditions de courantologie autour des sites parentaux modulent la morphologie et la survie des descendants. Enfin, j'ai montré que la variation de l'habitat causée par les changements globaux induits par l'homme, comme le blanchissement des anémones et l'exposition à la lumière artificielle nocturne (ALAN), peut modifier le phénotype des juvéniles de cette espèce, et ALAN peut aussi affecter leur survie. Ces résultats fournissent de nouvelles informations sur l'importance du phénotype parental et des conditions environnementales dans la détermination des traits associés à la dispersion des larves et à la survie post-installation.

À la lumière de ces résultats, au sein de cette thèse je discute de l'importance du phénotype maternel dans la détermination des traits larvaires associés à la dispersion; du rôle de la variabilité environnementale et des facteurs de stress, par effets directs ou via effets parentaux, dans la détermination du phénotype de la progéniture associé à la dispersion ou à la survie après l'installation; de l'importance, des atouts, ainsi que des limites associées aux expériences dans le milieu naturel, en utilisant une population sauvage; et, enfin, je présente les principales conclusions et implications de cette thèse (traduites ci-dessous).

Conclusion

Dans l'ensemble, cette thèse apporte de nouvelles perspectives sur le rôle de l'environnement et

des parents dans la détermination de la morphologie, de la physiologie et de la survie de la progéniture chez une espèce de poisson de récif corallien. Ces effets peuvent se traduire par des différences de dispersion et de survie après l'installation, avec des implications importantes pour la connectivité et la persistance de la population (Bernardo 1996a; Hixon et al. 2014). La capacité parentale d'ajuster le phénotype de la progéniture peut être un facteur clé impliqué dans la réponse aux changements environnementaux. Des études plus poussées sur le potentiel adaptatif des effets parentaux et environnementaux chez d'autres espèces, à la fois d'écologie similaire et différente (par exemple en termes d'habitat et de cycle de vie) au poisson-clown seraient utiles pour une meilleure compréhension de leur capacité à faire face aux changements globaux et dans optique d'optimisation des stratégies de conservation. Les nouvelles connaissances apportées dans ma thèse de doctorat peuvent fournir des informations supplémentaires pour mieux modéliser la dispersion larvaire, ainsi que la dynamique de population des poissons. Avec les résultats des effets directs du blanchissement des anémones et de ALAN sur les premiers stades du cycle de vie des poissons, ce doctorat aide également à mieux prédire comment les populations de poissons réagiront aux changements climatiques et aux nombreux stress d'origine anthropique auxquels ils font face. Ces prévisions sont essentielles pour atténuer les impacts et améliorer les stratégies de conservation, par exemple en ciblant les zones marines les plus importantes et les phénotypes de poissons à protéger en priorité.

Appendix 1. Manuscript: Anemone bleaching increases the metabolic demands of symbiont anemonefish

PROCEEDINGS B

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Research

Cite this article: Norin T, Mills SC, Crespel A, Cortese D, Killen SS, Beldade R. 2018 Anemone bleaching increases the metabolic demands of symbiont anemonefish. *Proc. R. Soc. B* **285**: 20180282.
http://dx.doi.org/10.1098/rspb.2018.0282

Received: 4 February 2018
Accepted: 16 March 2018

Subject Category:
Global change and conservation

Subject Areas:
physiology, behaviour, ecology

Keywords:
climate change, coral reef, fish, global warming, metabolic rate, temperature

Author for correspondence:
Tommy Norin
e-mail: tommy.norin@glasgow.ac.uk

Electronic supplementary material is available online at https://dx.doi.org/10.6084/m9.figshare.c.4041473.

THE ROYAL SOCIETY PUBLISHING

Anemone bleaching increases the metabolic demands of symbiont anemonefish

Tommy Norin[1], Suzanne C. Mills[2,3], Amélie Crespel[1], Daphne Cortese[2,3], Shaun S. Killen[1] and Ricardo Beldade[2,3,4]

[1]Institute of Biodiversity, Animal Health and Comparative Medicine, University of Glasgow, Graham Kerr Building, Glasgow G12 8QQ, UK
[2]CRIOBE USR 3278 EPHE-CNRS-UPVD PSL, BP 1013 Moorea, 98729 French Polynesia
[3]Laboratoire d'Excellence 'CORAIL', Paris, France
[4]MARE, Faculdade de Ciências da Universidade de Lisboa, Lisbon, Portugal

TN, 0000-0003-4323-7254; SCM, 0000-0001-8948-3384; SSK, 0000-0003-4949-3988

Increased ocean temperatures are causing mass bleaching of anemones and corals in the tropics worldwide. While such heat-induced loss of algal symbionts (zooxanthellae) directly affects anemones and corals physiologically, this damage may also cascade on to other animal symbionts. Metabolic rate is an integrative physiological trait shown to relate to various aspects of organismal performance, behaviour and locomotor capacity, and also shows plasticity during exposure to acute and chronic stressors. As climate warming is expected to affect the physiology, behaviour and life history of animals, including ectotherms such as fish, we measured if residing in bleached versus unbleached sea anemones (*Heteractis magnifica*) affected the standard (i.e. baseline) metabolic rate and behaviour (activity) of juvenile orange-fin anemonefish (*Amphiprion chrysopterus*). Metabolic rate was estimated from rates of oxygen uptake ($\dot{M}_{O_2}$), and the standard metabolic rate ($\dot{M}_{O_{2,min}}$) of anemonefish from bleached anemones was significantly higher by 8.2% compared with that of fish residing in unbleached anemones, possibly due to increased stress levels. Activity levels did not differ between fish from bleached and unbleached anemones. As $\dot{M}_{O_{2,min}}$ reflects the minimum cost of living, the increased metabolic demands may contribute to the negative impacts of bleaching on important anemonefish life history and fitness traits observed previously (e.g. reduced spawning frequency and lower fecundity).

1. Background

Mass bleaching of corals and sea anemones is occurring at unprecedented rates throughout the tropics as a consequence of climate warming and increased sea surface temperatures [1,2]. The frequency of severe bleaching events worldwide has increased from once every 27 years in the early 1980s to currently once every 5.9 years [3]. Furthermore, since 2000, a third of all bleaching events have occurred every 1–3 years [3]. Bleaching occurs following an environmental stress (elevated water temperature or solar radiation), causing the density of algal symbionts (zooxanthellae, *Symbiodinium* spp.) harboured within the coral or anemone tissue to decline, resulting in a loss of host coloration and a white appearance [4]. The ecological effects of bleaching are becoming increasingly clear, with negative impacts on, among others, fish communities associated with coral reefs [5,6].

Anemonefish (genus *Amphiprion*) form a symbiotic relationship with sea anemones and spend most of their lives in close association with their host anemone [7]. The anemone's stinging cells offer the fish protection against predators, especially in the juvenile stage where the fish are most vulnerable. The anemonefish also attach their eggs close to the base of the anemone

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where they are well protected [7]. The fish thus rely on their host for both defence and reproduction. Studies have shown negative effects of anemone bleaching on anemonefish, including reduced reproductive success and decreased abundance [8–10]. The underlying causes for these negative effects are not clear, but changes in the fish's physiology may be involved. Indeed, recent work has found increased levels of stress hormones in anemonefish associated with bleached anemones [10]. Such physiological responses to bleaching may also be reflected in other fundamental traits such as whole-animal metabolic rate, which on other occasions have been found to be negatively affected by increased temperatures (e.g. reduced scope to increase metabolic rate above baseline requirements [11]) and which is believed to be a key pathway through which climate change impacts ectothermic animals [12,13].

Using wild-caught orange-fin anemonefish (*Amphiprion chrysopterus*), we investigated if heat-induced bleaching of their host, the magnificent sea anemone (*Heteractis magnifica*), directly affected the anemonefish's standard metabolic rate, which is a measure of the baseline (resting) energetic requirements of an ectothermic animal and represents the cost of living. For diurnally active animals, such as the anemonefish, standard metabolic rate is usually reached at night when the fish are resting. We therefore also evaluated if the anemonefish from bleached and unbleached anemones differed in their day-time metabolic rates, which represents the elevation in metabolic rate caused by increased activity in response to daylight. In addition to being an indicator of stress, increases in metabolic rate among anemonefish could have effects on energy allocation and a range of behaviours, particularly those associated with foraging and risk-taking, and affect the fish's fitness and ability to cope with changing environmental conditions. We therefore also measured if activity levels during the day differed between anemonefish from bleached and unbleached hosts.

2. Methods

Ten juvenile orange-fin anemonefish were collected from unbleached magnificent sea anemones in and around Opunohu Bay, Moorea, French Polynesia, in November 2016 using hand nets while snorkelling or scuba diving. The fish were transported by boat to the CRIOBE research station located at inner Opunohu Bay and subsequently placed in individual aquaria (25 × 29 × 52 cm; approx. 30 l of water) containing either bleached ($n = 5$ fish) or unbleached ($n = 5$ fish) *H. magnifica* (mean ± s.e. anemone surface area: bleached = 388.3 ± 52 cm^2, unbleached = 383.4 ± 84 cm^2). This was done over 2 days, with fish being placed on bleached anemones on the first day and unbleached anemones on the second day, to ensure that the fish experienced the same amount of time in each treatment before experimental measurements. To increase the sample size, another six fish were collected in November 2017 and placed in bleached ($n = 3$ fish) or unbleached ($n = 3$ fish) anemones as for the fish from 2016. The fish were initially maintained on their bleached or unbleached anemones for 2 weeks in filtered, protein-skimmed and UV-treated seawater in a closed re-circulation system (mean ± s.e. temperature in aquaria: bleached = 28.54 ± 0.11°C, unbleached = 28.55 ± 0.11°C), during which time fish (and anemones) were fed granulated food twice daily and also supplemented with live artemia. After 2 weeks in either bleached or unbleached anemone treatments, metabolic rate was recorded overnight followed by activity in the morning (only for fish from 2016), as detailed below, after which all fish were swapped between aquaria and anemones, such that fish from bleached anemones were transferred to unbleached anemones and vice versa. The fish were then allowed another 2 weeks with their bleached or unbleached hosts before metabolic rate and activity were measured again in the same fish. This paired design produced a total sample size of 16 fish, of which all 16 were measured for metabolic rate while 10 were also measured for activity, all in both treatments (bleached versus unbleached anemones).

