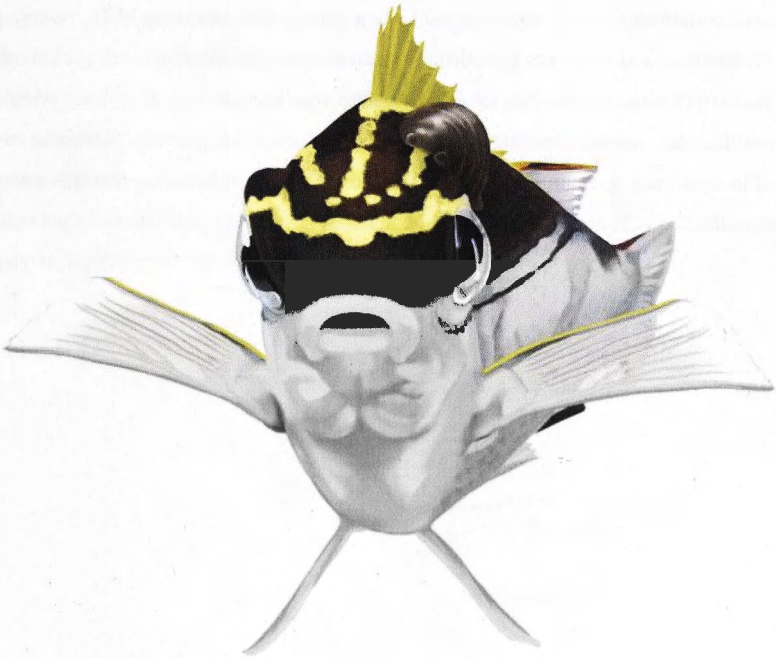
EFFECTS OF BIOTIC AND PHYSICAL STRESSORS ON FISH SWIMMING PERFORMANCE AND BEHAVIOUR



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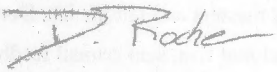
Cover page: *Scolopsis bilineata* and its ectoparasite isopod, *Anilocra nemipteri*.
(Illustration by Erin Walsh, ANU)

Declaration

The papers presented in this thesis are my own original work and no part has been submitted for a previous degree elsewhere. I am the lead author on all six chapters that make up the main body of this thesis. My supervisor, Michael Jennions, closest collaborator, Sandra Binning, and many other collaborators contributed valuable ideas, equipment, field assistance and writing and editorial advice. Their contributions are acknowledged in each chapter, and the main contributors are listed as co-authors. Five chapters have been published as peer-reviewed articles and one (Chapter 5) has just been submitted: journals are indicated at the beginning of each chapter. Six additional manuscripts are included in the appendix section to which I made at least three of the following contributions: ideas, experimental and statistical design, data collection, data analysis, and/or write-up.



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My supervisor, Michael Jennions, was by far the best advisor I could have hoped for. Although my project had nothing to do with the research in his lab, he took me on as a student, helped every time help was needed, and showed unwavering confidence in my abilities throughout the best and the worst of times. I will be ever grateful for that, Michael. Scott Keogh and Pat Backwell were also instrumental in making this dissertation a reality. As part of my thesis committee, they contributed much needed assistance and guidance. John Steffensen and Paolo Domenici thought me almost everything I know about fish swimming, respirometry and escape responses. My former supervisor, Brian Leung, continued to be a fantastic mentor and supporter even after I had finished my masters and left his lab. Don Kramer, who instigated my passion for research on coral reef fish, sent critical feedback on manuscripts and grant applications. Finally, Hanna Kokko provided a great source of distractions and entertainment (sometimes even scientific advice!), a constant flow of pre-loved New Scientists to read, and ample opportunities to satisfy my online shopping addiction.

Many collaborators and colleagues also helped immensely throughout this PhD: Cayne Layton, Rob Lanfear, Tonya Haff, Loeske Kruuk, Jodie Rummer, Jacob Johansen, Jaclyn Davies, Siobhan Heatwole, Rosie Sheb'a, Cassie Juan, Laura Strong, Mark Taylor, Jessica Pink, Simon Gingins, Alex Vail, Yolly Bosigner, Lisa Schwanz, Kristal Cain, Katrine Turgeon, and Edgard Fernando-Mendoza. Thank you also to A. Muir., J. Rucksa, S. Green, T. Wilson, P. McKinlay, R. Phillips and D. Barwick from the ANU RSB workshop for their help designing and building various experimental (and sometimes very imaginative) setups. None of the experiments in this thesis would have been possible without their ingenuity and engineering skills.

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Abstract

Locomotion is essential for all aspects of a fish's ecology and directly influences individual fitness by facilitating reproduction, food acquisition and predator avoidance. The effect of environmental factors on swimming fundamentally shapes the distribution of species. The first five chapters of this thesis examine how biotic and abiotic stressors influence fish swimming performance and behaviour. Because data (not just fish!) are essential for answering these questions, the last chapter discusses public data archiving, and proposes improvements to this practice.

Chapter 1 investigates the prevalence of a large ectoparasitic isopod, *Anilocra nemipteri*, and how it affects its fish host, *Scolopsis bilineata*, at Lizard Island on the Great Barrier Reef. I show that *A. nemipteri* is common and appears to affect the population size structure of *S. bilineata*. I suggest that this system is ideal to answer questions about host-parasite interactions and co-evolution (Roche *et al.* 2013, *Aust J Zool*).

In **Chapter 2**, I test whether *A. nemipteri*, which attaches asymmetrically on *S. bilineata,* affects lateralization (the preferential use of one side of the body for behavioural tasks). I show that parasitised fish are more lateralised than non-parasitised fish, and that removing the parasite from infected fish decreases the strength of lateralisation to the level of uninfected fish. These results suggest that side-biased behaviours are more plastic than previously thought (Roche *et al.* 2013, *Behav Ecol Sociobiol*).

Chapter 3 focuses on the host, *S. bilineata,* and compares two common respirometry methods for estimating metabolic rates in fishes. I argue that a single approach might not produce the most accurate parameter estimates for all fishes, and that researchers should carefully consider which apparatus and method are most appropriate for their species and question of interest (Roche *et al.* 2013, *J Exp Biol*).

Chapter 4 examines how cyclic changes in water flow velocity influence the swimming performance and energetics of a pectoral-fin (labriform) swimming fish. The results suggest that the costs of swimming in wave-like, unsteady flow are context

dependent, and are influenced by individual differences in the ability of fishes to adjust their fin beats to the flow environment (Roche *et al.* 2014, *J Exp Biol*).

Chapter 5 explores the effect of unsteady, wave-driven water motion on fast-start escape responses, a key behaviour used by fishes to evade predators. I found that water motion had a very strong negative effect on juvenile fishes' response time to a threatening stimulus, with deeper-bodied species being less affected. Since response latency is a key determinant of escape success, I argue that postural disturbances from unsteady water motion might reduce the ability of some coral reef fishes to evade predation (Roche submitted, *J Roy Soc Interface*).

Despite benefits for the wider society, many scientists are reluctant to share their data publicly. In **Chapter 6**, I explain why. I then offer practical solutions to increase the net benefits for individual researchers and to encourage public data archiving (Roche *et al.* 2014, *PLoS Biology*). I also argue against charging fees for researchers to archive their data in public repositories (Roche *et al.* 2013, *Nature*).

TABLE OF CONTENTS

INTRODUCTION

This thesis comprises chapters on a diversity of topics, loosely connected by fish and data. Each topic is briefly presented below, and introduced in detail at the start of each chapter. The appendices contain related papers to which I have contributed, but am not the first author.

Host-parasite interactions and fish behaviour

Parasites make up approximately 40% of all known species, and are ubiquitous in terrestrial and aquatic systems (Dobson *et al.* 2008; Poulin 2008). Parasites can have community-wide effects that include altering community structure via changes to the connectedness, number and length of links in food chains (Marcogliese 2002; Kuris *et al.* 2008; Lafferty *et al.* 2008). Parasites exploit their hosts for nutrition, shelter, and transport, and can negatively affect host fitness (Barber, Hoare & Krause 2000; Lafferty & Kuris 2009), alter population dynamics (Dobson & Hudson 1986; Marcogliese 2002; Hudson *et al.* 2006), modify host behaviour (Poulin 1995; Kuris 1997; Barber *et al.* 2000), and change species interactions (Minchella & Scott 1991). However, basic data on many host-parasite interactions remain scarce (Dobson *et al.* 2008; Lafferty *et al.* 2008).

Although parasites are extremely abundant in nature (Kuris *et al.* 2008), their cryptic nature often makes it difficult to assess their prevalence and impact on host populations. However, some ectoparasites are large and highly conspicuous, making them ideal systems in which to study the effects of parasitism (Lehmann 1993). The isopods are one of the largest and most diverse orders of crustaceans, approximately 10% of which are parasites of fishes (Bliss & Provenzano 1983). Many infect their hosts temporarily, but members of one family, the Cymothoidae, are obligate parasites (Bunkley-Williams & Williams 1998). Cymothoids are the largest and most conspicuous parasitic isopods (Adlard & Lester 1994), measuring up to one third of their host's standard length (Roche, unpublished data). Species in the genus *Anilocra* are widespread and commonly infect fishes in the Pacific Ocean and Caribbean Sea (Brusca 1981; Ketmaier *et al.* 2008). They exhibit high host specificity and can inflict serious harm upon their hosts (Bunkley-Williams & Williams 1998). For example, *Anilocra* species can cause mild anaemia, severe tissue damage, and inhibit the growth

and reproduction of their hosts (Adlard & Lester 1994; Adlard & Lester 1995; Fogelman 2005; Fogelman *et al.* 2009). Despite the important physiological consequences of carrying these large ectoparasites, few studies have examined their effects on host behaviour (but see Meadows & Meadows 2003).

Lateralisation, the preferential use of one side of the body for behavioural or cognitive tasks, is common in many animals, including humans (Rogers & Andrew 2002; Vallortigara & Rogers 2005; Schaafsma *et al.* 2009; Jozet-Alves *et al.* 2012). Contrary to previous beliefs, recent studies suggest that the strength of lateralisation can be context-dependent, rather than fixed (Mandel *et al.* 2008). For instance, in some fishes, a preference for using the left or right eye depends on whether individuals are viewing predators or conspecifics (Bisazza *et al.* 1997). However, there is still limited evidence that side biases vary in different ecological contexts (Brown *et al.* 2004). Given that cymothoid isopods attach asymmetrically on their host, they provide an ideal study system to examine possible effects of parasites on side preferences, and test the extent to which lateralisation varies depending on the ecological context.

Waves and fish swimming performance

In nature, water movement is influenced by numerous physical variables including wind, gravity, and obstructions below the surface (Webb *et al.* 2010). This creates complex flows characterised by turbulence and/or unsteadiness (Liao 2007). Turbulence refers to the creation of vortices of variable strengths and sizes in flowing water, whereas unsteady flows can be near-laminar but are characterized by changes in fluid velocity over time at a given point in space (Liao 2007; Webb *et al.* 2010). In shallow marine habitats, wave-driven water motion contains both turbulence and unsteadiness, and is an important physical stressor for sessile and mobile organisms (Denny 2006; Denny & Gaylord 2010). On coral reefs, for example, waves influence the ability of adult fishes to swim and occupy shallow, windward habitats, which leads to strong patterns of community structuring based on the ability of species to withstand ambient flow conditions (Bellwood & Wainwright 2001; Bellwood *et al.* 2002).

Historically, the energetic costs of swimming in fishes have been examined in laboratory swim tunnels under laminar flow conditions, at constant speeds (e.g.,

Steffensen *et al.* 1984; Claireaux *et al.* 1995; Farrell *et al.* 2003; Clark *et al.* 2011). Unfortunately, these measures may underestimate the true costs of swimming in nature, where the speed and complexity of water flows can vary considerably. Therefore, to better understand the energetic demands that fishes face in nature, we need to test swimming performance in settings that are more representative of wild conditions (Liao 2007; Lacey *et al.* 2012).

To date, only a few studies have measured energy consumption rates of fishes in complex, variable flows. Turbulent flows that have an element of predictability can be exploited by swimming fish (Liao *et al.* 2003a; Liao *et al.* 2003b; Liao 2004; Beal *et al.* 2006; Cook & Coughlin 2010; Taguchi & Liao 2011), whereas water flows with unpredictable and/or wide fluctuations in velocity tend to increase the costs of locomotion (Pavlov *et al.* 2000; Enders *et al.* 2003; Enders *et al.* 2005; Lupandin 2005; Webb & Cotel 2010). Whether complex flows are advantageous or disadvantageous to swimming fishes remains an important area of research. For instance, previous studies have all focused on fishes that use body-and-caudal fin (BCF) locomotion. Virtually nothing is known about how complex water flows influence the swimming performance and energetics of median and/or paired fin (MPF) swimmers, despite their abundance in freshwater and marine systems. MPF swimming fishes represent roughly 15-20% of all living fishes and over 60% of fish species on some coral reefs (Fulton 2010). These fishes employ a fundamentally different swimming mode to BCF swimmers: rather than undulating their body and tail, they create thrust with their paired (pectoral) or median (dorsal and caudal) fins, and maintain a rigid body. As a result of these biomechanical differences, complex flows could have dramatically different influences on the energetics of swimming in MPF fishes.

Beyond altering energy demands, waves can also perturb fish movements. For example, complex flows are suggested to have destabilizing effects on important swimming behaviours used by fishes during predator-prey interactions (Webb et al 2010). Specifically, waves could alter fast-start escape responses, a common behaviour used by prey fishes to escape from predators (Domenici & Blake 1997; Domenici 2011). These rapid accelerations are particularly important for juvenile coral reef fishes since predation is a dominant factor influencing their survival as they transition from a pelagic larval phase to a demersal phase during settlement on the reef (Fisher & Leis

2010). Juvenile reef fishes are highly sensitive to environmental variables (Leis & McCormick 2002), and biophysical interactions can have important effects on their distribution and abundance (Munday *et al.* 2008). To date, various stressors have been shown to affect fish fast-start swimming performance (e.g. temperature, hypoxia, salinity, turbidity, light cycles and pollutants; reviewed in Domenici 2010; Wilson *et al.* 2010). However, the importance of postural disturbances from wave-driven water motion has been completely overlooked.

Public data archiving

Most of the data collected, particularly in ecology and evolutionary biology, is quickly lost to science (Whitlock 2011). For this reason, an increasing number of journals are adopting policies that require data from papers to be publicly accessible (e.g. Parr & Cummings 2005; Vision 2010; Molloy 2011). Data archiving in public repositories has many benefits other than data preservation, namely encouraging good metadata production to ensure that datasets are interpretable (Whitlock *et al.* 2010); increasing the ability to evaluate and reproduce studies (Molloy 2011; Reichman *et al.* 2011; Tenopir *et al.* 2011); encouraging a stronger sharing culture (Huang & Qiao 2011); improving returns per research dollar (Piwowar *et al.* 2011; Tenopir *et al.* 2011); and increasing opportunities for teaching and learning (Tenopir *et al.* 2011; Whitlock 2011). Therefore, publishers and funding agencies are taking on greater roles to ensure that data are archived and available after publication (Guttmacher *et al.* 2009; Hanson *et al.* 2011).

Despite considerable group benefits, decisions to archive data are made by individuals, and it is less clear that the benefits currently outweigh the costs for individual researchers (see Tenopir *et al.* 2011). In fact, many researchers are reluctant to make their data publicly accessible (Savage & Vickers 2009; Tenopir *et al.* 2011; Milia *et al.* 2012; Drew *et al.* 2013), and can simply avoid journals that require public data archiving. For example, the benefits of public data archiving, such as an increased citation rate for an initial paper (Piwowar & Vision 2013), may not compensate for future publication loss by renouncing priority access to data (i.e. 'being scooped'; Brown 2013). Given intense competition for grants and academic positions, where publications are the major currency for assessing performance (Duke & Porter 2013;

Stodden *et al.* 2013), individuals are likely to make decisions that maximise their publication rate rather than benefits for science at large (Brown 2013; Stodden *et al.* 2013). Consequently, there is a substantial risk of these concerns affecting the rate and quality of public data archiving. For instance, journals do not police the quality of archived data (Noor *et al.* 2006; Roberts 2013), and archiving can be done in a form or format that makes data difficult, if not impossible, to re-use (Rivers 2013). While the benefits of data sharing have been discussed at length in the literature, the real and perceived costs have received far less attention. Acknowledging and discussing ways of decreasing these costs is essential to improve public data archiving practices across all disciplines.

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CHAPTER - 1

Prevalence of the parasitic cymothoid isopod *Anilocra nemipteri* on its fish host at Lizard Island, Great Barrier Reef

Roche DG, Strong LE and Binning SA (2013) *Australian Journal of Zoology* 60: 330-333.

Keywords

Anilocra nemipteri, Cymothoid, Great Barrier Reef, isopod, Lizard Island, parasite prevalence, *Scolopsis bilineata*

Abstract

Parasites are ubiquitous in nature but assessing their prevalence in wild fish populations is often challenging due to their cryptic nature. Low abundance can also hinder detailed studies. Here, we report a relatively high prevalence (4.3%; range 0 - 28%) of an ectoparasitic cymothoid isopod (*Anilocra nemipteri*) infecting the bridled monocle bream (*Scolopsis bilineata*) on reefs surrounding Lizard Island on the Northern Great Barrier Reef (GBR). The prevalence of infected and previously infected fish at this location was nearly 15%, which greatly exceeds reports from other localities on the GBR. At least one parasitized fish was observed at 75% of the reefs surveyed, although prevalence varied across sites. Parasitized *S. bilineata* were on average 25% smaller than unparasitized or previously parasitized fish. Given that these parasites have known detrimental effects on host growth, survivorship and swimming ability, our observation suggests that *A. nemipteri* may influence the size structure of its host population in the wild. Since *A. nemipteri* is large, conspicuous and relatively abundant, it provides an ideal study system to examine a range of important questions about the evolutionary ecology of parasites.

Introduction

Parasites can substantially alter host fitness by negatively affecting physiological, behavioural and morphological traits (Minchella and Scott 1991; Lehmann 1993; Poulin and Thomas 1999; Barber *et al.* 2000; Wood *et al.* 2007). Despite their ecological importance (Poulin 1999; Wood *et al.* 2007; Kuris *et al.* 2008), assessing the prevalence of parasites can be challenging (Justine 2010) due to their often

cryptic nature (Minchella and Scott 1991; Kuris *et al.* 2008) and sometimes low abundance in natural populations (e.g., Grutter 1994; Grutter 1995). However, some ectoparasites are highly conspicuous (Adlard and Lester 1995; Bunkley-Williams and Williams 1998), making them ideal study systems to examine the potential effects of parasitism on host fitness (Lehmann 1993). In fishes, ectoparasites can pose additional challenges as streamlining is important to reduce the cost of locomotion in water (Vogel 1994). Therefore, parasites may increase host susceptibility to predation in addition to decreasing host nutritional status and growth, all of which can influence the population dynamics and structure of the host species (Minchella and Scott 1991; Barber *et al.* 2000).

On the Great Barrier Reef (GBR), the bridled monocle bream (*Scolopsis bilineata*) is parasitized by the cymothoid isopod *Anilocra nemipteri*, which attaches posterodorsally to the eye of its host on the right or left side of the midline using its pereopods (Bruce 1986; Grutter 1994) (Fig. 1a,b). Infections leave a scar that is visible long after the parasite has detached (Bunkley-Williams and Williams 1998) (Fig. 1c). Ectoparasitic isopods in the family Cymothoidae infect a wide range of fishes on coral reefs worldwide (Bruce 1986; Bunkley-Williams and Williams 1998). Their large size (up to 23mm or 30% of host total length; Adlard and Lester 1995, Grutter 1994, D.G. Roche unpublished data) and asymmetric attachment probably interfere with a range of fitness-enhancing activities (Adlard and Lester 1994; Östlund-Nilsson *et al.* 2005; Fogelman *et al.* 2009). However, the prevalence of infections on *S. bilineata* reported to date at various sites on the GBR are either low or nil (Lester and Sewell 1989; Grutter 1994; A.E. Boaden, personal communication). Here, we assess current and past infections of *S. bilineata* by *A. nemipteri* using counts of currently infected and parasite-scarred individuals at Lizard Island on the Northern GBR. We also examine possible effects of the parasite on the size structure of the host population.

Material and methods

We used 50 x 4 m belt transects (n = 3 to 7 per site) to record infections of *S. bilineata* by *A. nemipteri* on 12 reefs above six meters depth at Lizard Island, Northern Queensland (14° 40' S; 145° 28' E). Two snorkelers swimming at a constant speed of 0.2 ms^{-1} surveyed fish 2 m on either side of the transect tape. We recorded four variables for each *S. bilineata* observed: fish size (total length), colour phase (juvenile

or adult), condition (unparasitized, parasitized or scarred from a previous infection), and the location of parasite attachment or scarring (left or right side of the body). Juveniles were identified as having distinct yellow and black stripes on the upper half of the body (Randall *et al.* 1997).

Prior to data collection, we practiced estimating fish lengths to the nearest one cm using model fishes underwater (deviations from actual sizes were < 1 cm). We tested for differences in the proportion of infected or scarred individuals among sites using two generalized linear models (GLM) with binomial error terms to account for the underlying distribution of the data. We tested for differences in size among parasitized, unparasitized and previously parasitized fish using a one-way ANOVA and a post-hoc Tukey test. Normality and homoscedasticity were assessed with diagnostic plots of the residuals and size was power transformed using a boxcox function to meet the assumptions of the model. We used a binomial test to determine whether parasites preferentially attached to one side of the body midline, including data from both parasitized and parasite-scarred fish.

Results

Transects by snorkelers on 12 different reefs revealed an overall current *A. nemipteri* prevalence of 4.3% (12,800 m^2 surveyed; N = 374 fish). Prevalence on adults was 3.6% and 9.8% on juveniles. An additional 9.8% of adult fish had marks of past infection. Prevalence differed significantly across reef sites (range 0 - 28%; $F_{(11,51)} = 1.99$, $p < 0.05$) with the highest average prevalence of infected fish at Bird Islets (28%) and Bird Lagoon (23%) (Fig. 2). The proportion of previously infected fish also differed across sites (range 0 - 34%; $F_{(11,51)} = 2.95$, $p < 0.01$) with the highest average ratio of scarred individuals occurring at Big Vickies (34%), Mermaid (16%) and Watson's Bay (9%).

Juveniles (mancae) of *A. nemipteri* only infected juvenile hosts and not adult hosts. Host size differed among parasitized, unparasitized and previously parasitized fish ($F_{(2, 371)} = 8.76$, $p < 0.001$). Overall, parasitized *S. bilineata* tended to be approximately 25% smaller (TL 10.75 ± 0.79 cm; mean ± s.e.m) than unparasitized (TL 14.12 ± 0.19 cm; Tukey HSD $p < 0.001$) and previously parasitized (TL 14.84 ± 0.32 cm; Tukey HSD $p < 0.001$) fish. There were no size differences between parasitized and

previously parasitized fish (Tukey HSD $p > 0.65$). Finally, parasites did not attach on one side of the host more than the other (30 left vs 27 right; binomial test, $p > 0.75$).

Discussion

We found that overall infection of the bridled monocle bream, *S. bilineata,* by the cymothoid ectoparasitic isopod, *A. nemipteri*, was high at our study sites compared to other sites on the GBR. Although *S. bilineata* is an abundant coral reef fish (Boaden and Kingsford 2012), previous studies have reported nil or very few infections by *A. nemipteri* at a variety of locations along the GBR including Heron Island, Lizard Island, One Tree Island and Orpheus Island (Lester and Sewell 1989; Grutter 1994; A.E. Boaden, personal communication). In a survey of parasites infecting fishes at Lizard Island and Heron Island, Grutter (1994) reports a single *S. bilineata* parasitized by *A. nemipteri*. A prevalence of 4.3% for adult fishes and 9.8% for juveniles observed in our study at Lizard Island is therefore comparatively high for this species. An additional 9.8% of adults bore marks of past infection, raising the total number of fish affected by parasites to nearly 15%. Tissue damage is thought to result from a necrotic reaction of the host's tissues underneath the parasite or the host growing around the parasite, creating a deformation of the body (Bunkley-Williams and Williams 1998). Previous studies have examined the biology and host-parasite interactions of congeneric anilocrid species using laboratory and field experiments (Adlard and Lester 1994; Adlard and Lester 1995; Östlund-Nilsson *et al.* 2005; Fogelman and Grutter 2008; Fogelman *et al.* 2009); however, none of these studies reported prevalence in the wild, which is essential to assess the frequency of infections in host populations and to compare prevalence among different cymothoid species infecting fishes on the GBR (but see Grutter 1994).

Our reef sites at Lizard Island were separated by hundreds of meters to a few kilometres. Differences in prevalence across this small spatial scale likely result because cymothoids are highly site specific (Bunkley-Williams and Williams 1998) and tend to occur in aggregations (Adlard and Lester 1994). The fact that mancae probably have limited dispersal abilities (see Adlard and Lester 1995; Fogelman and Grutter 2008; Jones *et al.* 2008) and that *S. bilineata* is site-attached (Boaden and Kingsford 2012) suggests that there is little opportunity for infection to spread across sites that are separated by large sand patches (e.g., 50-100 m long) and therefore not well connected. Nonetheless, 75% of the reefs we surveyed harboured at least one parasitized fish (Fig.

2), and the prevalence of infections reached 28% at one site (Lagoon-Bird Is.; Fig. 2). The parasite had no preference for attaching on the right or left side of its host.

Infected fish were considerably smaller than unparasitized or scarred individuals at Lizard Island (Fig. 3). The skewed size distribution of infected individuals could result from a simple preference of the parasite for smaller hosts since mancae (larvae) of parasitic cymothoids preferentially infect juvenile fish and subsequently grow with their host (Adlard and Lester 1995; Fogelman and Grutter 2008). However, parasitic cymothoid isopods associate with their hosts for long periods of time (Bunkley-Williams and Williams 1998) and experimental studies have shown that they can inflict deep wounds, stunt growth (Adlard and Lester 1994; Fogelman and Grutter 2008), impair reproduction (Adlard and Lester 1994; Fogelman *et al.* 2009) and ultimately kill their host (Adlard and Lester 1994; Bunkley-Williams and Williams 1998; Fogelman and Grutter 2008). Therefore, by impeding growth and removing individuals from the population before they attain full maturity, the parasite may have notable effects on the size structure of the host population. Interestingly, scarred *S. bilineata* that were previously parasitized did not differ in size from unparasitized individuals, suggesting that compensatory growth might allow fish to reach their full size if the parasite detaches. The possibility of resumed growth is supported by recent data showing that parasite removal reverses the negative physiological effects of *A. nemipteri* on its host in as little as 24h (Binning *et al.* 2012). However, distinguishing between a preference of the parasite for small individuals and detrimental effects on growth and survivorship would require a detailed study using otolith microstructure analysis to age fish.

Finally, being abundant as well as easily observed and manipulated, parasitic cymothoid isopods such as *A. nemipteri* provide ideal systems to conduct observational and experimental studies and answer a range of ecological and evolutionary questions on host-parasite interactions. For example, due to its large size, *A. nemipteri* was recently found to decrease streamlining and host swimming abilities by directly altering the surface of its host's body and increasing drag (Binning *et al.* 2012). Further studies would greatly improve our understanding of the evolutionary ecology of parasites by using this system to examine the effects of ectoparasitism on host fast-start performance (escape response, burst swimming), handedness (lateralization), physiology (anaemia, cortisol levels), demography (age determination with otoliths), habitat use (distribution

across water flow gradients) as well as inter- and intra-sexual selection (female mate choice and male-male competition).

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Fig. 1 Bridled monocle bream (*Scolopsis bilineata*) with (a,b) an ectoparasitic isopod (*Anilocra nemipteri*) attached above the eye and (c) scarring from a past infection.

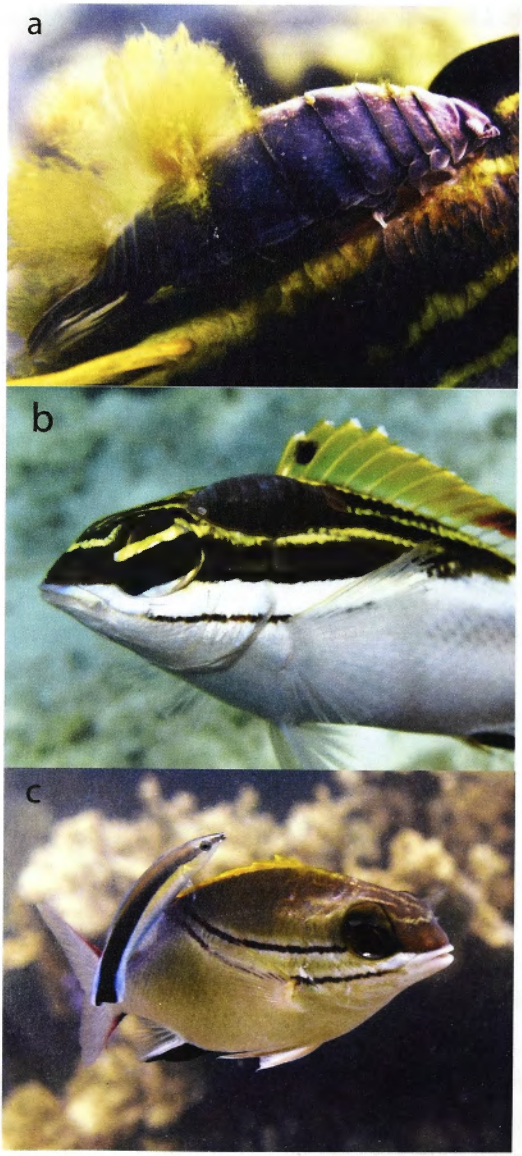


Fig. 2 Abundance (± s.e.m) of parasitized (black bars), previously parasitized (grey bars) and unparasitized (white bars) bridled monocle bream (*Scolopsis bilineata*) at 12 sites around Lizard Island.

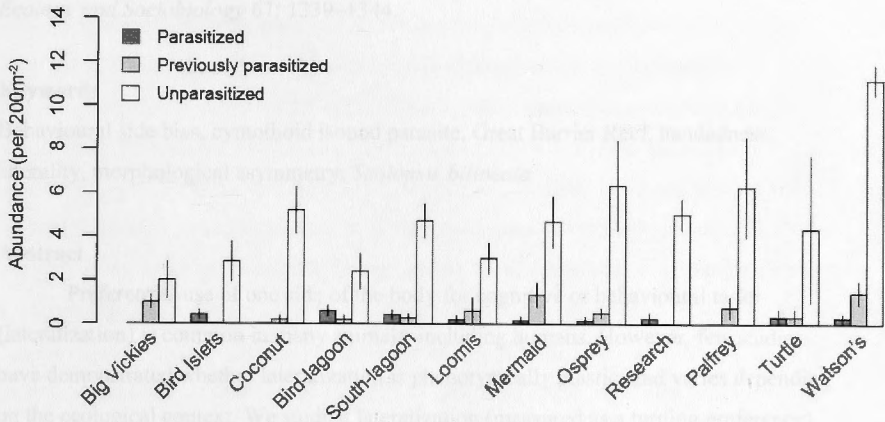
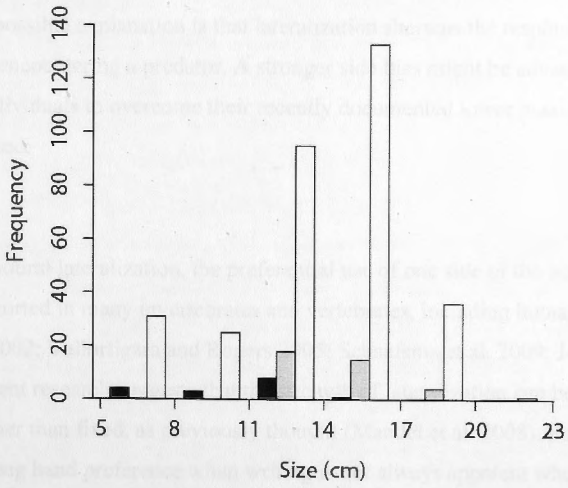


Fig. 3 Size distribution of parasitized (black bars), previously parasitized (grey bars) and unparasitized (white bars) bridled monocle bream (*Scolopsis bilineata*) at Lizard Island.



CHAPTER - 2

Increased behavioural lateralization in parasitized coral reef fish

Roche DG, Binning SA, Strong LE, Davies J and Jennions MD (2013) *Behavioural Ecology and Sociobiology* 67: 1339–1344.

Keywords

Behavioural side bias, cymothoid isopod parasite, Great Barrier Reef, handedness, laterality, morphological asymmetry, *Scolopsis bilineata*

Abstract

Preferential use of one side of the body for cognitive or behavioural tasks (lateralization) is common in many animals, including humans. However, few studies have demonstrated whether lateralization is phenotypically plastic, and varies depending on the ecological context. We studied lateralization (measured as a turning preference) in the bridled monocle bream (*Scolopsis bilineata*). This coral reef fish is commonly infected by a large, ectoparasitic isopod (*Anilocra nemipteri*) that attaches to the left or right side of its host's head. Fish that were parasitized showed no turning bias with respect to the side on which the parasite had attached. On average, however, parasitized fish were significantly more lateralized (i.e., had a strong side bias) than unparasitized fish. The extent of lateralization declined significantly when we experimentally removed the parasite. Our results indicate that lateralization can vary with the ecological context. One possible explanation is that lateralization shortens the response time until fish flee after encountering a predator. A stronger side bias might be advantageous for parasitized individuals to overcome their recently documented lower maximum swimming speed.

Introduction

Behavioural lateralization, the preferential use of one side of the body, is commonly reported in many invertebrates and vertebrates, including humans (Rogers and Andrew 2002; Vallortigara and Rogers 2005; Schaafsma et al. 2009; Jozet-Alves et al. 2012). Recent research suggests that the strength of lateralization can be context dependent rather than fixed, as previously thought (Mandel et al. 2008). For instance, in humans, a strong hand preference when writing is not always apparent when performing

other tasks, suggesting that handedness is a relatively plastic trait (Geuze et al. 2012). Such context-dependent or task-dependent changes in lateralization are, however, still poorly understood both in humans and non-humans.

Some of the strongest evidence for an adaptive role of behavioural lateralization comes from teleost fish. For example, lateralization has been shown to be advantageous in fish schooling (Bisazza and Dadda 2005), escape responses (Dadda et al. 2010), multitasking (Dadda and Bisazza 2006a; Dadda and Bisazza 2006b) and spatial reorientation (Sovrano et al. 2005). The enhanced performance of lateralized individuals in such cases is generally attributed to the specialization of certain cognitive or motor tasks by one hemisphere of the brain (Rogers and Andrew 2002). This potentially avoids the duplication of costly neural pathways, and allows for the simultaneous processing of information from different stimuli (Rogers and Andrew 2002; Rogers et al. 2004; Vallortigara and Rogers 2005). However, there are potential costs to favouring one side of the brain, since ecologically relevant stimuli generally occur equally often on both sides of the body (e.g., predator approach; Vallortigara and Rogers 2005; Dadda et al. 2009). The balance between such costs and benefits will determine how strongly each individual is lateralised. Frequency-dependent selection is then likely to determine the skew, or lack thereof, in the population distribution of lateralization (whether or not most individuals are biased towards the same side) (Hori 1993; Lee et al. 2012).

A few recent studies have shown that changes in abiotic (water pH) and biotic (presence of predator versus conspecifics) factors as well as changes in the environment (novel vs. familiar) can affect how strongly individuals are lateralized (Bisazza et al. 1997b; Brown et al. 2004; Reddon and Hurd 2009; Domenici et al. 2012), but there is still limited evidence that side biases vary in different ecological contexts (Brown et al. 2004).

On the Great Barrier Reef, the cymothoid isopod *Anilocra nemipteri* parasitizes the bridled monocle bream, *Scolopsis bilineata*, (Grutter 1994; Roche et al. 2013a) with up to 30 % of fish infected at some sites (Roche et al. 2013a). A single isopod typically attaches to a fixed location on one side of the host. Parasites can grow to almost one third of the fish's standard length (D.G. Roche, unpublished data) and reduce host growth and survivorship (Adlard and Lester 1994; Fogelman et al. 2009; Roche et al.

2013a). *A. nemipteri* does not exhibit any side bias in attachment preference on either the left or right side of the host's body (Roche et al. 2013a). These parasites impair the swimming ability of *S. bilineata*, mostly by increasing drag at high speeds and decreasing the host's critical swimming speed (U_{crit}) (Binning et al. 2013). Parasite attachment on one side of the body results in morphological asymmetry (Takeuchi et al. 2010), which can create uneven drag and/or weight distribution across the fish's body (Östlund-Nilsson et al. 2005), potentially affecting the host's centre of mass and pivot point. This could influence the fish's turning behaviour, causing parasitized individuals to turn more frequently towards/away from the side of their parasite. Since cymothoids significantly reduce swimming performance (Binning et al. 2013), increased lateralization could be particularly advantageous for parasitized fish when escaping from predators as lateralization has been associated with earlier responses to threatening stimuli (Dadda et al. 2010).

We tested the context dependency of lateralization by comparing the turning preferences of naturally unparasitized and parasitized *S. bilineata.* We then compared the extent of lateralization of fish before and after we experimentally removed their parasite. We hypothesized that parasitism would increase lateralization and predicted that: (1) experimentally removing parasites would reduce lateralization to the level of uninfected fish; (2) the side bias of a parasitized fish would depend on the side to which the parasite had attached.

Materials and methods

Parasitized (11.82 ± 2.50 cm; mean ± s.d.) and unparasitized (14.75 ± 1.47 cm; mean ± s.d.) *S. bilineata* were collected using barrier nets from the lagoon at Lizard Island, Northern Great Barrier Reef, Australia (14° 40' S; 145° 28' E), in March and April 2012 (Fig. 1). Fish were transported in buckets to the Lizard Island Research Station and held in flow through aquaria under a natural light and temperature regime. Fish were fed to satiation once a day with raw prawn and fasted for 24h prior to the experiments. All animals were kept in aquaria for a minimum of three days before performing swim trials to ensure all fish were healthy.