The *H. magnifica* were collected from the wild 2 weeks prior to the introduction of fish, and half were bleached by subjecting the anemones to 31°C for 5–6 days, at which point the anemones were still alive but had lost their symbiont algae and appeared white. Successful bleaching was clearly visible but also confirmed by measuring photosynthetic activity of the anemones (details and data in electronic supplementary material).

After each of the 2-week treatment periods, the fish from either bleached or unbleached anemones were transferred to a respirometry set-up (along with anemones from their respective treatments) for measurements of the fish's oxygen uptake rates ($\dot{M}_{O_2}$), which was used as a proxy for their metabolic rates. This was done over 2 consecutive days such that fish were measured after 14 days in both of the anemone treatments. Prior to transferring fish to the respirometry set-up, food had been withheld for 20 h to ensure the fish were post-absorptive for the metabolic rate measurements. The respirometry set-up was shielded from surrounding disturbances and comprised a 40 l (water volume) tank receiving flow-through normoxic seawater at 28.58 ± 0.12°C (mean ± s.e.), eight glass respirometry chambers (35 or 110 ml volume, depending on fish size) in which the $\dot{M}_{O_2}$ of the fish could be measured by oxygen meters and probes (FireStingO2; PyroScience GmbH, Germany), a peristaltic pump with gas-tight tubing which recirculated water through the chambers and past the oxygen probes, and a set of flush pumps which intermittently flushed fresh and fully aerated seawater through the respirometry chambers for 4 or 5 min in every 9 to 12 min intermittent-closed respirometry cycle (flush and close durations were adjusted based on chamber volumes and fish sizes; see [14] for details on respirometry). The respirometry chambers were supported by two plastic pipes in between which anemones from the respective treatment were placed such that their tentacles touched the bottom and sides of the respirometry chambers (figure 1). This allowed the fish to be surrounded by and see (but not touch) the anemones, and also receive olfactory cues from the anemones in the flush water. Fish were introduced to respirometry chambers a few minutes before the first automated $\dot{M}_{O_2}$ recordings were started in the afternoon and remained there for at least 14 h (average duration was 16.8 h) until the following morning, which produced between 77 and 99 $\dot{M}_{O_2}$ recordings per fish (figure 2).

After completion of the overnight $\dot{M}_{O_2}$ measurements, the 10 fish measured in 2016 were filmed for 27 min in both treatments, starting at approximately 11:00 (while the fish were still inside their respirometry chambers), by a camera mounted directly above the respirometry set-up. Fish activity was calculated from these 20 videos by digitally splitting the respirometry chambers into four sections of equal length and counting the number of times each fish crossed the divides between these areas. The fish were then removed from the respirometry chambers and their body masses recorded to the nearest 0.001 g, which meant that growth could be calculated over the final 2 weeks of the experiment for eight fish from each treatment. The exact food intake was not monitored, but all fish were manually fed approximately equal amounts. All fish and anemones survived and appeared healthy (except for the deliberate loss of zooxanthellae) throughout the experiment, and also after when they were returned to the lagoon.

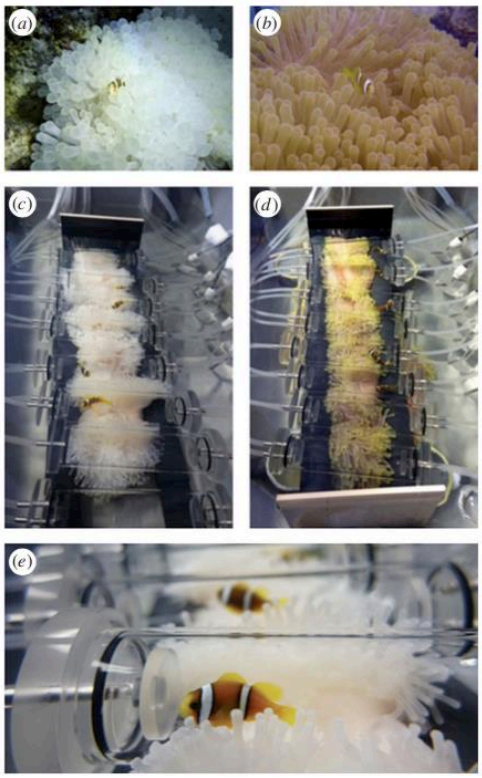

Figure 1. Photographs of juvenile orange-fin anemonefish (*Amphiprion chrysopterus*) in a (*a*) bleached or (*b*) unbleached anemone in the wild, and in the respirometry set-up used to measure the metabolic rates of the anemonefish in either (*c,e*) bleached or (*d*) unbleached anemones.

Fish $\dot{M}_{O_2}$ was calculated by multiplying the slopes (over 3–5 min) for the decline in oxygen inside the respirometry chambers during the closed phases of the respirometry cycles by the volume of the respirometry chamber after subtracting fish volume and background bacterial respiration (calculated from a respirometry chamber without fish). The standard metabolic rate ($\dot{M}_{O_{2,min}}$) of each individual fish was determined as the 0.25 quantile of the $\dot{M}_{O_2}$ data obtained over the entire respirometry trial [15]. One fish had to be excluded from the $\dot{M}_{O_2}$ data analyses because a detached piece of anemone had got sucked into the respirometry chamber at some point during the overnight measurements (fish 4 in figure 2), making the $\dot{M}_{O_2}$ recordings unreliable. As $\dot{M}_{O_{2,min}}$ primarily occurred during the night when the fish were resting, we also calculated day-time $\dot{M}_{O_2}$ as the mean of each fish's $\dot{M}_{O_2}$ values from 7:00 (coinciding with an increase in $\dot{M}_{O_2}$ as room lights came on) and until the respirometry trials were stopped later in the morning (figure 2).

As any differences in metabolic rates between fish would affect their oxygen demands while residing in their anemone hosts, we also measured the environmental oxygen availability among the tentacles of bleached and unbleached anemones in the field at both day (approx. 11:30; $n = 5$ bleached, $n = 10$ unbleached) and night (approx. 04:50; $n = 11$ bleached, $n = 18$ unbleached). Measurements were taken across two days in January 2018 using an oxygen probe (ProPlus multiparameter instrument; YSI Inc., Yellow Springs, USA) inserted between the anemone tentacles.

All statistical analyses were performed in R [16] as follows. Differences in $\dot{M}_{O_2}$ and activity between treatment groups were evaluated from linear mixed effect (LME) models (lme4 package) with either $\log_{10}$-transformed whole-animal $\dot{M}_{O_2}$ or $\log_{10}$-transformed activity as the response variable, trial (i.e. first round of measurements versus second round of measurements 2 weeks later), treatment, and $\log_{10}$-transformed body mass as the predictor variables (fixed effects), and fish ID and year of experiments (only for $\dot{M}_{O_2}$) as random effects. Differences in growth were evaluated from an LME with $\log_{10}$-transformed body mass gain as the response variable, $\log_{10}$-transformed initial body mass and treatment as fixed effects, and year as a random effect. Differences in oxygen availability among the anemone tentacles were evaluated from a linear model (LM) with oxygen as the response variable, and time (day versus night), treatment and site as predictor variables, including an interaction between time and treatment. Assumptions of homoscedasticity and normality of residuals were confirmed by visual inspection of residual-fit plots. Statistical significance (p-values) in LME models was evaluated from the lmerTest package, which uses Satterthwaite approximations of degrees of freedom.

Although overall mean ($\pm$s.e.) body mass was similar between treatment groups (bleached = 0.821 $\pm$ 0.183 g, unbleached = 0.836 $\pm$ 0.181 g), individual fish differed in mass, which (as expected) had a strong effect on both $\dot{M}_{O_{2,min}}$ (LME, effect of body mass: $t = 17.581$, d.f. = 12.965, $p < 0.0001$) and day-time $\dot{M}_{O_2}$ (LME, effect of body mass: $t = 12.844$, d.f. = 13.508, $p < 0.0001$). Each of the 77–99 $\dot{M}_{O_2}$ recordings for each fish was therefore adjusted to the overall mean fish body mass of 0.829 g (for graphical presentation in figure 2) using the calculation $\dot{M}_{O_{2,adjusted}} = \dot{M}_{O_{2,measured}} \cdot (M_{measured}/M_{mean})^{(1-b)}$, where M is body mass and b is the mean of the mass-scaling exponents for $\dot{M}_{O_{2,min}}$ (0.694) and day-time $\dot{M}_{O_2}$ (0.696), which was 0.695. $\dot{M}_{O_{2,min}}$ and day-time $\dot{M}_{O_2}$ are presented as model-derived estimates in both the text and in figure 3. Body mass had no effect on activity ($p = 0.781$) and these results are presented as raw (measured) values. Oxygen levels among the anemone tentacles are also presented as measured values.

3. Results

Estimated standard metabolic rate ($\dot{M}_{O_{2,min}}$) of the anemonefish was elevated in 12 out of the 15 fish in bleached anemones (figures 2 and 3), which led to $\dot{M}_{O_{2,min}}$ being significantly higher by 8.2% when living in bleached (0.335 $\pm$ 0.006 mg O_2 h^{-1}, mean $\pm$ s.e.) as compared with unbleached (0.310 $\pm$ 0.007 mg O_2 h^{-1}) anemones (LME, effect of treatment: $t = 2.986$, d.f. = 12.932, $p = 0.011$; figure 3). Day-time $\dot{M}_{O_2}$ did not differ between fish from bleached (0.495 $\pm$ 0.019 mg O_2 h^{-1}) and unbleached (0.480 $\pm$ 0.018 mg O_2 h^{-1}) treatments (LME, effect of treatment: $t = -0.982$, d.f. = 12.822, $p = 0.344$). Fish activity also did not differ between bleached (549 $\pm$ 43 line crossings, mean $\pm$ s.e.) and unbleached (599 $\pm$ 94 line crossings) treatments (LME, effect of treatment: $t = -0.090$, d.f. = 16.000, $p = 0.929$). The effect of trial (i.e. whether the fish were with bleached or unbleached anemones first) was not significant for $\dot{M}_{O_{2,min}}$ ($p = 0.860$), day-time $\dot{M}_{O_2}$ ($p = 0.359$) or activity ($p = 0.072$).

Fish residing on bleached anemones gained 0.088 $\pm$ 0.018 g in body mass, while fish from unbleached anemones gained 0.148 $\pm$ 0.020 g (means $\pm$ s.e.) over the 2 weeks. However, the seemingly lower growth of fish from bleached anemones did not reach significance (LME, effect of treatment: $t = 1.770$, d.f. = 13.000, $p = 0.100$).

Oxygen availability among the tentacles of bleached and unbleached anemones decreased from 105.5 $\pm$ 2.0 and 108.9 $\pm$ 1.9% air saturation during the day to 74.5 $\pm$ 0.5 and 72.5 $\pm$ 1.0% air saturation at night, respectively. The

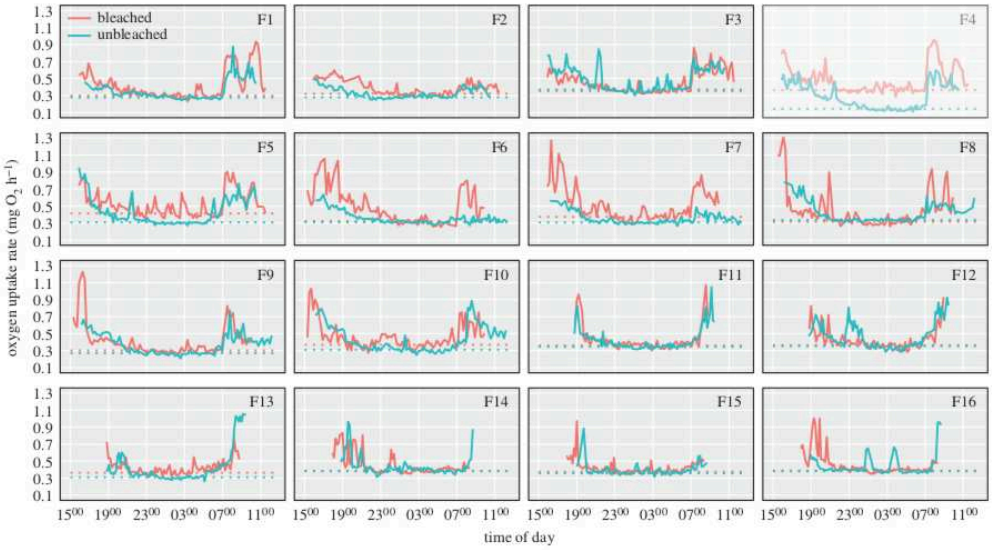

Figure 2. Body-mass-adjusted oxygen uptake rate ($\dot{M}_{O_2}$) traces for the overnight respirometry trials for the 16 anemonefish from bleached or unbleached anemones. Traces are based on individual $\dot{M}_{O_2}$ recordings (data points omitted for clarity) collected every 9–12 min depending on fish and respirometry chamber size. Dotted horizontal lines represent $\dot{M}_{O_{2,min}}$. Fish 4 (F4) was excluded from further data analyses (i.e. not included in the linear mixed effects models) because a detached piece of anemone had got sucked into the respirometry chamber at some point during the unbleached trial. Data for fish 1–10 are from 2016, while data for fish 11–16 are from 2017.

difference between day and night was the same for bleached and unbleached anemones (LM, time × treatment interaction: $t = 1.438$, d.f. = 38, $p = 0.159$) but, overall, night-time oxygen levels were significantly lower than day-time levels (LM, effect of time: $t = -14.193$, d.f. = 38, $p < 0.0001$).

4. Discussion

Anemonefish living with bleached host anemones had significantly higher $\dot{M}_{O_{2,min}}$ compared with fish from unbleached anemones, which is probably reflective of an autonomic stress response in fish exposed to bleached anemones [10]. This suggests that fish associated with bleached anemones are at an energetic disadvantage and may experience decreased growth rates as observed previously for fish with elevated $\dot{M}_{O_{2,min}}$ [17]. In the present study, fish exposed to unbleached anemones had growth rates that were 68% higher than when they were with bleached anemones, although this difference was not significant ($p = 0.100$). Activity levels did not differ between anemonefish from bleached and unbleached anemones, and this finding is corroborated by day-time $\dot{M}_{O_2}$ which also did not differ between treatments. In the wild, however, anemonefish with a higher $\dot{M}_{O_{2,min}}$ may be able to compensate for this elevated baseline energy demand with increased foraging activity, but this would also expose them to a greater risk of predation. Increased predation risk may be further exacerbated by the shrinking of anemones when bleached [9] due to reduced shelter area. Overall, the results here suggest that residing in bleached anemones may exacerbate the classical trade-off between foraging and growth on the one hand and predation risk on the other in juvenile anemonefish. The elevated $\dot{M}_{O_{2,min}}$ may also manifest as trade-offs with other life-history and fitness traits. Indeed, high basal or standard metabolic rate (i.e. elevated $\dot{M}_{O_{2,min}}$) has been linked to reduced reproductive success and reduced survival in a range of animal species, including fish [18–20]. This could explain the decreased fecundity and spawning frequency observed elsewhere for anemonefish associated with bleached anemones [9,10].

The higher oxygen demands of fish living with bleached anemones could also make the anemonefish more susceptible to aquatic hypoxia, if environmental oxygen levels dropped enough to affect the fish's $\dot{M}_{O_{2,min}}$. Night-time oxygen levels within the branches of coral colonies can decrease below 20% air-saturation on tropical reefs [21], but our measurements of oxygen levels among the anemone tentacles suggest that night-time reductions are modest. Despite this, the intermittent occurrence of hypoxic 'dead zones' on coral reefs has recently been reported to be widespread [22], suggesting that coral reef fish do experience reduced oxygen availability on a regular basis.

Sensing of bleached versus unbleached anemones could occur through visual, tactile or olfactory cues in the wild or in the fish's holding aquaria, while the anemonefish were able to use either visual or olfactory cues while in the respirometry chambers. Previous studies have shown that olfactory cues are pivotal for anemonefish recognition of their anemone host, and that anemonefish can distinguish between bleached and unbleached anemones through olfaction alone [23–25]. Despite this, anemonefish appear to settle on bleached anemones as much as on unbleached anemones [9]. This could be due to limited anemone (i.e. habitat) availability or simply because bleached anemones are encountered by larval anemonefish at the same rate as unbleached anemones during a bleaching event. It is also possible that, despite there being a fitness disadvantage to choosing a

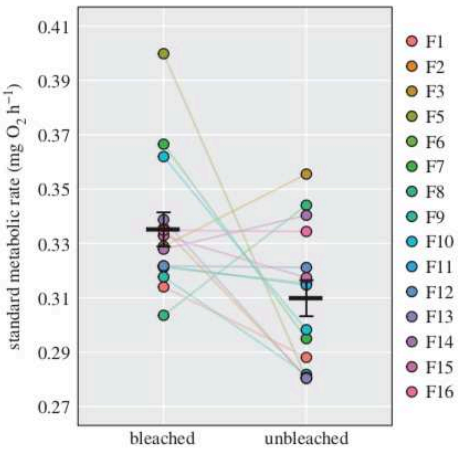

Figure 3. Body-mass-adjusted standard metabolic rates ($\dot{M}_{O_{2,min}}$) of the 15 anemonefish (F4 is excluded) after the two 2-week periods of living with either bleached or unbleached anemones. Mean $\dot{M}_{O_{2,min}}$ for each treatment is presented as wide horizontal black bars with black error bars (± 1 s.e.), while $\dot{M}_{O_{2,min}}$ values for each fish are presented as coloured circles with lines connecting the two data points from each fish.

bleached anemone, bleaching episodes are not frequent enough (currently) for selection to act on habitat choice; spending more time in search for a healthy host would increase predation risk, so settling quickly may outweigh the negative consequences of choosing a bleached host.

The anemonefish in our experiment were exposed to bleached anemones for only 2 weeks, while anemones remained bleached for 2–5 months during the 2016 bleaching event in Moorea [10]. It is possible that anemonefish exposed to bleached anemones over a longer period will acclimate in terms of their metabolic and stress responses. Another species of coral reef fish has been reported to show some capacity for developmental and trans-generational acclimation of metabolic rate to elevated temperatures [26,27], so acclimation to host bleaching may be possible but remains to be tested.

Our study evaluated the metabolic effect of host anemone bleaching at a temperature (28.6°C) equivalent to the current summer average sea surface temperature at the study location [10]. Future temperature increases will not only increase the chance of bleaching but also exert a direct effect on fish metabolic rate and hormonal stress levels [28]. As metabolic rate typically increases exponentially with temperature, it is likely that the combined stressors of bleaching and elevated temperature may increase the metabolic demands of future anemonefish far more than the 8.2% increase observed here for $\dot{M}_{O_{2,min}}$. Increasing carbon dioxide levels (i.e. ocean acidification) may add an additional stressor and elevate metabolic rate of anemonefish even further [29], although this may be relieved, to some extent, by trans-generational acclimation [29]. The combined effects of ocean acidification and elevated temperature can also cause disruptions to foraging behaviour and food consumption in juvenile anemonefish [30]. In addition, solar radiation may be a better discriminator of bleaching for inshore reefs [31] and, in a similar manner to temperature (and ocean acidification), may exert a direct effect on fish physiology, although this requires further studies.

Finally, our results show, for the first time, a causal link between host bleaching and a fish's increased energetic demand, adding to the accumulating evidence showing negative consequences of bleaching events, and of climate warming in general. In coral reef ecosystems, there are a considerable number of fish—as well as invertebrates—that strictly depend on hosts amenable to bleaching, such as corals and anemones [10]. The effects of bleaching on metabolic rate shown here for anemonefish may extend to a considerable number of other organisms and alter the trade-offs in such symbiotic associations. If predictions of future temperature regimes hold, mass bleaching is expected to occur every 1–3 years [1,3], which is within the lifespan of most coral reef fish. Selection on individuals that are able to acclimate should therefore increase, but whether or not this allows coral reef organisms to persist is unknown.

Ethics. Approval was granted from the institutional animal ethics committee at CRIOBE (permit no. 006725).

Data accessibility. Data are available in the electronic supplementary material.

Authors' contributions. S.C.M. and R.B. conceived the study and provided research facilities. T.N., S.C.M., R.B. and S.S.K. designed the experiments, and T.N. and S.S.K. provided experimental equipment. T.N., A.C. and D.C. conducted the experiments, and T.N., R.B. and S.C.M. analysed the data. T.N. drafted the manuscript and all authors revised it.