We used a detour test to assess behavioural lateralization (Dadda et al. 2010; Domenici et al. 2012). A single fish was introduced into a T-maze, consisting of a large

opaque tank (102 x 51 x 50 cm, length x width x height) with a runway down the middle (70 x 15 x 20 cm, length x width x height; Fig. 2). The water height in the tank was kept at 13 cm and water temperature was maintained at 27 °C ± 1 °C via a flow-through system with enough flow to ensure adequate aeration, but not to create a current in the tank. To initiate a trial, a fish was placed at the starting end of the runway and maintained there with an acrylic divider for three minutes. The experimenter then lifted the divider from behind the tank and gently pushed the fish with a dip net to initiate movement (Dadda et al. 2010; Domenici et al. 2012). The fish would swim to the end of the runway and reach a barrier (25 x 20 cm, width x height; Fig. 2), positioned perpendicular to the runway, forcing it to turn left or right. We ran ten consecutive trials per fish (3 min rest between trials) and recorded the fish's turning direction. We then calculated a relative lateralization index (L_R) (Bisazza et al. 1997b; Domenici et al. 2012): LR = ((Turn to the right - Turn to the left) / (Turn to the right + Turn to the left)) * 100. A score of -100 indicates an absolute preference for left turns and of 100, absolute preference for right turns (Domenici et al. 2012). If an entire population is lateralized in the same direction, we expect an extreme high (close to 100) or extreme low (close to -100) population-level L_R score (Bisazza et al. 1998). A mean L_R close to zero indicates that a population (or sample of a population) is neither left or right biased in its turning preference (Bisazza et al. 1998).

Even if a population as a whole is not left or right biased in its turning preference, it may still contain individuals that are themselves lateralized. In other words, individual fish may display distinct preferences for turning left or right irrespective of whether the population itself is lateralized. Therefore, we used the absolute lateralization index L_A ($L_A = |L_R|$) to calculated the strength of lateralization at the individual level, irrespective of the direction of lateralization. L_A ranges between 0 (individuals that turn left and right equally) and 100 (individuals that turn either left or right all the time) (Bisazza et al. 1998; Domenici et al. 2012).

Forty-one individuals were initially tested (25 unparasitized and 16 parasitized fish). We then removed parasites from the parasitized fish by holding the fish in a shallow water bath and gently unhooking the isopod with forceps (see Binning et al. 2013). We waited 24 hours and retested the fish in the T-maze.

We used goodness-of-fit G-tests to compare the proportion of left versus right turns per fish to the expected binomial distribution when $p = q = 0.5$ for unparasitized, parasitized and parasite-removed fish. Lack of fit could be due to overdispersion (i.e., individuals have side-biases) even if $L_R \neq 0$ and/or a systematic side-bias at the population level (i.e., $p \neq 0.5$, equivalent to $L_R \neq 0$). We then ran a generalized linear model (GLM) with quasibinomial errors (given overdispersion; see results) to test for differences in the mean side-bias (i.e., L_R) between parasitized and unparasited fish, and whether either mean differed from p = 0.5 (i.e., $L_R = 0$). Next, we calculated an absolute lateralization index L_A ($L_A = |L_R|$) for individuals in each of the three groups to evaluate the strength of lateralization of individuals, irrespective of the direction (Bisazza et al. 1997a; Domenici et al. 2012). We then compared L_A between unparasitized and parasitized fish using a Mann-Whitney U test. We also compared L_A between parasitized and parasite-removed fish with a Wilcoxon paired-sample test. Finally, we used a GLM with quasibinomial errors to test whether parasitized fish turned more often towards or away from the side to which their parasite was attached. Statistical analyses were conducted in R v2.15.0 (R Development Core Team 2010).

Results

The distribution of individuals' side biases (L_R) deviated from the null binomial distribution for unparasitized ($G = 44.8$, $p < 0.01$), parasitized ($G = 88.7$, $p < 0.001$) and parasite-removed fish ($G = 56.6$, $p < 0.001$) (Fig.3). In all three groups, fish were significantly more lateralized than expected by chance; the observed deviations from a binomial distribution ($p = 0.5$) were due to significant overdispersion ($\omega = 5.8$) because individual fish tended to show side-biases. Fitting a GLM showed no significant difference in mean L_R between unparasitized and parasitized fish ($t = 0.91$, $p = 0.37$), and the estimated mean L_R did not differ significantly from zero ($t = 0.70$, $p > 0.45$). The mean L_R for all three groups was close to zero: unparasitized -10.4 ± 13.5 (mean ± s.e.); parasitized 11.25 ± 21.7; and parasite-removed fish 16.3 ± 14.3 (Fig. 3). There was therefore no tendency for different fish to turn in the same direction (i.e., no population skew towards left or right turning fish). There was also no evidence that the turning bias of infected fish depended on which side their parasite was attached ($t = 0.077$, $p = 0.94$).

The absolute lateralization index L_A indicated individual-level side biases were significantly weaker for unparasitized than parasitized fish ($U = 119.5$, $p = 0.026$; Fig.

4). Intriguingly, L_A was also significantly smaller for parasite-removed fish compared to parasitized fish ($Z = 2.58$, $p = 0.01$; Fig. 4), indicating that the additional lateralization induced by a parasite is readily reversed (i.e., within 24 h of parasite removal). We found no significant relationship between host size and L_A ($p = 0.67$, $r^2 = 0.01$).

Discussion

Although there was no population level lateralization, individual bridled monocle bream tended to have a side-bias when turning at a barrier. The strength of this behavioural lateralization was associated with attachment of the ectoparasite *A. nemipteri*. Parasitized fish showed a significantly stronger side-bias than unparasitized fish, providing evidence that the strength of individual lateralization can vary with the ecological context.

A simple proximate explanation for these differences in lateralization could be that the asymmetric attachment of the parasite on one side of the fish creates a directional cue on the host's body (Takeuchi et al. 2010). When facing a barrier, some parasitized individuals might deal with the combination of having to turn and carrying a directional cue by consistently turning towards or away from the cue. Unparasitized fish that do not carry a directional cue, in contrast, would be less likely to show a strong turning bias when facing a barrier. Studies argue that directional bias in lateralization may lead to behavioural predictability in prey escape responses resulting in enhanced predator success (e.g., Reddon and Balshine 2010). This could explain why parasitized fish, although lateralized, did not show a consistent directional bias in lateralization towards or away from the parasite cue, which predators could learn to anticipate.

There are also adaptive explanations for the higher lateralization of parasitized fish. Notably, functional left–right cerebral asymmetries are important determinants of fish behaviour, particularly during predator-prey interactions (Dadda et al. 2010; Domenici et al. 2012). For example, behavioural lateralization helps avoid simultaneously initiating opposing directional responses when fleeing from a predator (Vallortigara and Rogers 2005). In a study of the surfperch *Cymatogaster aggregata*, Dadda et al. (2010) also found that lateralization likely increases escape success by lowering the response latency of fish to threatening stimuli. They attributed the increased reactivity of lateralized individuals to a lower sensory threshold required to

activate the Mauthner cells (Dadda et al. 2010), a pair of giant reticulospinal neurons responsible for short (< 50 ms) escape response latencies (Eaton et al. 2001).

Recently, Binning et al. (2013) showed that parasitized *S. bilineata* suffer from decreased swimming performance in critical swimming speed (U_{crit}) trials due to increased drag. U_{crit} measurements provide rough estimates of maximum sustained (aerobic) swimming speed (Plaut 2001), but typically involve burst (anerobic) swimming towards the end of the trial (Farrell 2007; Svendsen et al. 2010; Roche et al. 2013b). Prolonged and burst swimming are often used by fishes to escape from predators, particularly from ram feeders (as opposed to suction feeders) that chase their prey prior to capture. Faster responses owing to a stronger side bias might confer a fitness advantage to parasitized individuals that are poorer swimmers. In addition, *A. nemipteri* attaches very close to the eye, which might decrease the host's visual range. Rapid responses triggered by other sensory stimuli (e.g.,olfactory and mechano-acoustic stimuli; Domenici 2002; Stewart et al. 2013) might compensate for a reduced field of vision and otherwise elevated host vulnerability to predation.

Biologically relevant visual stimuli occur equally on both sides of the body, so strong lateralization is not always advantageous (Rogers and Andrew 2002; Vallortigara and Rogers 2005). The extent of lateralization probably represents a trade-off between faster decision making in lateralized individuals and the ability of non-lateralized fish to better process stimuli originating from all directions (Dadda et al. 2009; Reddon and Hurd 2009; Domenici et al. 2012). Reduced swimming performance presumably shifts the balance of this trade-off toward stronger lateralization and faster decision-making in parasitized hosts that are potentially more vulnerable to predators. In contrast, the balance would shift away from strong lateralization and towards the ability to process stimuli on both sides of the body for unparasitized individuals with good swimming abilities. This trade-off could explain why *S. bilineata* became more weakly lateralized once their parasite was removed. This change in lateralization strength unlikely resulted from testing fish twice since fish that were tested more than once in preliminary trails (n=8) displayed consistent lateralization indices.

Behavioural flexibility within different ecological contexts is often advantageous, especially for organisms living in complex habitats, such as coral reefs.

For instance, coral reef fishes adjust their swimming behaviour (fin use) in response to varying hydrodynamic conditions, presumably to save energy (Heatwole and Fulton 2012). Similarly, parrotfishes vary their flight-initiation distance depending on the perceived risk of predation (Gotanda et al. 2009). It is therefore plausible that the changes in lateralization of *S. bilineata* are adaptive in the context of predator-prey interactions. The observed plasticity in laterality is consistent with evidence that the preferential use of one eye is not necessarily fixed for individual fish (Brown et al. 2004). The rapid decrease in lateralization following parasite removal also supports recent findings that the negative physiological effects of *A. nemipteri* on *S. bilineata* are reversed within 24h post removal (Binning et al. 2013).

Predation is thought to play a key role in the evolution of lateralized behaviour. Previous studies have shown that lateralization varies between conspecific populations exposed to different predation pressures (Brown et al. 2004). Here, we show that parasitism might interact with the strong selection imposed by predators to favour phenotypic plasticity in behavioural lateralization. Further studies are needed that test the extent to which greater lateralization improves the fitness of parasitized *S. bilineata* when they encounter predators. More broadly, our results highlight the need to test for adaptive phenotypic plasticity in behavioural lateralization among other species.

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Ethical standards

Research was conducted under the Australian National University animal ethics permit A2012/02 and the Great Barrier Reef Marine Parks Authority collection permit G12/34805.1. Animals were released at their site of capture at the end of the study and none were harmed as a result of the parasite removal.

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Fig. 1 The parasitic cymothoid isopod *Anilocra nemipteri* infecting the bridled monocle bream, *Scolopsis bilineata* (photo D.G. Roche).

Fig. 2 Schematic representation of the T-maze used for the detour test. During each trial, the holding barrier was raised, allowing the fish to swim down the runway. The fish faced a barrier at the end of the runway and would turn either left or right. The direction chosen by the fish was then recorded by the experimenter.

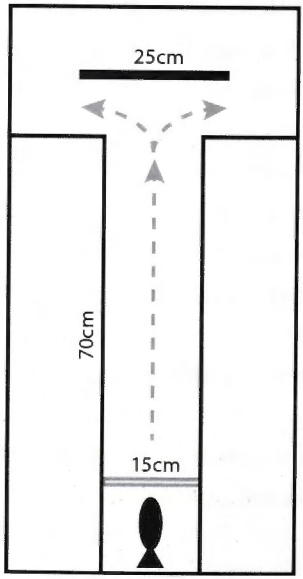


Fig. 3 The distributions of L_R in (A) unparasitized fish, (B) parasitized fish and (C) parasite-removed fish. The curve shows a binomial distribution with $p = q = 0.5$. Positive values are right turns; negative values are left turns. Values of |100| indicate fish that turned in the same direction (all left or all right) in all 10 trials.

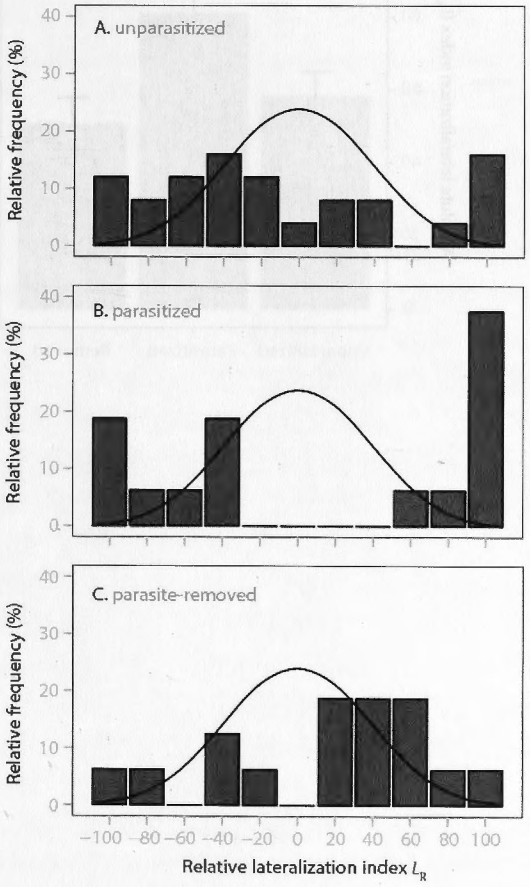
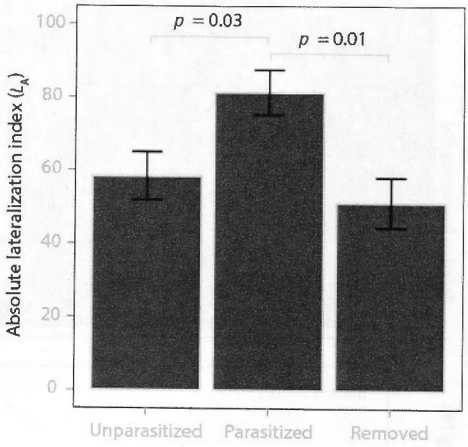


Fig. 4 L_A (mean ± s.e.) for unparasitized, parasitized and parasite-removed fish. Significance levels are shown for pairwise contrasts (see text).



CHAPTER - 3

Finding the best estimates of metabolic rates in a coral reef fish

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Keywords

Eco-physiology, oxygen consumption rate, maximum metabolic rate, standard metabolic rate, critical swimming speed, respirometry

Abstract

Metabolic rates of aquatic organisms are estimated from measurements of oxygen consumption rates ($\dot{M}O_2$) through swimming and resting respirometry. These distinct approaches are increasingly used in eco- and conservation physiology studies; however, few studies have tested whether they yield comparable results. We examined whether two fundamental $\dot{M}O_2$ measures, standard metabolic rate (SMR) and maximum metabolic rate (MMR), vary based on the method employed. Ten bridled monocle bream (*Scolopsis bilineata*) were exercised using (1) a critical swimming speed (U_{crit}) protocol, (2) a 15 min exhaustive chase protocol and (3) a 3 min exhaustive chase protocol followed by brief (1 min) air exposure. Protocol (1) was performed in a swimming respirometer whereas protocols (2) and (3) were followed by resting respirometry. SMR estimates in swimming respirometry were similar to those in resting respirometry when a three-parameter exponential or power function was used to extrapolate the swimming speed-$\dot{M}O_2$ relationship to zero swimming speed. In contrast, MMR using the U_{crit} protocol was 36% higher than MMR derived from the 15 min chase protocol and 23% higher than MMR using the 3 min chase 1 min air exposure protocol. For strong steady (endurance) swimmers, such as *S. bilineata*, swimming respirometry can produce more accurate MMR estimates than exhaustive chase protocols because oxygen consumption is measured during exertion. However, when swimming respirometry is impractical, exhaustive chase protocols should be supplemented with brief air exposure to improve measurement accuracy. Caution is warranted when comparing MMR estimates obtained with different respirometry methods unless they are cross-validated on a species-specific basis.

Introduction

Eco-physiology is the study of how organisms respond physiologically to environmental stressors (Fry, 1947; Fry, 1971; Schurmann and Steffensen, 1997; Claireaux and Lefrançois, 2007). Given the prevalence of anthropogenic stressors in natural systems, conservation physiology is rapidly growing as a discipline that aims to better understand and predict organisms' responses to these environmental changes (Wikelski and Cooke, 2006; Kieffer, 2010; Cooke et al., 2012). Respirometry, in particular, is increasingly used by eco-physiologists as advances in technology and equipment accessibility are facilitating studies (Kieffer, 2010), especially in less studied groups such as tropical fishes (e.g., Donelson et al., 2011; Munday et al., 2012). However, as conservation physiology and respirometry continue to grow in popularity, standardized methods must be used to ensure that physiological data are robust and comparisons among studies are valid.

In aquatic respiratory physiology, two types of respirometry chambers are commonly used to conduct either swimming (Fry and Hart, 1948; Blazka et al., 1960; Brett, 1964; Steffensen et al., 1984) or resting respirometry (Teal and Carey, 1967; Hemmingsen and Douglas, 1970; Fry, 1971). Resting respirometry is sometimes also referred to as static respirometry (e.g., Reidy et al., 2000; Brick and Cech, 2002; Barnes et al., 2011), but this terminology is much less common. Despite differences in their complexity and ease of use, both methods allow measuring oxygen consumption rates ($\dot{M}O_2$) to estimate metabolic rates during or following varying levels of activity (e.g., resting vs. active swimming) (Reidy et al., 1995; Peake and Farrell, 2006; Killen et al., 2007). Different calculations can also be used within each method to compute the same estimates of metabolic rate. This probably introduces variation in metabolic rate estimates but studies have yet to carefully examine whether data obtained in different ways produce comparable results (but see Reidy et al., 1995; Reidy et al., 2000).

Two key physiological parameters characterize the upper and lower bounds of a fish's capacity to uptake oxygen: standard (resting) metabolic rate (SMR or $\dot{M}O_{2,min}$), and maximum metabolic rate (MMR or $\dot{M}O_{2,max}$). SMR corresponds to the minimum maintenance metabolism of a resting fish in a post absorptive state (Fry, 1971; Brett and Groves, 1979; Schurmann and Steffensen, 1997), whereas MMR corresponds to a fish's maximum rate of oxygen consumption (Fry, 1971; Beamish, 1978; Schurmann and

Steffensen, 1997; Korsmeyer and Dewar, 2001; Clark et al., 2011). During exercise, MMR is measured at a fish's maximum swimming speed during prolonged swimming (Bushnell et al., 1994; Schurmann and Steffensen, 1997; Korsmeyer and Dewar, 2001), which requires anaerobic metabolism and typically ends in fatigue within 200 min (Beamish, 1978; Peake and Farrell, 2004). In contrast, active metabolic rate (AMR) is a term describing the oxygen consumption rate of fish at their maximum sustained swimming speed (U_{max}). Unlike prolonged swimming, sustained swimming can be maintained for > 200 min and is powered solely by aerobic metabolism (Beamish, 1978; Peake and Farrell, 2004). Beyond U_{max}, fish generally engage in burst-and-coast swimming and $\dot{M}O_2$ begins to asymptote (Sepulveda and Dickson, 2000; Claireaux et al., 2006). As a result, MMR often slightly exceeds AMR since fish are forced to swim beyond their maximum sustained swimming speed for a limited time (Bushnell et al., 1994; Schurmann and Steffensen, 1997). Once measured, SMR and MMR can be used to calculate a fish's aerobic scope for activity (AS), which determines the range of metabolic energy available for aerobic activities (Fry, 1947; Bushnell et al., 1994; Cutts et al., 2002; Claireaux and Lefrançois, 2007; Clark et al., 2011). SMR and MMR exclude metabolic activities powered anaerobically because anaerobic metabolism cannot be measured directly through oxygen consumption at the time of exercise (Korsmeyer and Dewar, 2001).

In swimming respirometry, the most common means of estimating a fish's metabolic rate is using a critical swimming speed (U_{crit}) protocol such as the one initially developed by Brett (1964) (Reidy et al., 1995; Plaut, 2001; Farrell, 2007). Fish are made to swim against a laminar water flow in a swimming respirometer while water velocity is increased incrementally, at regular intervals, until the fish fatigues. The U_{crit} is the swimming speed at which fish become exhausted and stop swimming. Because oxygen consumption is measured continuously while fish are exercised to exhaustion, swimming respirometry is thought to provide a very accurate estimate of MMR (Farrell and Steffensen, 1987; Plaut, 2001; Shultz et al., 2011). In contrast, SMR is not directly measured using this method, but can be calculated by extrapolating the non-linear swimming speed-$\dot{M}O_2$ relationship to a swimming speed of zero (Bushnell et al., 1994; Reidy et al., 2000; Korsmeyer and Dewar, 2001; Korsmeyer et al., 2002; Binning et al., 2013). Despite many advantages of this method for measuring MMR, U_{crit} protocols can

be time consuming and species that are poor swimmers (e.g., ambush predators) often lack the motivation to swim in a respirometer (Peake and Farrell, 2006).

To circumvent the limitations of swimming respirometers, exhaustive chase protocols have been developed to estimate MMR whereby fish are manually chased to exhaustion (Black, 1958; Milligan, 1996; Kieffer, 2000) and immediately placed into a resting respirometer (Cutts et al., 2002; Jordan and Steffensen, 2007; Norin and Malte, 2011). Variations of this method also exist in which fish are briefly held out of the water after chasing (Ferguson and Tufts, 1992; Donaldson et al., 2010; Clark et al., 2012). Air exposure contributes to increasing metabolic demands and has been argued to simulate exercise stress associated with catch-and-release fisheries, where fish are temporarily held out of the water to allow hook removal (Donaldson et al., 2010; Clark et al., 2012). Because the volume of resting respirometers is generally small relative to the size of the fish, individuals tend to remain immobile in the chamber and MMR is measured during recovery from exercise/chasing (Steffensen, 2005). This method relies on post-exercise oxygen consumption rates and MMR therefore corresponds to the sum of the fish's routine metabolic rate (RMR; $\dot{M}O_2$ during activities that elevate SMR (Schurmann and Steffensen, 1997; Steffensen, 2005)) and excess post-exercise oxygen consumption (EPOC) to repay the oxygen debt incurred from anaerobic metabolism during chasing (Killen et al., 2007). One major advantage of using resting respirometry is that SMR can be measured while fish have remained inactive in the chamber for several hours (typically between 2 to 24 hours, depending on the species), thus allowing both SMR and MMR to be calculated in one trial (Cutts et al., 2001; Brick and Cech, 2002; Cutts et al., 2002; Nilsson and Ostlund-Nilsson, 2004; Nilsson et al., 2009; Nilsson et al., 2010; Donelson et al., 2011; Norin and Malte, 2011; Clark et al., 2012).

Some studies suggest or anecdotally report that similar MMR measurements can be obtained using both exhaustive chase protocols and U_{crit} protocols (e.g., Killen et al., 2007; Gingerich et al., 2010). However, a comprehensive comparison of key metabolic parameters measured with different respirometry methods has yet to be conducted. Here, we compare data obtained using three common methods of measuring SMR and MMR in fishes: (1) a traditional U_{crit} protocol, (2) an exhaustive chase protocol by manual chasing, and (3) an exhaustive chase protocol by manual chasing followed by brief (1 min) air exposure. $\dot{M}O_2$ measurements for protocol (1) were carried out in a

swimming respirometer whereas measurements for protocols (2) and (3) were performed in resting respirometers.

Materials and methods

Study site and species

We chose the coral reef fish *Scolopsis bilineata* (Nempiteridae) for this study due to its high abundance on the Great Barrier Reef (Boaden and Kingsford, 2012; Roche et al., 2013), adequate size relative to the respirometry equipment used, and amenable behaviour in the swimming respirometer (Binning et al., 2013). Adult fish were collected by divers using barrier and hand nets between February and March 2012 from reefs surrounding Lizard Island, on the northern Great Barrier Reef, Australia (14° 40' S; 145° 28' E). Fish were transported live in buckets to the aquarium facilities at the Lizard Island Research Station within two hours of capture and held in individual aquaria (40.0W× 29.0L× 18.0H cm) with a flow-through water system directly from the reef. Fish were fed once daily with pieces of raw prawn (mean wet weight approx. 1g) and maintained in aquaria for a minimum of three days before the respirometry trials. Length measurements for individual fish were obtained by holding each fish in a plastic bag half-filled with water and measuring total length (TL), body width and body depth with handheld callipers. Body mass (M) was measured directly on a balance. Fish were fasted for 24h prior to the experimental trials (Johansen and Jones, 2011; Shultz et al., 2011) to evacuate the digestive tract and standardize a post-absorptive state that maximizes energy availability for swimming (Niimi and Beamish, 1974). Ten fish (TL= 17.6 ± 0.4 cm; M = 97.0 ± 7.7 g; mean ± s.d.) were subjected to each of three protocols in a random order: a critical swimming speed (U_{crit}) trial (Brett, 1964; Beamish, 1978; Eliason et al., 2011; Johansen and Jones, 2011), a 15 min exhaustive chase trial (see Cutts et al., 2002; Killen et al., 2007; Fu et al., 2009; Norin and Malte, 2011; Shultz et al., 2011), and a three minute exhaustive chase followed by one minute air exposure trial (Ferguson and Tufts, 1992; Donaldson et al., 2010; Clark et al., 2012). The same fish (n=10) were subjected to each protocol following a repeated measures design (Reidy et al., 1995) to minimize inter-individual variation in metabolic rates. Fish were fed and allowed a minimum of 48 hours to recover between trials. Prior to trials, individuals were starved for at least 24 h, but never more than 36 h. In all three protocols, oxygen consumption rates were measured using intermittent-flow respirometry (Steffensen et al., 1984; Steffensen, 1989).

Swimming respirometry

Swimming trials were carried out in an 11.9 L Loligo flow tank respirometer (swim chamber dimensions 40.0L× 10.0W × 10.0H cm) filled with well-aerated, filtered and UV-sterilized seawater and maintained at a constant temperature of 28 ± 0.1°C (mean ± actual range). Oxygen levels in the respirometer were recorded using a Fibox 3 fiber optic oxygen meter (PreSens, Regensburg, Germany) online feed into the AutoResp 1 Software (Loligo Systems, Copenhagen, Denmark). Flow in the working section of the respirometer was calibrated using a digital TAD W30 flow-meter (Höntzsch, Waiblingen, Germany). Solid blocking effects of the fish in the working section were corrected by the respirometry software (AutoResp, Loligo Systems, Copenhagen, Denmark) following Bell & Terhune (1970). We used 10 min determination periods with a 240s flush, 60s wait and 300s measurement cycle (Binning et al., 2013). Once an individual's length and mass were inputted into the software, three determinations were run without fish to measure initial background rates of respiration from bacterial load in the test chamber. The fish was then placed in the respirometer and left to habituate to the chamber for six to eight hours at a swimming speed of 0.75 BLs^{-1} until oxygen consumption rates stabilized (Johansen et al., 2010; Binning et al., 2013). This speed corresponded to the lowest water flow necessary to ensure constant swimming and minimize spontaneous activity in this species. To start the trial, the flow speed was slowly increased to 1.25 BL s^{-1} and maintained constant for three $\dot{M}O_2$ determinations (see Brett, 1964). Flow speed was incrementally increased by 0.5 BL s^{-1} every three determinations for the duration of the experiment. Trials were complete when fish could no longer maintain their position in the swim chamber and were forced to rest against the back grid of the chamber (U_{crit}) for > 5s (Johansen and Jones, 2011). The time and speed was recorded and the water flow was reduced to 0.75 BL s^{-1} to ensure the fish's recovery from oxygen debt. We calculated U_{crit} following Brett (1964):

$$U_{crit} = U + U_i * (t/t_i) \quad (1)$$

where U is the penultimate swimming speed before the fish fatigued and stopped swimming; U_i is the swimming speed at which the fish was unable to continue swimming; t is the length of time the fish swam at the final swimming speed where fatigue occurred; t_i is the amount of time fish were swam at each speed interval (i.e., 30

min). The fish was then removed from the test chamber and returned to its holding tank. Three additional determinations were run to measure final background rates of respiration in the chamber. Background oxygen consumption rates at the end of each cycle were determined from the slope of the linear regression between initial and final background rates, and were subtracted from each $\dot{M}O_2$ determination. All slopes aside from background respiration rates had an r^2 greater than 0.97. To reduce bacterial growth and respiration in the chamber, the respirometer was drained and rinsed in freshwater when the background consumption rates exceeded 15% of the resting metabolic rate of the fish.

We calculated oxygen consumption rate at $U = 0.75$ BL s^{-1} following three different methods commonly used in swimming respirometry studies: (1) by averaging the three lowest $\dot{M}O_2$ measurements (SMR_{swim_low}) before increasing U to 1.25 BL s^{-1} (Schurmann and Steffensen, 1997); (2) by averaging the three last $\dot{M}O_2$ measurements (SMR_{swim_last}) immediately before increasing U to 1.25 BL s^{-1} (Binning et al., 2013); and (3) by generating a frequency distribution of $\dot{M}O_2$ (bin size = 5 mg O_2 kg^{-1} h^{-1}) at U = 0.75 BL s^{-1} and averaging values in the lowest mode (SMR_{swim_hist}) to exclude elevated values resulting from spontaneous activity (Steffensen et al., 1994; Korsmeyer et al., 2002; Jordan and Steffensen, 2007; Svendsen et al., 2012). For this third method, a double normal distribution was fitted to a frequency histogram of the raw $\dot{M}O_2$ data: elevated values of $\dot{M}O_2$ corresponding to the first normal distribution were excluded and the second normal distribution with lower $\dot{M}O_2$ values was used to provide an estimate of $\dot{M}O_2$ at $U = 0.75$ BL s^{-1}. We averaged three $\dot{M}O_2$ measurements in (1) and (2) for consistency with $\dot{M}O_2$ calculations at higher swimming speeds. Oxygen consumption rate ($\dot{M}O_2$) was then plotted against swimming speed (U) to produce an oxygen consumption curve, including only speeds that resulted exclusively in aerobic activity (i.e., from $U = 0.75$ to $U = 3.25$ BL s^{-1}). The onset of anaerobic activity was determined as the swimming speed when fish transitioned gait from steady to unsteady (bursting-and-coasting) swimming (Peake and Farrell, 2004). Bursting and coasting was defined when the fish used caudal fin beats (typically 1, 2 or 3 beats) and a subsequent forward glide motion >5 cm. Standard metabolic rate (SMR) was obtained by extrapolating the curve to $U = 0$ BL s^{-1} (Steffensen et al., 1994; Schurmann and Steffensen, 1997; Korsmeyer et al., 2002) using either a traditional exponential function (Brett, 1964; Webb, 1975; Korsmeyer et al., 2002; Binning et al., 2013) with two (equation 2) or

three (equation 3) parameters, or the hydrodynamics-based power function with three-parameters (equation 4) (Wu, 1977; Videler, 1993; Korsmeyer et al., 2002; Johansen et al., 2010; Svendsen et al., 2010).

$$\dot{M}O_2 = a10^{bU} \quad (2)$$

$$\dot{M}O_2 = a + b10^{cU} \quad (3)$$

$$\dot{M}O_2 = a + bU^c \quad (4)$$

Therefore, for each of the three different functional forms, three SMR estimates were obtained (SMR_{swim_low}, SMR_{swim_last}, SMR_{swim_hist}) following different calculations of $\dot{M}O_2$ at $U = 0.75$ BL s^{-1}. Maximum metabolic rate (MMR_{swim}) was measured at the maximum swimming speed where fish completed at least one 10 min $\dot{M}O_2$ determination; we averaged $\dot{M}O_2$ values when fish completed more than one determination (up to three determinations).

Resting respirometry

Resting respirometry differs from swimming respirometry in that resting chambers are simpler and more affordable, allowing the benefit of testing multiple fish simultaneously. Individual chambers are connected to flush pumps that turn on intermittently after each $\dot{M}O_2$ determination to replenish the chamber with oxygenated seawater; a closed-loop recirculation pump also mixes the water inside the chamber during $\dot{M}O_2$ determinations. Our resting respirometry system consisted of four darkened cylindrical chambers 3.48 L in volume, fitted with fiber optic oxygen probes and immersed in a temperature-controlled aquarium (100 x 52 x 49 cm; length x width x height) filled with aerated seawater. The water temperature was maintained at 28 ± 0.5°C (mean ± actual range) and dissolved oxygen concentration was recorded with a four channel FireSting O_2 Optical Oxygen Meter (Pyroscience, Aachen, Germany). We used two different methods to estimate MMR. The first consisted of a 15 min exhaustive chase trial in which individual fish were placed in a 110 cm diameter circular tank and chased continuously with a 20 mm diameter pvc tube until exhaustion (i.e., all fish became unresponsive within 12-15 min) (see Cutts et al., 2002; Fu et al., 2006; Killen et al., 2007; Fu et al., 2009; Norin and Malte, 2011). The experimenter would only touch the tail of the fish if it slowed down or stopped swimming. Fish swam primarily with their caudal fin, occasionally bursting-and-coasting. The second method

consisted of a 3 min exhaustive chase followed by 1 min of air exposure (Ferguson and Tufts, 1992; Donaldson et al., 2010; Clark et al., 2012). Fish were chased in an identical manner and subsequently maintained out of the water in a rubber mesh net for 1 min. Following either procedure, each fish was immediately placed in a resting respirometry chamber and their oxygen consumption measured for 5 min. The measurement period started within 10 s from cessation of chasing for the 15 min chase protocol, and 10 s following the end of air exposure for the 3 min chase 1 min air exposure protocol. This $\dot{M}O_2$ measurement corresponded to the maximum metabolic rate: MMR_{chase} or MMR_{air}, depending on the method employed. Subsequently, $\dot{M}O_2$ was measured continuously following a 10 min measurement and 10 min flush cycle. SMR was obtained by leaving the fish in the chamber overnight between 6 and 12 hours, and calculated in one of three ways: (1) by averaging the three lowest $\dot{M}O_2$ measurements recorded (SMR_{rest_low}); (2) by averaging the three last $\dot{M}O_2$ measurements recorded (SMR_{rest_last}); and (3) by generating a frequency distribution of all $\dot{M}O_2$ measurements recorded (bin size = 5 mg O_2 kg^{-1} h^{-1}) and averaging values in the lowest mode (SMR_{rest_hist}). Previous resting respirometry studies have averaged either three (Cutts et al., 2001; Brick and Cech, 2002; Cutts et al., 2002) or six $\dot{M}O_2$ measurements to obtain SMR estimates (Schurmann and Steffensen, 1997; Gingerich et al., 2010; Norin and Malte, 2011; Shultz et al., 2011). We chose to average three measurements for consistency with $\dot{M}O_2$ calculations in the U_{crit} protocol. Oxygen consumption rate ($\dot{M}O_2$ in mg O_2 kg^{-1} h^{-1}) was calculated with LabChart v. 6.1.3 (ADInstruments, Dunedin, New Zealand) as the slope of the linear regression of oxygen concentration decline over time for each determination cycle using the equation (Bushnell et al., 1994; Schurmann and Steffensen, 1997):

$$\dot{M}O_2 = sV_{resp}\alpha M^{-1} \quad (5)$$

where s is the slope (mmHg h^{-1}), V_{resp} is the volume of the respirometer minus the volume of the fish (L), α is the solubility of oxygen in water (μgO_2 L^{-1} $mmHg^{-1}$) adjusted for temperature and barometric pressure and M is the mass of the fish (kg). Three determinations were run before and after each trial to measure background rates of bacterial respiration in individual chambers, which were subtracted from $\dot{M}O_2$ values upon calculation. The system was rinsed in freshwater every third day to insure that

background oxygen consumption rates remained below 15% of the resting metabolic rate of fish.

Statistical analysis

We used a linear mixed effects model (LMM; lme function in R) to compare values of maximum metabolic rate (MMR_{swim}, MMR_{chase}, MMR_{air}). We used a second LMM to compare SMR estimates obtained in resting respirometry (SMR_{rest_low}, SMR_{rest_last}, SMR_{rest_hist}) with those obtained in swimming respirometry (SMR_{swim_low}, SMR_{swim_last}, SMR_{swim_hist}) using three different functional forms to describe the relationship between swimming speed and $\dot{M}O_2$ (i.e., a two-parameter exponential function, a three-parameter exponential function and a three-parameter power function). Linear mixed models can be used to reduce inter-individual variation in metabolic rates and control for the non-independence of data points obtained on the same individuals (Bolker et al., 2009). Diagnostic plots and Shapiro-Wilk's test were used to ensure that the data met the assumptions of the models. We compared the fit of non-linear relationships by computing the proportion of variance explained. All analyses were performed in R v2.11.1 (R Development Core Team, 2010).

Results

The mean (± s.e.m.) critical swimming speed for all fish was 3.76 ± 0.10 BL s^{-1}, whereas the mean (± s.e.m.) maximum swimming speed at which fish completed at least one 10 min $\dot{M}O_2$ determination was 3.85 ± 0.10 BL s^{-1}. At 4.25 BL s^{-1}, only three out of ten fish completed one $\dot{M}O_2$ determination. MMR differed according to the respirometry method employed (LMM; $F_{2,18} = 19.2$, $p < 0.001$; Fig. 1A,B): MMR_{swim} was 36% higher than MMR_{chase} (estimate = -167.69, 95% CI = -221.35 to -114.03, t = -6.13, $p < 0.001$) and 23% higher than MMR_{air} (estimate = -107.15, 95% CI = -160.81 to -53.49, t = -3.91, $p = 0.001$). MMR_{air} was significantly higher than MMR_{chase} (estimate = 60.54, 95% CI = 6.88 to 114.20, t = 2.21, $p = 0.04$; Figs 2B, 3A).

SMR estimates obtained in resting respirometry differed based on the calculation method used: SMR_{rest_low} was significantly lower than SMR_{rest_last} (estimate = 23.75, 95% CI = 6.71 to 40.78, t = 2.73, $p < 0.01$) but not different from SMR_{rest_hist} (estimate = 7.84, 95% CI = -9.19 to 24.87, t = 0.90, $p > 0.3$); there was no significant difference between SMR_{rest_last} and SMR_{rest_hist} (estimate = -15.91, 95% CI = -32.94 to

1.12, t = -1.83, p = 0.07) (Fig. 2A,B). In contrast, when calculated for each of the three functional forms, SMR estimates obtained in swimming respirometry did not differ significantly, irrespective of the calculation method employed (LMM; all p values > 0.05; Fig. 2B).

Fitting a two-parameter exponential function produced SMR estimates 25% lower, on average, than the lowest SMR estimate obtained in resting respirometry (LMM; contrast group = SMR_{rest_low}; SMR_{swim_low} estimate = -27.49, 95% CI = -44.59 to -10.39, t = -3.15, p = 0.004; SMR_{swim_last} estimate = -24.29, 95% CI = -41.40 to -7.19, t = -2.78, p = 0.01; SMR_{swim_hist} estimate = -22.37, 95% CI = -39.47 to -5.27, t = -2.56, p = 0.016). Alternatively, SMR_{rest_low} did not differ from SMR_{swim_low} when we fit a three-parameter exponential function or a three-parameter power function to the swimming speed-$\dot{M}O_2$ relationship (LMM; all p values > 0.05; Table 1; Fig. 2B). When using either a three-parameter exponential or power function, most differences between SMR obtained in resting versus swimming respirometry occurred between SMR_{rest_last} and SMR_{swim_last} (Table 1; Fig. 2B); there were very few significant differences between SMR_{rest_hist} and SMR_{swim_hist} (Table 1; Fig. 2B). Including $\dot{M}O_2$ measurements at speeds that induced bursting-and-coasting (U = 3.75 and 4.25 BL s^{-1}) into the swimming speed-$\dot{M}O_2$ relationship did not change these results qualitatively.

Discussion

We found notable differences in MMR, a key metabolic rate parameter, measured using different respirometry methods (Fig. 1). Previous studies have suggested that resting and swimming respirometry produce similar MMR estimates (Gingerich et al., 2010), with some support from data on the lumpfish *Cyclopterus lumpus* (Killen et al., 2007). Although there was overlap in SMR estimates obtained with different methods, MMR estimated using a U_{crit} protocol was significantly higher than MMR obtained using two different exhaustive chase protocols combined with resting respirometry.

Using swimming respirometry, SMR is indirectly measured by extrapolating the swimming speed-$\dot{M}O_2$ relationship to U = 0 BL s^{-1} (Brett, 1964; Bushnell et al., 1994; Schurmann and Steffensen, 1997). Following this approach, we used three common calculations to estimate SMR, either by averaging 1) the lowest three $\dot{M}O_2$

measurements at $U = 0.75$ BL s^{-1} (SMR_{swim_low}), 2) the last three $\dot{M}O_2$ measurements at $U = 0.75$ BL s^{-1} (SMR_{swim_last}), or 3) all values in the lowest mode of an $\dot{M}O_2$ frequency distribution at $U = 0.75$ BL s^{-1} (SMR_{swim_hist}). Despite different calculations, the three SMR estimates did not significantly differ from each other (Fig. 2) and considerably overlapped SMR_{rest_low} and SMR_{rest_hist} estimates (Table 1) when extrapolated based on a three-parameter exponential or power function (Fig. 3B,C). SMR_{rest_last} differed from the two other SMR estimates in resting respirometry because spontaneous activity elevated $\dot{M}O_2$ values in the early morning, towards the end of the trials. This was not the case in swimming respirometry as U_{crit} trials began shortly before sunrise.

In a study on the Atlantic cod, *Gadus morhua,* Schurmann and Steffensen (1997) found similar results when comparing SMR estimated using both swimming and resting respirometry. Our findings also suggest that SMR can accurately be estimated by extrapolating the swimming speed-$\dot{M}O_2$ relationship obtained from U_{crit} protocols. Importantly however, when we fit a more simple, two-parameter exponential function to this data, SMR values estimated with the U_{crit} protocol were ~25% lower than SMR_{rest_low}, irrespective of the calculation employed (Table 1, Fig. 3C). This finding is in stark contrast with those of Korsmeyer et al. (2002), who recommend using the traditional two-parameter exponential function. While this simpler, function requires deriving only two constants (Korsmeyer et al., 2002), it may not be the most reliable functional form to extrapolate $\dot{M}O_2$ beyond the range of swimming speed values measured. In contrast, the hydrodynamics-based power function is believed to overestimate SMR since it places more weight on higher swimming speed values (Videler and Nolet, 1990; Korsmeyer et al., 2002). Our SMR estimates from the hydrodynamics-based power function were higher than estimates from the three-parameter exponential function, but this difference was not significant (Fig. 2).

We obtained higher MMR estimates in the swimming respirometry protocol compared to either exhaustive chase protocols. Several factors may explain this difference. Chasing by an experimenter may not have induced complete exhaustion in *S. bilineata* even if fish became unresponsive towards the end of the chase. However, the duration of our 15 min protocol greatly exceeded that of typical 1-5 min chases in the published literature (Cutts et al., 2002; Fu et al., 2009; Gingerich et al., 2010; Norin and Malte, 2011; Shultz et al., 2011; Clark et al., 2012). *S. bilineata* uses its pectoral and

caudal fins for swimming and has intermediate to high sustained swimming abilities (Fulton, 2007; Binning et al., 2013). In contrast, many of the fishes that have been subjected to exhaustive chase protocols thus far are body-caudal fin (BCF) swimmers with high unsteady (burst) swimming performance, such as trout (Ferguson and Tufts, 1992; Norin and Malte, 2011), Pacific salmon (Donaldson et al., 2010; Clark et al., 2012), bonefish (Shultz et al., 2011), and bass (Gingerich et al., 2010). Studies suggest that 1-2 min chases are sufficient to achieve complete fatigue in these species (Gingerich et al., 2010; Norin and Malte, 2011; Clark et al., 2012). Rapid exhaustion most likely occurs because manual chasing induces repetitive burst swimming (Clark et al., 2012), which is powered by white muscle fibres and anaerobic metabolism (Milligan, 1996; Kieffer, 2000). The use of fast, glycolytic muscles for escape swimming explains why these fishes fatigue rapidly and incur large oxygen debts, which can be measured as post-exercise metabolism in the resting respirometry chamber. Alternatively, some BCF swimmers, such as Atlantic salmon (Cutts et al., 2002), carp and catfishes (Fu et al., 2009) can sustain unsteady swimming for longer periods and require chases up to 5 minutes to reach full exhaustion. Pectoral (e.g., Labridae, Scaridae, Pomacentridae, Cichlidae, Embiotocidae) and pectoral-caudal (e.g., Chaetodontidae, Nemipteridae) swimmers may require even longer exhaustive chases, however, as we observed in the case of *S. bilineata*. Fishes that use their median-paired fins (MPF) for swimming may burst less frequently during chases (e.g., Gotanda et al., 2009) and utilize both red (aerobic) and white (anaerobic) muscle fibres to power their escape. As such, increased use of red muscle could lead to lower oxygen debts and reduced EPOC required to clear metabolites resulting from anaerobic activity. Since the magnitude of EPOC directly influences measurements of MMR in resting respirometry (Reidy et al., 1995), fishes that escape using a combination of white and red muscles will likely display lower $\dot{M}O_2$ values than fishes relying predominantly on white muscle and anaerobic metabolic pathways. Although swimming respirometry appears to be a better method for measuring MMR in fish that are good steady swimmers, the opposite may be true of fish with better unsteady swimming performance (see Peake and Farrell, 2006). For example, in a study of post-exercise metabolic rates in Atlantic cod, Reidy et al. (1995) found that MMR during recovery after exhaustive chasing significantly exceeded $\dot{M}O_2$ measurements at U_{crit}, which is contrary to our results.

Lastly, the short 3 min exhaustive chase and 1 min air exposure protocol yielded higher estimates of MMR than the prolonged 15 min chase. Brief periods of air exposure have been used in a number of studies to simulate fisheries encounters (Ferguson and Tufts, 1992; Donaldson et al., 2010; Clark et al., 2012), and likely push fish beyond their anaerobic threshold, leading to increased EPOC. Given the considerable variability in the duration of fish responses to chase protocols and the likelihood of additional variation from using different chasing techniques and intensity (e.g., tail pinching vs. manual or stick chasing), air exposure may provide a very effective method of standardizing exhaustive chase protocols and improving the accuracy of MMR estimates across species.

Conclusions and recommendations

Our experiment demonstrated that, for *S. bilineata*, the U_{crit} swimming protocol provided a more accurate estimate of MMR than chase protocols combined with resting respirometry. However, because swimming respirometry is impractical for some species (Reidy et al., 1995; Jordan and Steffensen, 2007), chasing followed by air exposure likely provides the best alternative. Furthermore, we found that SMR can accurately be estimated from data obtained using swimming respirometry. However, extrapolating the oxygen consumption curve depends on the functional form used to describe the swimming speed-$\dot{M}O_2$ relationship. As such, resting respirometers provide a reliable measure of SMR with which to compare estimates from U_{crit} protocols and should be used whenever possible. Additional studies are required to test how data produced with various respirometry methods compare across fish species with different life histories (e.g., demersal vs. pelagic, predatory vs. herbivorous) and swimming behaviours (e.g., pectoral, pectoral-caudal and caudal swimming). However, caution is warranted when comparing results obtained with different approaches, particularly in the case of MMR, unless cross-validation has been performed on a species-specific basis. Bearing in mind these results and the limitations of the methods used, researchers should carefully choose the apparatus and method most appropriate for their species and specific research questions before conducting respirometry studies.

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Table 1. *P* values from LMM contrasts between SMR obtained in resting respirometry (SMR_{rest}) and swimming respirometry (SMR_{swim}). SMR_{swim} was calculated using three different functional forms: a two-parameter exponential function (exp 2), a three-parameter exponential function (exp 3), and a three-parameter power function (power 3). Three different calculation methods were used for all SMR estimates: by averaging 1) the three lowest $\dot{M}O_2$ determinations (low), 2) the three last $\dot{M}O_2$ determinations (last), and 3) $\dot{M}O_2$ values in the lowest mode of an $\dot{M}O_2$ frequency distribution (hist). Significance is denoted by * with $\alpha = 0.05$.

	SMR_{swim} exp 2			SMR_{swim} exp 3			SMR_{swim} power 3		
SMR_{rest}	**low**	**last**	**hist**	**low**	**last**	**hist**	**low**	**last**	**hist**
low	<0.01*	<0.01*	0.01*	0.24	0.89	0.5	0.78	0.13	0.052
last	<0.001*	<0.001*	<0.001*	<0.001*	0.01*	0.04*	0.02*	0.22	0.44
hist	<0.001*	<0.001*	<0.001*	0.04*	0.44	0:82	0.54	0.55	0.29

Fig. 1 A) Maximum metabolic rate (MMR) for 10 *S. bilineata* obtained with three different methods: 1) in a swim respirometer (MMR_{swim}), 2) in a resting respirometer after a 15 min exhaustive chase trial (MMR_{chase}), and 3) in a resting respirometer after a three min exhaustive chase trial followed by one min air exposure (MMR_{air}); B) Paired difference between MMR_{swim} and MMR_{chase} as well as MMR_{air}, controlling for the non-independence of measurements on the same fish. Error bars are s.e.m.

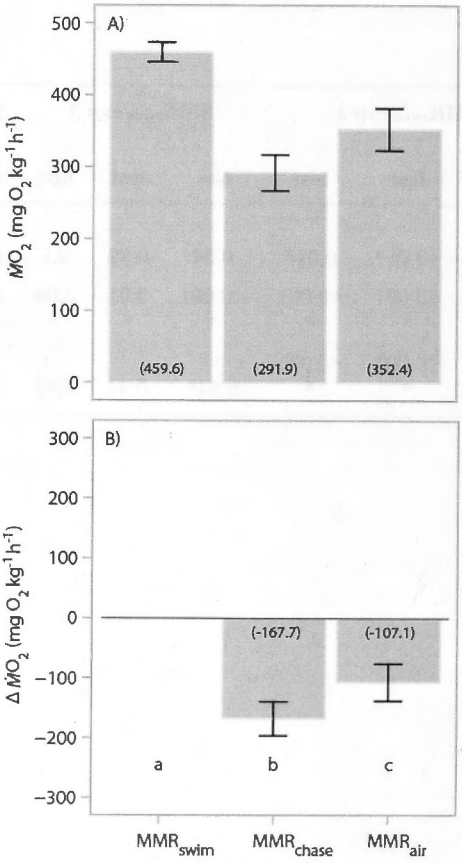


Fig. 2 A) Standard metabolic rate (SMR) for 10 *S. bilineata* obtained with two different respirometry methods and three distinct calculations: first, in a resting respirometer (resting) by averaging 1) the three lowest $\dot{M}O_2$ determinations (low, light grey bar), 2) the three last $\dot{M}O_2$ determinations (last, grey bar), and 3) $\dot{M}O_2$ values in the lowest mode of a $\dot{M}O_2$ frequency distribution (hist, dark grey bar); second, in a swimming respirometer (swim) by extrapolating the swimming speed-$\dot{M}O_2$ relationship to a speed of zero after averaging 1) the three lowest $\dot{M}O_2$ determinations at U=0.75 BL s^{-1} (low, light grey bar), 2) the three last $\dot{M}O_2$ determinations at U=0.75 BL s^{-1} (last, grey bar), and 3) $\dot{M}O_2$ values in the lowest mode of a $\dot{M}O_2$ frequency distribution at U=0.75 BL s^{-1} (hist, dark grey bar). Three different functional forms were fitted to the swimming speed-$\dot{M}O_2$ relationship in swimming respirometry: a two-parameter exponential function (swim exp 2), a three-parameter exponential function (swim exp 3) and a three-parameter power function (swim power 3). B) Paired differences controlling for the non-independence of measurements on the same fish using SMR_{rest_low} as a contrast. Error bars are s.e.m.

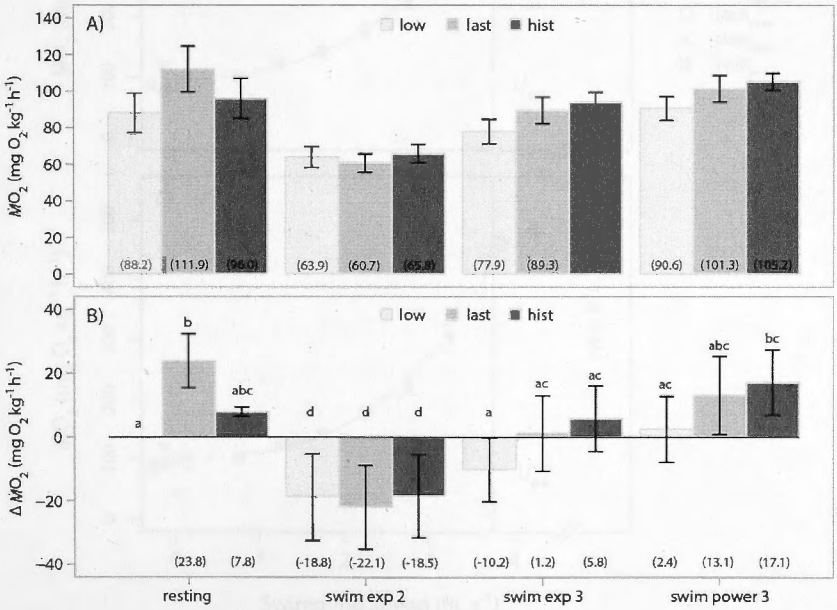
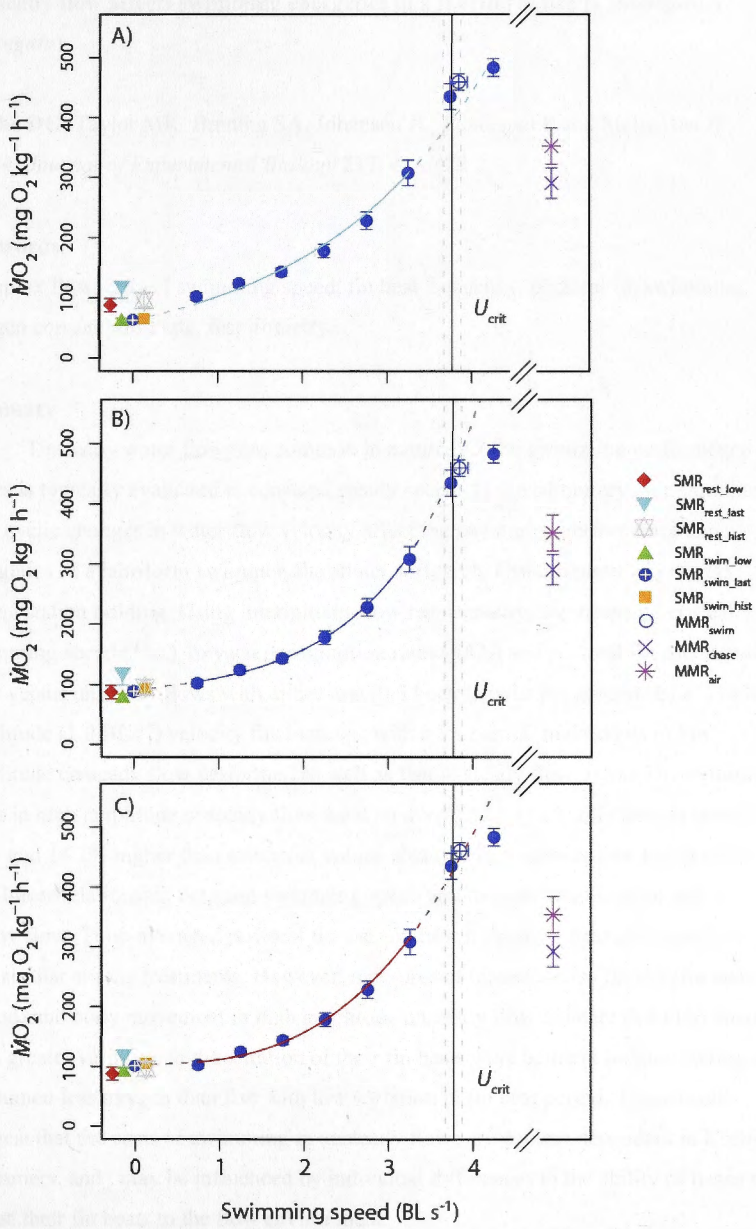


Fig. 3 The relationship between the rate of oxygen consumption ($\dot{M}O_2$) and swimming speed (U) for the coral reef fish, *S. bilineata* (n=10). SMR estimates were calculated by extrapolation of A) a two-parameter exponential function (turquoise line; $\dot{M}O_2 = 62.9$ (± 6.45 s.e.m.) * $10^{0.21(\pm 0.02)\,U}$, $r^2 = 0.77$); B) a three-parameter exponential function (blue line; $\dot{M}O_2 = 77.72$ (± 25.70 s.e.m.) + 14.12 (± 11.50 s.e.m.) * $10^{0.38(\pm 0.10)\,U}$, $r^2 = 0.78$); and C) a three-parameter power function (red line; $\dot{M}O_2 = 104.13$ (± 11.99 s.e.m.) + 7.26 (± 4.75 s.e.m.) * $U^{2.86(\pm 0.54)}$, $r^2 = 0.78$). Intercepts are slightly different from those displayed in Fig. 2, which are mean intercepts for ten relationships. Solid lines indicate relationships based on aerobic swimming only ($0.75 \geq U \leq 3.25$ BL s^{-1}); broken lines indicate extrapolations to $U = 0$ BL s^{-1} and beyond $U = 3.25$ BL s^{-1}, at speeds that resulted in bursting-and-coasting (anaerobic metabolism). Symbols are described in the legend and error bars are s.e.m. Values of SMR are offset at $U = 0$ BL s^{-1} to avoid overlap. Mean U_{crit} is shown as a vertical solid line with broken grey lines indicating s.e.m. Sample sizes are n = 10 at $U \leq 3.25$ BL s^{-1}, n = 9 at $U = 3.75$ BL s^{-1} and n = 3 at $U = 4.25$ BL s^{-1}.


A)
B)
C)
MO_2 (mg O_2 kg^{-1} h^{-1})
0
100
200
300
400
500
U_{crit}
0
1
2
3
4
Swimming speed (BL s^{-1})
SMR_{rest_low}
SMR_{rest_last}
SMR_{rest_hist}
SMR_{swim_low}
SMR_{swim_last}
SMR_{swim_hist}
MMR_{swim}
MMR_{chase}
MMR_{air}

CHAPTER - 4

Unsteady flow affects swimming energetics in a labriform fish (*Cymatogaster aggregata*)

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Keywords

Complex flow, critical swimming speed, fin beat frequency, pectoral fin swimming, oxygen consumption rate, respirometry.

Summary

Unsteady water flows are common in nature, yet the swimming performance of fishes is typically evaluated at constant, steady speeds in the laboratory. We examined how cyclic changes in water flow velocity affect the swimming performance and energetics of a labriform swimmer, the shiner surfperch, *Cymatogaster aggregata*, during station holding. Using intermittent-flow respirometry, we measured critical swimming speed (U_{crit}), oxygen consumption rates ($\dot{M}O_2$) and pectoral fin use in steady flow versus unsteady flows with either low (0.5 body lengths per second; BLs^{-1}) or high amplitude (1.0 BLs^{-1}) velocity fluctuations, with a 5 s period. Individuals in low amplitude unsteady flow performed as well as fish in steady flow. However, swimming costs in high amplitude unsteady flow were on average 25.3 % higher than in steady flow and 14.2% higher than estimated values obtained from simulations based on the non-linear relationship between swimming speed and oxygen consumption rate in steady flow. Time-averaged pectoral fin use (fin beat frequency measured over 300 s) was similar among treatments. However, measures of instantaneous fin use (fin beat period) and body movement in high amplitude unsteady flow indicate that individuals with greater variation in the duration of their fin beats were better at holding station and consumed less oxygen than fish with low variation in fin beat period. These results suggest that the costs of swimming in unsteady flows are context dependent in labriform swimmers, and may be influenced by individual differences in the ability of fishes to adjust their fin beats to the flow environment.

Introduction

The energetic costs of locomotion are a large and variable component of the energy budgets of mobile organisms (Boisclair and Sirois, 1993). Environmental factors that influence locomotor costs can therefore have profound effects on individual fitness (Arnold, 1983; Irschick and Garland, 2001). In fishes, the energetic costs of swimming have traditionally been estimated by measuring oxygen consumption rates in steady (i.e., constant speed) water flows (e.g., Steffensen et al., 1984; Claireaux et al., 1995; Farrell et al., 2003; Clark et al., 2011). However, these measures may not reflect the true costs of swimming in nature, where the velocity of water flows can vary dramatically over short time-scales (Liao, 2007; Webb et al., 2010). Increasingly, fish biologists and eco-physiologists are aware of the need to measure swimming costs in settings that approximate wild conditions both to improve our understanding of fish locomotion and for practical applications such as water and habitat management (Enders et al., 2003; Liao, 2007; Lacey et al., 2012).

In nature, water flow can be influenced by numerous physical variables including wind, gravity, and obstructions below the surface, creating complex water flows (Liao, 2007; Webb et al., 2010). Terms used to describe flow hydrodynamics with regard to swimming are often not clearly defined, which makes generalizations about the effects of these complex flows on fishes difficult (see Liao 2007). Turbulence usually refers to the creation of vortices of variable strengths and sizes in flowing water, whereas unsteady flows can be near-laminar and are characterized by changes in fluid velocity over time at a given point in space (Liao 2007, Webb et al. 2010). Depending on the causal agent, turbulent flows may have an element of predictability that can be exploited by swimming fish (Liao et al., 2003a, b; Liao, 2004; Beal et al., 2006; Cook and Coughlin, 2010; Taguchi and Liao, 2011). However, water flows with unpredictable and/or wide fluctuations in velocity are known to increase the costs of locomotion (Pavlov et al., 2000; Enders et al., 2003, 2005; Lupandin, 2005; Webb and Cotel, 2010). Whether unsteady flows represent advantages or disadvantages to swimming fish remains an important area of research.

One major impediment to studying the effects of complex flows on fish locomotion is the challenge of creating describable and/or repeatable hydrodynamic perturbations in an experimental setting (Liao, 2007; Lacey et al., 2012). As a result,

only a handful of studies have directly examined the metabolic costs of swimming in complex flows (Enders et al., 2003; Liao et al., 2003a; Cook and Coughlin, 2010; Tritico and Cotel, 2010; Taguchi and Liao, 2011). While extremely useful, these studies are restricted to fishes such as trout, salmon and minnows that use their body and caudal fin for propulsion (BCF swimmers). However, roughly 15-20% of all living fishes, including a large proportion of fishes in shallow marine (e.g., labrids, pomacentrids) and freshwater (e.g., cichlids, centrarchids) habitats, use their pectoral or paired fins for swimming (MPF or labriform swimmers) (Westneat, 1996; Bellwood and Wainwright, 2001). MPF swimmers are commonly found in habitats associated with complex flows (Fulton, 2010), especially inshore coastal habitats where wave-driven water motion varies considerably across local and regional gradients (Webb et al., 2010). For example, on the West Coast of the United States, bays and sounds that are sheltered from large storm waves regularly experience wave-driven water flows ranging from 0 to 50 cm s^{-1} (Finlayson, 2006; Gaylord et al., 2008). Similarly, shallow coral reef habitats in the tropics are routinely subjected to wind-driven water motion (Denny and Gaylord, 2010) and can harbour over 60% of fish species that use labriform swimming as a primary means of locomotion (Fulton, 2010). Currently we do not know how unsteady flows affect the swimming performance and energetics of labriform swimming fishes in coastal habitats.

We used intermittent-flow respirometry to compare the energetics, swimming performance and kinematics of a marine labriform swimmer, the shiner surfperch (*Cymatogaster aggregata* Gibbons), holding station in steady versus unsteady water flows. Our unsteady flow treatments mimicked a repeatable, unilateral wave surge scenario (sinusoidal variations in water flow velocity in a single direction, around a constant mean velocity) with low and high amplitude fluctuations in flow velocity. These two treatments are hereafter referred to as low and high unsteady flow. The vertical component of orbital waves was absent from the flow changes imposed, which therefore mimicked the horizontal component of waves. This movement (i.e., parallel to the seabed) is the dominant flow for travelling waves in shallow, coastal waters (Denny, 2006; Webb et al., 2010).

First, we tested whether fish swimming in unsteady flows incur greater swimming costs than fish in steady flow at the same mean velocity. Second, we

estimated the values of oxygen consumption rate ($\dot{M}O_{2\text{-}E}$) for fish swimming in the two unsteady flow treatments using simulations based on the non-linear relationship between swimming speed (U) and $\dot{M}O_2$ obtained in steady flow. Third, we compared observed ($\dot{M}O_2$) and estimated ($\dot{M}O_{2\text{-}E}$) oxygen consumption rates, where their difference may result from additional costs of accelerating and decelerating as well as maintaining stability in unsteady flow. Finally, we examined whether observed differences in $\dot{M}O_2$ between the three flow treatments were related to pectoral fin kinematics measured on different time scales (time-averaged over 300s vs. instantaneous over the duration of one fin beat cycle) and body movements in the swim chamber to understand possible mechanisms underlying differences in oxygen consumption rates.

Materials and methods

Fish collections and husbandry

Adult *Cymatogaster aggregata* were collected using a beach seine net at Fourth of July and Jackson's Beach on San Juan Island, Washington, USA, in August 2011. Fish were held in flow-through aquaria at the University of Washington's Friday Harbour Laboratories at an ambient light regime. Tanks were continuously supplied with filtered seawater (salinity 34 ppt) at a mean temperature of 12 °C (range 11 to 13 °C). Given the proximity of the laboratory to the collection site, fish were not fed and tested shortly after their capture, ensuring near-wild conditions during the experiments. Fish were fasted for a minimum of 24 h before the experimental trials to ensure that satiation was standardized across individuals (Niimi and Beamish, 1974; Johansen et al., 2010; Roche et al., 2013).

Respirometry

We measured oxygen consumption rates ($\dot{M}O_2$; $mgO_2\ kg^{-1}\ h^{-}1$) for 20 fish (total length L_T= 14.84 ± 0.49 cm; mass = 46.3 ± 6.3 g; means ± s.d.) swimming in an 8.31 L clear Plexiglas Steffensen-type respirometer (Steffensen et al., 1984; Methling et al., 2011) with a working section of 9.0×26.0×10.0 cm (width × length × depth) (electronic supplementary material; ESM, Fig. S1). Oxygen levels in the respirometer were recorded using a fiber optic oxygen meter (PreSens Fibox 3, Regensburg, Germany) monitored with AutoResp ™ V1 (Loligo Systems, Copenhagen, Denmark). We calibrated the flow in the working section of the respirometer from 0 to 80 ± 0.5 cm s^{-1}

(mean ± s.e.m) using a digital TAD W30 flow-meter (Höntzsch, Waiblingen, Germany) at 1 cm intervals. The flow velocity profile varied less than 5% across the full cross-section of the working chamber and we did not observe fish favouring one corner or particular side of the working section during the swim trials. Changes in flow speed inside the swim chamber can lag behind changes in the rotational speed of the propeller; however, we measured flow velocity during oscillations in propeller speed and observed minimal dampening and attenuation of intended flow speed minima/maxima in the swim chamber. Solid blocking effects of the fish were corrected by the respirometry software (AutoResp ™ V1); the mean fish cross sectional area was 8.1% of the swim chamber cross sectional area, corresponding to a 3.5-4% greater effective water velocity around the fish compared to the water velocity in the empty swim chamber (Webb, 1975). We used UV filtration to reduce bacterial growth in the system and regularly rinsed the respirometer in freshwater to ensure that bacterial respiration rates remained below 15% of the standard metabolic rate of fish. Three $\dot{M}O_2$ determinations were run without fish before and after each trial to measure bacterial respiration in the test chamber. Background respiration rates were determined from the slope of the linear regression between initial and final measurements of background respiration rates and subtracted from each $\dot{M}O_2$ determination.

At the start of a trial, fish were placed in the respirometer and left to acclimate for six to eight hours at a swimming speed of 0.5 BLs^{-1} until their oxygen consumption rate stabilized. This speed corresponded to the lowest water flow necessary to ensure constant swimming and minimize spontaneous activity. Using six of the 20 test subjects, we measured $\dot{M}O_2$ as a function of steady swimming speed (U) starting at 1.0 BLs^{-1} and increasing flow speed by increments of 0.5 BLs^{-1} every 30min, following a standard critical swimming speed (U_{crit}) protocol (Brett, 1964; Plaut, 2001). $\dot{M}O_2$ (in mg O_2 kg^{-1} h^{-1}) was calculated by the respirometry software (AutoResp ™ V1) as the slope of the linear regression of oxygen concentration decline over time for each determination cycle using the equation:

$$\dot{M}O_2 = sV_{resp}\alpha M^{-1} \quad (1)$$

where s is the slope (mmHg h^{-1}), V_{resp} is the volume of the respirometer minus the volume of the fish (L), α is the solubility of oxygen in water (μgO_2 L^{-1} $mmHg^{-1}$)

adjusted for temperature and barometric pressure and M is the mass of the fish (kg). $\dot{M}O_2$ was determined every 10 minutes following a 225 s flush, 75 s wait and 300 s measure cycle. The trial stopped when fish could no longer swim unassisted or were forced to rest on the back grid of the flow chamber for five or more seconds. Both time and speed at this occurrence were recorded and the water flow was reduced to 0.5 BLs^{-1}. The fish was then removed from the test chamber and returned to its holding tank.

We repeated the same step-wise procedure for the remaining 14 test subjects, but varied flow velocity by an amplitude of either 0.5 BLs^{-1} (low unsteady flow; n=7) or 1.0 BLs^{-1} (high unsteady flow; n=7) around the mean at each speed increment (e.g., 1.0 ± 0.5 BLs^{-1}). These velocity fluctuations were adjusted for each individual fish based on body length and approximated natural, wave-induced changes in flow speed on the coasts of Puget Sound (0 – 50 cm s^{-1}; Finlayson 2006). We used a computer generated sine function with a period of 5 s (TracerDAQ Pro™, Measurement Computing, Norton, MA, USA) to create repeatable variations in water flow speed by continuously controlling the voltage to the motor and the rotational speed of the propeller in the respirometer. Five seconds corresponds to moderate wave periods in the San Juan Islands (Finlayson, 2006). The computer was connected to a motor controller (Movitrac AC VFD, SEW Eurodrive, Lyman, SC, USA) via a USB-1208 ADDA converter (USB DAQ Data Acquisition, Measurement Computing, Norton, MA, USA). Flow straighteners were used to dissipate gross turbulence and produce flow with uniform micro-turbulence (Beamish, 1978). Eliminating micro-turbulence in flumes is practically impossible (Beamish, 1978); however, very small orbits with diameters much less than a fish's body length are unlikely to have notable effects on swimming performance (see Tritico and Cotel, 2010; Webb et al., 2010). We used flow visualisation with neutrally buoyant particles (expandable polystyrene beads diameter < 1 mm, Foamex Polystyrene, Revesby, Australia) in a similar respirometer to confirm the absence of large vortices. Given their small size, these particles allowed us to detect perturbations greater than 1 mm. Flow visualizations were filmed in high definition at 30 fps. Frame-by-frame measurements using the plugin MtrackJ in ImageJ (Meijering et al., 2012) revealed a relatively uniform flow profile and the absence of vortices with a diameter > 0.5 cm (most were much smaller or almost nil), both in steady and unsteady flow conditions. Examples of flow characterisations in steady and high unsteady flow are provided in the supplementary material (Fig. S2, Fig. S3, Table S1).

A computer generated sine function that determined the rotational speed of the swim tunnel's propeller allowed us to precisely control four key parameters in the different flow conditions: the period, amplitude and wave-length of the water velocity fluctuations, and the mean flow velocity. Prior to the experiments, we calibrated the voltage from the computer generated sine function with changes in water velocity in unsteady flow using a digital Höntzsch TAD W30 flow-meter (Höntzsch, Waiblingen, Germany). We increased the mean water flow velocity from 1 BLs^{-1} to 4.5 BLs^{-1} by increments of 0.5 BLs^{-1}, following a standard U_{crit} protocol. The period was 5 seconds for all treatments and the amplitude varied between 0 BLs^{-1}, 0.5 BLs^{-1} or 1 BLs^{-1} depending on the treatment. The wave-length varied between 5 BL and 22.5 BL since wave-length is a product of the period and the water flow velocity. The variance in water flow velocity was 0 for steady flow, 0.125 for low unsteady flow, and 0.5 for high unsteady flow. The variance remained the same within each flow treatment since the amplitude of water velocity fluctuations was identical within treatment, across all mean swimming speeds. There was only small variation in the maximum and minimum flow velocities recorded around a given mean flow velocity, which indicated that the flow conditions were consistent (ESM Fig. S2, Table S1).

We used the hydrodynamics-based power function to describe the relationship between $\dot{M}O_2$ and U in the different flow treatments (Wu, 1977; Videler, 1993):

$$\dot{M}O_2 = a + bU^c \quad (2)$$

where a is the estimated $\dot{M}O_2$ at zero speed (standard metabolic rate; SMR), b is the linear coefficient and c is the exponent, which is indicative of aerobic swimming efficiency (Wardle et al., 1996). A three-parameter power or exponential function is preferred over a simpler, two parameter function ($\dot{M}O_2 = a10^{bU}$) for comparing swimming costs among groups when SMR is not measured directly (Roche et al., 2013). We restricted these analyses to aerobic swimming by excluding data for individual fish at swimming speeds that induced burst-and-coast swimming (see Korsmeyer et al., 2002; Svendsen et al., 2010). Burst-and-coast swimming was defined as an event that included caudal fin beats (1, 2 or 3 beats) and a subsequent forward

glide motion >5 cm relative to a fixed point in the swim chamber, without the use of pectoral fins (see Svendsen et al., 2010).

Swimming performance

We used a Canon Vixic HV30 to video the test subjects continuously during each trial and determine their pectoral–caudal gait transition speed ($U_{p\text{-}c}$) and critical swimming speed (U_{crit}). A mirror was placed at 45° adjacent to the working section to record the top and side view of the fish in a single frame. $U_{p\text{-}c}$ was reached when fish recruited their caudal fin to assist pectoral fin swimming more than once in five seconds (caudal fin beat frequency, $f_c > 0.2$ Hz); U_{crit} was reached when fish could no longer swim unassisted and were forced to rest on the back grid of the working section of the respirometer for more than five consecutive seconds (Johansen and Jones, 2011). We calculated $U_{p\text{-}c}$ and U_{crit} following the equation in Brett (1964):

$$U_{p\text{-}c} \text{ or } U_{crit} = U + U_i \text{ x } (t/t_i) \quad (3)$$

where U is the penultimate swimming speed before the fish changed gait from pectoral to pectoral-caudal swimming ($U_{p\text{-}c}$) or before the fish fatigued and stopped swimming (U_{crit}); U_i is the swimming speed at which the fish changed swimming gait or was unable to continue swimming (i.e., swimming speed at increment i); t is the length of time the fish swam at the final swimming speed where gait change or fatigue occurred; t_i is the amount of time fish were swam at each speed interval in the trial (30 min).

Fin beat kinematics (frequency and period) and body movement

For each fish (N=20) and each speed increment, we examined the three 5 minute video segments that corresponded to the $\dot{M}O_2$ measurement cycles. Using ODlog (Macropod Software), we recorded the number of (1) pectoral fin beats, (2) combinations of pectoral and caudal fin beats, and (3) caudle fin beats that resulted in burst-and-coast swimming. We calculated pectoral fin beat frequency (f_p) in Hertz as the number of pectoral fin beats performed divided by the time elapsed during the analysis period (300 s). Calculated in this way, fin beat frequency represents a time-averaged measure of fin oscillations (Drucker and Jensen, 1996).

To examine finer-scale effects of water speed fluctuations on pectoral fin kinematics, we measured the period of individual pectoral fin beats (T) during the five second cycle of sinusoidal water velocity fluctuations. We used field-by-field video analysis to record values of T three times per fish (once per $\dot{M}O_2$ determination) at each swimming speed. Fin beat period is the duration of a pectoral fin beat cycle, which begins and ends with consecutive onsets of pectoral fin abduction (Drucker and Jensen, 1996). It includes both the propulsive phase measured from the onset of abduction to the end of adduction, and a non-propulsive phase during which the fish glides until the onset of the next abduction (Drucker and Jensen, 1996). We calculated the mean and the coefficient of variation (CV; s.d./mean) of fin beat period across the three $\dot{M}O_2$ determinations for each fish and swimming speed. Since there was considerable individual level variation in oxygen consumption rates and fin beat period among fish in high unsteady water flow, we tested whether fish that had a high variation in fin beat period (i.e., longer and shorter fin beat cycles as denoted by the CV of fin beat period) in response to high fluctuations in water flow velocity consumed less oxygen than fish that had less variable fin beat periods (see video in ESM). We also tested whether these fish were better able to hold station in the swim chamber by maintaining their ground speed constant. We used field-by-field video analysis to measure horizontal and vertical body displacements during the five second cycle of sinusoidal water velocity fluctuation three times per fish (once per $\dot{M}O_2$ determination) at each swimming speed.

Estimated oxygen consumption and fin beat period

In fishes, the response curve of oxygen consumption rate vs. swimming speed is a positive, nonlinear function (Korsmeyer et al., 2002; Cannas et al., 2006); therefore, for the same mean swimming speed, fish experiencing cyclic changes in water flow velocity should consume more oxygen than fish swimming at a constant velocity (Fig. 1) (Ruel and Ayres, 1999). To compare observed and estimated oxygen consumption rates, we calculated estimated changes in $\dot{M}O_2$ values ($\dot{M}O_{2\text{-E}}$) as a function of swimming speed for the low and high unsteady flow treatments. This was achieved by integrating a sinusoidal function based on experimental variations in flow speed (period of 5 s, amplitude of 0.5 BLs^{-1} or 1 BLs^{-1}) into the equation for the $\dot{M}O_2$-swimming speed relationship in steady flow. Comparisons of $\dot{M}O_2$ and $\dot{M}O_{2\text{-E}}$ values allowed us to determine whether potential differences could be attributed to factors other than the mathematical properties (i.e., non-linearity) of the swimming speed- $\dot{M}O_2$ relationship.