Competing interests. The authors have no competing interests.

Funding. T.N. was funded by the Danish Council for Independent Research (Individual Post-doctoral Grant and Sapere Aude Research Talent Grant; DFF-4181-00297). S.S.K. was supported by Natural Environment Research Council Advanced Fellowship NE/J019100/1 and European Research Council starting grant 640004. S.C.M., R.B. and D.C. were supported by an Agence National de la Recherche grant (ANR-14-CE02-0005-01/Stay or Go) and S.C.M. from the Contrat de Projets Etat - Polynésie Française and Air Tahiti Nui.

Acknowledgements. We thank Zoe Scholz, Tara Cousins and Clara Diaz for their assistance with fish and anemone collection or oxygen measurements, as well as Gilles Siu and Pascal Ung for their help at CRIOBE. We also thank Laetitia Hedouin for use of her underwater fluorometer (diving PAM) for measuring anemone photosynthetic activity.

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Appendix 2. Manuscript: Physiological and behavioural effects of anemone bleaching on symbiont anemonefish in the wild

Received: 16 June 2020 | Accepted: 17 November 2020
DOI: 10.1111/1365-2435.13729

RESEARCH ARTICLE

Functional Ecology

Physiological and behavioural effects of anemone bleaching on symbiont anemonefish in the wild

Daphne Cortese[1] | Tommy Norin[2,3] | Ricardo Beldade[1,4] | Amélie Crespel[2] | Shaun S. Killen[2] | Suzanne C. Mills[1,5]

[1]PSL Université Paris: EPHE-UPVD-CNRS, USR 3278 CRIOBE, Papetoai, Moorea, French Polynesia

[2]Institute of Biodiversity, Animal Health and Comparative Medicine, University of Glasgow, Glasgow, UK

[3]DTU Aqua: National Institute of Aquatic Resources, Technical University of Denmark, Lyngby, Denmark

[4]Estación Costera de Investigaciones Marinas, Departamento de Ecología, Facultad de Ciencias Biológicas, Pontificia Universidad Católica de Chile, Santiago, Chile

[5]Laboratoire d'Excellence "CORAIL", France

Correspondence
Daphne Cortese
Email: daph.cortese@gmail.com

Funding information
Agence National de la Recherche, Grant/Award Number: ANR-14-CE02-0005-01/Stay or Go; Natural Environment Research Council, Grant/Award Number: NE/J019100/1; Danish Council for Independent Research, Grant/Award Number: DFF-4181-00297; European Union's Horizon 2020

Handling Editor: Diego Barneche

Abstract

1. Climate change causes extreme heat waves that have induced worldwide mass coral bleaching. The impacts of temperature-induced bleaching events on the loss of algal endosymbionts in both corals and anemones are well documented. However, the cascading impacts of bleaching on animals that live in association with corals and anemones are understudied.
2. We performed a field-based experiment to investigate how host anemone bleaching affected the metabolic rate, growth, behaviour and survival of wild juvenile orange-fin anemonefish *Amphiprion chrysopterus* over 1, 2 and (for survival) 9 months.
3. We found that the standard metabolic rate of anemonefish residing in bleached anemones decreased over time but was unaffected in fish from healthy anemones. Despite the reduced metabolic cost, the growth rate of fish from bleached anemones was significantly lower compared to fish from healthy anemones, suggesting that animals residing in bleached hosts are at an energetic disadvantage. This was corroborated by our finding that fish from bleached anemones spent more time out of their anemones, suggestive of a greater need to forage in the water column. However, fish from bleached anemones were overall less active and used less space around the anemone, resulting in a negative correlation between space use and survival after 4 weeks.
4. Our results provide insight into the physiological and behavioural effects of host bleaching on juvenile fish in the wild, and highlight how relatively short-term thermal anomalies can have long-lasting impacts beyond the bleached anemones or corals themselves.

KEYWORDS
acclimation, climate change, coral reef fish, environmental stressor, growth rate, standard metabolic rate, survival, temperature

1 | INTRODUCTION

Anthropogenic climate change and extreme weather events are global phenomena that impact terrestrial and aquatic organisms. The increasing frequency and duration of global episodes of coral reef bleaching (Hughes et al., 2017) are testament to the severity of climate-driven impacts on coral reef ecosystems. Corals are not alone, as other marine invertebrates, including sea anemones, also bleach in response to environmental stressors (McClanahan et al., 2009). If these organisms survive, the temporary loss of

Daphne Cortese and Tommy Norin contributed equally.

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endosymbiotic dinoflagellates can last several months before recovery (Jones, 1997; Lang et al., 1992; Saenz-Agudelo et al., 2011; Beldade et al., 2017). As such, short-term climatic stressors such as transient heat waves lasting from days to weeks (Glynn, 1996; Oliver et al., 2018) are outlived by the ensuing longer-term bleaching episodes. The impacts of these bleaching events may cascade onto other animals associated with corals and anemones for either shelter, foraging, or recruitment (Graham et al., 2007; Jones & Syms, 1998; Wilson et al., 2006). However, the physiological and behavioural impacts of bleaching on associated species, such as coral reef fish, have rarely been tested.

Fishes are essential components of coral reef ecosystems, due to their role in reef health, diversity, resilience and local economy (Bellwood et al., 2004; Graham et al., 2006; Moberg & Folke, 1999). Bleaching-induced coral mortality can drive fish mortality due to the degradation and loss of suitable habitats (Bonin et al., 2009), but recent evidence has shown that temporary bleaching of anemones also impacts the physiology and reproduction of associated anemonefish (Beldade et al., 2017; Norin et al., 2018). To cope with prolonged bleaching, fish may need to adjust their physiology and behaviour through phenotypic plasticity (e.g. acclimation) to enhance fitness while living in a bleached environment. For example, bleaching-associated decreases in anemonefish food sources (e.g. plankton in the water column, anemone waste products and symbiont algae; Piontkovski & Castellani, 2009; Tada et al., 2003; Verde et al., 2015) may require anemonefish to alter their behaviour to maximise foraging, or to adjust their energy expenditure to compensate for reduced food intake (Dill, 1983; Höjesjö et al., 1999). As fish associated with bleached (white) hosts are visually more conspicuous (Coker et al., 2009), the risk of predation potentially increases too, which may reduce foraging and other behaviours outside of the anemone (Lima & Dill, 1990). The previously observed increased metabolic demands of anemonefish after two weeks of bleaching in the laboratory (Norin et al., 2018) may also trade-off with growth due to competition for a finite energy budget (energy allocation trade-off; Weiner, 1992). However, to what extent these behavioural and physiological adjustments occur in nature remains unknown.

Here, we quantify the effects of varying bleaching exposure durations on a suite of life-history traits of anemonefish living in the wild. We conducted an extensive field-based study with wild site-attached juvenile orange-fin anemonefish *Amphiprion chrysopterus* transplanted onto healthy (unbleached) or bleached anemones in the lagoonal reef system of Moorea, French Polynesia. Anemonefishes live in a permanent obligatory mutualistic symbiosis with sea anemones (Fautin, 1991) and remain with the host anemone on which they settled as juveniles (Buston, 2003a; Hattori, 1994), even if it bleaches (Saenz-Agudelo et al., 2011), making them especially vulnerable to bleaching and an ideal model species (Beldade et al., 2017; Mills et al., 2018, 2020). We compared standard (resting) metabolic rate (SMR), behaviour (activity and space use) and growth rate of anemonefish after 4 and 8 weeks of living with unbleached or bleached anemones, and tracked the fish's survival for 9 months. These exposure durations are ecologically relevant both to bleaching and anemone recovery (Jones, 1997; Lang et al., 1992), and sufficient to observe the potential for acclimation (Fangue et al., 2014; Sandblom et al., 2014). We also explored correlations among the measured traits, as environmental disturbances are known to alter the relationship between physiological and behavioural traits (Killen et al., 2013).

2 | MATERIALS AND METHODS

2.1 | Experimental overview

Magnificent sea anemones *Heteractis magnifica* ($n = 53$) and juvenile orange-fin anemonefish *Amphiprion chrysopterus* ($n = 47$) were collected in the northern lagoon of Moorea, French Polynesia (17°32′19.8″S, 149°49′46.3″W) from September to November 2017.

Unbleached anemones were collected 2 to 3 weeks before the fish, during which time about half of the anemones were thermally induced to bleach in the laboratory at the CRIOBE research centre (see 'Host anemones' below). Two days before placing fish onto them, unbleached and bleached anemones of similar size were individually placed back into the field in 47 cylindrical cages (60 × 40 cm) randomly distributed across four sites in Moorea's northern lagoon (Figure 1). Environmental conditions (water flow, water temperature and depth) were measured at the sites (Table S1) consisting of a sandy flat, such that all cages were 5 m away in any direction from any larger coral structures ('bommies'). Cages were used to prevent anemone predation from, for example, turtles, and were placed at least 10 m apart.

Anemonefish were caught from unbleached anemones in the lagoon (there was no bleaching at this time) and placed individually into the 47 cages with either an unbleached ($n = 26$) or a bleached ($n = 21$) anemone.

The metabolic rate, growth rate and behaviour (see individual sections below) of the anemonefish were measured approximately 4 weeks (26–29 days; Table S2A) after residing in unbleached or bleached anemones, and a random subset of individuals ($n = 23$) were measured a second time after approximately 8 weeks (46–65 days; Table S2A), with no difference in exposure time between treatments for either exposure period (Table S3). While the experimental exposures to unbleached or bleached anemones varied around the target 4 and 8 weeks, we will, for simplicity, refer to the two measurement points as 'Week 4' and 'Week 8'. The first day of the experiment, when fish were placed onto anemones, is referred to as 'Week 0'.

Anemonefish survival and anemone bleaching status were monitored weekly over the first 8 weeks and then every 2 weeks over a further 7 months. At the end of the ninth month of monitoring, all anemonefish were returned to the un-caged host anemone from which they were originally collected, and anemones were returned to anemone patches in the lagoon.

Ethical approval for the study was granted from The Animal Ethics Committee, Centre National de la Recherche Scientifique (permit number 006725).

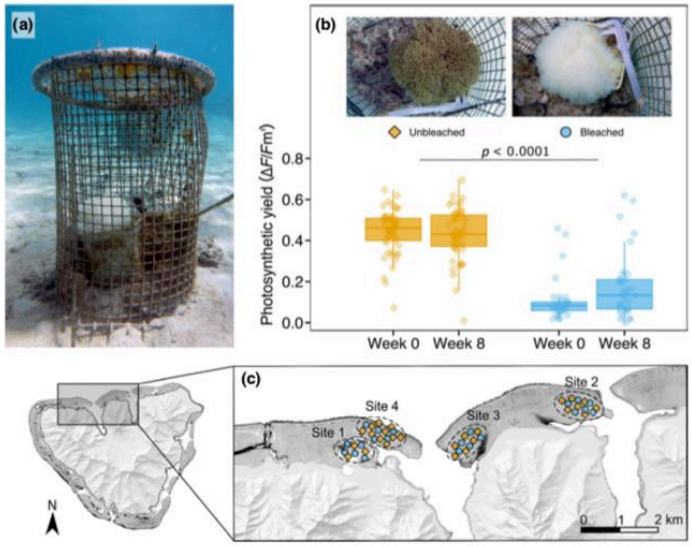

FIGURE 1 In situ experimental photographs and study sites. (a) Experimental set-up with a bleached anemone and a juvenile orange-fin anemonefish *Amphiprion chrysopterus* inside a cage (3 × 3 cm mesh size). The anemonefish could freely swim in and out of the cage through the mesh. The cage was used to prevent anemone predation and secured in place with steel rods. (b) Photosynthetic yield in unbleached and bleached anemones at the start (Week 0) and after 8 weeks (Week 8). ΔF is the difference between Fm' (maximum fluorescence yield) and F (fluorescence yield of a dark reference). The photos show the top-view from within a cage containing an unbleached (left) and a bleached (right) anemone, each with a juvenile anemonefish and 20-cm long callipers included for scale. (c) Map of the island of Moorea with an insert of the northern lagoon (17°32′19.8″S, 149°49′46.3″W) showing the location of the two treatments (unbleached and bleached anemones) distributed across four sites

2.2 | Host anemones

Anemones (containing no anemonefish) were collected by hand while SCUBA diving at depths of 3–6 m and brought to the CRIOBE laboratory in coolers filled with water from the collection sites. In the laboratory, the anemones were kept in aquaria receiving flow-through water from the lagoon and fed brine shrimp daily. About half were maintained at 28°C for 2 weeks and remained unbleached. The others were bleached by heating the aquaria water to 31°C for 2 weeks.

Once positioned in the field, anemone bleaching status was ascertained visually on a weekly basis and confirmed by measuring photosynthetic activity at Week 0 and Week 8 of the experiment. Photosynthetic activity in the anemones was measured in triplicate using an underwater diving pulse amplitude modulated fluorometer (DIVING-PAM; Heinz Walz GmbH). All the anemones from one site were measured on the same night, and measures were taken from all sites between 17:12 and 17:37. Photosynthetic activity was significantly higher in unbleached than bleached anemones both at the beginning of the experiment and after 8 weeks (linear mixed-effects models, LME: Treatment; $t = 11.793$, $p < 0.0001$; Time; $t = 1.000$, $p = 0.324$, $mR^2 = 0.583$, $cR^2 = 0.712$, Figure 1b; Table S4B). Anemone size (surface area) was measured at Weeks 0, 4 and 8 (details in Supporting Information).

Three out of the 21 bleached anemones started to show signs of recovery after 4 weeks and were replaced with other bleached anemones of approximately the same size. As replacing a recovering bleached anemone may disturb associated anemonefish, to ensure equal disturbance between treatments, we correspondingly replaced a healthy anemone at the same site with another healthy anemone of approximately the same size.

2.3 | Anemonefish

Anemonefish were caught by hand-netting while free or SCUBA diving between September and November 2017 (Table S2), transferred in water-filled coolers to CRIOBE's aquarium facilities, weighed and, on the same day, returned to the lagoon onto one of the caged anemones, with no significant difference in start date between treatments (Table S3). Fish were released within ~10 cm of the caged anemone and always swam straight to the anemone and hid within its tentacles.

Natural recruitment of *A. chrysopterus* is limited in Moorea (Beldade et al., 2012, 2016; Schmitt & Holbrook, 2000) and post-settlement mortality is high due to intraspecific aggression (Buston, 2003a). We therefore maintained the number of *A. chrysopterus* juveniles at one individual per anemone throughout the experiment, and any natural recruits (smaller in size than our focal fish) were noted and removed. *Dascyllus trimaculatus* also use anemones during a part of their life cycle (O'Donnell et al., 2017), however, they

are subjected to heterospecific aggression from *A. chrysopterus* (Mills et al., 2020), which depresses their density and survival (Holbrook & Schmitt, 2004; Schmitt & Holbrook, 1996), but their presence has little to no effect on anemonefish (Schmitt & Holbrook, 2000). Nevertheless, to control for any aggression, growth or density-dependent effects on focal experimental *A. chrysopterus*, all natural recruits of *D. trimaculatus* were removed.

Focal *A. chrysopterus* were monitored weekly. Even though the anemonefish were able to move through the netting of their cages, the distance between cages (>10 m) and high predation risk outside the anemone tentacles (Buston, 2003a; Elliott et al., 1995; Mariscal, 1970), especially for juveniles (Buston & Garcia, 2007), made the chance of fish exchanging anemone host unlikely. Nevertheless, to confirm individual identification during the experiment, focal fish were photographed and identified by colour patterns based on the second and third vertical white stripes (example photos in Figure S1).

2.4 | Metabolic rate

At the end of Week 4 and 8, the anemonefishes were caught and transferred by boat in a water-filled cooler to CRIOBE's aquarium facilities. The fish were initially held with unfed unbleached or bleached anemones (as per their respective treatments in the field) for ~20 hr in aquaria receiving filtered, flow-through water from the lagoon. This was done to ensure that the fish were not digesting during subsequent metabolic rate measurements. Each individual fish was then transferred to an intermittent-closed respirometry setup where its oxygen uptake rate could be recorded as a proxy for its metabolic rate (Svendsen et al., 2016).

The respirometry setup was shielded from surrounding disturbances and comprised a 40 L (water volume) tank receiving flow-through normoxic seawater from the lagoon at 29.16 ± 0.04°C (*M* ± *SE* temperature in the respirometry setup across all experiments), glass respirometry chambers (35 or 110 ml volume, depending on fish size) in which the oxygen uptake rate of the fish could be measured by use of fibre-optic oxygen meters with probes (FireStingO_2; Pyro Science GmbH) and accompanying software (Pyro Oxygen Logger; Pyro Science GmbH), a peristaltic pump (Masterflex L/S; Cole-Parmer) with gas-tight tubing that recirculated water through the respirometry chambers and past the oxygen probes and a set of flush pumps (EHEIM Compact; EHEIM GmbH & Co) which intermittently flushed fresh and fully aerated seawater through the respirometry chambers for 5 min in every 9 to 10 min intermittent-closed respirometry cycle (flush and close durations were adjusted based on chamber volumes and fish sizes). The respirometry chambers were supported by two plastic pipes in between which anemones from the respective treatment were placed such that their tentacles touched the bottom and sides of the respirometry chambers (photos in figure 1 in Norin et al., 2018). This allowed the fish to be surrounded by and see (but not touch) the anemones, and also receive olfactory cues from the anemones in the flush water. Fish were introduced to respirometry chambers a few minutes before the first automated oxygen uptake rate recordings were started in the afternoon and remained there for ~17 hr until the following morning. The fish were then removed from the respirometry chambers and their body mass recorded.

Fish oxygen uptake rates were calculated by multiplying the slopes (over 3–4 min) for the decline in oxygen inside the respirometry chambers during the closed phases of the respirometry cycles by the volume of the respirometry chamber after subtracting fish volume and background bacterial respiration (calculated from a respirometry chamber without fish). The SMR of each individual fish was then estimated by first calculating the mean of the lowest 10% of its oxygen uptake rate measurements from the ~17 hr respirometry trial, then excluding any outliers (data points outside the mean ± 2 *SD*s, or data points with r^2 values for the linear regressions of the decline in oxygen over time lower than the mean r^2 − 2 *SD*s), and finally re-calculating the mean of the remaining data points.

Due to electrical problems at the research station, some of the scheduled respirometry trials could not be completed, resulting in a reduced sample size for metabolic rate measurements (n = 15 and n = 14 fish from unbleached and bleached anemones, respectively, at Week 4, and n = 12 and n = 9 fish, respectively, at Week 8; Table S2A).

2.5 | Behaviour

A GoPro camera was placed on the top of each cage in the lagoon (~50 cm from the anemone) in the afternoon of an experimental day (between approximately 14:00 and 15:00), and 20 min videos were recorded. The first 10 min of these videos were discarded as the acclimation period (Nanninga et al., 2017), while the following 10 min were used to quantify: (a) *Time spent out of the anemone* – defined as the percentage difference between the total observation time and the time that at least 50% of the fish's body was within the anemone tentacles from observations of a fish's location every 3 s (n = 200 observations); (b) *Activity* – the total number of times a gridline was crossed per minute, calculated by digitally separating the video frame into a grid of 10 × 6 sections of equal size (Figure S2) and counting each time the fish crossed a gridline within three haphazardly selected periods of 30 s; and (c) *Space use* – the total number of unique squares a fish occupied per minute, calculated from the number of unique squares on the digital grid (Figure S2) that the fish occupied within three haphazardly selected periods of 30 s.

Due to time restrictions in the field, only a subset of the fish was filmed at Week 4 (n = 11 and n = 12 in unbleached and bleached treatments, respectively; Table S2A). At Week 8, 23 fish were filmed but three videos were unusable due to poor camera placement on the cage, resulting in n = 12 and n = 8 in unbleached and bleached anemones, respectively (Table S2A).

2.6 | Growth rate

Anemonefish were weighed to the nearest 0.001 g at the start of the experiment (Table S2A), at which point body masses were not

significantly different between treatment groups (Wilcoxon-test; $W = 209$, $p = 0.81$, $n = 40$). Fish were re-weighed at Week 4 ($n = 20$ and $n = 20$ in unbleached and bleached treatments, respectively; Table S2A) and Week 8 ($n = 13$ and $n = 9$ in unbleached and bleached treatments, respectively; Table S2A).