Such factors could include costs associated with stability and acceleration-deceleration. We used this same procedure to calculate the variation (CV) in estimated fin beat period (T_E) as a function of swimming speed, based on the relationship between swimming speed and fin beat frequency in steady flow. In steady flow, we considered variability in pectoral fin beat period to be the natural amount of variation for individuals swimming in our flow chamber; therefore, we adjusted the CV of T_E for fish in the two unsteady flow treatments by adding the background variations in fin beat period observed for fish in steady flow.

Statistical analysis

We used a general linear mixed effects model (LMM; lme function in R) to test for differences in the $\dot{M}O_2$-swimming speed relationship across flow treatments. We specified the relationship between speed and $\dot{M}O_2$ as a second degree polynomial and individual fish as a random effect. Mixed models are useful to control for temporal autocorrelation among data points in physiological response curves (Peek et al., 2002; Bolker et al., 2009; Nakagawa et al., 2013). This same model was used to test for differences between estimated ($\dot{M}O_{2\text{-E}}$) and observed $\dot{M}O_2$ values in the two unsteady flow treatments. We tested for differences in swimming performance ($U_{\text{p-c}}$, U_{crit}) across flow treatments with two one-way ANOVAs and subsequent Tukey HSD tests. We used five distinct LMMs with fish as a random factor to examine (1) differences in the relationship between swimming speed and pectoral fin beat frequency across flow treatments, (2) the relationship between pectoral fin beat frequency and $\dot{M}O_2$ across flow treatments, (3) the relationship between swimming speed and pectoral fin beat period, (4) whether individual variation in fin beat period explained differences in $\dot{M}O_2$ for fish swimming in the low and high unsteady flow treatments, and (5) the relationship between variation in fin beat period and fish displacement (i.e., body movement) in high unsteady flow. We specified random intercepts and a first order autoregressive covariance structure to account for equally spaced points in time. Where needed, we used logarithmic (base 10) and exponential transformations to linearize the data and meet the assumptions of normality and homoscedasticity. For mixed effects models, we determined $R^2_{\text{GLMM(m)}}$, the proportion of variance explained by fixed factors, and $R^2_{\text{GLMM(c)}}$, the proportion of variance explained by both fixed and random factors (Nakagawa et al., 2013). $R^2_{\text{GLMM(m)}}$ values were almost identical to overall R^2 obtained for linear (non-mixed) models. We used within group-centering to compare estimates of

within-group slope vs. between-group slope and test relationships between variation in fin beat period and $\dot{M}O_2$ (van de Pol and Wright, 2009). All analyses were conducted in Rv2.11.1 (R Development Core Team, 2010). Data are deposited in figshare (public data repository): http://dx.doi.org/10.6084/m9.figshare.789064.

Results

Respirometry

The hydrodynamics-based power functions describing the $\dot{M}O_2$-swimming speed relationship in the three flow treatments (Fig. 2) were:

Steady flow $\dot{M}O_2 = 129.91\ (\pm 10.63) * 4.56\ (\pm 2.39)\ U^{(3.37 \pm 0.42)}$ (4)

Low unsteady flow $\dot{M}O_2 = 115.74\ (\pm 10.93) * 9.78\ (\pm 4.45)\ U^{(2.57 \pm 0.35)}$ (5)

High unsteady flow $\dot{M}O_2 = 155.44\ (\pm 20.10) * 6.37\ (\pm 6.14)\ U^{(3.36 \pm 0.84)}$ (6)

There were no significant differences in the shape of the relationship among flow treatments (LMM; quadratic term x treatment interaction, contrast group = steady flow; both *P*s>0.10; Fig. 2). The linear coefficient (which shifts the axis of symmetry away from the y-axis) of the $\dot{M}O_2$-swimming speed relationship in steady flow differed from that of the low (LMM; t=-2.1, P=0.04) and high unsteady flow treatments (LMM; t=3.5, P<0.001; Fig. 2). Importantly, fish in high unsteady flow had consistently higher oxygen consumption rates than fish in steady flow by 25.3% on average (range 20.5% - 34.4%) (LMM; t=4.3, P<0.001; Fig. 2). Fish swimming in the low unsteady flow treatment consumed on average 8.3% (range 1.8% - 23.3%) less oxygen than fish in steady flow, but this difference was not significant (LMM; t=-1.25, P=0.23; Fig. 2).

Calculations of $\dot{M}O_{2\text{-}E}$ indicated that fish swimming in the low unsteady flow treatment should consume on average 2.75% (range 1.63-3.1%) more oxygen than fish in steady flow, whereas fish in high unsteady flow should consume on average 11.1% more (range 6.7-13.3%) (Fig. 2). Observed $\dot{M}O_2$ did not differ significantly from $\dot{M}O_{2\text{-}E}$ for fish in low unsteady flow (LMM; t=-1.76, P=0.08) but was significantly higher than $\dot{M}O_{2\text{-}E}$ for fish in high unsteady flow (LMM; t=2.05, P=0.04) (Fig. 2).

Swimming performance

Fish in the different flow treatments transitioned from a pectoral to a pectoral-caudal swimming gait ($U_{p\text{-}c}$) at different swimming speeds (ANOVA; $F_{2,17}$=7.18, P <0.01) (Fig. 3). Fish in high unsteady flow reached $U_{p\text{-}c}$ at lower swimming speeds than fish in steady flow (Tukey HSD, P<0.05) and in low unsteady flow (Tukey HSD, P<0.01) (Fig. 3). There was no difference in $U_{p\text{-}c}$ between fish in low unsteady and steady flow (Tukey HSD, P=0.85). Critical swimming speed was also different among treatments (ANOVA; $F_{2,17}$=3.87, P <0.05). Fish in high unsteady flow reached U_{crit} at lower swimming speeds than fish in low unsteady flow (Tukey HSD, P<0.05). The U_{crit} of fish in steady flow did not differ from that in either high (Tukey HSD, P=0.13) or low (Tukey HSD, P=0.88) unsteady flow.

Time-averaged fin beat frequency

Pectoral fin beat frequency (f_p) increased with swimming speed (LMM slope; $F_{1,79}$=392.9, P<0.001) in a similar fashion for all three flow treatments (LMM interaction ns; $F_{1,79}$=0.78, P=0.46) (Fig. 4a). In turn, f_p was a good predictor of oxygen consumption rates in all three treatments (LMM slope; $F_{1,79}$=315.6, P<0.001; LMM interaction ns; $F_{2,79}$=0.07, P>0.90; Fig. 4b), but fish in high unsteady flow consistently consumed more oxygen for a given f_p than fish in either steady or low unsteady flow (LMM intercept; $F_{2,16}$=6.55, P<0.01; Fig. 4b).

Instantaneous fin beat period

Pectoral fin beat period (T) decreased with swimming speed (LMM slope; $F_{1,79}$= 931.1, p<0.001) similarly for fish in all three flow treatments (LMM interaction: $F_{2,79}$= 1.07, P=0.35). However, variation (CV) in T across swimming speeds differed among flow treatments and increased with the degree of flow unsteadiness (Fig. 5). Observed variations in T were consistently higher than the estimated variation (T_E) for fish in high unsteady flow, but not for fish in low unsteady flow (Fig. 5). There was a significant overall negative association between variation in T and $\dot{M}O_2$ (LMM slope: $F_{1,14}$=66.0, P<0.001) for fish in high unsteady flow and the slope of this relationship did not differ among swimming speeds (LMM interaction: $F_{3,14}$=0.18, p=0.91; Fig. 6b). The 95% confidence interval of the estimates for both the within-speed slope (estimate = -0.261, 95% CI = -0.400 to -0.122) and between-speed slope (estimate = -0.556, 95% CI = -0.930 to -0.182) did not overlap zero, indicating that these slopes differed from zero. In

contrast, associations between variation in T and $\dot{M}O_2$ were generally non-significant and inconsistent across swimming speeds for fish in low unsteady flow (Fig. 6a). The 95% confidence interval for the estimate of the between-speed slope did not overlap zero (estimate = -0.997, 95% CI = -1.516 to -0.478); however, the within-speed slope estimate overlapped zero (estimate = -0.154, 95% CI = -0.340 to 0.032), indicating no effect of variation in T on $\dot{M}O_2$.

Body movement

There was a significant overall negative association between fish displacement (horizontal and vertical body movement) and variation in T (LMM slope: $F_{1,14}$=7.16, P=0.02) for fish in high unsteady flow, and the slope of this relationship did not differ among swimming speeds (LMM interaction: $F_{3,14}$=0.20, p=0.89; Fig. 7).

Discussion

Swimming performance and oxygen consumption

Many labriform fishes live in marine environments and experience variable speed flows created by travelling waves in inshore habitats. Our experimental flow treatments mimicked unidirectional wave surge with either low or high amplitude velocity fluctuations and a period of 5 s, similar to waves in Puget Sound, Washington (Finlayson, 2006). We found that high unsteady flow tended to decrease fish swimming performance (Fig. 3) and increase swimming costs by an average of 25.3% compared to steady flow (Fig. 2). This increase in oxygen consumption rate also exceeded the 11.1% average increase expected based on calculations of $\dot{M}O_{2\text{-}E}$ (i.e., $\dot{M}O_2$ estimated using the non-linear relationship between swimming speed and $\dot{M}O_2$ in steady flow) (Fig. 2). Together, these results suggest that estimates of fish swimming energetics based on steady flow conditions underestimate the costs of swimming in water flow with large velocity fluctuations (in the order of one body length). This discrepancy may be due to additional energy expenditure from fish accelerating and decelerating (Kramer and McLaughlin, 2001; Minetti et al., 2001) as well as correcting for postural disturbances (see Webb, 2006; Webb et al., 2010) to maintain their position and stability during flow velocity changes. In contrast, swimming performance and oxygen consumption rates did not increase in low unsteady flow relative to steady flow. This result is consistent with the small estimated increase in oxygen consumption rate of only 2.75%, on average, based on $\dot{M}O_{2\text{-}E}$. Further experiments are necessary to establish the relative

costs of maintaining stability and varying acceleration for fish swimming in unsteady flows.

Spatial and/or temporal fluctuations in water flow velocity can result in both energetic challenges and benefits for fishes (Lacey et al., 2012). Enders et al. (2003) found that juvenile Atlantic salmon, *Salmo salar* Linnaeus, incurred higher energetic costs even at relatively low water velocity fluctuations in turbulent flow compared to laminar flow. In contrast, other studies have shown that BCF swimmers can exploit vortices that have an element of predictability; by bending their body around vortices, fish can generate forward thrust with less energy expenditure (e.g., Liao et al., 2003b; Taguchi and Liao, 2011). Our results support the general finding that disturbances from flow variations are important only if they are large relative to the size of the fish (Pavlov et al., 2000; Lupandin, 2005; Liao, 2007; Tritico and Cotel, 2010; Webb et al., 2010): irrespective of its mean swimming speed, *C. aggregata* appeared unaffected by relatively small water velocity fluctuations of 0.5 BLs^{-1} while experiencing significantly higher energetic demands when subjected to larger fluctuations of 1 BLs^{-1}.

Time-averaged pectoral fin kinematics and oxygen consumption

The relationship between mean swimming speed and pectoral fin beat frequency (measured over 300 s) did not differ among flow treatments (Fig. 4A). Despite these similarities, for a given fin beat frequency, fish in high unsteady flow consumed significantly more oxygen than fish in both low unsteady or steady flow (Fig. 4B). This suggests that a time-averaged measure of fin use is independent of observed differences in $\dot{M}O_2$. Within a given 5 s period of water velocity fluctuations, we observed individuals in low and high unsteady flows altering their fin beat movements: fish were beating their fins less frequently as the flow velocity decreased and, conversely, increased their fin beat frequency as the flow velocity increased (see video in ESM). Despite these adjustments in fin kinematics, the mean fin beat frequency (f_p) was the same at any given mean swimming speed, regardless of the flow treatment. Since f_p was calculated as the average number of fin beats over a time scale of minutes, this time-averaged measure of fin use did not capture adjustments in the timing of fin beats made by fishes in unsteady flow. Therefore, measurements of fin kinematics on a shorter time scale are needed to explain differences in $\dot{M}O_2$ among treatments (see below - variation in instantaneous pectoral fin kinematics).

Pectoral fin beat frequency is positively related to swimming speed and/or oxygen consumption in several species of labriform fishes (e.g., Mussi et al., 2002; Kendall et al., 2007; Tudorache et al., 2009; Johansen et al., 2010). Some authors have suggested that the relationships between fin beat frequency, oxygen consumption rate and swimming speed in a given fish species may provide useful indicators of swimming energetics in the wild, which are extremely difficult to estimate in aquatic species (Steinhausen et al., 2005; Ohlberger et al., 2007; Tudorache et al., 2009; Layton, 2011). However, our results suggest that the use of time-averaged fin kinematics to predict oxygen consumption rates depends on the hydrodynamic context in which these estimates are made. As such, caution is warranted when inferring oxygen consumption rates from time-averaged measures of fin beat frequency.

Variation in instantaneous pectoral fin kinematics

Although time-averaged f_p was similar across flow treatments, fin beat period (T) clearly differed. The consistently-timed beats of fish swimming in steady flow resulted in low variation in T, whereas the rapid speeding up and slowing down of fin beats in unsteady flow resulted in higher variations in T (Fig. 5). As mean swimming speed increases, fish must beat their fins faster in order to keep up with increasing flow speed, resulting in a shorter refractory or gliding period between fin beats. As a result, both the observed and predicted variation in T decreased with increasing swimming speed for fish in the unsteady flow treatments (Fig. 5). In contrast, variation in T remained constant in steady flow (Fig. 5), despite increases in f_p with higher swimming speeds (Fig. 4a).

In low unsteady flow, observed variations in T were greater than the estimated variation (T_E) only at low swimming speeds, where water velocity fluctuations would have had a minimal effect on fish (Fig. 5). In high unsteady flow, however, observed variations in T were consistently greater than estimated variations, across all swimming speeds (Fig. 5). These differences in fin beat period variability between treatments are consistent with observed differences in $\dot{M}O_2$ (Fig. 2).

Similarly, there was a clear relationship between $\dot{M}O_2$ and variation in T in high unsteady flow (Fig. 6B) but not in low unsteady flow (Fig. 6A): fish with greater

variation in T consumed less oxygen for a given mean swimming speed in high unsteady flow (Fig. 6A). While we did not quantify whether, within one 5 s wave period, accelerations in water velocity coincided with shorter fin beat periods and vice versa, these relationships clearly indicate that larger variations in T were energetically advantageous in conditions of high flow variability. This also suggests that some fish are capable of adjusting the duration of their fin beats to changes in water flow velocity to maintain a relatively constant ground speed in the swim chamber (Fig. 7). Specifically, by increasing the refractory period of fin beats as the water decelerates, fish may be able to conserve energy by increasing their glide towards the end of the wave cycle. Fish with lower variation in T may not take advantage of decelerations in water flow velocity and may resort to more energetically costly behaviours to try and maintain their position in the swim chamber. Such behaviours were not assessed in this study but could include modifying the amplitude and/or the power output of fin strokes to accelerate and overcome resistance at various swimming speeds, as well as braking to control posture and position during deceleration.

In conclusion, our results suggest that swimming costs in unsteady flows depend on the magnitude of the water velocity fluctuations. When velocity fluctuations were relatively large, the energetic costs of swimming in unsteady flow exceeded the costs of swimming in steady flow at the same mean velocity. It is important to note that these costs apply to station-holding fishes, which swim to remain stationary relative to the substrate. In contrast, traveling fishes exposed to wave surge might be able to conserve energy by taking advantage of forward surges and varying their ground speed while maintaining a constant velocity relative to the water. Our results are also conservative since hydrodynamic perturbations in our experiments were unidirectional and designed to minimize turbulence. Coastal habitats are often characterized by turbulent, oscillatory wave-driven water motion, which may require fishes to expand more energy for postural control and stability. Swimming costs also depended on the ability of individual fish to adjust their fin kinematics to the flow environment and avoid displacement while station holding in the swim chamber. Individual variability in swimming performance has previously been observed in a number of species and shown to be both repeatable and biologically important (Kolok, 1999). It is possible that inter-individual differences in our study relate to differences in habitat use among fish; for example, individuals foraging high in the water column may experience greater water

flows than individuals that remain closer to the substrate. Further studies should examine the learning potential of individual fish to modify their fin beat kinematics via repeated exposure to variable water flows.

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Symbols and abbreviations

A: amplitude (BLs^{-1})

f_c: caudal fin beat frequency (Hz)

f_p: pectoral fin beat frequency (Hz)

L_T: Total length (cm)

$\dot{M}O_2$: Oxygen consumption rate ($mg\ O_2\ kg^{-1}\ h^{-1}$)

$\dot{M}O_{2\text{-}E}$: Estimated oxygen consumption rate ($mgO_2\ kg^{-1}\ h^{-1}$)

T: fin beat period (s)

T_E: Estimated fin beat period (s)

U: swimming speed (BLs^{-1})

$U_{p\text{-}c}$: gait transition speed to pectoral-caudal swimming (BLs^{-1})

U_{crit}: critical swimming speed (BLs^{-1})

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Fig. 1 Theoretical effects of sinusoidal variations in water flow velocity (or swimming speed) around a constant mean speed (2 BLs^{-1} in this example, indicated by a dashed vertical line) on oxygen consumption rate ($\dot{M}O_2$). Since this relationship is nonlinear, increases in $\dot{M}O_2$ from swimming at speeds above the mean outweigh decreases in $\dot{M}O_2$ from swimming below the mean. These effects are greater for large (1 BLs^{-1}) than small (0.5 BLs^{-1}) amplitude variations in water flow speed. A = amplitude.

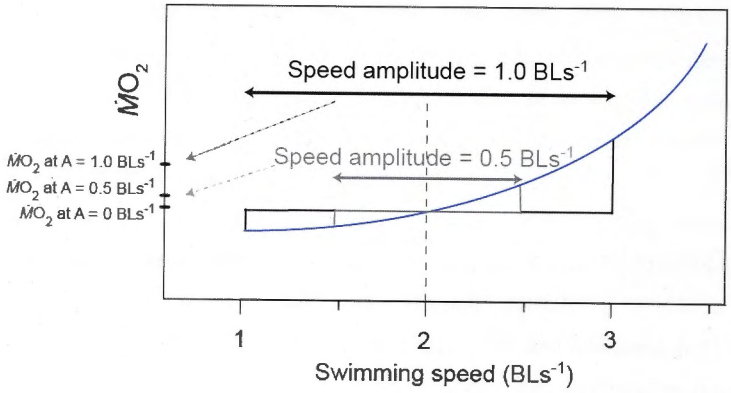


Fig. 2 Oxygen consumption rate ($\dot{M}O_2$ in $mgO_2\ kg^{-1}\ h^{-1}$) in relation to mean swimming speed for *C. aggregata* in three water flow conditions: steady flow (A=0; blue triangles), low amplitude unsteady flow (A=0.5 BLs^{-1}; green circles) and high amplitude unsteady flow (A=1.0 BLs^{-1}; red squares). $R^2_{GLMM(m)} = 0.86$ and $R^2_{GLMM(c)} = 0.90$. Error bars are plus or minus one s.e.m. Relationships are based on aerobic swimming (i.e., $\dot{M}O_2$ measurements at speeds that did not induce bursting-and-coasting). The black dotted lines indicate estimated oxygen consumption rates ($\dot{M}O_{2\text{-}E}$) for fish in low and high amplitude unsteady flow, respectively. $\dot{M}O_{2\text{-}E}$ was calculated by integrating a sinusoidal function based on experimental variations in flow speed (period of 5 s, amplitude of 0.5 BLs^{-1} or 1 BLs^{-1}) into the equation for the $\dot{M}O_2$-swimming speed relationship in steady flow (see Fig. 1). A = amplitude.

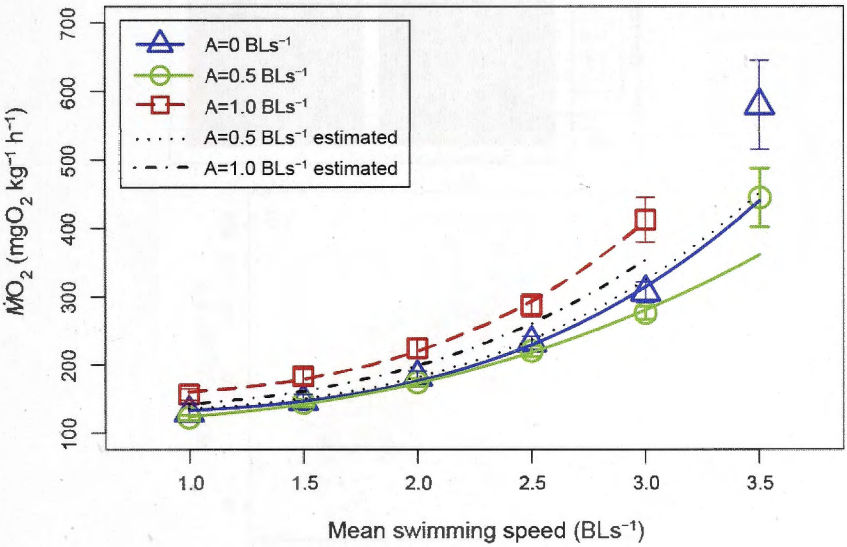


Fig. 3 Swimming performance of *C. aggregatta* in steady flow (blue bars), low unsteady flow (A = 0.5 BLs^{-1}; green bars) and high amplitude unsteady flow (A = 1.0 BLs^{-1}; red bars). $U_{p\text{-}c}$ = gait transition speed (fish transition to caudal assisted pectoral swimming), U_{crit} = critical (or maximum) swimming speed. Error bars are plus or minus one s.e.m. * denotes significance, *ns* = non-significant (α=0.05).

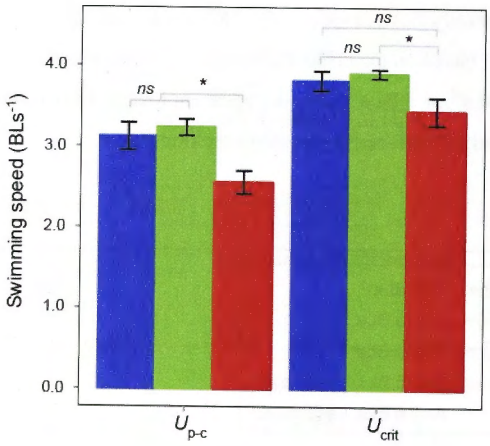


Fig. 4 (A) Pectoral fin beat frequency (f_p) in relation to swimming speed ($R^2_{GLMM(m)}$ = 0.77; $R^2_{GLMM(c)}$ = 0.81); and (B) oxygen consumption rate ($\dot{M}O_2$; log scale) in relation to fin beat frequency ($R^2_{GLMM(m)}$ = 0.76; $R^2_{GLMM(c)}$ = 0.79) for *C. aggregata* swimming in steady flow (blue triangles), low unsteady flow (0.5 BLs^{-1}; green circles) and high amplitude unsteady flow (1.0 BLs^{-1}; red squares). A = amplitude.

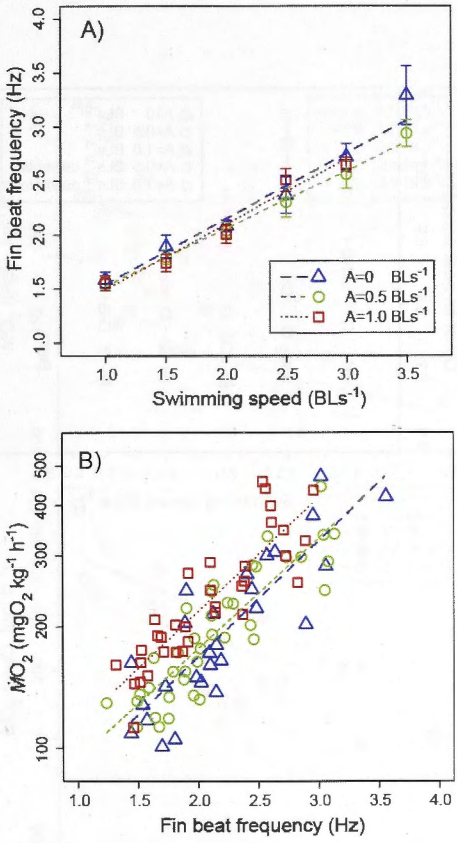


Fig. 5 Mean variation (CV) in pectoral fin beat period (T) within a 5 s wave period in relation to swimming speed for *C. aggregata* in steady flow (A=0 BLs^{-1}; blue triangles), low unsteady flow (A=0.5 BLs^{-1}; green circles), and high unsteady flow (A=1.0 BLs^{-1}; red squares). Black circles and squares represent the estimated variation in fin beat period for low and high amplitude unsteady flow, respectively. Estimated variations were adjusted by adding the background variations in T observed for fish in steady flow (i.e., values represented by the blue triangles). A = amplitude.

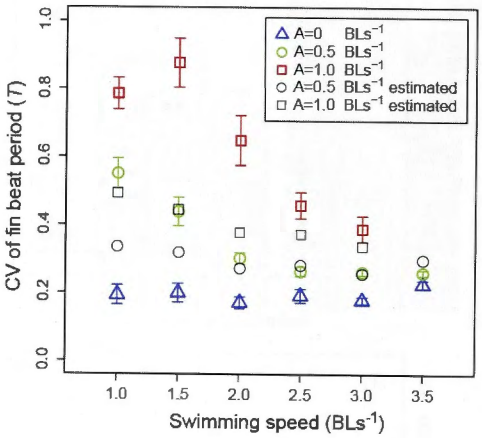


Fig. 6 Oxygen consumption rate ($\dot{M}O_2$; $\log_{10}$ scale) in relation to variation in pectoral fin beat period within a 5 s wave cycle for individual *C. aggregata* swimming A) at five different speeds (1.0, 1.5, 2.0, 2.5 and 3.5 BLs^{-1}) in low amplitude (0.5 BLs^{-1}) unsteady flow and B) at four different speeds (1.0, 1.5, 2.0 and 2.5 BLs^{-1}) in high amplitude (1.0 BLs^{-1}) unsteady flow ($R^2_{GLMM(m)} = 0.81$; $R^2_{GLMM(c)} = 0.90$). Data are shown for swimming speeds that induced only aerobic metabolism and pectoral fin swimming exclusively (i.e., below $U_{p\text{-}c}$).

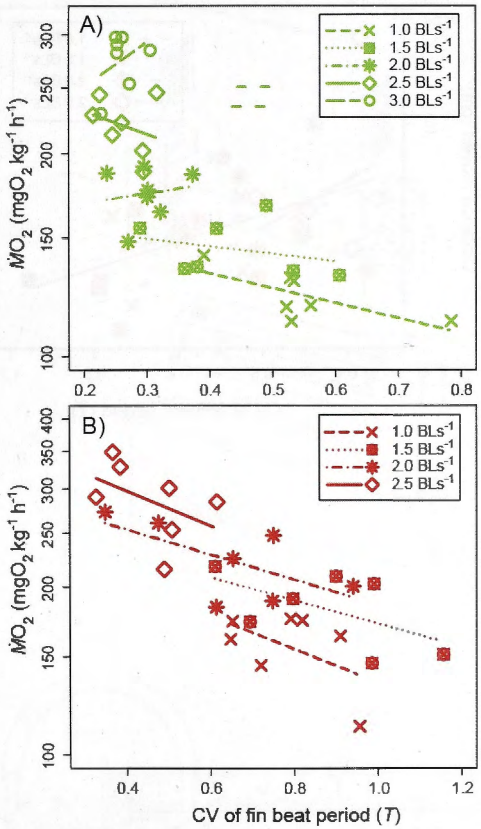


Fig. 7 Distance moved (cm) in relation to variation (CV) in pectoral fin beat period within a 5 s wave cycle for individual *C. aggregata* swimming at four different speeds (1.0, 1.5, 2.0, and 2.5 BLs^{-1}) in high amplitude (1.0 BLs^{-1}) unsteady flow. $R^2_{GLMM(m)}$ = 0.17 and $R^2_{GLMM(c)}$ = 0.64. Data are shown for swimming speeds that resulted only in aerobic activity (i.e., swimming speeds that induced burst-and-coast swimming are excluded). Regression lines show relationships within the four distinct high amplitude swimming speeds; the overall relationship is shown as a solid black line.

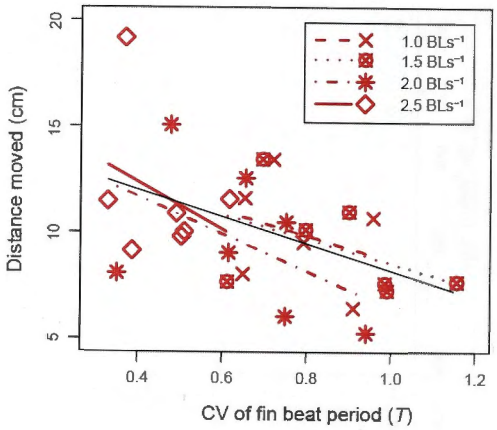
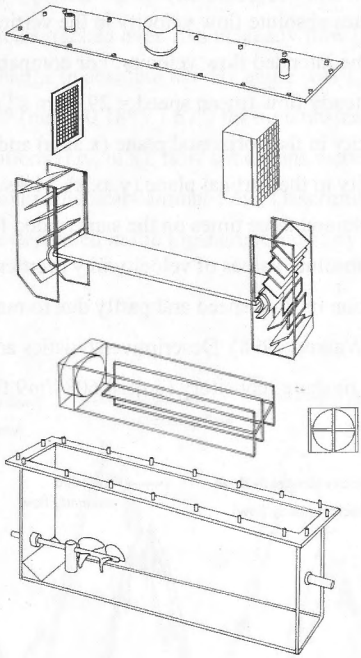


Fig. S1 Steffensen-type swimming respirometer to conduct intermittent-flow respirometry. Panel A shows the different components of the respirometer, including the baffles, flow straightener and honeycomb used to produce near-laminar flow. Panel B shows the assembled respirometer with an external motor powering the propeller. Arrows indicate the direction of the flow.

A)



B)

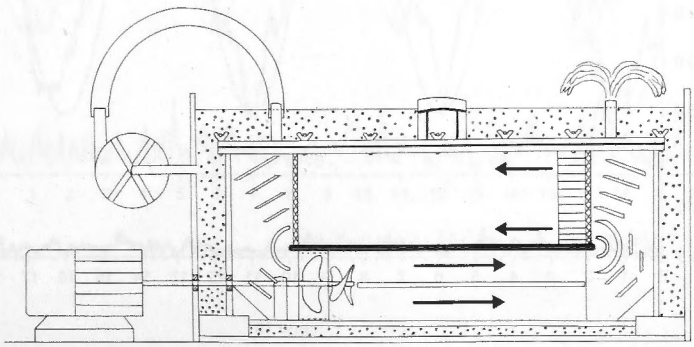


Fig. S2 Example of flow characteristics in steady flow versus high unsteady flow for an average size fish (14.8 cm total length). Water flow velocity (cm s^{-1}) was obtained by tracking passive particles (neutrally buoyant expandable polystyrene beads with diameter < 1 mm, Foamex Polystyrene, Revesby, Australia) in the test section of the swim chamber at 0.033 Hz. Particles were tracked using the manual object tracking plugin MtrackJ for ImageJ (Meijering et al., 2012). Particles were tracked for 20 s in high unsteady flow (mean speed = 29.6 cm s^{-1} or 2 BLs^{-1}; amplitude = 1 BLs^{-1}; period = 5 s): the thick blue line indicates flow velocity in the horizontal plane (x axis) and the thick red line indicates absolute flow velocity in the vertical plane (y axis). The dashed grey line indicates the intended flow velocity. For comparative purposes, particles were tracked for 10 s in steady flow (mean speed = 29.6 cm s^{-1} or 2 BLs^{-1}): the thin blue line indicates flow velocity in the horizontal plane (x axis) and the thin orange line indicates absolute flow velocity in the vertical plane (y axis). Measures were obtained by averaging values obtained three times on the same video for both steady and unsteady flow; we averaged absolute values of velocity in y (vertical plane). Noise is partly due to small scale variation in flow speed and partly due to magnification of errors from the digitizing process (Walker, 1998). Descriptive statistics are presented in Table S1. Data are deposited in the figshare repository (doi:10.6084/m9.figshare.789064).

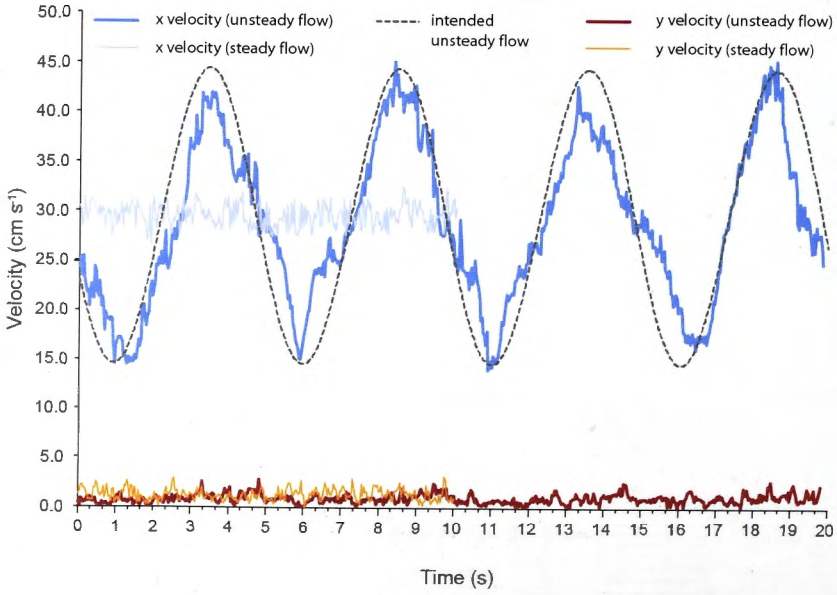


Fig. S3 Same as Fig. S2, but showing (in green) the change in absolute vector angle of tracked passive particles (Fig. S2) at 0.033 Hz relative to the expected flow direction (i.e. measured vector vs. horizontal vector angle; Mv-Hv angle). Particles were tracked using the manual object tracking plugin MtrackJ for ImageJ (Meijering et al., 2012). Measures were obtained by averaging absolute values obtained three times on the same video for both steady and unsteady flow. The thick green line indicates the absolute Mv-Hv angle for particles tracked over 20 s in high unsteady flow (mean speed = 29.6 cm s^{-1} or 2 BLs^{-1}; amplitude = 1 Bl s^{-1}; period = 5 s). The thin green line indicates the absolute Mv-Hv angle for particles tracked over 10 s in steady flow (mean speed = 29.6 cm s^{-1} or 2 BLs^{-1}). The mean change in absolute Mv-Hv angle was 1.98° (range 0.00° – 4.76°) for steady flow and 2.47° (range 0.18° - 7.67°) for high unsteady flow. Other than horizontal velocity fluctuations (i.e., in x), flow conditions were therefore similar in the two treatments and approximated near-laminar flow. Descriptive statistics are presented in Table S1. Data are deposited in the Figshare Repository (DOI: 10.6084/m9.figshare.789064).

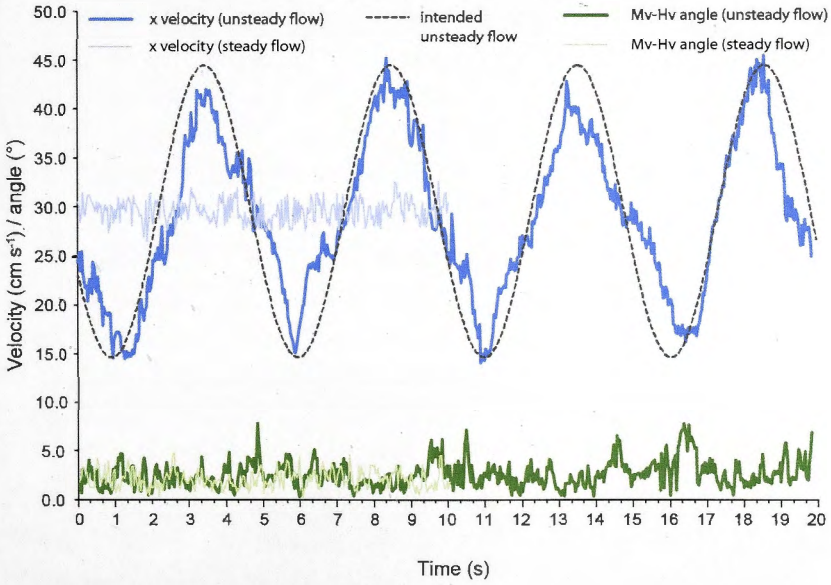


Table S1 Intended vs. observed flow characteristics (mean ± s.d.) for steady (mean speed = 29.6 cm s^{-1} or 2 BLs^{-1}) and high unsteady (mean speed = 29.6 cm s^{-1} or 2 BLs^{-1}; amplitude = 1 BLs^{-1}; period = 5 s) flow visualized in Figs S1 and S2. Abs = absolute.

Descriptor	**Steady flow**		**High unsteady flow**	
	Intended	*Observed*	*Intended*	*Observed*
Velocity in x (cm s^{-1})	29.60 ± 0.00	29.63 ± 1.18	29.60 ± 0.00	28.98 ± 7.98
Amplitude (cm s^{-1})	N/A	N/A	14.80 ± 0.00	15.03 ± 2.01
Velocity in y (cm s^{-1})	0.00 ± 0.00	-0.24 ± 0.84	0.00 ± 0.00	0.10 ± 0.70
Abs velocity in y (cm s^{-1})	0.00 ± 0.00	1.27 ± 0.59	0.00 ± 0.00	0.93 ± 0.49
Mv-Hv angle (°)*	0.00 ± 0.00	-0.37 ± 1.37	0.00 ± 0.00	0.28 ± 2.04
Abs Mv-Hv angle (°)*	0.00 ± 0.00	1.98 ± 0.92	0.00 ± 0.00	2.47 ± 1.36
Period (s)	N/A	N/A	5.00 ± 0.00	4.98 ± 0.28

* Vector angle relative to intended flow direction.

CHAPTER - 5

Waves affect the escape response of juvenile coral reef fish

Roche DG (submitted) *Journal of the Royal Society Interface*

Keywords

Body morphology; complex flow, fast-start, fineness ratio, postural disturbance, predator-prey interactions, unsteady flow, unsteady swimming

Abstract

Fish often escape from predators with a burst of speed (fast-start responses). In laboratory studies, these responses are always measured in stationary water. However, water motion due to waves and currents is ubiquitous in many aquatic systems. In shallow marine habitats, wave action creates unsteady water motion that varies spatially and temporally. This likely affects the ability of fish to escape, especially when they are small. Here, I examined whether wave-driven water flow differentially affects the escape response of juvenile coral reef fish by comparing three species of damselfishes (Pomacentridae) with different body morphologies. Body depth is thought to improve postural control and stability. Escape responses were elicited under static or unsteady flow conditions. In unsteady flow, all fish covered a greater distance and reached a higher speed and acceleration when escaping in the direction of the water flow. However, compared to static flow, unsteady flow had no detectable effect on maximum escape performance, although fish took longer to respond to the stimulus in unsteady compared to static flow. This effect differed between species. The fusiform-shaped species responded more slowly in unsteady than in static flow. This difference was less pronounced in the species with an intermediate body depth and absent in the species with a deep body. The deep-bodied species also tended to orient itself more directly into the water flow and was less displaced by water motion. Response latency is a major determinant of escape success, so postural disturbances from unsteady water motion might reduce the ability of some fishes to evade predators during juvenile settlement. This could have important implications for the distribution, abundance and recruitment of reef fishes across spatial and temporal scales.

Introduction

Fleeing from predators is one of the most important tasks faced by animals (Ydenberg & Dill 1986). Effective locomotion is, therefore, a key component of predator-prey interactions and fundamental to the survival of mobile organisms (Howland 1974; Domenici, Claireaux & McKenzie 2007; Higham 2007). Fast-start escape responses are the primary behaviour used by fishes to evade predators (Domenici & Blake 1997; Domenici 2011). These rapid accelerations are particularly important for juvenile coral reef fish (Fisher & Leis 2010) since predation is a fundamental factor influencing their survival (Almany 2003; Almany & Webster 2006; Holmes & McCormick 2009). Predation in reef fish is greatest during and shortly after larvae metamorphose from a planktonic to a demersal life stage as they settle on the reef (Steele & Forrester 2002; Almany & Webster 2006). Due to their small size, newly-settled coral reef fish are targeted by many generalist and piscivorous predators (Stewart & Jones 2001; Holmes & McCormick 2010). Recent work suggests that over 50% of juveniles can be eaten within 48 hours of settlement (Almany & Webster 2006). Consequently, environmental factors that affect juvenile escape performance might have a substantial influence on the recruitment of coral reef fish to adult populations (Rice, Crowder & Marschall 1997; Fisher & Leis 2010).

In aquatic systems, several environmental parameters tend to fluctuate dramatically (Abrahams, Mangel & Hedges 2007). Recent studies have examined the effect of temperature, dissolved oxygen, turbidity, light and pH on fish escape performance (reviewed in Domenici, Claireaux & McKenzie 2007; Wilson *et al.* 2010). However, the importance of water motion has been largely overlooked. This oversight is surprising because water flow is a ubiquitous and highly variable physical property of most aquatic systems (Denny 1988; Webb, Cotel & Meadows 2010). In shallow marine habitats, wave-driven water motion is an important stressor for both sessile and mobile organisms (Denny 2006; Denny & Gaylord 2010; Webb, Cotel & Meadows 2010). On coral reefs, for example, waves influence the ability of adult fishes to swim and occupy shallow, windward habitats, which leads to strong patterns of community structuring based on the ability of species to withstand ambient flow conditions (Bellwood & Wainwright 2001; Bellwood *et al.* 2002). Wave-driven water motion is characterized by unsteadiness and turbulence due to rapid changes in water flow velocity and flow interacting with the reef structure (Liao 2007; Webb, Cotel & Meadows 2010). Waves

can be energetically demanding for fish (e.g., Roche *et al.* 2014), and have been suggested to perturb their swimming movements and have destabilizing effects on important behaviours, including those associated with predator-prey interactions (Webb 2002; Webb, Cotel & Meadows 2010). Currently, however, the extent to which wave-driven water flow affects fish escape performance is completely unknown. Such biophysical interactions could be of considerable ecological importance for coral reef fish since predator-prey relationships are so critical in shaping their distribution and abundance. Wave intensity and frequency is also increasing in ocean basins worldwide as a result of climate change (Young, Zieger & Babanin 2011; IPCC 2013), with anticipated impacts on marine communities (Harley *et al.* 2006; Byrnes *et al.* 2011). Therefore, basic knowledge of how waves influence key swimming behaviours is essential to improve our understanding and our ability to predict how environmental change will affect fish communities.

Here, I examined whether unsteady, wave-driven water flow affects the escape performance of post-settlement juvenile coral reef fish in the family Pomacentridae. The damselfish are a species-rich, morphologically diverse group, widely distributed throughout temperate and tropical waters around the world (Allen 1991; Cooper, Smith & Westneat 2009). Many are small (< 5 cm total length) and important prey items for predatory coral reef fish (Kingsford 1992; Beukers-Stewart & Jones 2004). This family is characterized by broad differences in body depth, a trait known to influence fast-start behaviours (Domenici, Claireaux & McKenzie 2007) and linked to differences in the sustained swimming performance of juvenile reef fish (Fisher *et al.* 2005; Fisher & Hogan 2007). A deep, laterally compressed body is thought to improve fast-start performance and postural control (Domenici & Blake 1997; Eidietis, Forrester & Webb 2002; Domenici *et al.* 2008). Although suboptimal from a hydrodynamic perspective, lateral compression has the advantage that it allows for greater expansion of the dorsal and anal fins (Webb 2004; Webb 2006) and reduces rolling (Weihs 2002; but see Webb 2004). Therefore, I predicted that fish with this body shape would maintain better stability in unsteady flow. I studied three species of damselfish to test whether postural disturbances from unsteady water motion decrease escape performance and if this effect varies among species with different body morphologies.

Materials and methods

Animal collections and experimental set-up

Post-settlement juvenile fishes were collected on SCUBA in March 2012, using Aqui-S solution and hand nets on reefs adjacent to the Lizard Island Research Station on the Northern Great Barrier Reef, Australia (14°40'S; 145°28'E). I caught *Neopomacentrus azysron*, *Chromis atripectoralis* and *Dascyllus reticulatus* (family Pomacentridae). These species co-occur on the reef but differ in their body morphology. The fineness ratio (FR) is a measure of how elongate a fish is relative to its transverse sectional diameter (Fisher & Hogan 2007), and differs significantly among species (one-way ANOVA, $F_{2,81} = 1726$, $p < 0.001$): *N. azysron* has a shallow, fusiform body (SL = 12.7 ± 0.5 cm, FR = 1.67 ± 0.08; means ± s.d.), *C. atripectoralis* a body of intermediate depth (SL = 12.4 ± 1.0 cm, FR = 2.42 ± 0.07) and *D. reticulatus* a deep, laterally compressed body (SL = 12.6 ± 0.9 cm, FR = 3.10 ± 0.11) (Fig. 1). Pectoral fins are also important for stability (Drucker & Lauder 2003; Lauder & Drucker 2004; Webb 2004), but are small and transparent in juvenile reef fish, which makes them impossible to view on whole photographs and difficult to dissect and pin (Fisher & Hogan 2007). Additionally, pectoral fin shape tend to be similar in juveniles and differentiates throughout ontogeny (Fulton & Bellwood 2002).

Captured fish were placed in holding aquaria (40 × 29 x 18 cm) with seawater pumped directly from the reef. The water temperature was 29 ± 1 °C (mean ± actual variation) and fish were exposed to a natural photoperiod of 12 h for at least three days prior to the experiments. Fishes were fed once a day with commercial pellets (INVE NRD 2/4, Primo aquaculture, Australia) and fasted for a minimum of 24 h prior to the experiments. Animals were returned to their site of capture at the end of the study.

Experiments were conducted in a rectangular acrylic tank (70 x 60 cm x 12 cm) (Fig. S1). Water depth was maintained at 12 cm. Due to their small size and to facilitate filming, individual fish were placed in a fine mesh enclosure (large net breeder, Aqua One, Australia ; 26.5 x 15 x 15.5 cm) at the center of the tank. Four programmable pumps (Vortech MP10wES, EcoTech Marine, USA) were positioned at the back of the aquarium and wirelessly synchronized to create unsteady, wave-driven flow. Under conditions of static flow (water velocity < 0.2 cm s^{-1}), the pumps remained on, but the propellers were removed to control for any effects of noise and/or vibrations from the

pumps. A mirror was inclined at 45° below the aquarium to film escape responses and avoid image distortion from surface water movements (see Domenici & Blake 1991). Floodlighting was provided by three 150 W spotlights, 70 cm above the water level. The experimental tank was continuously supplied with recirculating seawater, which kept the water temperature constant (29.0 ± 0.7 °C, mean ± actual variation).

Escape performance and body fineness measures

Prior to each fast-start trial, a focal fish was transferred to the experimental tank and left undisturbed for 30 min (Domenici & Blake 1991). Escape responses were induced by mechano-acoustic stimulation (Dadda, Koolhaas & Domenici 2010; Marras *et al.* 2011). A 50 ml cylindrical container filled with lead weights was released by an electromagnet from 45 cm above the water surface, approximately 5 cm from the focal fish. To avoid visual stimulation, the stimulus fell inside a PVC tube (11 cm diameter) positioned 1 cm above the water surface (Lefrançois, Shingles & Domenici 2005). The stimulus was attached by a string to the stand holding the electro-magnet so that it did not hit the aquarium floor. The exact time of contact with the water surface could be observed in the mirror below the tank (see Marras *et al.* 2011).

Escape responses were recorded at 420 Hz by a camera (Casio Exilim EX-FH100, Casio Computer Co., Tokyo, Japan) mounted on a tripod in front of the aquarium, facing the mirror. Typically, fast-start trials are conducted in shallow water to limit vertical displacement and facilitate kinematic measurements of fish movements in two dimensions (e.g., Langerhans 2009). Here, a minimum water depth was necessary to create unsteady flow. I therefore used a second camera to film directly through the aquarium front wall. If the fish moved a vertical distance greater than its body depth, it was excluded from the analyses.

Individual fish were tested three to six times at 30 min intervals (Marras *et al.* 2011). Two to three trials were subsequently selected for video analysis to standardize the orientation and the distance of the fish relative to the stimulus. Fourteen fish were tested per species in each of the two flow conditions in a full factorial design (N_{total} = 84). Immediately following experiments, individual fish were sedated by submersion in a cool water bath for 3 s and then photographed on wetted, plasticized gridded paper. Photographs were analyzed in ImageJ v.1.45 to measure size and FR. Fish in the six

treatment groups (3 species x 2 flow conditions) were of similar size (SL) (one-way ANOVA, $F_{5,78} = 0.57$, $p > 0.7$). FR is typically calculated by dividing body length by the average of body width and body depth (Bainbridge 1960). However, I calculated FR as standard length divided by body depth because the body width of these juveniles is similar and very difficult to measure accurately without handling and harming these small animals.

Flow visualizations

I used Digital Particle Image Velocimetry (DPIV; Ryerson & Schwenk 2012) to characterize the flow conditions in the unsteady flow treatment (Fig. 2). Flow velocity and vorticity were estimated by filming neutrally buoyant particles (Fluorescent Green Polyethylene Microspheres 1.025 g cc^{-1} 63-75 µm, Cospheric LLC, Santa Barbara, CA USA) at 30 Hz in high definition with a Casio Exilim EX-FH100 camera. Microspheres were illuminated using a NOVAlaser X100 laser pointer (NOVAlasers Inc., Toronto, Canada - power output 100 mW at 532 nm) fitted with a collimating lens to create a light sheet 2 mm thick. The light sheet intersected the breeding net in mid-water, 6 cm above the aquarium floor. Image sequences were pre-processed in the video editor Avidemux v.2.5.4 (www.avidemux.org) to maximize the contrast between the particles and the black background then imported into PIVlab v.1.32 (Thielicke & Stamhuis 2012). PIVlab estimates the probable shift of particles by cross-correlation between the same interrogation areas in image pairs. Vector maps and velocity or vorticity fields were generated using a 256 × 256 pixel interrogation area and a 128 pixel step (i.e., the vertical and horizontal distance between the centre of the interrogation areas). Frame sequences showing changes in velocity and vorticity are available as electronic supplementary material (ESM). The wave frequency was 0.85 ± 0.01 Hz (mean ± s.d.) and the average (absolute) flow velocity in the experimental arena ranged between 0.5 ± 0.4 and 17.5 ± 2.5 cm s^{-1} (mean ± s.d.) (Fig. S2). These conditions are representative of water flow speeds recorded under winds of 15 knots and intermediate wave heights on semi-exposed reefs at Lizard Island (Roche D.G. unpublished data; Fulton & Bellwood 2005). The maximum flow velocity is 66% of the critical swimming speed of juveniles of similar pomacentrids species after settlement (26.3 cm s-1; Stobutzki & Bellwood 1994).

Escape response measurements

Escape responses typically consist of a unilateral contraction of the axial musculature (stage 1), which bends the body into a 'C' shape, and a subsequent contralateral contraction, resulting in a half tail-beat (stage 2) (Domenici and Blake 1997, Eaton et al. 2001). Escape sequences were analyzed using the software ImageJ v1.45 and the plugin MtrackJ (Meijering, Dzyubachyk & Smal 2012). The two-dimensional X-Y coordinates of the fish's centre of mass (CoM) were plotted every 2.4 ms starting 12 ms before and ending 48 ms after the onset of the stimulus (25 frames in total). Since fish were too small to mark their CoM directly on the body, the CoM was visually determined at a fixed distance from the tip of the head during video analysis (Langerhans 2009). Measurement error on displacement data from visually estimating the CoM was assessed by digitizing videos from five fish, two times each (Langerhans 2009). This error was < 4 % for all videos tested. Seven escape performance variables were measured following Lefrançois & Domenici (2006): responsiveness (the percentage of fish that performed an escape response when stimulated); response latency (the time between the moment when the stimulus contacted the water and the first reaction of the fish); cumulative distance traveled (D_{esc}); maximum escape speed (U_{max}); maximum acceleration (A_{max}); stage 1 turning angle (the angle between the straight line joining the tip of the head and the CoM at the onset and end of stage 1); stage 1 turning rate (stage 1 turning angle divided by stage 1 duration). Distance-time variables (D_{esc}, U_{max}, A_{max}) were evaluated within a fixed time period of 24 ms, corresponding to the mean duration of stages 1 and 2 for all three species. Measuring distance-time variables within a fixed time period avoids performance biases related to differences in the duration of escape responses (Domenici 2011). A five-point quadratic polynomial regression (Lanczos, 1956) was used to obtain smoothed values of speed and acceleration, the first and second derivatives of distance (Walker 1998; Lefrançois & Domenici 2006).

Statistical analysis

I used a one-way ANOVA to test for differences in D_{esc} among species in static flow. In unsteady flow, the timing of the stimulus relative to the water movement in the experimental tank differed between trials. Therefore, I used a linear mixed model (LMM) to test the effect of species and water flow direction relative to the escape trajectory on distance-time variables (D_{esc}, U_{max}, A_{max}) while controlling for the non-

independence of data points obtained from the same individual. Then, for each individual fish, I computed the best value (e.g., highest U_{max} or shortest latency) of all escape performance variables across the two or three stimulus presentations chosen in the static and unsteady flow treatments (see Domenici 2010b; Domenici 2011). I used a two-way MANOVA to test for an overall effect of flow and species on distance-time and maneuverability/agility variables (D_{esc}, U_{max}, A_{max}, stage 1 turning rate and stage 1 turning angle). This was followed by separate analyses to look at the effect of predictors on each response variable. I used a two-way ANOVA to test for the effect of water flow and fish species on escape latency. Finally, I tested for differences in displacement distance due to water flow among the three species using a one-way ANOVA. Assumptions of normality and homoscedaticity were assessed graphically with diagnostic plots. Escape latency was transformed with a boxcox function to meet the assumptions of the model. Analyses were conducted in R v3.0.1 (R Core Team 2013).

Results

The best value of cumulative escape distance (D_{esc}) differed among species in static flow (one-way ANOVA, $F_{2,39} = 9.52$, $P < 0.001$): the deep- and shallow-bodied species escaped farther, in a fixed amount of time, than the species with an intermediate body depth (Fig. 4A). In unsteady flow, all species covered a greater distance when escaping with rather than against the direction of the water motion (LMM, $F_{3,68} = 17.3$, $P < 0.001$; interaction ns, $P > 0.15$; Fig. 3). The results for maximum swimming speed and acceleration were similar (graphs not shown).

Several escape variables were similar for all species in both flow treatments. Responsiveness was high in all three species, with fish responding to the stimulus > 94% of the time in unsteady flow and 100% of the time in static flow. Compared to static flow, unsteady flow had no significant effect on the best value of escape performance (D D_{esc}, U_{max}, A_{max}, turning rate, turning angle) (MANOVA, Pillai = 0.10, approx $F_{5,74} = 1.67$, $P = 0.15$; interaction ns, $P > 0.4$). Results for individual response variables are presented in Table S1. Escape distance was consistently greater (6 % on average, across species) in unsteady than static flow but this difference was non-significant (Fig. 4A).

The timing of escape responses was the factor most affected by unsteady flow. The effect of water motion on fish response latency differed among the three species (two-way ANOVA, species × flow: $F_{2,78} = 4.4$, $P < 0.02$) (Figs 4B, 5): unsteady flow increased latency by 230 % for *N. azysron* (FR=3.10, fusiform body), 190 % for *C. atripectoralis* (FR=2.42, intermediate body depth), and 2 % for *D. reticulatus* (FR=1.67, deep body). All species moved less (i.e., were less displaced by water motion) than a passive particle of similar size in unsteady flow (one-way ANOVA, $F_{3,52} = 53.0$, $P < 0.001$) but displacements differed among species (one-way ANOVA, $F_{2,39} = 3.6$, $P < 0.05$), with the deep-bodied species being the least displaced by water motion (Fig. 6).

Discussion

Fast-starts in unsteady flow

Various environmental stressors affect fish fast-start swimming performance: temperature, hypoxia, salinity, turbidity, light cycles and pollutants (Domenici 2010a; Wilson *et al.* 2010). Clearly, water motion also influences escape responses. In unsteady, wave-driven flow, fish performed better (i.e., reached greater speeds and travelled further) when escaping in the direction of, rather than against or perpendicular to, the water motion (Fig. 3). Since maximum muscle power output is be the same irrespective of flow conditions, differences in swimming speed between static and unsteady flow should equal the water flow velocity. Although non-significant, maximum escape distance and velocity were consistently greater in unsteady than static flow for all three species (Table 1; Fig. 4A). Various factors could explain why these differences were minimal. Maximum flow velocities were small (approximately 20%) relative to the maximum escape speeds achieved by fish, and the timing of escapes – even the ones leading to the best performance - did not always occur in maximum flow (i.e., at the wave crest). In addition, the recruitment of muscles, otherwise used to generate thrust, for postural control in unsteady flow could have further reduced differences in burst speed between flow conditions. Future studies should test fish in stronger flows to disentangle these effects.

Burst speed and escape distance are important performance abilities for evading predators, but escape success also depends on the ability of a prey to quickly respond to a threat (Walker *et al.* 2005; Fuiman *et al.* 2006; Domenici 2010b). Unlike escape kinematics, the timing of responses by juvenile fish differed considerably between flow

conditions and among species (Fig. 4B). The shallow-bodied *N. azysron* were more than twice as slow and *C. atripectoralis* (intermediate body depth) took almost twice as long to respond in unsteady flow. In contrast, the latency of the deep-bodied *D. reticulatus* was unchanged. These differences in response time could be due to the destabilizing effect of unsteady water motion and the added challenge of maintaining an upright posture and adequate orientation relative to the flow. All else being equal, a more laterally compressed body should improve resistance to rolling disturbances (Eidietis, Forrester & Webb 2002; Weihs 2002). The large pelvic fins of *D. reticulatus* (Fig. 1) could also have improved its stability under wave disturbances. However, the effect of unsteady flow on response latency was inversely related to the body depth of these species, which suggests that body morphology is an important trait for postural control. Indeed, the deep-bodied *D. reticulatus* tended to orient more directly into the flow and were significantly less displaced by water motion (i.e., had a shorter excursion distance) than the fusiform-shaped *N. azysron* (Fig. 6). Reactivity to potential threats (responsiveness, response latency) is a major factor in the escape success of prey fish (Fuiman *et al.* 2006; Domenici 2010b). Even mild unsteady flow, as shown here, therefore has the potential to reduce the survivorship of juvenile fish that are most susceptible to postural disturbances.

Fast-starts in fish are typically controlled by the Mauthner cells, a pair of large reticulospinal neurons that receive various sensory inputs, such as visual and mechano-acoustic signals (Eaton, Lee & Foreman 2001). High speed neural transmission and processing via these cells allows for rapid responses to imminent threats within 5-20 ms. Interestingly, the shallow-bodied *N. azysron* exhibited much greater variation in escape latency in unsteady versus static flow than did the other two species (Fig. 4B). The strong positive skew in the distribution of its response latencies (Fig. 5A) suggests that some escape responses in unsteady flow were not Mauthner cell mediated. Other pathways, through different reticulospinal, cells can control escape responses but have longer latencies (Domenici 2010a). Slower responses are generally associated with lower performance (e.g., slower turning rates), and are observed both in healthy fish and in fish with an ablated Mauthner system (Domenici 2011). In unsteady flow, postural disturbances from waves and associated stability control issues seemingly led to some non-Mauthner-cell escapes, with longer latencies (>40 ms).

Fast-starts in static flow

There is a dearth of information on the fast-start escape performance of early-stage coral reef fish. Studies in the last 10-15 years have shown that pre- and post-settlement larvae can achieve extremely high sustained swimming speeds of 30 to 50 body lengths s^{-1}, suggesting that reef fish larvae are not simply passive organisms adrift in the plankton (e.g., Stobutzki & Bellwood 1994; Leis & Carson-Ewart 1997; Fisher *et al.* 2005; Nilsson, Hobbs & Östlund-Nilsson 2007). Here, I show that juvenile pomacentrids can also achieve impressive burst speeds in excess of 100 cm s^{-1} (85 SL s^{-1}; Table 1). This is almost twice the maximum velocity achieved by similar-sized temperate species, including salmonids (Fig. 11.5 in Fisher & Leis 2010). Burst swimming is closely related to the ability of juvenile reef fish to avoid predation and capture food, and is therefore directly relevant to their ecology (Fisher & Leis 2010). The impressive escape response of small pomacentrids is perhaps not surprising given that damselfishes are among the fastest swimming coral reef fish larvae (in relative swimming speeds), both in nature and in laboratory swim tunnels (Leis & Carson-Ewart 1997; Nilsson, Hobbs & Östlund-Nilsson 2007).

Escape distance differed among juveniles in static flow: both the shallow- and deep-bodied species escaped faster and farther than the intermediate species (Fig. 4A). This could result because different, and sometimes opposite, morphological features are suggested to improve fast-start performance (Domenici 2003; Walker 2004). On the one hand, species with an elongated body (with depth distributed evenly along the body axis), small head and large dorsal and anal fins such as pike, *Esox lucius,* represent features of good fast-start performers (Webb 1984; Domenici 2003; Langerhans *et al.* 2004). On the other hand, a deep body allows a lateral profile that increases the mass of water accelerated by body movements, which also improves fast-start performance (Weihs 1973; Domenici & Blake 1991). For example, the deep body of crucian carp (*Carassius carassius*), induced in the presence of predators, results in higher locomotor performance (escape distance, speed, acceleration and turning rate) than that of the shallow body morphs found in predator-free habitats (Domenici *et al.* 2008). These two contrasting body profiles roughly correspond to the two pomacentrids at opposite ends of our body depth spectrum, which performed best. Response latency was also distinctly higher in the deep-bodied *D. reticulatus* than in the other two species (Fig. 4B). Beyond improving fast-start performance, increased body depth is also associated with reduced

vulnerability to gape-limited predators, which are abundant on the reef (e.g., Pseudochromidae, Labridae) (Rice, Crowder & Marschall 1997; Holmes & McCormick 2010; Domenici 2011). Since fleeing is costly, less vulnerable prey might assess potential threats for longer, possibly explaining the slower response time of *D. reticulatus*.

Conclusion

Hydrodynamic stability is clearly advantageous in high energy coral reef environments, which experience rapid variations in flow velocity (Bartol *et al.* 2003). Here, I found that there were differences in escape performance among juvenile damselfish, and that unsteady, wave-driven flow increased the response latency of fish that were more displaced, and potentially more destabilized, by water motion. Juvenile reef fish are highly sensitive to environmental variables (Leis & McCormick 2002), and any negative effects on their survival and settlement patterns will have important consequences for adult populations (Munday *et al.* 2008). Wave intensity and frequency is increasing across ocean basins worldwide as winds and severe weather events become more frequent with climate change (Webster *et al.* 2005; IPCC 2013). Strong winds and waves coinciding with juvenile recruitment pulses could affect not only larval dispersal (e.g., Burgess, Kingsford & Black 2007), but also predator-prey interactions and the survivorship of post-settlement juveniles. Many damselfishes recruit seasonally (Russell, Anderson & Talbot 1977; Williams 1983), and are initially limited by habitat as they settle on the reef (Sale 1978). Once the recruitment pulse is over, post-settlement mortality becomes important because juveniles that are consumed can no longer be replaced even if habitat becomes available. Future research should examine whether relationships exist between wave intensity, predation rates and recruitment patterns in the wild, as well as differences in the effect of wave-driven flow on predators and prey due to size differences (see Abrahams, Mangel & Hedges 2007).

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Data accessibility

The data for this paper are archived in figshare: doi10.6084/m9.figshare.862938.

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Table 1. Escape performance variables (mean ± s.e.m.) for three damselfish species (Pomacentridae) with different body fineness ratios in conditions of static flow and unsteady flow.

	N. azysron		*C. atripectoralis*		*D. reticulatus*	
Variable	static	unsteady	static	unsteady	static	unsteady
Latency (ms)	7.0 ± 0.6	16.2 ± 3.0	7.1 ± 0.5	13.4 ± 1.2	10.7 ± 1.5	10.9 ± 1.0
D_{max} (cm)	1.68 ± 0.08	1.82 ± 0.11	1.23 ± 0.07	1.26 ± 0.09	1.54 ± 0.05	1.61 ± 0.06
D_{max} (SL)	1.32 ± 0.06	1.43 ± 0.09	1.01 ± 0.06	1.02 ± 0.07	1.21 ± 0.04	1.29 ± 0.05
U_{max} (cm s^{-1})	78.6 ± 5.0	109.8 ± 6.4	80.8 ± 3.8	86.7 ± 7.8	101.5 ± 4.7	91.5 ± 3.0
U_{max} (SL s^{-1})	78.6 ± 3.6	87.3 ± 5.3	65.9 ± 3.2	69.5 ± 5.9	72.2 ± 2.7	80.8 ± 3.0
A_{max} (cm s^{-2})	77.3 ± 3.6	80.2 ± 5.0	54.4 ± 3.0	65.3 ± 7.4	62.2 ± 2.1	74.2 ± 3.9
$S1_{duration}$ (s^{-1})	5.4 ± 0.4	4.8 ± 0.0	5.8 ± 0.5	5.4 ± 0.6	4.9 ± 0.2	4.8 ± 0.0
T_{angle} (°)	84.8 ± 6.2	76.0 ± 5.0	94.9 ± 6.2	112.6 ± 8.0	74.9 ± 5.2	87.9 ± 8.6
T_{rate} ($°s^{-1}$)	13.6 ± 0.7	14.1 ± 0.6	10.8 ± 0.6	10.8 ± 0.8	11.9 ± 0.5	1.5 ± 0.6

Fig. 1. Indo-Pacific damselfishes (Pomacentridae) with different body fineness ratios (FR): *Neopomacentrus azysron*, *Chromis atripectoralis*, *Dascyllus reticulatus*. Scale bar = 10 mm.

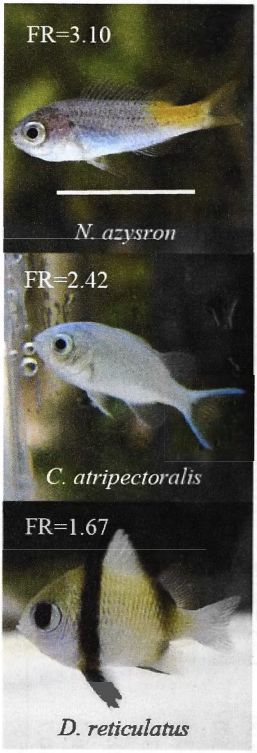


Fig. 2. PIV analysis output showing patterns of water movement and flow velocities (black arrows) in the experimental arena in unsteady flow conditions: A) during flow direction change and B) at the wave crest. The colour map represents the magnitude of flow velocity in m s^{-1}. White vectors are values interpolated by the software due to spurious or missing data points. Scale bar for vectors = 0.20 m s^{-1}. For frame sequences of velocity vector and vorticity fields, see ESM.

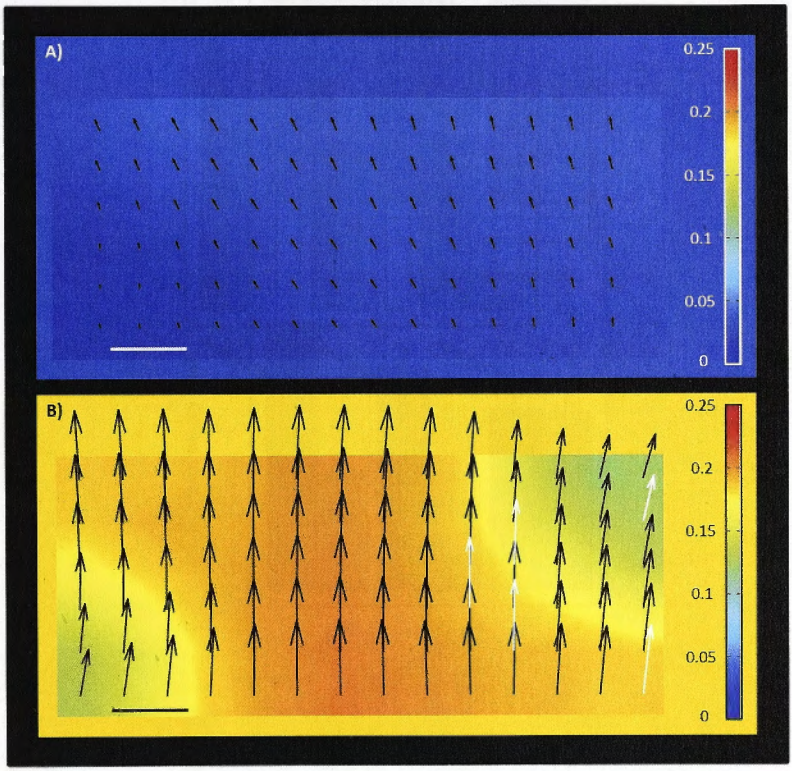


Fig. 3. Distance travelled (in standard lengths; SL) in a fixed time interval (24 ms) for three species of Pomacentridae in unsteady flow conditions. Light bars are escape responses against the traveling wave and dark bars are escapes in the direction of the traveling wave. Bars of intermediate shades represent escapes at the wave trough (low flow velocity) or perpendicular to the direction of the water flow. Error bars are s.e.m.

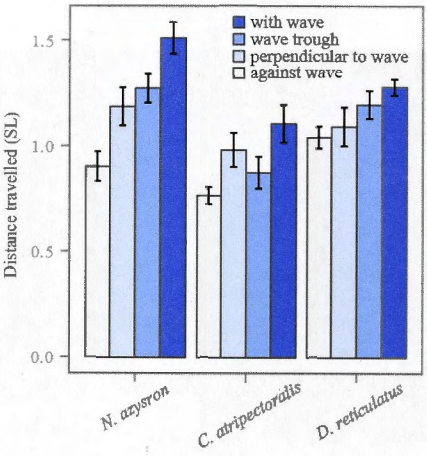


Fig. 4. A) Maximum distance travelled (in standard lengths; SL) and B) minimum response latency (ms) for three species of Pomacentridae in unsteady water flow (dark bars) and static flow (light bars). Error bars are s.e.m.

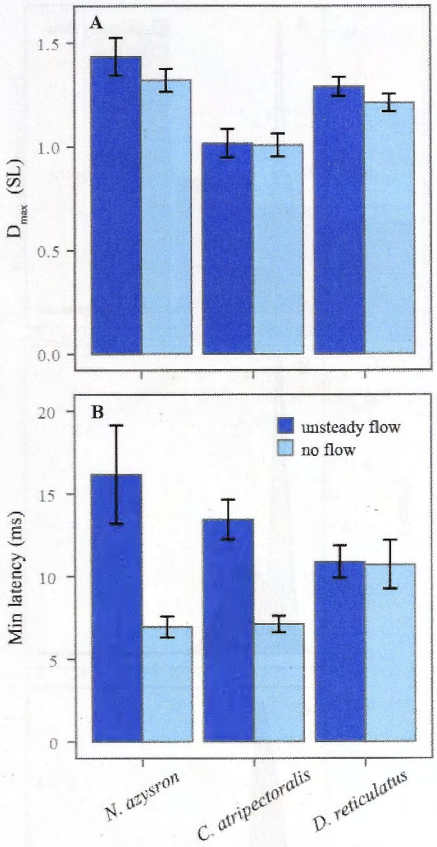


Fig. 5. Kernel density plots showing the distribution of response latencies (ms) for A) *Neopomacentrus azysron*, B) *Chromis atripectoralis*, and C) *Dascyllus reticulatus* in unsteady water flow (dark blue) and static flow (light blue).

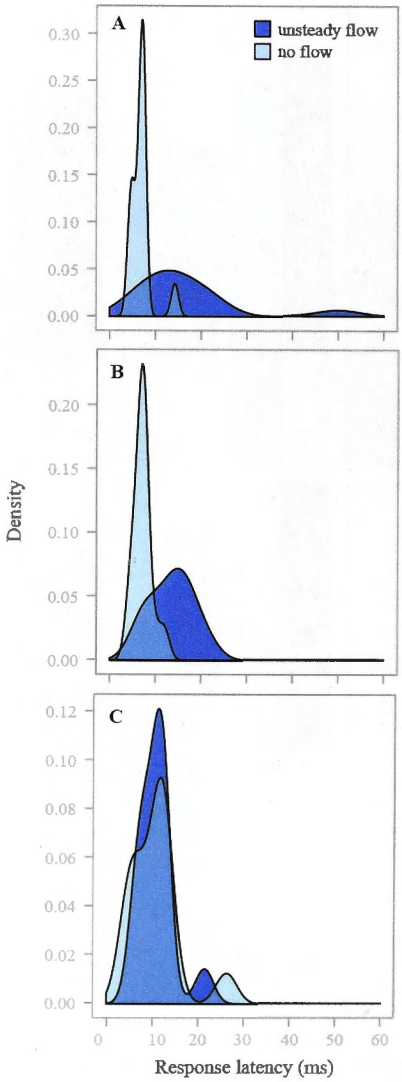


Fig. 6. Excursion distance (cm) of a passive, neutrally buoyant particle and three species of Pomacentridae in unsteady water flow. Error bars are s.e.m.

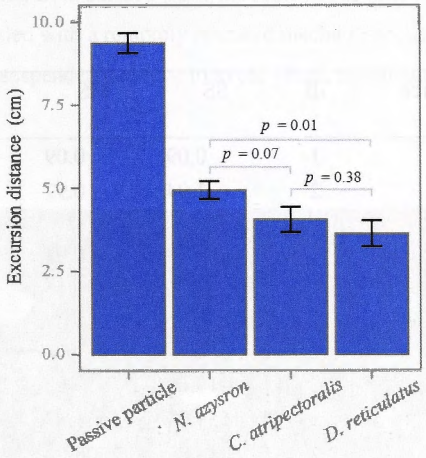


Table S1. Effects of species, water flow and size on kinematic performance variables in three damselfish species (Pomacentridae) with different body morphologies. Differences in overall kinematic performance in conditions of static flow vs. unsteady flow were tested with a MANOVA. Values presented are for subsequent univariate tests; bold *p*-values denote significance.

Response variable	Source	df	SS	MS	*F*	*P*
D_{max}	Flow	1	0.09	0.09	1.70	0.20
	Species	2	1.94	0.97	17.9	**< 0.001**
	Flow × species	2	0.04	0.02	0.37	0.69
U_{max}	Flow	1	1016.20	1016.16	4.27	**0.04**
	Species	2	3278.10	1639.07	6.88	**< 0.01**
	Flow × species	2	119.60	59.82	0.25	0.78
A_{max}	Flow	1	1551.90	1551.87	5.54	**0.02**
	Species	2	5013.80	2506.92	8.95	**< 0.001**
	Flow × species	2	342.9	171.46	0.61	0.54
T_{angle}	Flow	1	1120.00	1120.20	1.80	0.18
	Species	2	9783.00	4891.60	7.84	**< 0.001**
	Flow × species	2	2793	1396.3	2.24	0.11
T_{rate}	Flow	1	10.01	10.01	1.78	0.19
	Species	2	132.64	66.32	11.79	**< 0.001**
	Flow × species	2	9.31	4.65	0.83	0.44

Fig. S1. Experimental setup for fast-start experiments. Four programmable pumps (Vortech MP10wES, EcoTech Marine, USA) on the back wall created unsteady water flow that travelled back and forth in the aquarium at a frequency of 1.7 ± 0.09 Hz (mean ± s.d.). The mirror at 45° below the aquarium allowed the filming of escape responses without image distortion from surface water movement. Juvenile fish were placed in the central arena and startled with a remotely operated mechano-acoustic stimulus. The stimulus fell inside a suspended pvc tube to avoid visual stimulation before contact with the water surface.