Specific growth rate (SGR) was determined as the percentage increase in individual body mass per day as $SGR = \ln(BM_{t_2}) - \ln(BM_{t_1}) \times t^{-1} \times 100$, where BM is body mass at t_2 (final time) and t_1 (initial time), and t is the time (days) between the two consecutive measures (Hopkins, 1992).

2.7 | Survival

Anemonefish survival (absence/presence) was recorded weekly over the first 8 weeks and every 2 weeks over the following 7 months. Given that juvenile anemonefish are unlikely to voluntarily leave their anemone due to risk of predation (Buston, 2003a; Elliott et al., 1995), the absence of a fish from its anemone was equated to mortality. Despite the use of cages, seven anemones (and thus also the anemonefish) disappeared during the nine months survival study, but these absent fish were not included in the survival analyses. In addition, non-natural mortality linked to electrical problems at the research station was also excluded from survival analyses (final $n = 29$, Table S2B).

2.8 | Statistical analysis

Statistical analyses were performed in R version 3.6.1 (R Core Team, 2019).

Linear mixed-effects models (LMEs) were used to explore the effect of the explanatory variables – anemone treatment (categorical), exposure time (continuous), fish body mass (continuous) and site (categorical) – on fish SMR, growth rate and behaviour. Fish ID (categorical) was included as a random effect to account for non-independence of data (i.e. when fish were measured twice over time). Time was included as a continuous variable since not all measurements were taken at exactly four or eight weeks but spread around these target time points for logistical reasons. All LMEs were fitted using the LMERTEST package (Kuznetsova et al., 2017), while marginal (mR^2) and conditional (cR^2) R^2 were obtained with the package PIECEWISESEM (Lefcheck, 2016). The selection of the best-fit model was determined using likelihood ratio tests (LRTs), starting from the most complex model and subsequently removing non-significant interactions and explanatory variables (Tables S6A, S7A, S8A, S9A and S10A) *via* the LMTEST package (Zeileis & Hothorn, 2002). In addition, SMR and fish body mass were $\log_{10}$-transformed to account for their nonlinear (power) relationship. The 'ns' function in the SPLINES package was used in the growth rate model to account for nonlinear body mass effects on fish growth. Space use was $\log_{10}$-transformed to alleviate non-normality. In each model, residuals were visually inspected to ensure that all assumptions were met. For graphical representation, regressions were fitted on predicted values obtained using the 'ggemmens' function in the GGEFFECT package (Lüdecke, 2018).

The consistency of each individual fish's behaviour across the three haphazardly chosen sections of 30 s video was evaluated by calculating the adjusted repeatability (R_{adj}) of activity and space use using the RPTR package (Stoffel et al., 2017). We calculated overall R_{adj} using the same model structure as for the LMEs (Table S5A), with chronological order of the three 30 s sections added as an additional explanatory variable. We also calculated R_{adj} for each anemone treatment separately by sub-setting the data for each treatment and removing treatment as a fixed effect in these models. Behaviour was significantly repeatable in all cases ($R_{adj} = 0.318$ to 0.464, $p \leq 0.0072$; Table S5B).

We tested for differences in survival between treatments using a Cox proportional hazard model *via* the 'coxph' function in the SURVIVAL package (Therneau, 2020; Therneau & Grambsch, 2000). Site was used as a covariate. Cox proportional hazard models calculate survival as the probability that an individual survives from the time origin (start of experiment) to a specified future time (end of experiment). To do this, the hazard function (risk of death over time) is used as a response variable.

Correlations among all traits were performed using Pearson's correlation coefficient or Spearman's rank correlation (parametric and nonparametric data, respectively). For each trait where fish body mass had an effect, body-mass-adjusted values were used for correlation analyses. Body-mass-adjusted values were obtained by calculating model partial residuals (i.e. fixing body mass and removing its partial effect) using the 'remef' function from the REMEF package (Hohenstein & Kliegl, 2020).

3 | RESULTS

3.1 | Physiology

The SMR of anemonefish varied over time depending on anemone treatment (bleached or unbleached, Figure 2), as indicated by the significant interaction between treatment and exposure time (LME: Time × Treatment; $t = 2.024$, $df = 45$, $p = 0.049$; $mR^2 = 0.904$, $cR^2 = 0.904$; Table S6B). SMR was the same between treatment groups after approximately 4 weeks of anemone exposure, but decreased in anemonefish from bleached anemones between 4 and 8 weeks of exposure (Figure 2).

3.2 | Behaviour

3.2.1 | Time spent out of the anemone

Anemone treatment had a significant effect on the time fish spent out of the anemone (LME: Treatment; $t = -2.946$, $df = 29.69$, $p = 0.006$; $mR^2 = 0.331$, $cR^2 = 0.592$; Table S7B), with fish from

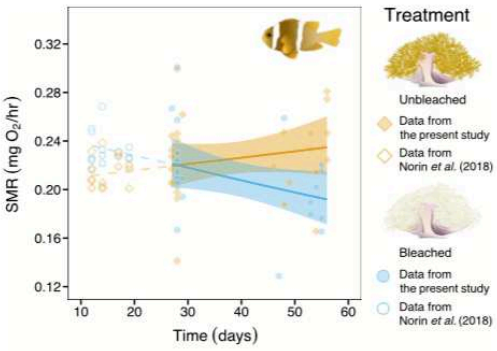

FIGURE 2 Standard metabolic rate (SMR) of *Amphiprion chrysopterus* juveniles residing in unbleached or bleached anemones over time. Solid orange and blue lines are model predicted regression lines fitted to the data from the present study, surrounded by 95% confidence bands. Data shown in open symbols at 12–19 days are from a previous laboratory study by Norin et al. (2018) and the regression lines have been extended to these data (dashed lines) for visual comparison only [i.e. the data from Norin et al. (2018) are not part of the fit]. The presented SMR data, including those from Norin et al. (2018), have all been adjusted (using model residuals) to the overall mean body mass of the fish from the present study (0.59 g), to allow direct comparisons between data [note that the SMR data from Norin et al. (2018) were adjusted to a body mass of 1 g in their figure 3]

bleached anemones spending 22.7% more time out of the anemone compared to fish from unbleached anemones (Figure 3a). In addition, the amount of time fish spent out of the anemone increased over time by 0.77% each day (LME: Time; $t = -3.893$, $df = 23.61$, $p < 0.001$; Figure 3a; Table S7B).

3.2.2 | Activity

Anemone treatment had a significant effect on fish activity (LME: Treatment; $t = 2.276$, $df = 38$, $p = 0.029$; $mR^2 = 0.339$, $cR^2 = 0.339$; Table S8B), with fish from unbleached anemones being more active than fish from bleached anemones (Figure 3b). Exposure duration did not have a significant effect on fish activity (LME: Time; $t = 1.795$, $df = 38$, $p = 0.081$; Table S8B), but larger fish tended to be more active than smaller fish (LME: Body mass; $t = 1.928$, $df = 38$, $p = 0.061$; Table S8B).

3.2.3 | Space use

Anemone treatment also had a significant effect on fish space use (LME: Treatment; $t = 2.495$, $df = 38$, $p = 0.017$; $mR^2 = 0.492$; $cR^2 = 0.492$; Table S9B), with fish from unbleached anemones using more space over and around the anemone than fish from bleached anemones (Figure 3c). Fish also increased space use over

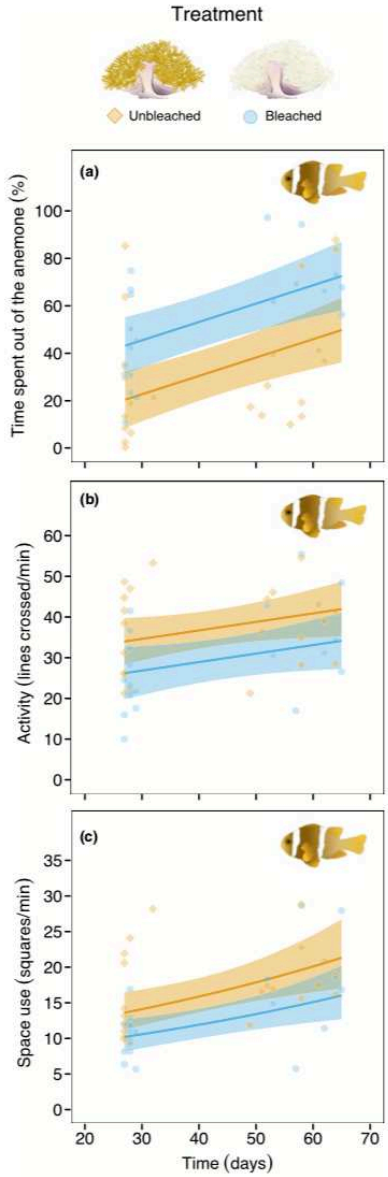

FIGURE 3 Behaviours of *Amphiprion chrysopterus* juveniles residing in unbleached or bleached anemones over time. (a) Time spent out of the host anemone, (b) movement activity and (c) horizontal space use above the anemone. Symbols represent individual raw data points in (a) while in (b) and (c) data points are adjusted (using model residuals) to the overall mean body mass of the fish from the present study (0.59 g), as body mass significantly affected activity and space use but not time spent out of the anemone. Lines are model predicted regression lines surrounded by 95% confidence bands

time by ~1.2% each day (LME: Time; $t = 3.027$, $df = 38$, $p = 0.004$; Table S9B). As expected, larger fish used more space around the anemone (LME: Body mass; $t = 2.627$, $df = 38$, $p = 0.012$; Table S9B).

3.3 | Growth rate

Anemone treatment had a significant effect on fish growth rate (LME: Treatment; $t = 4.047$, $df = 57$, $p < 0.001$; $mR^2 = 0.707$, $cR^2 = 0.707$; Table S10B), with fish of a given mass growing 0.85% per day faster when residing in unbleached than bleached anemones across the entire experiment (Figure 4a). Treatment exposure time had no significant effect on fish growth rate (LME: Time; $t = 1.217$, $df = 57$, $p = 0.229$; Figure 4a; Table S10B).

3.4 | Survival

Although survival appeared to diverge between treatments after 10 weeks (Figure 4b), there was no significant difference in survival between fish from unbleached and bleached anemones throughout the 9 months of treatment exposure (coxph model: Treatment; coef = −0.56, $z = -1.11$, $p = 0.268$, Table S11B). However, study

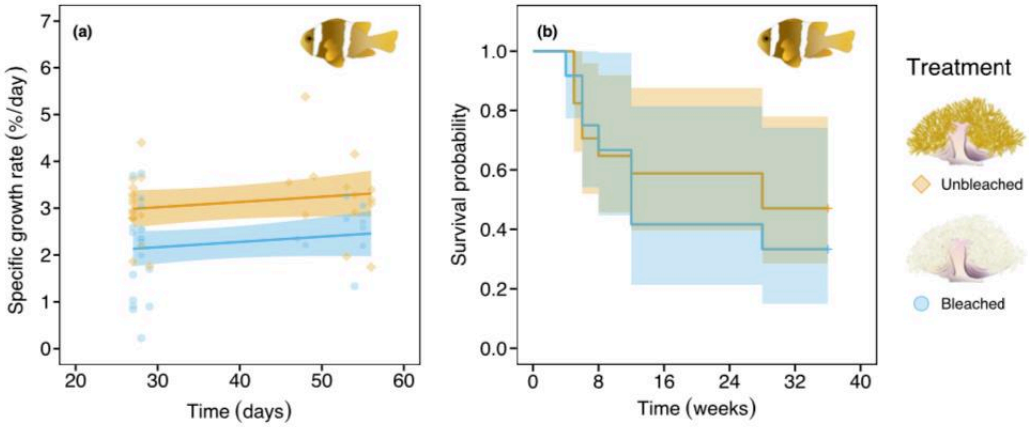

FIGURE 4 Growth and survival of *Amphiprion chrysopterus* juveniles residing in unbleached or bleached anemones over time. (a) Specific growth rate. Symbols represent individual data points adjusted (using model residuals) to the overall mean body mass of the fish used in the growth analyses at the initial time (t_1, 0.23 g). Solid orange and blue lines are model predicted regression lines fitted to the data, surrounded by 95% confidence bands. (b) Survival over 9 months (initial 8 weeks of the main experiment plus an additional 28 weeks of monitoring the fish in the same treatments). Solid orange and blue lines are survival probability curves, surrounded by 95% confidence bands

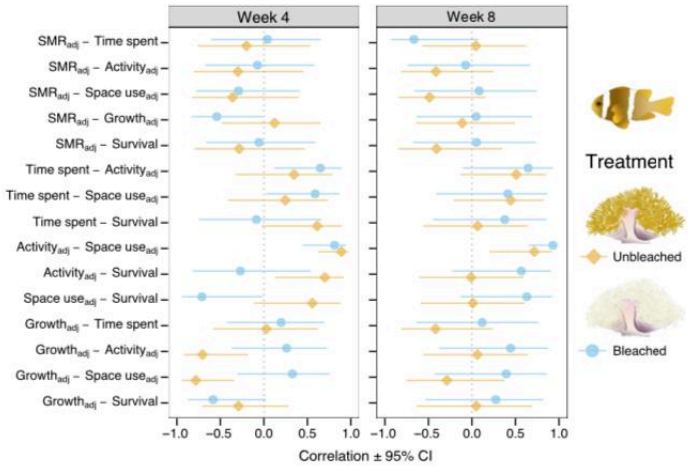

FIGURE 5 Correlations between all measured traits after 4 and 8 weeks of residing in unbleached or bleached anemones. Correlations (circles and diamonds) where the 95% confidence interval (horizontal error bars) does not cross zero (dashed vertical lines) are significant. Body mass significantly affected standard metabolic rate (SMR), activity, space use and growth rate, so body-mass-adjusted ($_{adj}$) values were used in correlations. For time spent (out of the anemone), raw values were used as fish body mass had no effect on this trait (Figure 3a). All test statistics are summarised in Table S12

site had an impact on fish survival with Site 3 showing higher survival probability compared to Sites 2 and 4 (coxph model: Site 3; coef = −1.66, $z = -2.31$, $p = 0.021$; Table S11B).

3.5 | Correlations among traits

Standard metabolic rate correlated negatively with growth rate after 4 weeks in bleached anemones (Figure 5). Behaviours correlated positively with each other: time spent out of the anemone was positively correlated with fish activity and space use after 4 weeks in bleached anemones, and activity and space use were highly positively correlated in both treatments after both 4 and 8 weeks (Figure 5). Activity was also positively correlated with survival in fish inhabiting unbleached anemones after 4 weeks, but did not correlate in fish from bleached anemones at either 4 or 8 weeks (Figure 5). Space use was negatively correlated with survival in bleached anemones after 4 weeks (Figure 5). Growth rate was negatively correlated with activity and space use in unbleached anemones after 4 weeks, but there were no significant correlations among these traits in fish from bleached anemones (Figure 5). All test statistics are summarised in Table S12.

4 | DISCUSSION

Our field-based experiment allowed us to test the indirect effects of climate change and warming-induced bleaching on wild coral reef fish in the absence of elevated temperature, while also exposing fish and anemones to natural variability arising from selection and environmental variables, including water current, food availability, solar radiation and inter- and intra-specific interactions (all small organisms <4 cm body depth could traverse the mesh cages). We found that bleaching induced a reduction in the fish's activity, a reduction in hiding as fish spent more time out of bleached anemones, a decrease in SMR over time and lower growth rates. However, despite these differences in fish behaviour and physiology, after 9 months there was no impact of host anemone bleaching on fish survival.

At first glance, our results for SMR appear to contradict a previous laboratory study showing that bleaching increased the metabolic demands of juvenile anemonefish after two weeks (Norin et al., 2018). However, the increased SMR observed by Norin et al. (2018) fits well with our SMR results when extrapolated back to a 2-week exposure period (Figure 2), despite the different experimental conditions (laboratory vs. field). Combined, these results suggest that anemonefish initially experience increased SMR in response to the short-term stressor of bleaching, but that metabolic rates decrease over a longer, and more ecologically relevant, exposure to bleaching. Reduced food intake is a likely contributing factor to the observed decrease in SMR, and is known to cause a reduction in SMR in other fish species (Auer et al., 2015; O'Connor et al., 2000; Van Leeuwen et al., 2012). The lower growth rate of anemonefish from bleached anemones further corroborates that reduced food intake is likely causing the decrease in SMR, which is also supported by the negative correlation between SMR and growth in fish from bleached, but not unbleached, anemones after 4 weeks.

The decrease in SMR over time in fish from bleached anemones suggests that individuals with a relatively high SMR were energetically disadvantaged, had little excess capacity for growth, and were forced to down-regulate their SMR. While a down-regulated SMR can be advantageous in food-limited environments (Auer et al., 2015; Metcalfe, 1998; Metcalfe et al., 1995; O'Connor et al., 2000), there is a limit to how much SMR can be reduced without affecting basic physiological functioning, and reduced tissue-level metabolic rates, as a consequence of reduced food intake, carry an oxidative cost in the form of harmful reactive oxygen species (Salin et al., 2018). The indication of a decreasing SMR up to 8 weeks of exposure to bleached anemones provides no evidence for acclimation (stabilisation) in SMR, unlike other studies on coral reef fish that have reported physiological and behavioural acclimation to the thermal stressor itself (Donelson et al., 2011). Therefore, our observed decrease in SMR between 4 and 8 weeks of bleaching is more likely to reflect a deteriorating condition rather than acclimation. An interesting next step would be to investigate if fish are able to back-regulate their metabolic physiology after anemones have recovered from bleaching. Moreover, we conducted our study on bleaching-naïve wild juveniles about 1-month-old and, as such, phenotypically plastic responses over multiple bleaching events remain to be explored.

The negative impact of bleaching is further supported by our behavioural data, as survival correlates positively with space use and activity in unbleached anemones (Figure 5), but negatively in bleached anemones. High activity and space use are likely important for foraging on planktonic prey in the water column and for territorial behaviour and competition with both con- and hetero-specifics (e.g. *D. trimaculatus*), behaviours that are all energetically costly (Barry, 2014; Biro & Stamps, 2010; Koteja, 2000; Schmitz, 2005; Yeates et al., 2007). Anemonefish receive their nutrients from plankton, whose density declines under thermally induced bleaching (Piontkovski & Castellani, 2009; Tada et al., 2003), and from anemone waste products and symbiont algae (Verde et al., 2015); both of these food sources may be reduced or lost entirely when anemones bleach. Under normal, unbleached conditions, the positive correlation between survival and behaviour (space use and activity) indicates that foraging and territoriality are balanced with food intake from the territory. However, during bleaching episodes, even though fish from bleached anemones spent more time out of their anemone (Figure 3a), they were less active and used less space around the anemone compared to fish from unbleached anemones (Figure 3b,c), with no indication that they maximised foraging or compensated energetically for the diminished food availability. The absence of energetic compensation can have detrimental outcomes (Brown & Kotler, 2004; Werner & Anholt, 1993), emphasised here by the negative correlation between survival and space use in bleached anemones. The energetic cost of spending more time outside the anemones might be balanced with other benefits, such as finding a better, unbleached habitat. However, the anemones were placed 10 m apart in our study and, as anemonefishes are sedentary species (Hattori, 1994), we did not observe any movement between cages.

The loss of symbiont algae and the likely decreased availability of waste products in bleached anemones, coupled with an absence of compensatory foraging, are two likely causes of the lower growth rate observed for fish from bleached anemones (Figure 4a). A third cause may be initially higher metabolic rates, which can occur 2 weeks after the onset of bleaching (Norin et al., 2018; Figure 2). Although fish with a higher SMR can digest and grow faster if enough food is available to cover their increased maintenance costs (Millidine et al., 2009; Reid et al., 2012), in the absence of any compensatory foraging (as our behavioural data suggest), the initially higher SMR may have impacted growth for the remaining 6-week exposure period through competition for a finite energy budget and an inability to catch up on growth later (Metcalfe & Monaghan, 2001). This is supported by the observed negative correlation between SMR and growth after 4 weeks in bleached anemones. In addition, the subsequent decrease in SMR of fish from bleached anemones may have further reduced their growth rate. Growth is an especially important trait in anemonefishes, as they live in size-dependent hierarchies, which determine the timing of sex change and reproductive status (Buston, 2003b; Fricke, 1979). The finding that bleached anemone hosts lower the growth of associated fish is therefore likely to have cascading and life-long consequences for individual anemonefish (in addition to reduced reproduction; Beldade et al., 2017), but also for other fish species associated with hosts that bleach. Indeed, a poor start in life during bleaching episodes, with lower growth and reduced size-at-age, results in smaller fish more likely to lose in competition for space and more vulnerable to predation (Arendt, 1997; Sogard, 1997).