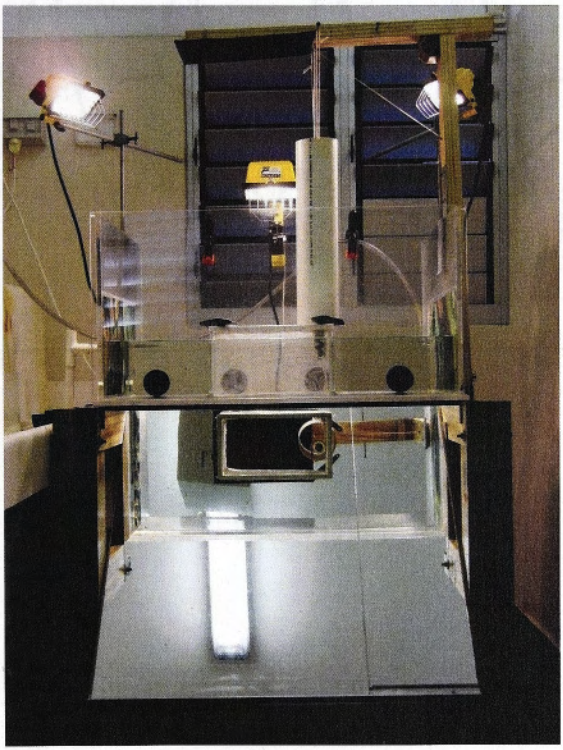
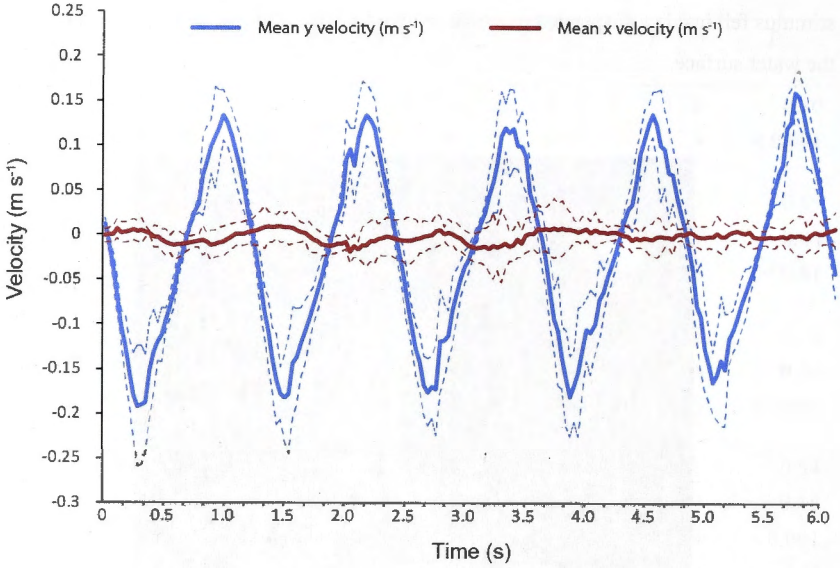


Fig. S2. Example flow profile showing X and Y components of mean flow velocity in the experimental arena over six seconds. Means were computed by averaging all vectors per frame in the software PIVlab v.1.32. Dotted lines represent one standard deviation from the mean.



CHAPTER - 6

Troubleshooting public data archiving: suggestions to increase participation

Roche, D.G., Lanfear R., Binning, S.A., Schwanz, L.E., Cain, K.E., Haff, T.M., Kokko, H., Jennions, M.D., Kruuk, L.E.B. (2014) *PLoS Biology* 12: e1001779

Keywords

Altmetrics, data repository, data sharing, online database, open access, open data

Abstract

An increasing number of publishers and funding agencies require public data archiving (PDA) in open access databases. PDA has obvious group benefits for the scientific community, but many researchers are reluctant to share their data publicly because of real or perceived individual costs. Improving participation in PDA will require lowering costs and/or increasing benefits for primary data collectors. Small, simple changes can enhance existing measures to ensure that more scientific data are properly archived and made publicly available: (1) facilitate more flexible embargoes on archived data; (2) encourage communication between data generators and re-users; (3) disclose data re-use ethics; and (4) encourage increased recognition of publicly archived data.

Main text

Good science relies on transparent, reproducible results, and scientific data are often collected with public funds [1-3]. For these reasons, funding agencies, publishers and researchers are increasingly encouraging public data archiving (PDA) into open access databases [1-8]. It is widely accepted that the benefits of PDA to the scientific community greatly outweigh the costs [6-10]. However, decisions to archive data are currently made by individual researchers, and it is less obvious that the benefits of PDA outweigh the costs for all individuals [10]. This probably explains why PDA is far from universal in the biological sciences [e.g., 11,12] (but see major initiatives in genomics [13]), and why many researchers still harbour concerns about making their data publicly available [10,14-17]. This is particularly true in fields such as ecology and evolutionary biology, where datasets are often complex, have a long shelf-life and can be used to test multiple hypotheses [3,7,18]. The benefits of data sharing have been extensively

discussed [1,3,5,7,10,19], but the real and perceived costs have received far less attention in the literature. Acknowledging and discussing how to ameliorate these costs is critical to promoting PDA in all disciplines. Here, we hope to stimulate discussion by briefly reviewing the costs and benefits of PDA, and suggesting practical solutions to reduce the costs and increase the benefits for individual researchers.

The value of PDA can be viewed either from the perspective of the scientific and broader community as a whole (group), or from that of individual researchers. **Group benefits** are substantial and have driven the formulation of policies aimed at establishing a culture of data archiving and sharing. PDA increases data preservation by avoiding losses from hardware malfunction or obsolescence [7], or from researchers moving on to different projects or retiring. PDA also encourages good metadata production to ensure that datasets are interpretable [8]. In turn, open access to data increases the ability to evaluate and reproduce studies [1,9-10], encourages a stronger sharing culture [5], improves the return per research dollar [10,19] and increases opportunities for teaching and learning [7,10]. Currently, **group costs** include the financial costs of maintaining public databases such as figshare, Dryad, TreeBASE and GenBank [7,20]. Potential future costs might arise if large amounts of freely available primary data online lead to the publication of misinterpretations of datasets, which is more likely when the intricacies of data collection and biological considerations are difficult to convey in metadata files [21]. Additionally, spurious conclusions may arise due to Type I errors from data dredging (i.e., exploratory analyses) and subsequent publication bias [22]. Finally, if data re-use has perceived advantages over collecting primary data for individual researchers (see below), this could decrease the overall amount of primary data collected and potentially create long-term group costs.

At the **individual** level, there are various **benefits** to open data archiving for researchers who collect primary data. These include increased citation of the original study and/or of the archived datasets [7,23], recognition through metrics such as 'altmetrics' [24] and the proposed new Data Usage Index [25] and 'data deposition' metric [16], potential co-authorship of new studies [7], improved data management requirements (which ultimately make it easier for researchers to re-use their own data) [7,10], and prizes for pursuing 'open science' initiatives (e.g., http://asap.plos.org). **Individual costs** include the time required to generate appropriate metadata and data

descriptors to facilitate re-use [7,9], the modest financial costs of submitting data to some archives [26], and the need to monitor how one's data are used [e.g., 27,28] because of concerns regarding misinterpretation of data by researchers with less experience of the study system [29]. In our experience, however, individuals are most concerned about the loss of priority access following PDA, which could generate competition with others when conducting subsequent analyses [see 3,16-17,30]. Many individuals judge that the benefits of PDA, such as an increased citation rate for an initial paper [31], will not compensate for the future publications lost by renouncing priority access to the data they collected [32] – the fear of being 'scooped'. Given intense competition for grants and academic positions, where publications are the major currency for assessing performance [20-21], it is rational for an individual to make decisions that primarily maximise his/her publication rate rather than benefits for science at large [20,32], and there is therefore substantial risk of these concerns affecting rates of PDA.

Many journals and funding agencies (e.g., NSF, NIH, NERC) now require PDA following publication [7,33] - for specific policies of journal and funding agencies see [33-35]. This provides an effective 'stick' [36], but authors who are concerned about PDA can simply avoid these journals, or can archive data in a way that makes them difficult to re-use. Currently, most journals do not police the quality of archived data [36-37], making it easy to circumvent the system if desired (e.g., by not archiving data at all or by archiving either incomplete data or data in inappropriate formats) [16-17,38]. Unfortunately, in biology, the concerns regarding PDA are possibly strongest for large-scale studies conducted over multiple geographic locations, seasons or years, which require substantial financial and logistic resources (e.g. those in ecology, evolutionary biology and climate change science). These datasets may be vital for elucidating trends in species distributions, phylogenetic relationships or selection pressures through time, as well as the wider effects of climate change, habitat loss and invasive species [18,39]. Where such data involve large teams of researchers, additional concerns arise as to overlap of data re-users' activities with on-going work, particularly by graduate students. PDA of these data is costly for authors in a system that requires rapid release into the public domain (e.g., figshare offers no embargo option), making it difficult for the original authors to reap sufficient rewards (i.e., publications) for their substantial initial investment in data collection. Consequently, many valuable datasets

are improperly archived or not archived at all [e.g., 16,38], and therefore never enter the public domain.

A slight shift in the protocols for the use of public data could complement existing measures to promote PDA by lowering costs and increasing benefits for individual data collectors. In essence, more (or larger) 'carrots', not 'sticks', are needed to increase participation in PDA [40]. Our proposed measures are four-fold: (1) facilitate more flexible data embargoes; (2) encourage better communication between data re-users and data collectors; (3) disclose data re-use ethics; and (4) encourage the recognition of publicly archived datasets by academics, funding bodies and hiring committees.

1. Facilitate more flexible embargoes on archived data

By default, public repositories release archived datasets when an article is published [7-8]. However in adopting the Joint Data Archiving Policy (JDAP) [33], the American Genetic Association (Journal of Heredity) emphasized the importance of the 'right of first use' by data providers given the substantial investments of individual researchers in generating and curating datasets [41]. This right can be facilitated by embargoing data for a certain period. The question then becomes: how long is a reasonable embargo? Some journals that follow the JDAP allow data to be placed under embargo for up to a year [8,21]. For example, 7.4% of authors that archived data in Dryad prior to September 2013 chose a one year no-questions-asked embargo when this option was available (Fig. S1) [42]. Longer embargoes can be obtained upon appeal to editors, but currently anything longer than one year requires special agreement. A recent analysis of re-use of gene expression data suggested that a two-year embargo is sufficient to outlive most re-uses of published data by the original authors [31]. Arguably, however, this timeframe is too short for many subdisciplines of ecology and evolution (e.g., with field data collected across multiple years and datasets with multiple potential uses) ,where data less often become obsolete due to new technologies, and where records collected years or decades previously may still be re-used [e.g., 43]. In such cases, embargoes of up to five years may be more appropriate to allow data generators sufficient time to use the data fully for their planned purpose: for example, when a project involves an extensive period of data collection followed by, or

concurrent with, analysis and publication of several aspects of the data; intended extensions of a dataset to include additional species, seasons, years, etc.; when the data constitute a significant portion of a student's dissertation; and situations such as interruption of research due to parental or sick leave. Readily granting embargoes of up to five years in such cases could reduce the motivation for avoiding proper archiving of complete datasets, and thereby increase participation in PDA.

To assess current policies on embargoes in data archiving, we conducted an informal survey of journals that follow the JDAP [44]. Of the 33 journals contacted, 21 responded. All but one indicated that requests for extended embargoes are currently rare: authors ask for embargoes exceeding 1 year in less than 1% of cases. The opinion of editors on extended embargoes varied. Four cited 'sensitive' data as the only reason for embargo extensions (e.g., endangered species locations, commercial clauses, human subject data); one said they require authors to seek approval from funding agencies before granting extended embargoes. Three journals had very positive views, for example stating that any reason authors make is a good one; only one journal had a formal policy on extended embargoes up to five years that supported PhD research, long-term datasets, etc. Overall, the editors who responded to our survey were receptive to longer embargoes where sufficient justification could be given. Requesting longer embargoes could therefore ease one of the most significant concerns regarding PDA: the loss of priority access to data for sufficient time to generate additional publications using the same data.

Offering longer embargoes need not impede data sharing if most authors continue to opt for shorter or no embargoes (Fig. S1). Authors opting for a longer embargo period could be required to release metadata, with encouragement for interested data re-users to contact them directly to request access to datasets prior to the embargo expiry (see point 2 below). The TRY Plant Trait Database is an excellent example of how metadata can facilitate data sharing of private or embargoed data (http://www.try-db.org). Clearly, open data are preferable to embargoed data, but properly archived, searchable data under a temporary embargo are better than un-archived data that will never become open.

2. Encourage communication between data generators and re-users

We need to encourage a culture of, and an agreed-upon etiquette for, communication between data collectors and data re-users. In a recent case, an unfortunate situation arose in which sequences placed in the Global Initiative on Sharing All Influenza Data (GISAID) database were unwittingly used before the original researchers had submitted their own paper. Fortunately the problem was rapidly resolved by open and reasonable discourse [45]. Basic etiquette and open communication also help to avoid duplicated effort between data collectors and re-users. Of equal importance, good communication reduces the risk of alternative interpretations of data being published by researchers with widely different degrees of knowledge of the study system. This concern is particularly relevant for extensive datasets from complex ecological systems [e.g., 27,28]. Good communication also has the mutually advantageous benefit that it often facilitates new collaborations: most data collectors are likely to be pleased to hear suggestions for novel ways to use their hard-earned data.

Good communication is the responsibility of all parties, and sensible guidelines have been proposed. White et al. (2013) suggest nine simple ways to facilitate data re-use by making data understandable, easy to analyse, and readily available [46]. If data collectors wish to be informed of further uses of their archived data, a request to be contacted should be included with the archived files. Those re-using data are also encouraged to offer co-authorship of any resulting papers if the data provide a 'non-trivial' input to the new project [7]. Arguably, data that have been carefully collected, managed, and archived are themselves a 'non-trivial' contribution if they constitute a sizable portion of the data used for a publication. However, attributing co-authorship will obviously be challenging in many cases – especially if the original study has multiple authors, or if a dataset integrates pre-existing data [21]. Clearly, there is a need for consensus ethical rules for co-authorship attribution when an analysis uses data from multiple studies (e.g., a meta-analysis, synthesis paper) [47]. Further discussion is required to establish workable guidelines [21,45], but in principle the problems are no more intractable than many that arise over authorship of primary data papers [see 48]. As a useful starting point, Duke and Porter (2013) suggest four criteria that must be met for data providers to merit co-authorship: whether the data are integral to the analysis;

whether they are novel or unique; whether the data provider is willing to share authorship; and whether the provider is able to participate [21].

3. Disclose data re-use ethics

Ultimately, measures that reduce conflict among parties early on in the data sharing process will promote PDA. Publishers have a key role to play in establishing cultural norms for data re-use [4,7]. One measure is to require ethical statements about data re-use. Many journals currently require statements about author contributions, conflicts of interest and animal ethics approval. Journals could similarly require the details of data re-use: a brief summary of any effort made to contact the primary researchers, their response, and any discussion about results, interpretation, co-authorship and consent of re-use of any data under embargo. Journal editors could also consider offering data generators the option to review any paper using their data or to publish a response, with these policies being clear to data re-users on submission of a paper. Similar procedures could apply for grant applications to funding agencies.

4. Encourage increased recognition of publicly archived data

Following any embargo period, archived datasets generally enter the public domain under the Creative Commons Zero (CC0) license [49]. CC0 does not legally require data to be cited when re-used [50]. Adequate recognition of PDA therefore relies on scientific ethics and good practice – citing open datasets is one of the best ways to reward their publication and encourage participation in PDA. Journals can directly contribute to this if their instructions to authors require citing both the dataset and the original article in studies that use publicly accessible data. For example, phylogenetic studies using sequence data from GenBank are encouraged to cite originating papers in addition to accession numbers [16]. In practice, this is challenging because journals often restrict reference lists and references in supplementary information are not indexed by the main citation services. Because of this, we reiterate a recent call for citation services to recognise references in supplementary information [51].

Ultimately, encouraging funding bodies and employers to recognize data-use metrics will be fundamental to increasing individual-level incentives for PDA.

Reassuringly, some funding bodies already have policies that recognize 'altmetrics' [52] and research outputs such as datasets, software, code and patents [24]. Recognition of publicly archived datasets would also be enhanced if academics routinely included information about their published datasets in their curriculum vitae. This effort will be helped by recent initiatives such as ORCID (www.orcid.org), which collects information on publicly archived datasets in the figshare database (www.figshare.com). Integration of data from other repositories such as Dryad and Genbank would facilitate quantification of the impact of each researcher's publicly archived data. Importantly, the recent San Francisco Declaration on Research Assessment (DORA) makes key recommendations for improving the way individual scientist's research outputs, including datasets, are evaluated [53].

In conclusion, the trend towards PDA and greater data sharing has many benefits, but it also generates tensions. Meaningful solutions require frank acknowledgement of the potential differences between the interests of individual researchers versus those of the broader scientific community. We hope that researchers, publishers and databases will consider these issues when deciding on the best practices for PDA.

Acknowledgements

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Data re-use

The data on embargo selections of Dryad data authors [42] were kindly compiled and archived by Todd J. Vision for this paper. We are grateful for the contribution. Co-authorship was not offered as the data are only a small part of the study.

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Fig. 1 Illustration by Ainsley Seago

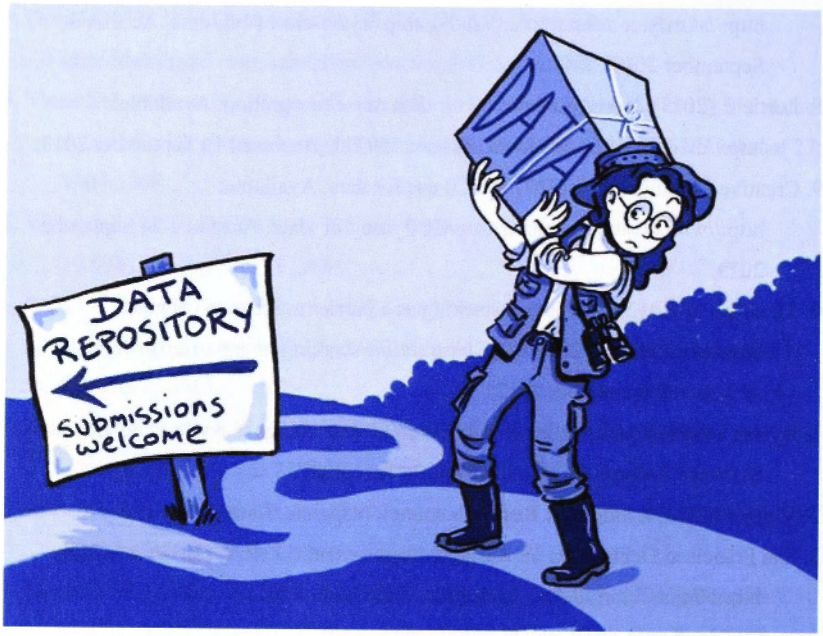
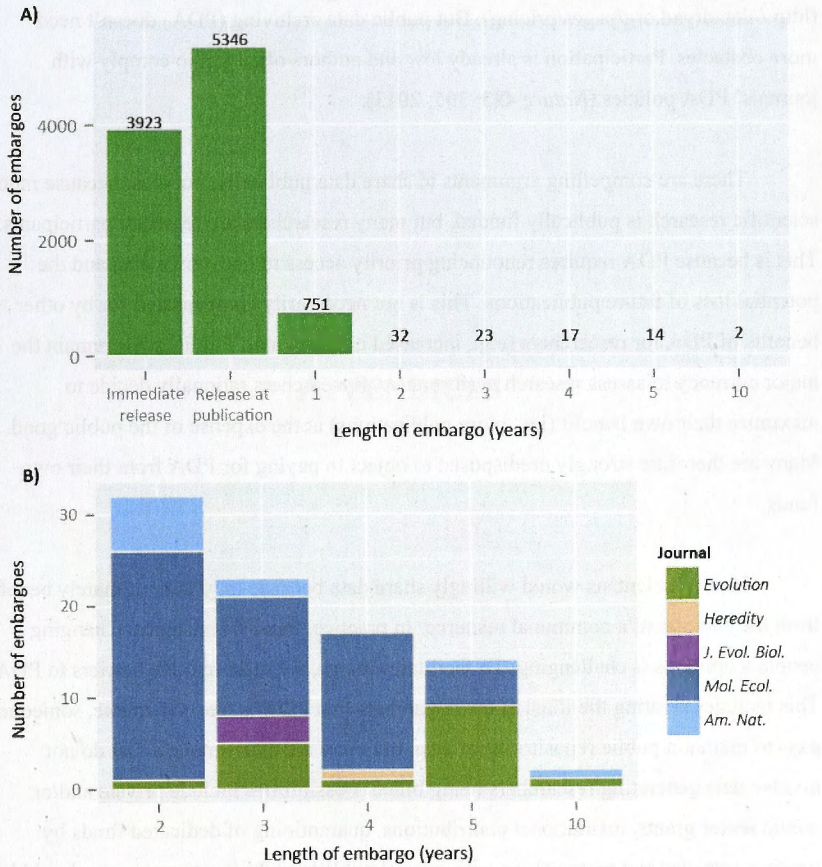


Fig. S1 (A) Embargo selections of Dryad data authors for the 10,108 files in Dryad (datadryad.org) deposited from inception to September 20 2013. Data include only journals for which the authors had the option of selecting an embargo. **(B)** Long-term embargoes (> 1 year) by journal that granted them. Data were obtained from [42].



Data deposition: Fees could damage public data archives

Roche DG, Jennions MD and Binning SA (2013) *Nature* 502: 171

Dryad is among the largest public data repositories in the life sciences. On September 1st, it implemented modest fees to archive datasets (http://datadryad.org/pages/pricing). But public data archiving (PDA) doesn't need more obstacles. Participation is already low and authors often fail to comply with journals' PDA policies (*Nature* **493**: 305; 2013).

There are compelling arguments to share data publically, not least because much scientific research is publically funded, but many researchers are reluctant participants. This is because PDA requires renouncing priority access to one's own data and the potential loss of future publications. This is not necessarily compensated for by other benefits of PDA for researchers (e.g., increased citation rate). Publications remain the major currency to assess research performance. Researchers rationally decide to maximise their own benefit (i.e., more publications) at the expense of the public good. Many are therefore strongly predisposed to object to paying for PDA from their own funds.

Ideally, scientists would willingly share data because they will ultimately benefit from the creation of a communal resource. In practice, there is resistance. Changing people's opinions is challenging. To facilitate change, we must remove barriers to PDA. This includes creating the illusion for researchers that PDA is free. Of course, someone pays to maintain public repositories of data, but there are many models that do not involve data generating researchers being billed. Possibilities include private and/or public sector grants, institutional contributions, quarantining of dedicated funds by funding agencies and partnerships with journal publishers. In short, payments should be directly from funders' budgets (or publishers' profits) to PDA providers, to circumvent researchers being billed. Like 'free' restaurant bread or hotel internet access, costs can be hidden to keep people happy.

Reference

Drew B (2013) Data deposition: missing data mean holes in tree of life *Nature* 493: 305

APPENDICES

APPENDIX - I

Shelters and their use by fishes on fringing coral reefs

Ménard A, Turgeon K, **Roche DG**, Binning SA and Kramer DL (2012) *PLoS One* 7: e38450.

Keywords

Caribbean, coral reefs, corals, fishes, habitats, reefs, shelters, species diversity

Abstract

Coral reef fish density and species richness are often higher at sites with more structural complexity. This association may be due to greater availability of shelters, but surprisingly little is known about the size and density of shelters and their use by coral reef fishes. We quantified shelter availability and use by fishes for the first time on a Caribbean coral reef by counting all holes and overhangs with a minimum entrance diameter ≥3 cm in 30 quadrats (25 m^2) on two fringing reefs in Barbados. Shelter size was highly variable, ranging from 42 cm^3 to over 4,000,000 cm^3, with many more small than large shelters. On average, there were 3.8 shelters m^{-2}, with a median volume of 1,200 cm^3 and a total volume of 52,000 cm^3m^{-2}. The number of fish per occupied shelter ranged from 1 to 35 individual fishes belonging to 66 species, with a median of 1. The proportion of shelters occupied and the number of occupants increased strongly with shelter size. Shelter density and total volume increased with substrate complexity, and this relationship varied among reef zones. The density of shelter-using fish was much more strongly predicted by shelter density and median size than by substrate complexity and increased linearly with shelter density, indicating that shelter availability is a limiting resource for some coral reef fishes. The results demonstrate the importance of large shelters for fish density and support the hypothesis that structural complexity is associated with fish abundance, at least in part, due to its association with shelter availability. This information can help identify critical habitat for coral reef fishes, predict the effects of reductions in structural complexity of natural reefs and improve the design of artificial reefs.

Introduction

In coral reef ecosystems, structural complexity is frequently associated with greater abundance and number of fish species [1]–[4]. One hypothesis for this association is that complex structures offer more shelters or refuges such as holes, caves, and crevices, which provide protection from predators, competitors, currents, and strong light as well as sites for reproduction and foraging [5]–[8]. Supporting evidence comes from a small number of observational studies demonstrating that measures of shelter availability such as density or total volume of holes on natural reefs predict abundance or species richness better than other measures of physical complexity such as rugosity [2], [9], [10]. In addition, there is support from experimental studies on a variety of small, artificially constructed reefs showing an increase in fish density with increasing shelter availability [7], [11]–[15]. Yet, despite the potential importance of shelters for fishes, information about their distribution and abundance is remarkably scarce for natural coral reefs and nonexistent in the Caribbean region (but see [16]). This gap in knowledge may be due in part to the challenges of defining shelters (e.g., [17]) and to the time and effort required to measure and count them [2], [10]. Among the studies that did measure and count shelters on reefs, very few presented the data and instead provided only correlations, qualitative indices or integrated measures from ordination analyses [2], [10],[18]–[21], preventing comparisons among studies. To the best of our knowledge, no studies have examined variables that influence shelter availability other than coral cover and most studies of shelter use by coral reef fishes have focused on one or a few related species (e.g.,[17], [22]–[26]). No study has attempted to identify the whole assemblage of shelter-using fishes or to document the variables that influence which of the available shelters are used. Assessing the variables influencing shelter availability and occupation by fishes is important for identifying critical habitat for conservation, for understanding the ecological implications of reductions in reef complexity [27], and for improving the design of artificial reefs. This information is particularly critical for Caribbean reefs which, despite being among the best studied in the world, are also among the most threatened [28].

The goals of our study were (1) to assess the size distribution of two types of shelters (holes and overhangs) in a fringing reef system in Barbados, (2) to examine how shelter occupancy by fishes was related to shelter size and type, (3) to determine how spatial variation in shelter availability, as measured by shelter density, shelter size,

and total shelter volume, was related to structural complexity, reef zone and water depth, (4) to determine how spatial variation in the proportion of shelters occupied and in the density of shelter-using fishes was related to shelter availability and to structural complexity, reef zone and water depth, (5) to examine evidence that shelters are a limiting resource as indicated by the relationship between shelter availability and fish density, and (6) to characterize the species richness and diversity of the shelter-using fish assemblage.

Methods

Ethic Statement

This study involved no capture or handling of fishes or corals and only brief disturbance of fishes when sampling shelter characteristics. The procedures were approved by the McGill University Animal Use Committee, Animal Use Protocol and Permit 5039 and conformed to all guidelines of the Canadian Council on Animal Care.

Study Sites

We sampled shelters in three zones of two fringing reefs on the west coast of Barbados, West Indies. Barbados has well-defined fringing coral reefs on its west (leeward) coast, which have become partially degraded since first described in the 1960s [29]–[31]. The reefs sampled were North Bellairs (13°11′33″ N, 59°38′30″ W) and Chefette (13°10′53″ N, 59°38′25″ W), both in the Barbados Marine Reserve (Figure 1). The data were collected using SCUBA in June – August 2006 between 08:30 hrs and 16:30 hrs on days when the visibility was at least 5 m.

We sampled only the reef crest, spur and groove and fragmented spur zones. The back reef zone (termed reef flat by Lewis [29]) was excluded because we wished to focus our efforts on areas with greater densities of shelters and fishes. Zones were identified by observations of the physical characteristics of the substrate prior to sampling and comparisons with distance from shore measurements of each zone from previous research in Barbados ([29], [31], [32]; Figure 1). The reef crest extends seaward, approximately 40 m from the edge of the back reef. Unlike offshore or exposed reefs, the reef crest on the leeward side of Barbados seldom experiences heavy wave action. The surface is exposed in places during extreme low tides but usually remains about 1 m below the surface. The substrate is composed mainly of dead coral

rock with irregular surfaces and small pinnacles of coralline algae (predominantly *Porolithon*) coating the remains of coral skeletons. The crest as defined in this study combines parts of the reef crest and the coalesced spur zone described by Lewis [29] and Tomascik and Sander [31] and corresponds to the reef flat of Stearn et al. [32]. The spur and groove zone consists of a series of ridges projecting seaward and alternating with winding valleys of sand and rubble [29], [31],[32]. The spurs support substantial live coral cover and reach depths of 3–4 m at the seaward edge. We distinguished a fragmented spur zone immediately seaward from the end of the continuous spurs. Here, the continuous ridges disappear and are replaced by scattered patches of coral heads, often a single massive colony of *Montastrea*, *Siderastrea* or *Diploria* surrounded by sand. Although this habitat was considered part of the spur and groove zone by previous authors, we distinguished it because of its greater depth and patchy structure.

We used gridded aerial maps of the sites to delimit the study areas and select GPS coordinates of potential sampling locations. We chose fifteen quadrats on each reef by randomly selecting GPS coordinates from the maps. A marker was dropped from a boat above each selected position and a diver located the marker (representing the center of the quadrat) underwater to determine whether the site was suitable for sampling. Since our purpose was to focus on habitat dominated by hard substrate within each zone, we excluded quadrats for which the estimated sand cover exceeded 50%. In the very few instances when this occurred, we chose another quadrat by randomly selecting a new GPS coordinate. This randomized sampling did not produce a balanced design across reef zones; instead, it reflected the relative contribution of each zone to the habitat on the two reefs. Of the 30 quadrats selected, 17 were in the reef crest, 7 in the spur and groove zone and 6 in the fragmented spurs.

Data Collection

When the center of a quadrat was selected, we determined its boundaries (5×5 m) with a measuring tape and marked the corners with flagging tape. Following a 10-min habituation period for fishes to resume normal activity, two divers began measuring shelters and recording their occupants. A shelter was defined as any enclosed or semi-enclosed space, including holes, crevices and spaces under overhanging structures and between branches of living coral. We only sampled shelters for which the smallest

diameter of the entrance was at least 3 cm because of the time and effort necessary to sample the numerous very small shelters in our relatively large quadrats. For shelters with more than one entrance, we used the largest entrance for measures of location, size and depth. Shelters were classified into two types based on the amount of lateral protection offered: holes had walls on all but one side, whereas overhangs were spaces under projections without front or lateral walls. For each shelter, we recorded the XY coordinates of the shelter entrance within the quadrat (±5 cm) and the number and species of all fishes occupying it. A shelter was considered occupied if a fish was at least partially inside it when the sampling began. We then measured (±1 cm) the width and height of the entrance (holes) or the width and height of the covered space (overhangs) as well as the distance from the entrance of a hole or front of an overhang to the end of the shelter (length) using a graduated PVC tube. We estimated shelter volume using the formula for the area of an ellipse (0.7854 width×height) multiplied by the length of the shelter [2]. Fish that swam out during measurements were included in the count, but the occasional fish that entered a shelter during a measurement was not. If a fish swam between multiple shelters, we recorded all shelters used by the individual during the sampling period and randomly assigned the fish to a single shelter for the analyses. For each quadrat (25 m^2), we combined holes and overhangs and calculated three measures of the amount of space available in shelters: the mean density of shelters (number m^{-2}), the median volume of shelters (cm^3), and the total volume of all shelters combined (cm^3 m^{-2}). We used the median volume because shelter volume was log-normally distributed. We calculated two measures of shelter use by fishes: the proportion of shelters occupied by one or more fishes and the density of shelter-using fishes (number m^{-2}).

We recorded the mean water depth and structural complexity for each quadrat. Mean water depth was calculated from readings on a dive computer (Suunto Gekko Watch) at all intersections of a 1×1 m grid (36 measurements per quadrat). Structural complexity was estimated using a modification of rugosity measurements described by Luckhurst and Luckhurst [1]. A 5 m chain was laid along three length and three width positions (2.5 m apart) on the quadrat (six measurements in total), and the horizontal distance covered by the chain at each position was determined. Rugosity was calculated as the stretched length of the chain (500 cm) divided by the horizontal distance the chain covered when laid along the contour of the reef. Two divers required an average

of 4.5 h (range 1.6–8.9 h) to complete the measurements on a quadrat, depending on the number of shelters present.

Data Analysis

For quantitative descriptions of shelters and shelter-using fishes, we provide means where the data were approximately normally distributed and medians where the data were approximately log-normally distributed.

Variation in shelter volume

To test for differences in volume between holes and overhangs, we used a generalized linear mixed model (GLMM) (glmer function in lme4 package in Rv2.12.2 [33]) with a Gaussian structure of error terms. Quadrat, reef zone (crest vs. spur and groove vs. fragmented spur) and reef identity (North Bellairs vs. Chefette) were treated as random factors, and we controlled for spatial autocorrelation by blocking shelters (n = 2,863) by quadrat and nesting quadrats within reef zone and reef identity.

Occupancy and number of occupants in relation to shelter volume

We examined the relationships between shelter volume and (1) shelter occupancy and (2) the number of fish per occupied shelter, including the effect of shelter type. We used GLMMs with a binomial or a Gaussian error term structure, as appropriate. We controlled for spatial autocorrelation by blocking shelters (n = 2,863) or occupied shelters (n = 1,266) by quadrat and nesting quadrats within reef zone and reef identity.

Predictors of shelter availability

We used the Information Theoretic approach [34] to determine which set of physical variables best explained variation in shelter availability as measured by (1) shelter density, (2) median shelter volume and (3) total shelter volume, using quadrats as replicates (n = 30). We used GLMMs with three predictors: rugosity, reef zone, and reef identity. For these analyses, reef zone and identity were used as both fixed and random factors, where zone was nested within reef identity, to account for the spatial autocorrelation of quadrats. The fragmented spur zone was used as the treatment contrast for the factor reef zone, and North Bellairs was used as the treatment contrast

for the factor reef identity. Because the previous analyses had revealed few significant differences between shelter types, we combined holes and overhangs for these analyses. Prior to each analysis, for model simplicity and parsimony and following recommendation from Burnham and Anderson [34], we reduced the number of candidate models in our analysis by excluding single terms and two-way interactions that had no apparent effect on the response variable as determined from graphical examination of all biologically meaningful two-way interactions. To select the best candidate models for each response variable, we used the Akaike Information Criterion modified for small sample sizes (AICc) and performed model averaging when the normalized Akaike weight values (w_{im}) of the best models were <0.9 ([34], [35]; Text S1). We built distinct sets of models with water depth and reef zone to avoid problems of multicollinearity, but present only the results of models with reef zone which had consistently higher predictive power and support, based on AICc scores. We used the percent deviance explained to evaluate each model's goodness-of-fit. We allowed a two-way interaction term between rugosity and reef zone. Rugosity, median shelter volume and total shelter volume were $\log_{10}$-transformed prior to the analyses. Rugosity and water depth were z-standardized (i.e. mean = 0, SD = 1) to remove non-essential collinearity between single predictors and interaction terms [36], to facilitate comparison among predictors by converting them to a similar scale, and to make single terms more interpretable in the presence of an interaction [37].

Predictors of occupancy and density of shelter-using fishes

We performed similar analyses as above (*Predictors of shelter availability*) to test which physical characteristics of reefs and shelters best explained variation in (1) the proportion of shelters occupied and (2) the density of shelter-using fishes, using quadrats as replicates (n = 30). Six predictors were included in each analysis: shelter density, median shelter volume, total shelter volume, rugosity, reef identity, and reef zone or water depth. Two-way interactions were allowed between reef zone and shelter density, median shelter volume and rugosity, total shelter volume and rugosity, water depth and rugosity, shelter density and median shelter volume, and shelter density and total shelter volume. We only present models with reef zone because zone consistently explained a higher percentage of the total deviance and had better support than water depth. Rugosity, median shelter volume, and total shelter volume were $\log_{10}$-

transformed and z-standardized prior to the analyses. Shelter density was also z-standardized.

Shelters as a limiting resource for fishes

We examined whether shelters are a limiting resource for fishes on the reef by determining the shape of the relationships (linear vs. asymptotic) between fish density and (1) shelter density, (2) median shelter volume, and (3) total shelter volume. A linear relationship between population density and resource availability would be expected for a limiting resource whereas population density should have no relationship or an asymptotic relationship with a non-limiting resource. We estimated the parameter value b for the linear relationships ($y = b*x$) and the parameters $b$and d for the asymptotic relationships ($y = (b*x/[1+(b/d*x)])$ using a maximum likelihood approach (function mle2 in R, bbmle package v1.0.0; [38]). We chose a normal distribution (dnorm) to model our data and assessed the likelihood of each model with AICc scores and normalized Akaike weights (w_{im}).

Species richness

Species richness estimates typically increase with increasing sample size before reaching an asymptote [39]. To test whether the number of shelters sampled in each quadrat was sufficient to assess species richness at this scale, we produced a rarefaction curve [39], which related the mean and standard deviation of the expected number of species observed to the number of occupied shelters sampled in all 30 quadrats combined. The curve was calculated based on random permutations of the entire dataset using the specaccum function in R (vegan package; [40]).

Results

Variation in Shelter Volume

Individual shelter volumes varied by nearly 5 orders of magnitude, with the smallest shelter measuring 42 cm^3 and the largest over 4,000,000 cm^3 (4 m^3). There were nearly three times as many holes (n = 2,134) as overhangs (n = 729). The volumes of both holes and overhangs were approximately log-normally distributed, indicating that there were many more small than large shelters (Figure 2). Although the size of holes and overhangs overlapped extensively, holes were smaller (median = 898 cm^3), on average, than overhangs (median = 2,205 cm^3; t-value = 187.52, estimate ± SE =

3.055±0.441, 95% CI = 0.377 to 0.504). Because some previous studies measured only shelter diameter, we examined the relationships among the measures of shelter size. For both holes and overhangs, width, height, and length of shelters were correlated with each other (Pearson correlations, r = 0.586–0.709) and with shelter volume (r = 0.840–0.907). Shelter volume was related to the largest diameter (width or height) by the relationship: $\log_{10}$ volume (cm^3) = 0.553+2.252 $\log_{10}$ diameter (cm) (r^2 = 0.847, 95% CI for intercept = 0.512 to 0.594, 95% CI for slope = 2.217 to 2.287).

Median shelter volumes per quadrat were log-normally distributed and varied 33-fold, ranging from 484 to 16,136 cm^3 (median = 1,211 cm^3). Mean shelter volumes were also log-normally distributed, but higher and more variable, with a 57-fold range from 1,125 to 64,517 cm^3(median = 12,492 cm^3). Quadrat 5 on the crest on Chefette Reef had a much higher median shelter volume than the other 29 quadrats. Without this quadrat, the variation in median shelter volume was reduced to 9-fold, ranging from 484 to 4,432 cm^3 (median = 1,188 cm^3). Total shelter volume per quadrat was also log-normally distributed and varied 127-fold, ranging from 2,436 to 309,679 $cm^3 \cdot m^{-2}$ (median = 51,567 $cm^3 \cdot m^{-2}$).

Occupancy and Number of Occupants in Relation to Shelter Volume

Of the 2,863 shelters examined, 44.2% were occupied by at least one fish, including 900 holes (42.2%) and 366 overhangs (50.2%). The proportion of occupied shelters increased from about 0.22 to 1.00 as shelter volume increased from about 100 cm^3 to about 100,000 cm^3 (Figure 2). Shelter volume (n = 2,863) explained 16.0% of the total deviance in occupancy (estimate ± SE = 0.637±0.046, z-value = 14.00, 95% CI = 0.691 to 0.905). There were no differences in shelter occupancy between holes and overhangs when controlling for shelter volume (estimate ± SE = 0.002±0.096, z-value = 0.026, 95% CI = −0.185 to 0.190). The relationship between occupancy and shelter volume also did not differ between holes and overhangs, although there was a trend toward a faster increase in occupancy with increasing volume for holes than for overhangs (estimate ± SE = −0.187±0.100, z-value = −1.867, 95% CI = −0.384 to 0.009).

The number of fishes per occupied shelter was log-normally distributed and ranged from 1 to 35, with a median of 1 fish per occupied shelter. Larger shelters were

occupied by more fish. For example, the largest holes (upper quartile) contained approximately 50% of fishes found in holes, whereas the smallest holes (lower quartile) contained only 12% of these fishes (Figure 3A). A similar trend was observed for overhangs (Figure 3B). Shelter volume (n = 1,266) explained 7.8% of the total deviance in the number of fishes per occupied shelter (estimate ± SE = 0.2499±0.017, t-value = 14.58, 95% CI = 0.216 to 0.283). There were no differences in the number of fishes occupying holes versus overhangs when controlling for shelter volume (estimate ± SE = −0.102±0.041, t-value = −2.500, 95% CI = −0.182 to −0.022). The relationship between the number of fishes per occupied shelter and shelter volume also did not differ between holes and overhangs.

Predictors of Shelter Availability among Quadrats

Mean shelter density per quadrat varied more than 13-fold, ranging between 0.6 and 8.2 shelters m^{-2} (mean = 3.8 shelters m^{-2}) across reefs and reef zones. Shelter density was negatively correlated with median shelter volume (r = −0.228) but positively correlated with total shelter volume per quadrat (r = 0.498). Median and total shelter volumes were uncorrelated (r = 0.010).

Two models had support in explaining shelter density, based on AICc scores. The best model, which included the predictors reef zone, rugosity and their interaction, was highly supported (w_{im} = 0.80) and explained 76.9% of the total deviance in shelter density. The second best model (w_{im} = 0.20) included reef identity, reef zone, rugosity, the interaction between reef zone and rugosity and explained 77.7% of the total deviance. Based on predictor estimates, there was strong support for an increase in shelter density with increasing rugosity (estimate ± SE: 2.443±0.396, t-value = 6.171, 95% CI = 1.667 to 3.219, Figure 4A). However, shelter density increased more slowly with increasing rugosity in the reef crest (estimate ± SE: −2.3642±0.420, t-value = −5.624, 95% CI = −3.188 to −1.540) and in the spur and groove zone (estimate ± SE: −1.990±0.454, t-value = −4.379, 95% CI = −2.880 to −1.099) than in the fragmented spur zone (Figure 4A). Shelter density also varied with reef zone and reef identity. Differences among reef zones were due to lower shelter density in the fragmented spur zone than in the reef crest and the spur and groove zones (Figure 4A). The 95% CI of the estimate for the effect of the reef crest versus the fragmented spur zone did not overlap zero (estimate ± SE: −1.443±0. 464, t-value = −3.113, 95% CI = −2.251 to

−0.534), whereas the estimate of the effect of the spur and groove versus the fragmented spur zone did (estimate ± SE: −0.034±0.817, t-value = −1.626, 95% CI = −1.786 to 0.166). There was a trend toward higher shelter density on North Bellairs Reef than on Chefette Reef, but the 95% CI of the estimate for the effect of reef identity overlapped zero (estimate ± SE: 0.1934±0. 222, t-value 0.871, 95% CI = −0.242 to 0.628). Repeating the analysis without Quadrat 5 (very high rugosity but low shelter density; Figure 4A, point a) did not change the results.

Much like shelter density, total shelter volume per quadrat increased with rugosity (estimate ± SE: 0.597±0.152, t-value = 3.924, 95% CI = 0.298 to 0.893). The model that included only rugosity had extremely high support based on AICc scores (w_i = 0.93) and explained 35.5% of the deviance in total shelter volume (Figure 4C). In contrast, the relationships between median shelter volume per quadrat and physical predictors were less clear. There was evidence for an interaction between zone and rugosity, whereby median shelter volume increased more slowly with increasing rugosity in the spur and groove zone than in the fragmented spur and the reef crest zones. Overall, there was strong support for an increase in median shelter volume with increasing rugosity as well as evidence for an effect of reef zone - the median shelter volume was lower in the spur and groove than in the reef crest and fragmented spur zones (Table S1A). However, these trends were affected by the high median shelter volume and high rugosity of Quadrat 5 (Figure 4B). When we repeated the analysis without Quadrat 5, rugosity had very little influence on median shelter volume, and the 95% CI of all predictor estimates overlapped zero (Table S1B).

Predictors of shelter occupancy and density of shelter-using fishes among quadrats

The proportion of shelters occupied varied more than 3-fold among quadrats (n = 30), ranging from 0.22 to 0.72 (median = 0.40). The mean density of shelter-using fishes at the quadrat scale varied 23-fold, ranging from 0.4 to 9.2 fish m^{-2} (median = 2.3 fish m^{-2}).

Predictors of the proportion of shelters occupied in quadrats (n = 30) were ambiguous because the results were affected by the extreme shelter volumes in Quadrat 5. When the analysis included all quadrats, shelter occupancy appeared to be affected by median shelter volume and the interaction between median shelter volume and shelter

density. Occupancy increased with median shelter volume and was higher when shelter density was also high (Table S2A, Figure S1A). However, after excluding Quadrat 5 from the analysis, total shelter volume was the strongest predictor, although none of the predictors had strong support because the 95% CI of all estimates overlapped zero (Table S2B).

Three models had support in explaining variation in fish density among quadrats (n = 30) based on AICc scores, explaining 74.4% to 83.9% of the total deviance (Table 1). Unlike the analysis for shelter occupancy, this analysis was not strongly affected by the extreme shelter volumes recorded in Quadrat 5. Median shelter volume, shelter density and the interaction between median shelter volume and shelter density were the most influential predictors; they were present in all models included in the best subset and the 95% CI of their estimates after model averaging did not overlap zero. Fish density increased with increasing median shelter volume and shelter density and was higher when both predictors were high. The univariate relationships between fish density and the three measures of shelter availability are shown in Figure 5, and the interaction is graphed in Figure S1B. Fish density was greater in the reef crest and spur and groove zones than in the fragmented spur zone; it was also higher on North Bellairs Reef than on Chefette Reef. The 95% CI for the estimates of reef zone and reef identity did not overlap zero. The model relating fish density to shelter density, median shelter volume and their interaction had over 1000 times more support than the models based on rugosity combined with any one of the three individual measures of shelter volume. It also had over 100 times more support than the model that included shelter density and median shelter volume, without their interaction.

Shelters as a Limiting Resource for Fishes

Fish density increased linearly with increasing shelter density (Figure 5A). Based on maximum likelihood estimation, the linear relationship between fish density and shelter density with a slope of 0.62 (ΔAICc = 0.0, w_{im} = 0.686, total deviance explained = 26.3%) was 2.2 times more likely than the asymptotic relationship (ΔAICc = 1.6, w_{im} = 0.314). Fish density also increased linearly with increasing median shelter volume (Figure 5B). The linear relationship with a slope of 0.01 had extremely high support (ΔAICc = 0.0, w_{im} = 0.999) compared to the asymptotic relationship (ΔAICc = 23.9, w_{im} = <0.001), but explained only 2.24% of the total deviance in fish density.

After excluding Quadrat 5, the linear relationship had a slope of 0.04 and explained 52.9% of the total deviance. In contrast to shelter density and median shelter size, fish density increased non-linearly with increasing total shelter volume per quadrat. The asymptotic relationship had extremely high support (ΔAICc = 0.0, w_{im} = 0.999) compared to the linear relationship (ΔAICc = 14.2, w_{im} = <0.001). Fish density increased rapidly with increasing total shelter volume, reaching a density of approximately 3 fish m^{-2} at an approximate total shelter volume of 100,000 cm^3 m^{-2} (Figure 5C). The asymptote for this relationship was approximately 3.9 fish m^{-2}.

Species Richness

In total, we recorded 1,845 shelter-using fishes belonging to 66 species (Table S3). The rarefaction curve (Figure S2) indicated that the number of species recorded rose steeply with the number of shelters sampled. On average, detecting 50% of the total number of species recorded in this study required sampling 140 occupied shelters. These numbers suggest that 5×5 m quadrats containing 4–82 occupied shelters (mean = 42.2) were too small a sampling area to provide a reliable estimate of species richness in the system. However, the large number of occupied shelters sampled in this study (n = 1,266) is sufficient to suggest that 66 species is a reliable estimate of species richness in the system.

Discussion

Shelter Sizes

Our study provides the first published data on the size distribution of shelters on a Caribbean reef, the first detailed documentation of the individual and total volumes of shelters on a reef, and the first comparison between the abundance and sizes of holes and overhangs. Larger shelters were much less abundant than smaller shelters. Frequency consistently decreased as shelter size increased for holes above 10,000 cm^3 and for overhangs above about 32,000 cm^3. Similar patterns were apparent in studies on natural reefs [9], coral heads [16], and small experimental reefs created with living coral [7]. Because we did not measure shelters <3 cm in minimum entrance diameter, we likely underestimated the frequency of shelters with very small volumes. Therefore, the decreasing frequency of shelters smaller than 10,000 cm^3 does not provide evidence for a lower abundance of smaller shelters.

Shelter size and abundance differed between holes and overhangs. Holes were more numerous but smaller, on average, than overhangs. The correlations among the linear measurements and between the linear measures and volumetric estimate suggest that measuring only diameter may provide an approximate estimate of shelter volumes. This may be sufficient in many cases, especially since the volumetric calculation is only an approximation based on idealized geometry and not able to account for curving passages that penetrate deeper into the reef [41]. However, different types of shelters may differ in their relationship between entrance diameter and volume. Indeed, additional ecological insights might be gained by further refining the classification of shelter types, for example, by distinguishing shelters among the branches of living coral colonies from cavities of different origin within the reef structure [41].

Surprisingly few studies have quantified the size and availability of shelters on natural coral reefs, and even fewer have presented data allowing comparison among shelter types, reef types or geographical regions. Studies that included all potential shelters on reefs have been carried out on the Great Barrier Reef [18]–[21], the Red Sea [9], Hawaii [2], Moorea [42], and the Seychelles [10]. The only Caribbean observations have come from Nemeth's [16] studies of how shelters in isolated heads of boulder coral *Montastrea annularis* and *Porites porites* rubble influence survival of newly settled damselfish *Stegastes partitus* in the U.S. Virgin Islands and Forrester and Steele's [17] estimation of the abundance of crevices at the sand-reef interface suitable as refuges for the bridled goby *Coryphopterus glaucofraenum*. Of the studies that included all potential shelters, only two presented detailed size and density information [9],[16]. One study aggregated the data into a subjective ordinal scale comprising both shelter density and diversity [21], and the others presented the data only synthesized by means of a Principal Components Analysis [2], [10], [20], [42].

Shelter Occupancy

Larger shelters were more likely to be occupied and, if occupied, to contain a larger number of fishes. The pattern did not differ between holes and overhangs when the difference in size between the two shelter types was taken into account. Holes in the two smallest size classes (<1,000 cm^3) were occupied less than one third of the time, whereas holes in the two largest size classes (>100,000 cm^3) were always occupied.

Only 12% of shelter-using fishes were found in the smallest 25% of holes whereas 50% of fishes were found in the largest 25% of holes. While shelter size obviously limits the maximum number of occupying fish, this constraint seems unlikely to explain the observed pattern because many of the smaller shelters were large enough to be used by additional individuals. Although the effect of shelter volume was large, it only explained 8–16% of the deviance in occupancy and number of occupants, indicating the importance of other factors. Such factors might include aspects of shape, position, location on the reef and whether the shelter occurred in the reef matrix or in live coral. All these variables have been indicated as important in other studies [22], [23], [25].

We are not aware of any other studies that provide comparable data on shelter occupancy by fishes. Studies of occupation of artificial shelters by spiny lobsters *Panulirus argus* [43] and an assemblage of smaller invertebrates [44] have also found that larger shelters were generally occupied by more individuals. However, our results contrast with suggestions from previous studies emphasizing the value of a close match between shelter size and fish size [2], [12],[22]. Several possibilities may explain the higher rate of occupancy and higher number of fishes observed in larger shelters. Obviously, small shelters may physically exclude large individuals. Additionally, large shelters are rarer than small shelters and may therefore be heavily used by fish species or size classes that require large shelters. Larger shelters may also facilitate the formation of aggregations that provide antipredator defenses or other benefits as has been shown in spiny lobsters [43]. In addition, larger shelters may provide a greater range of microhabitats and may be harder to defend by territorial species that actively exclude other individuals. Nevertheless, more detailed studies are required to understand how shelter size and fish size are related for different taxa and different contexts.

Predictors of Shelter Availability

Our study shows a considerably lower density of shelters than the few previous reports and is the first to examine predictors of spatial variation in shelter availability. On fringing reefs in Barbados, shelter density averaged 3.8 shelters m^{-2}, median shelter volume about 1,200 $cm^3$and median total volume about 51,500 cm^3. Only two studies provide data with which we can compare our measures, and both recorded only shelter density as a measure of shelter availability. Roberts and Ormond [9] recorded a much

higher density of shelters, about 120 shelters m^{-2} (estimated from their Figure 7) on fringing reefs in the Red Sea. Even after excluding their 1–5 cm size class to make the data more comparable to our 3 cm threshold, there were still about 20 holes m^{-2}. Because Roberts and Ormond [9] counted spaces between coral branches as shelters (C. Roberts, personal communication), the difference may be related to richer coral cover at their sites or to a higher proportion of solid substratum. Nemeth[16] reported that isolated *Montastrea* coral heads in the Virgin Islands averaged about 14 shelters m^{-2}, but this probably included some shelters smaller than the size threshold we used. Shelter availability showed important spatial variation among quadrats, with more than a 10-fold range for density and more than a 100-fold range for total volume. It appears that some quadrats had many small shelters whereas others had fewer but larger shelters, resulting in the weak negative correlation between shelter density and median size. However, density appeared to be more important than median size as an influence on the total volume of shelters because density and total volume were positively correlated whereas median size and total volume were not. Shelter density and total volume were clearly associated with rugosity, indicating that structural complexity reflects, in part, the presence of shelters. It is not clear whether median shelter size was also associated with rugosity because the positive association depended on a single data point. In the fragmented spur zone, shelter density was lower than in the other two zones, but increased more rapidly with increasing rugosity, indicating that rugosity cannot be taken as an absolute proxy for shelter density but needs to be related to zone. Variation in the relationship between rugosity and shelters could be a result of differences between zones in the amount of vertical relief and live coral. On the reefs examined, the reef crest included large eroded coral heads with high vertical relief whereas the fragmented spurs had low relief but more live coral and more shelter-rich interfaces between the reef and the sandy substratum. Recent studies indicate that rugosity varies with the type of coral cover and that changes in rugosity vary among processes that affect coral cover [45], [46]. To clarify the relationships between shelter availability and structural complexity, it may be useful to differentiate shelters located within the reef matrix from shelters formed among branches of living coral on the reef surface and to increase our understanding of the processes that create and destroy shelters. It appears that little is known about such processes, especially those occurring within the reef matrix (but see [41], [47]). We are aware of only one previous study that has attempted to examine the relationships between structural complexity and shelter

availability. For the crevices at the reef-sand interface used by bridled gobies, Forrester and Steele [17] found that shelter density was associated with the proportion of solid substrate but only weakly with live coral cover and not at all with rugosity.

Predictors of Shelter Occupancy and Fish Density

Our data suggest that the density of shelter-using fishes was directly related to shelter availability rather than to some other variable associated with structural complexity. Variation in the density of shelter-using fishes was quite well explained by a strong association with the density of shelters, their median size, and the interaction between density and median size, as well as some effect of reef zone and identity. Although rugosity was associated with shelter density, rugosity did not appear in the selected models and was less successful than shelter availability in predicting the density of shelter-using fishes. On the other hand, our models did not reveal robust predictors of occupancy, with the clearest pattern due to the effect of one extreme value. While many studies have found an association between rugosity and coral reef fish density [1], [4], [48], only a few have compared the predictive power of shelters with that of rugosity. Roberts and Ormond [9] found that a surface index similar in concept to rugosity but estimated from photographs had little predictive power for explaining the density of fishes at several sites and depths in the Red Sea; however, a multiple regression based on three size classes of holes explained much of the variance. Friedlander and Parrish [2] reported that fish density in Hawaii was much more strongly associated with the total volume of holes than with either rugosity or alternative measures of shelter availability. Wilson et al. [10] also found that fish density was more closely associated with principal components related to the density of holes than with rugosity on Seychelles reefs. Thus, our study confirms for Caribbean reefs and for the fish actually observed in shelters the positive associations between shelter availability and fish density that have been identified in several other regions. However, generalizations concerning which measures of shelter availability best predict fish density are not yet possible.

In addition to shelter characteristics, there was evidence of an effect of reef zone on the density of shelter-using fish. While spatial variation in fish density is not surprising, and reef zones and water depth are well known to influence reef fish abundance [49], our study shows that these spatial differences are not explained by

differences in shelter availability and rugosity alone. Lower densities of fish in the fragmented spur zone may have been related to greater depth, lower vertical relief or the smaller proportion of continuous reef in this zone as well as to differences in shelter type. In addition, other variables that affect overall fish abundance such as the amount of live coral [50] may influence the density of shelter-using fishes.

Shelters as a Limiting Resource for Fishes

The linear increase in the density of shelter-using fishes with increasing shelter density supports the hypothesis that shelters are a limiting resource for fishes on coral reefs [12], [13],[51]. Changes in the availability of a limiting resource are expected to have a linear effect on population size [52]. An asymptotic relationship would have indicated a reduction in the average number of fishes per shelter with increasing shelter density in quadrats, providing evidence that other resources or processes limited fish abundance [53]. The considerable number of unoccupied shelters is not evidence for a lack of limitation because some of the shelters may have been too small or unsuitable in other ways. Furthermore, territorial species such as some pomacentrids and holocentrids may defend multiple shelters, preventing some from being used[25], [54].

Previous studies have shown a positive association between shelter availability and coral reef fish density [2], [9], [10], but the shape of the relationship was not examined. Using observations plus experimental removal of shelters and increases in fish density, Robertson and Sheldon [22] did not find evidence for limitations in the availability of nocturnal shelters in the diurnal bluehead wrasse *Thalassoma bifasciatum*. On the other hand, studies of shelter addition [26], [55], density-dependent mortality in relation to shelter density [17], [56], and small, experimental artificial reefs [7], [11]–[14] have provided evidence that population density does increase with greater shelter availability in coral reef fishes. Thus, our study adds support for the hypothesis that shelters are sometimes limiting by applying it to the assemblage of shelter-using fishes and to spatial variation in fish density within larger, natural reefs.

Species Richness

Most previous research on shelter use by coral reef fishes have either included all fish of broad taxonomic groupings without documenting whether or not they used shelters or have focused on one or a few related species. This study appears to be the

first to survey shelters systematically and to record the associated fish assemblage. Of the 66 species of fish identified in shelters, the most commonly found were pomacentrids, especially the genus*Stegastes*. Pomacentrids are diurnal species that use shelters as refuges from predators during the day [57] and night [22] as well as for nest sites [58]. The abundant diurnal acanthurids, labrids and scarids found on Barbados fringing reefs [59] were never or very rarely observed in shelters. It is important to note that by sampling only during the daytime, our sample may have underrepresented shelter use by species that use shelters primarily at night[22], [60]. Other than pomacentrids, the majority of the fishes in shelters were apogonids, haemulids, holocentrids and serranids, taxa that are mostly nocturnal or crepuscular [25],[60]–[62]. Pempherids were rare in this sample but do occur in large aggregations in a few locations on these reefs. Because of the shelter size criterion we used, we did not record species associated with much smaller holes such as the chaenopsids [23]. Some taxa such as muraenids that spend much time within shelters will be underrepresented because they are often not visible to observers [63], probably because they spend time in deeper, narrow, or curving holes.

Conclusions

Quantifying the size, number and use of shelters on two fringing reefs in Barbados has highlighted the importance of this component of habitat structure for the reef fish community. However, a lack of standardization in sampling methods, variables and the definition of a shelter on coral reefs make comparisons among studies difficult. We found that the rare, large shelters used by aggregations of several species have a disproportionate effect on fish densities and may be a valuable characteristic to assist in the selection of sites for conservation. This is even more important given the heavy impact and rapid changes occurring on coral reefs, particularly in the Caribbean region. The loss of structural complexity is a clear trend in the Caribbean, and possibly other regions [27]. If this loss reduces shelter availability, it may have profound effects on fish assemblages. However, our ability to predict such effects is limited because we know little about the processes responsible for the formation and loss of shelters, especially the larger caves, holes and crevices within the reef matrix. More detailed studies at the community level are also needed to help determine species preferences and their use of these important and limiting resources. We envisage considerable

potential benefits from using artificial reefs to experimentally test the role of shelters of various sizes in the recovery of fish assemblages on damaged reefs.

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Author Contributions

Conceived and designed the experiments: AM DLK KT. Performed the experiments: AM. Analyzed the data: DGR KT SAB. Wrote the paper: DLK DGR KT SAB.

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Table 1. Fish density.

Predictors	Model Rank			β	SE	95% CI	wip
	1	2	3				
Constant	•	•	•	−0.750	−0.938	−0.220 to 1.189	1.00
Median shelter volume	•	•	•	0.886	0.132	0.628 to 1.144	1.00
Shelter density	•	•	•	0.631	0.116	0.404 to 0.857	1.00
Zone RC vs. FS	•	•		0.767	0.214	0.348 to 1.185	0.93
Zone SG vs. FS	•	•		1.105	0.356	0.406 to 1.803	0.93
Reefs NB vs. CH	•			0.296	0.150	0.003 to 0.589	0.64
Shelter volume * shelter density	•	•	•	0.545	0.118	0.313 to 0.779	1.00
No. of parameters (K)	8	7	5				
AICc	38.371	49.992	52.680				
ΔAICc	0.000	1.621	4.309				
w_{im}	0.641	0.285	0.074				
Deviance explained	83.9	79.7	74.4				

Predictors and interaction terms included in the three best models explaining variation in fish density in thirty 25 m^2 quadrats located in three reef zones (RC = reef crest, SG = spur and groove, FS = fragmented spurs) and two reefs (NB = North Bellairs, CH = Chefette). Variables included in each model are denoted with "•". Predictors for which the 95% confidence interval (CI) did not overlap zero are indicated in bold font. The number of parameters (K) used in each model, the AICc, the ΔAICc (AIC of model$_i$ − AIC of best model), the w_{im} (normalized Akaike weights for each candidate model) and the deviance explained are shown at the bottom of the table. Model averaged estimates of parameters (β), unconditional standard errors (SE), 95% CI and the normalized Akaike weight for each predictor (w_{ip}) are also shown. All models include a constant.

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Figure 1. Schematic representation of the two study reefs on the west coast of Barbados, West Indies. The three reef zones examined are indicated as follows: reef crest (RC, light grey), spur and groove (SG, dark grey), and fragmented spurs (FS, black). The back reef (white), located inshore of the reef crest, was not sampled in this study. The Barbados Marine Reserve is indicated by the dotted polygon.

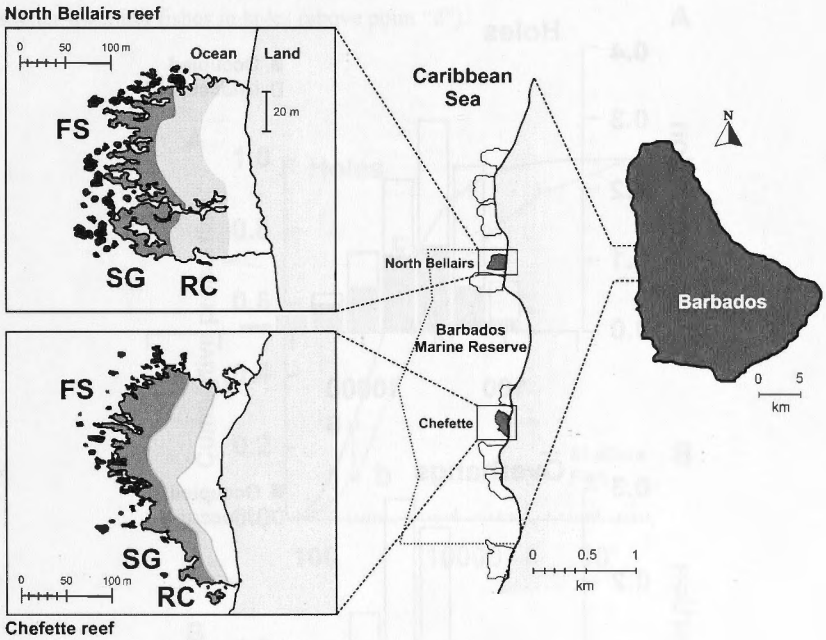


Figure 2. Frequency of holes and overhangs in relation to shelter volume. The proportional distribution of (A) holes (n = 2,134) and (B) overhangs (n = 729) in relation to shelter volume. Occupied shelters are shown as black bars and unoccupied shelters as white bars. Each bin has a width of 0.5 $\log_{10}$ cm^3. Bins with values smaller than 100 cm^3 are not shown because proportions were less than 0.001.

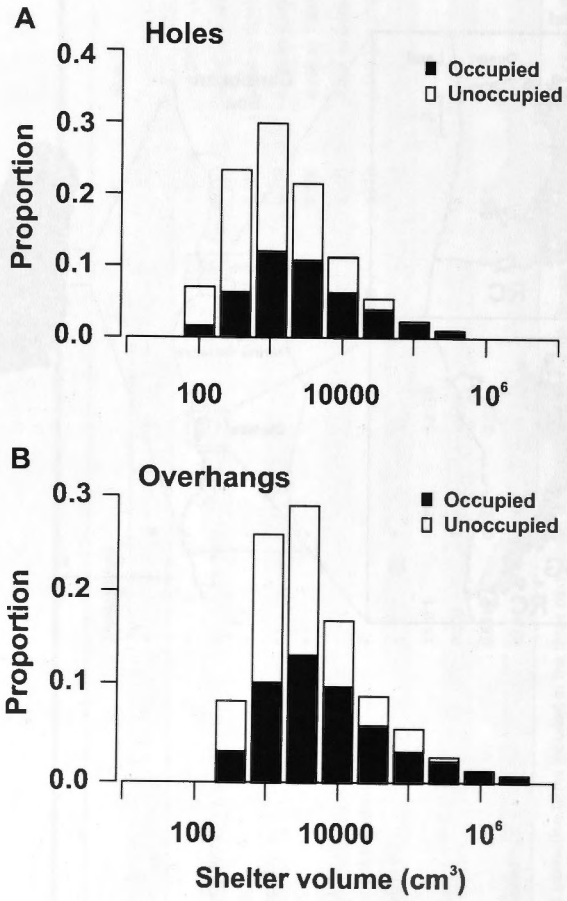


Figure 3. Cumulative proportion of shelters and shelter-using fishes in relation to shelter volume. The cumulative proportion of shelters with minimum entrance diameter >3 cm (solid lines) and the cumulative proportion of shelter-occupying fishes (dotted lines) in relation to $\log_{10}$ shelter volume for (A) holes (N = 2,134) and (B) overhangs (N = 729). The grey dot (labelled "a") indicates the smallest 25% of shelters and corresponds to only 12% of the shelter-dwelling fishes in holes (labelled "b"). The largest 25% of shelters (above point "c") correspond to approximately 50% of the shelter-dwelling fishes in holes (above point "d").

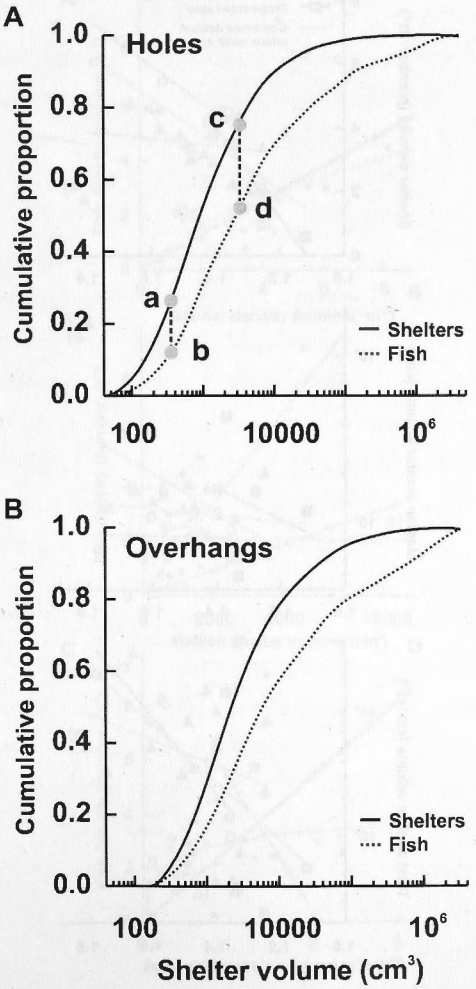


Figure 4. Shelter availability variables in relation to structural complexity. The relationship between A) shelter density (number m^{-2}), B) median shelter volume (cm^3), C) total shelter volume (cm^3 m^{-2}) and mean rugosity index across the 30 quadrats sampled. Lines represent the best fit linear regressions for each zone considered separately (reef crest – dotted, spur and groove – short dashes, fragmented spurs – long dashes). The solid gray line represents the best fit linear regression for the entire dataset, excluding the data point recorded at the highest value of rugosity (Quadrat 5, point a).

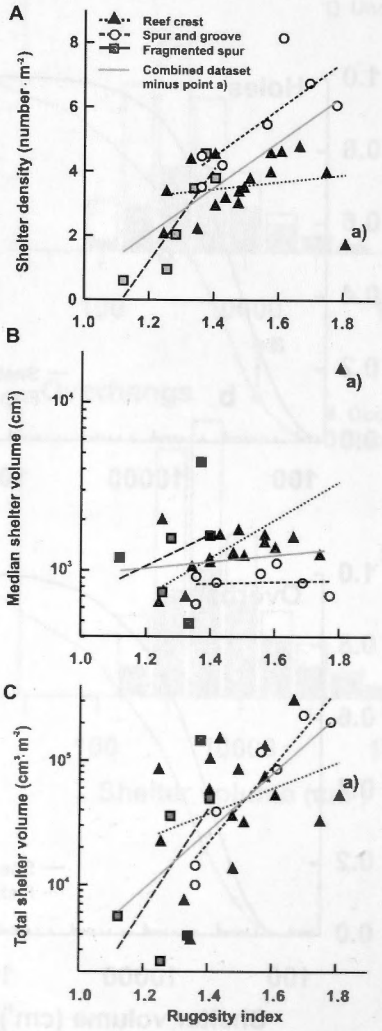
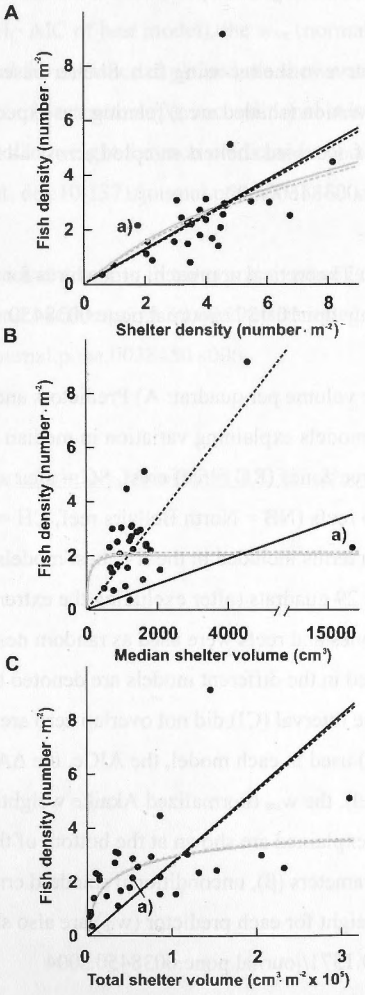


Figure 5. Relationship between fish density and shelter availability characteristics. The relationship between fish density (number m^{-2}) and A) shelter density (number m^{-2}), B) median shelter volume (cm^3) and C) total shelter volume ($cm^3 \cdot m^{-2}$) for the 30 quadrats sampled. The solid black line represents the best fit linear regression to the entire dataset, whereas the dashed black line represents the best fit linear regression without Quadrat 5 (point a). The solid grey line represents the best fit asymptotic curve to the entire dataset, whereas the dashed grey line represents the best fit asymptotic curve without Quadrat 5 (point a).



Electronic supplementary material

Figure S1. Shelter occupancy and fish density in relation to shelter density and median shelter volume. Three-dimensional plots showing A) the proportion of shelters occupied and B) fish density as a function of shelter density (y-axis) and median shelter volume (x-axis) for the 30 quadrats sampled. Black dots represent individual quadrats and the relationships shown by the colored grid were extracted from a general linear model. Median shelter volume was $\log_{10}$transformed and all variables were z-standardized. Point a) represents the extreme value of median shelter volume in Quadrat 5, which is discussed in the text. doi:10.1371/journal.pone.0038450.s001

Figure S2. Rarefaction curve in shelter-using fish. Shelter-based rarefaction curve (solid line) ± standard deviation (shaded area) relating the expected number of species observed to the number of occupied shelters sampled across all 30 quadrats. doi:10.1371/journal.pone.0038450.s002

Text S1. The Information Theoretical approach: procedures for model selection with AICc and model averaging. doi:10.1371/journal.pone.0038450.s003

Table S1. Median shelter volume per quadrat: A) Predictors and interaction terms included in the four best models explaining variation in median shelter volume in 30 25-m^2 quadrats located in three zones (RC = reef crest, SG = spur and groove, FS = fragmented spurs) on two reefs (NB = North Bellairs reef, CH = Chefette reef). B) Predictors and interaction terms included in the five best models explaining variation in median shelter volume in 29 quadrats (after excluding the extreme median shelter volume of Quadrat 5). Zones and reefs were used as random nested factors in the models. Variables included in the different models are denoted by "•". Predictors for which the 95% confidence interval (CI) did not overlap zero are indicated in bold. The number of parameters (K) used in each model, the AICc, the ΔAICc (AIC of model$_i$−AIC of best model), the w_{im} (normalized Akaike weights for each candidate model) and the deviance explained are shown at the bottom of the table. Model averaged estimates of parameters (β), unconditional standard errors (SE), 95% CI and the normalized Akaike weight for each predictor (w_{ip}) are also shown. All models include a constant. doi:10.1371/journal.pone.0038450.s004

Table S2. Shelter occupancy: A) Predictors included in the four best models explaining variation in shelter occupancy in 30 25-m^2 quadrats located in three reef zones (RC = reef crest, SG = spur and groove, FS = fragmented spurs) on two reefs (NB = North Bellairs reef, CH = Chefette reef), B) Predictors and interaction terms included in the best two models explaining variation in shelter occupancy in 29 quadrats (after excluding the extreme median shelter volume value found in Quadrat 5). Zones and reefs were used as random nested factors. Variables included in the different models are denoted by "•". Predictors for which the 95% confidence interval (CI) did not overlap zero are indicated in bold. The number of parameters (K) used in each model, the AICc, the ΔAICc (AIC of model$_i$−AIC of best model), the w_{im} (normalized Akaike weights for each candidate model) and the deviance explained are shown at the bottom of the table. Model averaged estimates of parameters (β), unconditional standard errors (SE), 95% CI and the normalized Akaike weight for each predictor (w_{ip}) are also shown. All models include a constant. doi:10.1371/journal.pone.0038450.s005

Table S3. Fish abundance and diversity in holes and overhangs. **A**bundance of fishes found in shelters (holes and overhangs) in 30 quadrats sampled on two fringing reefs in Barbados. doi:10.1371/journal.pone.0038450.s006

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http://www.plosone.org/article/info%3Adoi%2F10.1371%2Fjournal.pone.0038450#s5

APPENDIX - II

Ectoparasites increase swimming costs in a coral reef fish

Binning SA, **Roche DG** and Layton C (2013) *Biology Letters* 9: 20120927.

Keywords

Aerobic capacity, critical swimming speed, drag, isopod, metabolic rate, respirometry

Abstract

Ectoparasites can reduce individual fitness by negatively affecting behavioural, morphological and physiological traits. In fishes, there are potential costs if ectoparasites decrease streamlining, thereby directly compromising swimming performance. Few studies have examined the effects of ectoparasites on fish swimming performance and none distinguish between energetic costs imposed by changes in streamlining and effects on host physiology. The bridled monocle bream (*Scolopsis bilineata*) is parasitized by an isopod (*Anilocra nemipteri),* which attaches above the eye. We show that parasitized fish have higher standard metabolic rates (SMR), poorer aerobic capacities and lower maximum swimming speeds than non-parasitized fish. Adding a model parasite did not affect SMR, but reduced maximum swimming speed and elevated oxygen consumption rates at high speeds to levels observed in naturally-parasitized fish. This demonstrates that ectoparasites create drag effects that are important at high speeds. The higher SMR of naturally-parasitised fish does, however, reveal an effect of parasitism on host physiology. This effect was easily reversed: fish whose parasite was removed 24 h earlier did not differ from unparasitized fish in any performance metrics. In sum, the main cost of this ectoparasite is probably its direct effect on streamlining, reducing swimming performance at high speeds.

Introduction

Ectoparasites can substantially affect hosts by impacting physiological, behavioural and morphological traits, and damaging the host's integument [1-3]. In fishes, ectoparasites pose additional challenges since streamlining is important to reduce the costs of locomotion [4]. Changes to fish morphology created by ectoparasites potentially reduce streamlining and increase friction drag along the fish's body, which may considerably reduce host performance [3, 5]. Although the consequences of

carrying ectoparasites can be high, few studies have examined the effects of ectoparasitism on the swimming performance and energetics of infected fishes [3, 5-6]. Crucially, there have been no attempts to separate out costs due to the hydrodynamic effects of reduced streamlining from the effects of parasites on host physiology.

Cymothoid isopods are ectoparasitic crustaceans that infect fishes throughout the tropics [1]. These abundant and relatively large (4.2 – 23.0 mm) parasites either attach themselves to a fixed location on their host, or move around freely on the host's body [1]. On coral reefs, these isopods parasitize several species including the bridled monocle bream (*Scolopsis bilineata*), with approximately 4 % of the population infected by *Anilocra nemipteri* at some locations on the Great Barrier Reef (S.A. Binning, unpublished data). This isopod can typically grow to over 15% of its host's total length. It attaches itself firmly above the eye of the fish, and has the potential to reduce host swimming performance.

Here, we measured the effects of *A. nemipteri* on the swimming performance and energetics of the bridled monocle bream, *S. bilineata*. To separate the physiological and hydrodynamic effects this ectoparasite, we evaluated aerobic swimming performance and swimming speed in fish that were (a) parasitized, (b) unparasitized (c) parasitized but had the parasite experimentally removed, and (d) unparasitized but had a model parasite experimentally added. We compared (a) and (c) to test for physiological effects of the parasite on host performance as well as (b) and (d) to test for hydrodynamic effects of parasitism. We compared (a) and (b) to quantify the net effect of parasitism on hosts. We predicted that the negative effects of parasitism on the swimming performance of *S. bilineata* are mainly due to physiological effects at slow swimming speeds, but that hydrodynamic effects become important at high swimming speeds.