Despite all the indications that host anemone bleaching affects the resident anemonefish negatively, bleaching did not significantly reduce anemonefish survival over 9 months. The use of cages (to reduce anemone predation) could have reduced natural predation on anemonefish by predators larger than the cage mesh size, especially considering that juveniles from bleached anemones spent less time in their anemone, which should render them more vulnerable to predation (Buston, 2003a; Elliott et al., 1995; Mariscal, 1970). Moreover, the bleached, white coloration of anemones enhances the visual contrast between anemone and fish, rendering the fish more visible to predators (Coker et al., 2009). However, our results corroborate those of previous natural field observations in which neither anemonefish densities nor adult survival were affected by bleached anemones (Beldade et al., 2017; Saenz-Agudelo et al., 2011).

Overall, the evidence of detrimental effects of bleaching, together with the strong habitat dependency of coral reef species (~12% of coral reef fishes live in symbiosis with hosts that bleach; Beldade et al., 2017) emphasises the importance of mitigating and regulating human actions that contribute to climate-induced bleaching events.

ACKNOWLEDGEMENTS

Financial support was provided by the Agence National de la Recherche to Glenn Almany, S.C.M., and R.B. (ANR-14-CE02-0005-01/Stay or Go) and to S.C.M. (ANR-11-JSV7-012-01/Live and Let Die), by Pacific Funds to S.C.M. and by LabEx 'CORAIL' to R.B. and S.C.M. ('Where do we go now?'). T.N. was supported by the Danish Council for Independent Research (DFF-4181-00297) and the European Union's Horizon 2020 research and innovation programme under the Marie Skłodowska-Curie grant agreement No. 713683. S.S.K. was supported by Natural Environment Research Council Advanced Fellowship (NE/J019100/1) and European Research Council starting grant (640004). We particularly thank Till Deuss for his support and help in the CRIOBE anemonefish aquarium facility. We thank Laetitia Hédouin for the use of the diving pulse amplitude modulated fluorometer. We are thankful to Paul Johnson for his suggestions on statistics. We are also thankful to Gilles Siu, Pascal Ung, Anne Haguenauer, Frederic Zuberer, Franck Lerouvreur and all the CRIOBE community for support in the field. We would like to express our gratitude to Glenn Almany (RIP) for his support and unwavering enthusiasm for this project.

CONFLICT OF INTEREST

The authors declare no conflicting interests.

AUTHORS' CONTRIBUTIONS

Designed the study: D.C., T.N., R.B., A.C., S.S.K. and S.C.M.; Collected the data: D.C., T.N., R.B., A.C., S.S.K. and S.C.M.; Analysed the data: D.C. and T.N.; Wrote the manuscript: D.C., T.N., R.B. and S.C.M.; Revised the manuscript: all authors.

DATA AVAILABILITY STATEMENT

Data and code are available on Zenodo: https://doi.org/10.5281/zenodo.4167473 (Cortese et al., 2020).

ORCID

Daphne Cortese https://orcid.org/0000-0002-5746-3378
Tommy Norin https://orcid.org/0000-0003-4323-7254
Ricardo Beldade https://orcid.org/0000-0003-1911-0122
Amélie Crespel https://orcid.org/0000-0002-6351-9008
Shaun S. Killen https://orcid.org/0000-0003-4949-3988
Suzanne C. Mills https://orcid.org/0000-0001-8948-3384

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SUPPORTING INFORMATION

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How to cite this article: Cortese D, Norin T, Beldade R, Crespel A, Killen SS, Mills SC. Physiological and behavioural effects of anemone bleaching on symbiont anemonefish in the wild. *Funct Ecol*. 2021;00:1–11. https://doi.org/10.1111/1365-2435.13729

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ABSTRACT

Environmental changes induced by human activities impact marine and terrestrial ecosystems worldwide. While facing these perturbations, species may respond in a variety of ways, including migration to a more suitable habitat via dispersal, acclimation via phenotypic plasticity or adaptation via genetic changes. The main opportunity for dispersal in sedentary marine organisms such as coral reef fish is the pelagic larval phase, during which time larvae journey in open water before settling onto a suitable habitat. Marine larval dispersal depends on physical factors, such as ocean currents, but is also largely determined by larval biological traits, such as physiology, behaviour and morphology. However, in which way offspring traits associated to dispersal are influenced by either their local environment or their parental legacy, remains less well studied. After larval settlement, phenotypes can still be affected by environmental stressors and thus it is vital to also understand their ability to acclimate to environmental stressors induced by human activities. To answer these questions, I used the orange fin anemonefish *Amphiprion chrysopterus* as a model species and combined *in situ* and laboratory analyses. First, by using laboratory reared larvae of wild spawned eggs I explored the influence of maternal size on dispersal-associated traits throughout ontogeny (swimming performance and body size) and demonstrated that larger mothers produce larvae that swim faster (Chapter 2). Then, I investigated how anemonefish parental environmental conditions, such as anemone habitat size (proxy of habitat quality and degradation) affect offspring phenotype (i.e. egg size, larval size and larval swimming performance) and found that parents living in larger habitats produce larger eggs, while parents living in degraded habitats produce smaller offspring but with better swimming abilities (Chapter 3). In addition, I explored the transgenerational and developmental effects of water flow regimes on larval and juvenile morphology, physiology and survival. Results show that early life survival can be affected by the water flow regime that their parents live in, suggesting differential investment (Chapter 4). In the last part of the thesis, I focused on the direct effects of chronic exposure to environmental stressors, such as anemone bleaching (Chapter 5) and artificial light at night (ALAN, Chapter 6) on juvenile *A. chrysopterus* phenotypes and survival in the wild. I demonstrated that anemone bleaching affects the physiology and behaviour of fish but not survival, suggesting that wild fish may trade-off growth with survival, even though reduced growth at early stages may have life-long fitness effects. ALAN had direct effect on fish survival and also affected its growth. The results of this PhD provide a better understanding of the origin of variability in the factors influencing the dispersal process as well as the post-settlement phenotype in an iconic coral reef fish. This knowledge may help to better predict how fish populations will respond to climate change. Such predictions are key to mitigating the impacts of climate change and improving conservation strategies, for example by targeting the most important marine areas and fish phenotypes to protect.

KEYWORDS

Environmental stressors, larval traits, phenotypic plasticity, coral reef fish, parental effects, survival

RÉSUMÉ

Les changements environnementaux induits par les activités humaines impactent l'ensemble des écosystèmes marins et terrestres de la planète. Les espèces peuvent faire face à ces perturbations de différentes manières, par exemple en se déplaçant vers des habitats plus adaptés (migration et dispersion), en s'acclimatant (plasticité phénotypique) ou en s'adaptant (changements génétiques). Chez les organismes marins sédentaires comme les poissons des récifs coralliens, la principale opportunité de dispersion se situe lors de la phase larvaire, durant laquelle les larves se développent en milieu océanique avant de revenir s'installer dans les habitats récifaux. Cette dispersion larvaire dépend de facteurs physiques, tels que les courants océaniques, mais est également largement déterminée par les traits biologiques des larves, tels que leur physiologie, comportement et morphologie. Cependant, l'influence de l'environnement et des traits biologiques des parents sur ces traits larvaires associés à la dispersion reste moins bien étudiée. De plus, les jeunes poissons peuvent aussi être affectés par les stress environnementaux après s'être installés dans les récifs, soulignant l'importance de mieux comprendre leur capacité à s'acclimater à ces stress. Pour répondre à ces problématiques, ma thèse utilise le poisson clown à nageoires orange *Amphiprion chrysopterus* comme espèce modèle, et combine analyses *in situ* et en laboratoire. En collectant des œufs d'*A. chrysopterus* dans l'environnement puis en élevant les larves qui en découlent, j'ai d'abord étudié l'influence de la taille maternelle sur les traits larvaires associés à la dispersion (vitesse de nage et taille). J'ai pu observer que les mères les plus grandes produisent des larves qui nagent plus vite (Chapitre 2). J'ai ensuite examiné comment les conditions environnementales liées au milieu parental, telles que la taille des anémone (proxy de la qualité et de la dégradation l'habitat parental), affectent le phénotype des jeunes poissons. J'ai ainsi constaté que les parents vivant dans des habitats plus grands produisent des œufs plus grands. Au contraire, les parents qui vivent dans des habitats dégradés produisent des œufs et larves plus petites mais qui nagent plus vite (Chapitre 3). De plus, j'ai exploré les effets transgénérationnels et développementaux de la courantologie sur la morphologie, la physiologie et la survie des larves et des juvéniles, et observé qu'un fort courant autour des anémones influence négativement la survie des juvéniles. Ce résultat suggère notamment que l'investissement parental peut dépendre des conditions environnementales (Chapitre 4). Dans la dernière partie de ma thèse, je me suis intéressée aux effets d'expositions chroniques à deux stress environnementaux, le blanchissement des anémones (Chapitre 5) et la présence de lumière artificielle la nuit (ALAN, Chapitre 6), sur le phénotype et la survie des juvéniles d'*A. chrysopterus* en milieu naturel. J'ai démontré que le blanchissement des anémones affecte la physiologie et le comportement des poissons mais pas leur survie. Bien que des compromis entre croissance et survie peuvent se mettre en place, une réduction de croissance durant ces premières phases du cycle de vie pourrait avoir des effets à plus long terme sur leur fitness. De plus, j'ai observé que l'exposition à ALAN affect la survie des poissons et réduit leur croissance. Ma thèse permet donc de mieux comprendre comment et quels sont les facteurs et stress influençant le processus de dispersion et le phénotype post-installation chez un poisson emblématique des récifs coralliens. Ces connaissances aident à mieux prédire comment les populations de poissons réagissent aux changements climatiques. De telles prévisions sont essentielles aux stratégies de conservation, et permettent de cibler les zones marines et les phénotypes des poissons les plus importants à protéger.

MOTS-CLÉS

Facteurs de stress environnementaux, traits larvaires, plasticité phénotypique, poissons de récif coralliens, effets parentaux, survie