Materials and methods

Experimental swimming and respirometry trials

Adult *Scolopsis bilineata* were collected using ultrafine barrier nets and hand nets between February and March 2012 from reefs surrounding Lizard Island, northern Great Barrier Reef, Australia (14° 40' S; 145° 28' E). Fish were transported live in buckets to the aquarium facilities at the Lizard Island Research Station within 2h of

capture. Eighteen unparasitized (total length L_T= 13.27 ± 0.17 mm; mass =38.2 ± 0.8 g; means ± s.e.) and 20 parasitized (L_T= 12.66 ±0.18 mm; mass = 34.8 ± 0.9 g; means ± s.e.) fish were divided into four treatment groups: unparasitized (eight fish), parasitized (10 fish), parasite-removed (10 fish) and model parasite-added (10 fish; ESM Fig. 1). Parasites were removed using forceps 24h before the start of swim trials. The average length, width and mass of the isopods were used to mould model parasites from Instamorph® polyester thermoplastic (L_T= 2.48 ± 0.06 mm; body width W_b= 0.99 ± 0.03 mm; mass =0.6 ± 0.0 g; means ± s.e.). Model parasites were attached with EA Cyberbond 2610 instant adhesive. Swimming trials were carried out in an 11.9 L Loligo swimming respirometer at a constant temperature of 28 ± 0.1°C. We measured oxygen consumption rate ($\dot{M}O_2$) as a function of swimming speed (U) following a standard U_{crit} protocol [7]. Trials were stopped when fish could no longer swim unassisted or were forced to rest against the back grid of the flow chamber (U_{crit}) for > 5s (See ESM for supplementary materials and methods).

Oxygen consumption curves and aerobic scope

We used an exponential function to describe the relationship between $\dot{M}O_2$ and U for each site [7-8]:

$$\dot{M}O_2 = a10^{(bU)}$$

which in its log-transformed linear form becomes

$$\log\dot{M}O_2 = \log a + bU$$

where a is the estimated $\dot{M}O_2$ at zero speed (standard metabolic rate: SMR) and b is the slope of the semi-logarithmic regression. Maximum metabolic rate (MMR) was measured at U_{crit}. We calculated the factorial aerobic scope (AS) as the ratio of MMR to SMR.

Statistical analyses

We used one-way ANOVAs with Tukey HSD post-hoc tests to examine differences in swimming (U_{crit}) and metabolic performance (SMR, MMR, AS) among treatments. Factorial aerobic scope was $\log_{10}$ transformed to meet the assumptions of the model. The linear forms of the oxygen consumption rate curves were used to test for

differences in the relationship between fish swimming speed (U) and oxygen consumption rate ($\log_{10}\dot{M}O_2$) among treatments using a general linear mixed effect model (lme function in R). We used a mixed model to control for temporal autocorrelation among data points in the physiological response curves [9]. All analyses were performed in R v2.11.1 [10].

Results

Parasitized fish had higher standard metabolic rate ($F_{3,34}$= 7.152, p< 0.001) and lower aerobic scope ($F_{3,34}$ = 8.897, p= 0.001) than fish from the other three treatments (Tukey's HSD, p<0.01 for all contrasts; Fig. 1). Maximum metabolic rate did not differ among treatment groups ($F_{3,34}$ = 0.992, p=0.408), with differences in aerobic scope resulting from an increased standard metabolic rate in parasitized fish. Both parasitized fish and fish with a model parasite added swam slower than unparasitized and parasite-removed individuals ($F_{3,34}$ = 4.922, p< 0.01; Tukey's HSD, p<0.01 for all contrasts; Fig. 1). Parasitized individuals consumed oxygen at a consistently higher rate than individuals in other treatments (LMM intercept: $F_{3,34}$= 4.20, p= 0.013; Fig. 2). There was no difference in the rate of oxygen consumption at any swimming speed between parasite-removed and unparasitized individuals (intercept estimate = -0.0014, 95% CI = 0.0733 to -0.0760, p=0.97; slope estimate = 0.0060, 95% CI = 0.0218 to -0.0099, p=0.46). However, the costs of swimming increased at higher speeds in fish with a model parasite added (LMM slope: $F_{3,234}$= 4.68, p< 0.01; Fig. 2).

Discussion

The ectoparasitic isopod *Anilocra nemipteri* probably increases friction drag along the fish's body surface. Although this effect is non-lethal, the consequences of a reduced maximum swimming speed and lower aerobic scope, as well as a higher standard metabolic rate and a greater overall oxygen consumption rate are potentially significant for individual fitness and population demographics.

Hydrodynamic effects of the parasite were more pronounced at speeds above approximately 2.5 body lengths s^{-1} (Fig. 3). At lower speeds, drag effects appeared to be minimal. Parasitized fish, however, continued to consume an average of 24 % more oxygen on average when at rest than fish in the other three treatments. Ostlund-Nilsson et al. [5] found similar increases in the resting metabolic rate of the cardinalfish,

Cheilodipterus quinquelineatus parasitized by a congeneric isopod, *Anilocra apogonae*. The authors attributed this increase in energetic cost to the destabilizing effect of the asymmetrically-attached parasite rather than to any physiological effects of parasitism. Indeed, they found that parasitized fishes used their pectoral fins more while at rest, presumably in order to maintain stability [5]. Our results suggest otherwise since standard metabolic rate did not increase when a model parasite was added, and we did not observe any change in pectoral fin beat frequency when fish were at rest (S.A. Binning, unpublished data). Physiological effects due to parasitism are probably responsible for the elevated standard metabolic rate observed in our study. Interestingly, these physiological effects appear to be rapidly reversed. Fish that had their parasite removed 24 h earlier performed as well as unparasitized individuals across all performance measures. There appears to be no detectable long term physiological damage from this ectoparasitic isopod once removed.

High-speed swimming is required during anaerobic burst events such as predator escapes and for sustained aerobic swimming such as during severe weather events. In these situations, parasitized individuals will be strongly disadvantaged as their maximum swimming speed is significantly reduced. Parasitized fish also had a reduced capacity for aerobic activity, which compromises their ability to engage in multiple energy-expending activities at one time. Although burst swimming involves anaerobic metabolic pathways, these activities are negatively affected by a reduced aerobic capacity: bursting incurs an oxygen debt that must be repaid at the expense of other fitness-enhancing activities. Additionally, since water flow velocity varies dramatically across sites at Lizard Island [11], an inability to cope with high water flows could severely limit the range of habitats that parasitized fish can exploit. Furthermore, the metabolic rates of parasitized individuals were higher at all swimming speeds suggesting that energy requirements of parasitized fish exceed that of uninfected fish. As a result, parasitized individuals either need to spend more time foraging, putting them at greater risk of predation, or may suffer from reduced growth and/or reproduction [1, 12-13].

Although parasites are known drivers of morphological change in hosts, many of these changes are the result of underlying physiological modifications [e.g. 14, 15]. Here we show that parasites can alter the hydrodynamic profile of hosts with

measurable consequences on swimming performance in the absence of any physiological effects. By interfering with streamlining via increased friction drag, large ectoparasites potentially compromise important activities such as sustained swimming, habitat use, foraging and predator evasion.

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Supplementary Material and Methods

We measured critical swimming speed (U_{crit}) and oxygen consumption rates ($\dot{M}O_2$: mg $O_2 kg^{-1} h^{-1}$) in adult *Scolopsis bilineata* collected using ultrafine barrier nets and hand nets between February and March 2012 from reefs around Lizard Island, northern Great Barrier Reef, Australia (14° 40' S; 145° 28' E). Fish were transported to the aquarium facilities at the Lizard Island Research Station within 2h of capture, and held in individual aquaria (40.0W× 29.0L× 18.0H cm) with a flow-through water system directly from the reef. Fish were fed once a day with pieces of raw prawn (mean wet weight apx. 1g). Parasites were removed from fish in the removal treatment with tweezers 24h before the start of swim trials by holding the fish in a shallow water bath

and gently unhooking the isopod. Parasites were also removed in the same manner from fish in the parasitized treatment immediately following swim trials. The average length, width and mass of these isopods were used to mould model parasites from Instamorph® polyester thermoplastic. Model parasites were attached by holding the fish firmly in two hands while another researcher blotted a spot on the head dry and applied the model with a tiny amount of EA Cyberbond 2610 instant adhesive. The fish was then immediately put in the respirometry chamber and left to rest. The entire parasite application procedure took approximately 45 seconds. A handling control found no differences between fish handled in this way with glue application but without model parasite attachment and unparasitized fish (n=3 fish for glue control). Fish were kept in aquaria for a minimum of three days before performing swim trials to ensure only healthy individuals were used. Fish were fasted for 24h prior to the swimming trials to standardize a post-absorptive state that maximizes energy availability for swimming. Length measures for individual live fish were obtained before swimming trials by holding each fish in a plastic bag half-filled with water and measuring total length (body length; BL), body width (BW) and body depth (BD) with handheld callipers. Mass (M) was measured directly on a scale. These measures were used to calibrate the respirometer and calculate the flow rate of water measured in body lengths per second (BLs^{-1}).We measured oxygen consumption rate ($\dot{M}O_2$) as a function of swimming speed (U) following a standard U_{crit} protocol [1, 2]. Swimming trials were carried out in a 11.9 L Loligo flow tank respirometer (swim chamber dimensions 40.0L× 10.0W × 10.0H cm) filled with fully aerated, filtered and UV sterilized seawater at a constant temperature of 28 ± 0.1°C. Oxygen levels in the respirometer were recorded using a fibre optic oxygen meter (Presense Fibox 3) online feed into the AutoResp 1 Software (Loligo Systems, Denmark). The flow in the working section of the respirometer was calibrated using a digital TAD W30 flow-meter (Höntzsch, Germany). Solid blocking effects of the fish in the working section were corrected by the respirometry software (AutoResp, Loligo Systems) following Bell & Terhune [3]. We used ten minute loops with a 240s flush, 60s rest and 300s measure cycle. Once an individual's length and mass measures were inputted into the respirometry software, three determinations were run without fish to measure initial background rates of respiration due to bacterial load in the test chamber. The fish were then placed in the respirometer and left to acclimate for six to eight hours at a swimming speed of 0.75 BLs^{-1} until their oxygen consumption stabilized. This speed corresponded to the lowest water flow necessary to

ensure constant swimming and minimize spontaneous activity in this species. We measured oxygen consumption at 0.75 BLs^{-1} by averaging the three $\dot{M}O_2$ measurements immediately prior to the onset of the first trial. To start the trial, the flow speed was slowly increased to 1.25 BL s^{-1} for three loops. Flow speed was incrementally increased by 0.5 BL s^{-1} every three loops for the duration of the experiment. The trial stopped when the fish could no longer swim unassisted or was forced to rest on the sides or back of the flow chamber for five or more seconds. The time and speed at this occurrence was recorded and the water flow was reduced back to 0.75 BL s^{-1} to ensure the fish's recovery from oxygen debt, which we determined had been reached when the fish's oxygen consumption fell back to near its initial rate at 0.75 BLs^{-1} as measured at the start of the experiment. The fish was then removed from the test chamber and returned to its holding tank. Three additional loops were run to measure final background rates of respiration in the chamber. Background consumption rates at the end of each trial were determined from the slope of the linear regression between initial and final background rates, and were subtracted from each $\dot{M}O_2$ determination. For parasitized treatment fish, isopods were removed after the swim trial before returning the fish to its holding tank. The isopod was then placed in the chamber during the final background measurements to account for the oxygen consumption due to the isopod. The average of these final background measures was subtracted from all $\dot{M}O_2$ loops in this treatment. To reduce bacterial growth and respiration in the chamber, the respirometer was drained and rinsed in freshwater when the background consumption rates exceeded 20% of the resting metabolic rate of the fish (approximately every six fish). Data are deposited in the Dryad Repository (http://dx.doi.org/10.5061/dryad.r73v3).

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Fig. 1 Bar plots with average values (± SE) for parasitized (black), unparasitized (white), parasite removed (dark grey) and model parasite added (light grey) fish for a) standard metabolic rates (SMR), b) factorial aerobic scope and c) experimental (U_{crit}) swimming speeds (U). Different letters (a, b) indicate significant differences between treatment groups ($p<0.01$).

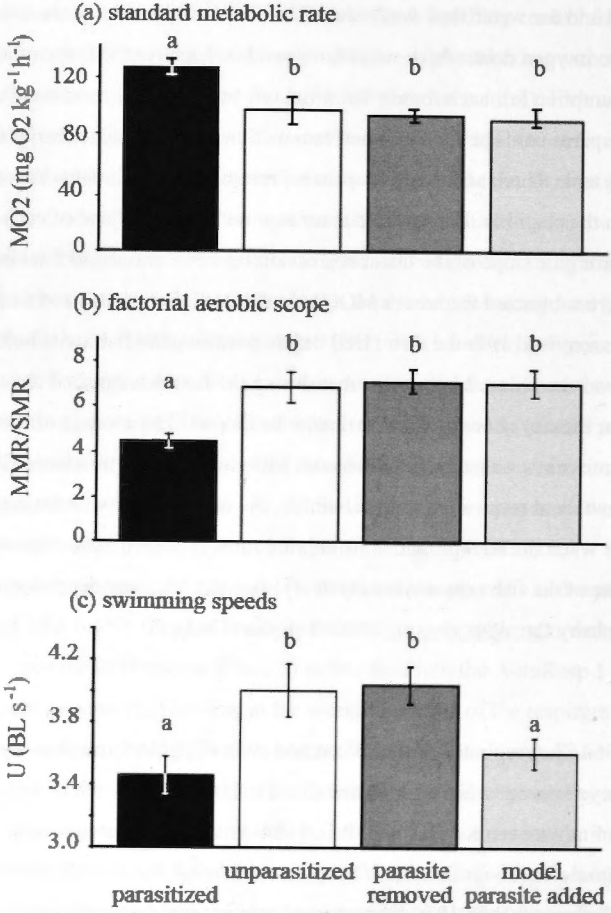
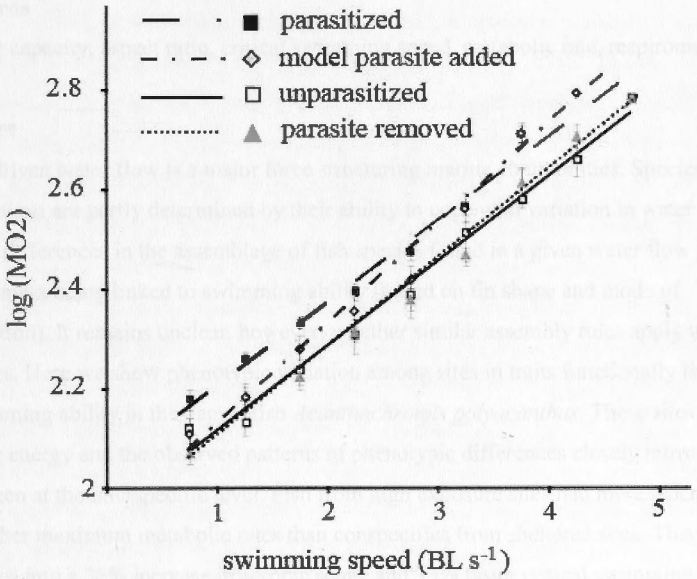


Fig. 2 Metabolic rate ($\dot{M}O_2 \pm$ SE) as a function of swimming speed (U, body lengths second^{-1}) for fish from each of the four treatments (N = 38). Regressions are as follows: $\log\dot{M}O_2 = 1.95 + 0.17\ U$, $r^2 = 0.74$ for unparasitized fish; $\log\dot{M}O_2 = 2.04 + 0.16\ U$, $r^2 = 0.90$ for parasitized fish; $\log\dot{M}O_2 = 1.95 + 0.19\ U$, $r^2 = 0.86$; for model parasite added fish; and $\log\dot{M}O_2 = 1.94 + 0.17\ U$, $r^2 = 0.88$ for parasite removed fish.



APPENDIX - III

Localised intraspecific variation in the swimming phenotype of a coral reef fish across different wave exposures

Binning SA, **Roche DG** and Fulton CJ (2014) *Oecologia* 174: 623-630

Keywords

Aerobic capacity, aspect ratio, critical swimming speed, metabolic rate, respirometry

Abstract

Wave-driven water flow is a major force structuring marine communities. Species distributions are partly determined by their ability to cope with variation in water flow, such as differences in the assemblage of fish species found in a given water flow environment being linked to swimming ability (based on fin shape and mode of locomotion). It remains unclear, however, whether similar assembly rules apply within a species. Here we show phenotypic variation among sites in traits functionally linked to swimming ability in the damselfish *Acanthochromis polyacanthus*. These sites differ in wave energy and the observed patterns of phenotypic differences closely mirrored those seen at the interspecific level. Fish from high exposure sites had more tapered fins and higher maximum metabolic rates than conspecifics from sheltered sites. This translates into a 36% increase in aerobic scope and 33% faster critical swimming speeds for fishes from exposed sites. Our results suggest that functional relationships among swimming phenotypes and water flow not only structure species assemblages, but can also shape patterns of phenotypic divergence within species. The close links between locomotor phenotype and local water flow conditions appear to be important to understand species distributions as well as phenotypic divergence across environmental gradients.

Introduction

Organisms frequently appear to be adapted to their local environment. As environments are often highly variable, organisms must (a) have evolved locally due to selection for fitter genotypes, (b) have environmentally-induced means of adjusting their phenotype to match the prevailing conditions (i.e. show adaptive phenotypic plasticity; West-Eberhard 1989), or (c) have moved to more suitable habitats for their

phenotype. In both terrestrial and aquatic ecosystems, variation in the physical environment is a major force influencing patterns of phenotypic distribution through time and space (e.g. Leonard et al. 1998; Fulton et al. 2005; Desrochers 2010).

In aquatic habitats, numerous physical factors structure communities across a range of spatial scales (e.g. Bertness et al. 1999; Kaandorp 1999; Bellwood et al. 2002; Chapman et al. 2002). In particular, water motion, caused by waves and currents, influences species distributions, and selects for various functional traits that are needed to exploit these environments (e.g. Kaandorp 1999; Denny and Gaylord 2002; Fulton et al. 2005; Langerhans 2008; Mims and Olden 2012). For example, in fish, functional traits that affect swimming performance predict interspecific patterns of habitat use. Over 60% of coral reef fishes primarily use their pectoral fins for swimming (labriform swimming, Webb 1994; Fulton and Bellwood 2005). In this group, pectoral fin shape affects swimming performance likely due to biomechanical constraints: species with tapered, high aspect ratio (AR) fins are faster steady swimmers (i.e. straight line swimming at a constant velocity) than those with rounded, low AR fins, which are better at maneuvering in low-flow environments (Vogel 1994; Fulton et al. 2005). Since reef fish often need to swim at speeds dictated by the ambient water velocity, fishes with high AR fins tend to dominate high-flow wave-swept habitats whereas low AR-finned species are abundant on sheltered, low-flow reefs (Webb 1994; Bellwood et al. 2002). Living in high-flow habitats may also require a higher aerobic capacity to support a wider range of swimming speeds. Aerobic scope (AS) is a measure of the metabolic range within which an animal can sustain aerobic activities (Claireaux and Lefrancois 2007). A large AS may enable fishes to sustain energetically-demanding activities across a wider range of water flow conditions. As such, AS could be a key trait driving the exploitation of wave exposed habitats by coral reef fishes.

The functional relationship between swimming phenotype and wave-driven flow has been well explored in freshwater and marine fishes by looking at variation in habitat use among species (e.g. Bellwood et al. 2002; Fulton et al. 2005; Langerhans 2008). However, we have yet to fully resolve whether similar processes are responsible for structuring the distribution of individuals of the same species in marine environments (Fulton et al. 2013). Evidence for local adaptation and/or plastic responses to water flow

conditions would suggest important, currently overlooked mechanisms that influence species distributions and the potential for expansion into new environments.

The Pomacentridae are a large family of pectoral-fin swimming fishes, ideal for functional studies due to their diversity and abundance in coastal systems worldwide (Cooper et al. 2009). On the Great Barrier Reef, *Acanthochromis polyacanthus* is one of the few cosmopolitan species able to thrive in a range of reef habitats including shallow reef crests and lagoons (Williams 1982). Unlike other Pomacentrid fishes, *A. polyacanthus* lacks a pelagic larval stage (Doherty et al. 1994; Kavanagh 2000). This has important implications for gene flow. Population divergence occurs over spatial scales as little as 3 km when sites are separated by deep-water (> 10m) channels providing a foundation for exploring variation in functional traits at small spatial scales (Doherty et al. 1994; Kavanagh 2000; Planes et al. 2001; Bay 2005; Bay et al. 2008; Miller-Sims et al. 2008).

We tested the hypothesis that populations of the widespread coral reef damselfish, *Acanthochromis polyacanthus* at sites with high versus low exposure to waves differ in their swimming phenotypes. More specifically, we predicted that fish at high exposure sites will: (1) display higher AR pectoral fins; (2), have faster steady swimming speeds, and (3) have a greater aerobic scope. These predictions are based on patterns seen when comparing these traits among species that vary in their preference for habitats with high and low water flow.

Materials and methods

Study system and abundance surveys

Fish were collected between Feb-2011 and Mar-2011 at six sites around Lizard Island, northern Great Barrier Reef, Australia (14° 40' S; 145° 28' E). Three sites were chosen on the windward (wave exposed) and three on the leeward (sheltered) side of the island (Fig. 1). Patterns of water motion at these sites have been previously measured, with wave height and water flow velocity differing more than 6-fold between sites of different exposure during windy conditions (Fulton and Bellwood 2005): wave exposed sites experience average water flows of approximately 38 cm s^{-1}, whereas sheltered sites experience flows of about 6 cm s^{-1}. Windward and leeward sites around Lizard Island are relatively similar in terms of canopy cover, structural complexity, fish habitat use,

and other (non-wave related) abiotic variables (Crossland and Barnes 1983; Goatley and Bellwood 2011; Heatwole and Fulton 2013).

The abundance of adult *Acanthochromis polyacanthus* was recorded at all study sites by divers using underwater visual censuses. Censuses were conducted on calm weather days (winds <5 knots) on reef crests (1.5 - 4m depth). All individuals > 5 cm total length (TL) were counted within four replicate 50m × 4m belt transects at each site. We surveyed each site extensively, covering approximately 800 m^2 of habitat per site. The sites surveyed encompassed a range of habitat types typical of mid-shelf reefs. The average fish density (# individuals m^{-2}) was calculated for each transect.

Fin morphology

Ten to 12 fish per site were collected at all six sites using micro- spears or barrier nets, sedated with 10% Aqui-S solution and euthanized in an ice slurry so that we could measure their pectoral fin morphology. Pectoral fins were dissected at the base of the fin, spread out and pinned onto a foam sheet, fixed into position using a concentrated formalin solution (39% formaldehyde), and digitally photographed (Panasonic Lumix DX3) on gridded paper (Binning and Fulton 2011). The length of the leading edge and total fin surface area were measured using ImageJ software (V 1.43). Fin AR was then calculated as the length of the leading edge squared divided by the total fin area. In total, we measured fins from 63 adult individuals (total length L_T= 120.5 ± 2.5 mm; mass = 31.5 ± 1.6 g; means ± s.e.m.).

Experimental swimming and respirometry trials

We measured experimental swimming speeds and estimated aerobic metabolic rates (~ oxygen consumption rate; $\dot{M}O_2$ in mg $O_2 kg^{-1} h^{-1}$) in adults from two wave exposed and two sheltered sites (Fig. 1). Five fish of similar size from each site (20 fish in total, L_T = 129.4 ± 1.1 mm; mass = 41.5 ± 0.9 g; no significant differences in wet fish mass among sites; $F_{1,3} = 0.293$, $P = 0.83$) were collected with ultra-fine barrier nets and transported to the aquarium facilities at the Lizard Island Research Station within 2h of capture. Fish were held in individual holding aquaria (40.0W× 29.0L× 18.0H cm) with a flow-through water system directly from the reef. Fish were fasted for 24h prior to the swimming trials to ensure a post-absorptive state that maximizes energy availability for swimming (Niimi and Beamish 1974; Johansen and Jones 2011). Length measures were

obtained by gently holding each fish on a wet surface and measuring total length (TL), body width (BW) and body depth (BD) with handheld calipers. Mass (M) was measured by placing the fish into a tared container of water on a scale, which minimized air exposure and stress prior to each trial. For this species, this method is as accurate as weighing the individual directly on the scale. These measures were used to calibrate the respirometer and calculate the flow rate of water in body lengths per second (BL s^{-1}). Swimming trials were carried out in an 11.9 L Loligo swimming respirometer (swim chamber dimensions 40.0L× 10.0W × 10.0H cm) filled with aerated, filtered and UV sterilized seawater at a constant temperature of 28 ± 0.1°C. We used intermittent-flow respirometry (Steffensen 1989) and oxygen levels in the respirometer were recorded using a fibre optic oxygen meter (Presens Fibox 3) online feed into the AutoResp 1 Software (Loligo Systems, Denmark). The flow in the working section of the respirometer was calibrated using a digital TAD W30 flow-meter (Höntzsch, Germany). The flow profile varied less than 3% across the full cross-section of the swim chamber, with the lowest flow occurring in the central part of the chamber. We did not observe individuals favouring one corner or particular side of the working section during the swim trials. Solid blocking effects of the fish in the working section were corrected by the respirometry software (AutoResp, Loligo Systems) following the equations of Bell & Terhune (1970).

We measured $\dot{M}O_2$ as a function of swimming speed (U) following a standard critical swimming speed (U_{crit}) protocol (Brett 1964; Steffensen et al. 1984; Rouleau et al. 2010; Binning et al. 2013; Roche et al. 2013). We used ten minute cycles with a 240s flush, 60s wait and 300s measure cycle. During the flush phase, oxygenated water from the surrounding water bath is pushed into the chamber to replenish the oxygen depleted by the swimming fish. The flush period ensured that the oxygen concentration throughout the trial did not decrease below 80% air saturation, and avoided CO_2 build up. The short wait period ensured that water in the chamber was sufficiently well mixed before measurements of $\dot{M}O_2$ commenced. Once an individual's length and mass measures were inputted into the respirometry software, three cycles were run without a fish to measure initial background rates of respiration due to the bacterial load in the test chamber. Fish were then placed in the test chamber of the respirometer and left to acclimate for five to eight hours at a swimming speed corresponding to one body length per second (BL s^{-1}) until their oxygen consumption rate stabilized. This speed ensured

constant swimming while minimizing spontaneous activity in this species. We measured oxygen consumption rate at 1.0 BL s^{-1} by averaging the three $\dot{M}O_2$ measurements immediately prior to the onset of the first trial (Roche et al. 2013). To start the trial, the flow speed was then slowly increased to 1.5 BL s^{-1} for three 10-minute $\dot{M}O_2$ measurement cycles. Flow speed was then incrementally increased by 0.5 BL s^{-1} every three cycles (i.e. every 30 minutes) for the duration of the experiment. Fish were continually monitored for a gait change from pectoral-fin swimming to pectoral-caudle swimming (Drucker and Jensen 1996) for more than five seconds continuously, at which point the flow speed and time into the interval was recorded (Johansen and Jones 2011). The trial stopped when the fish could no longer swim unassisted or was forced to rest on the back of the flow chamber for five or more seconds (Johansen and Jones 2011). The time and flow speed was recorded and the water flow was then reduced to 1.0 BL s^{-1} to ensure the fish's recovery from oxygen debt. We determined that a fish had recovered when its oxygen consumption rate fell back to near its rate at the start of the experiment at 1.0 BL s^{-1}. The fish was then returned to its holding tank. Three additional cycles were run to measure final background rates of respiration in the chamber (Clark et al. 2013). Background oxygen consumption rates at the end of each cycle were determined from the slope of the linear regression between initial and final background rates, and were subtracted from each $\dot{M}O_2$ cycle. To reduce bacterial growth and respiration in the chamber, the respirometer was drained and rinsed in freshwater when the background oxygen consumption rates exceeded 20% of the resting metabolic rate of the fish.

$U_{p\text{-}c}$ and U_{crit} calculation

We calculated a fish's gait transition speed ($U_{p\text{-}c}$) and critical swimming speed (U_{crit}) following the equation in Brett (1964):

$$U_{p\text{-}c} \text{ and } U_{crit} = U + U_i \times (t/t_i)$$

where U is the penultimate swimming speed before the fish changed gait ($U_{p\text{-}c}$) or fatigued and stopped swimming (U_{crit}); U_i is the swimming speed at which the fish changed swimming gait or was unable to continue swimming (i.e. swimming speed at increment i); t is the length of time the fish swam at the final swimming speed where fatigue or gait change occurred; t_i is the amount of time fish were swam at each speed interval in the trial (i.e. 30 min).

Oxygen consumption rate curves and aerobic scope

We used a hydrodynamics-based power function with three parameters to describe the relationship between $\dot{M}O_2$ and swimming speed (U) for each site (Roche et al. 2013):

$$\dot{M}O_2 = a + bU^c$$

where a is the estimated $\dot{M}O_2$ at zero speed (standard metabolic rate: SMR). In another species of coral reef fish, fitting a three parameter power function to the oxygen consumption curve obtained in a swimming respirometer was shown to provide accurate estimates of SMR which did not differ significantly from estimates obtained in a resting respirometer (Roche et al. 2013). Maximum metabolic rate (MMR) was measured at the maximum swimming speed where fish completed at least one 10 min $\dot{M}O_2$ determination; we averaged $\dot{M}O_2$ values when fish completed more than one determination (up to three determinations; Roche et al. 2013). We calculated the aerobic scope (AS) as MMR minus SMR (Clark et al. 2013). We also calculated factorial aerobic scope as (MMR-SMR)/SMR. We obtained similar results using both calculations; therefore we only present AS.

Statistical analyses

We used two-way mixed-effect ANOVAs to test for differences in fish density, fin morphology (AR), swimming performance ($U_{p\text{-}c}$, U_{crit}) and metabolic performance (SMR, MMR, AS) among sites (random factor) and wave exposures (fixed factor) using SPSS v.19. Fish body mass did not differ among sites ($P = 0.83$) and including this predictor in the metabolic performance analyses did not qualitatively change the results. Oxygen consumption rates and swimming performance data were normally distributed (Shapiro-Wilks test, $P > 0.18$ in all cases). Fish density was $\log_{10}$ transformed to meet the assumptions of the model. We used a general linear mixed effects model (LMM; lme function in R v3.0.1) to test for differences in the $\dot{M}O_2$-swimming speed relationship across wave exposures. We specified the relationship between swimming speed and $\dot{M}O_2$ as a second degree polynomial; site and fish identity were included as random factors.

Results

Acanthochromis polyacanthus were equally abundant at high and low wave exposures ($F_{1,22} = 0.07$, $P = 0.80$) with an average density of 0.1 individuals m^{-2}. Pectoral fin shape differed between wave exposures with fish from exposed sites having higher aspect ratio pectoral fins (mean ± SE; 1.63 ± 0.02) than fish from sheltered sites (mean ± SE; 1.44 ± 0.04; $F_{1,4} = 12.85$, $P < 0.05$; Fig. 2a). Fish from sheltered sites exhibited more variation in pectoral fin AR between individuals than fish from exposed sites (Table 1).

There was a significant difference in the shape of the $\dot{M}O_2$-swimming speed relationship between exposures (LMM interaction term; $F_{2,97} = 7.82$, $P < 0.01$), with sheltered fish consuming more oxygen than exposed fish at higher swimming speeds (Fig. 3). In addition, fish from wave exposed sites out swam those from sheltered sites during the trials: wave exposed fish had a significantly higher MMR ($F_{1,18} = 8.87$, $P < 0.01$), greater AS ($F_{1,18} = 6.43$, $P < 0.05$), higher gait transition ($U_{p\text{-}c}$) speeds ($F_{1,2} = 21.29$, $P < 0.05$) and faster critical (U_{crit}) speeds ($F_{1,2} = 21.29$, $P < 0.05$) than those from sheltered sites (Table 1, Fig. 2b,c). SMR did not differ between fish from high and low wave exposure sites ($F_{1,18} = 0.05$, $p = 0.83$). Differences in AS are therefore due to a higher MMR in fish from exposed sites, consistent with the higher critical swimming speed observed in these individuals.

Discussion

Water motion is a critical force structuring coral reef communities leading to predictable effects of wave exposure on the presence or absence of species (e.g. Kaandorp 1999, Bellwood et al. 2002; Fulton et al. 2005). This predictable influence of water flow on community structure is widely attributed to the functional relationship between phenotypic traits that affect swimming performance and the ability to swim efficiently at different water flow rates. Here, we show that this functional relationship might also explain the spatial distribution of phenotypic variation within a single species. Differences in wave-driven flows experienced by *A. polyacanthus* at exposed and sheltered sites are a plausible factor responsible for differences among sites in several traits that are known to be related to swimming ability. Across populations of *A. polyacanthus* separated by < 10km along a mid-shelf island, we found significant differences in fin shape, swimming performance and aerobic scope that mirror patterns

observed in interspecific studies. That is, fish from sites exposed to higher wave energy possessed traits that are thought to enhance swimming capabilities under fast water flow conditions.

Although *A. polyacanthus* displays relatively high levels of genetic diversity across our study sites (Bay 2005; Bay et al. 2008), our ability to assert large-scale trends is limited since we only examined four to six sites on a single mid-shelf island. While we observed a strong relationship between water flow and the morpho-physiological traits we measured, this evidence is correlational. Nonetheless, our results likely apply to other reef locations where similar differences in wave energy occur. Two additional studies support this view. First, Fulton et al. (2013) found similar patterns in fin-shape and swimming speed across flow gradients in *A. polyacanthus* distributed across a 40 km cline in wave-driven water motion. Second, another study at similar sites around Lizard Island found that many fishes, including *A. polyacanthus,* respond to varying wave conditions by changing their patterns of fin use and their body orientation relative to the flow in exposed sites during rough weather conditions (Heatwole and Fulton 2013). These results were not attributable to differences in habitat use. Specifically, *A. polyacanthus* occupies a similar mean water column height (approx. 50 cm above the substrate) on both the leeward and windward sides of the island in all weather conditions (Heatwole and Fulton 2013). In combination, both these studies suggest that water motion rather than other environmental parameters is driving phenotypic differences between exposed and sheltered sites in *A. polyacanthus*.

Acanthochromis polyacanthus use their fins to produce lift-based thrust similar to the wing flapping pattern of swimming penguins (Vogel 1994). With this form of locomotion, high AR fins can increase the lift-to-drag ratio on the fin surface and enhance the ability to generate forwards thrust, especially in the presence of water flow (Vogel 1994). Conversely, high AR fins are less useful in low-flow habitats since generating thrust through lift is less effective in these conditions (Vogel 1994). In addition to morphological adaptations, *A. polyacanthus* from wave exposed sites presumably require a greater range of swimming speed performances. They likely achieve this through various modifications to their cardiovascular system leading to a higher aerobic scope than their sheltered conspecifics. Our swimming trials found that fish from wave exposed sites swam up to 1:1 BL s^{-1} or 33 % faster than fish from

sheltered sites. This difference appears to be fueled by a 36 % increase in aerobic scope. A large aerobic scope presumably allows these fish to carry out several different activities simultaneously without incurring an oxygen debt (Claireaux and Lefrancois 2007). Rates of plankton delivery and detritus deposition are often greatest in high flow habitats (Wilson et al. 2003; Clarke et al. 2005), so the increased supply of food at these sites may offset any costs of increased energy requirements for swimming faster in wave exposed habitats. Interestingly, our census indicated that *A. polyacanthus* was equally abundant across the six study sites, suggesting no clear preference for wave exposure. Fully understanding these results requires additional studies exploring the costs of maintaining a large aerobic scope, as well as bioenergetic models exploring relative rates of energy gain and expenditure across different habitats.

Adaptive radiations in coral reef fishes have generally been attributed to divergence in trophic structures to exploit different food niches (e.g. Wainwright 1991). Our results suggest that distinct flow habitats created by reef locations (windward vs. leeward) might also promote additional phenotypic divergence among populations based on swimming ability. Changes in locomotor traits facilitate range expansions into novel environments in a range of taxa. For instance, the recent adaptive radiation of *Anolis* lizards into habitats of varying complexity in the Caribbean has been linked to divergence in hindlimb length affecting sprint speed, jumping and perching ability (Losos 1990a; Losos 1990b). Similarly, the morphological and physiological adaptations displayed by *A. polyacanthus* might partly explain its widespread distribution throughout the Great Barrier Reef and Coral Sea. Patterns of fin-shape and swimming speed across flow gradients similar to those observed here also occur at larger spatial scales (gradient from inner to outer reef sites) in *A. polyacanthus* (Fulton et al. 2013). One interpretation is that selection for genes expressing phenotypes suited to the local wave environments have occurred (Langerhans 2008; Fulton et al. 2013). However, *A. polyacanthus* also shows relatively quick physiological adaptation (i.e. adaptive phenotypic plasticity) in response to changing thermal conditions in the laboratory (Donelson et al. 2011; Donelson et al. 2012). Consequently, a role for environmentally-induced plasticity in driving population variation in traits that affect swimming ability cannot be ruled out. This is an intriguing possibility. If true, it would represent some of the first empirical evidence of adaptive plasticity to natural environmental gradients in a coral reef fish. The degree to which phenotypic plasticity

and natural selection on genetic variation drive intraspecific variation in the swimming ability of *A. polyacanthus* (and of other labriform swimmers) should be further explored through comparisons across multiple islands to test the extent to which the pattern observed on Lizard Island can be generalised. In addition, split brood and/or common garden experiments are needed to determine the relative contribution of genetic and environmental factors to phenotypic variation in *A. polyacanthus*. Ultimately, the strong functional relationship among swimming morphology, physiology, performance, and flow habitat that we have observed within a single species closely mirrors the well-documented pattern seen when looking across species of coral reef fishes. Given the known effect of this functional relationship on community assemblages globally, it is a remarkable pattern that warrants further investigation. We need to determine whether it can also explain spatial variation in phenotypes within a wide range of individual species.

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Table 1 Mean fish total length (TL), mass (M), pectoral fin aspect ratio (AR), gait transition speed ($U_{p\text{-}c}$), critical swimming speed (U_{crit}), maximum metabolic rate (MMR) and aerobic scope for swimming (AS) of *A. polyacanthus* collected from sites around Lizard Island (± SE).

Site	Exposure	TL (mm)	M (g)	AR	$U_{p\text{-}c}$ (BL s^{-1})	U_{crit} (BL s^{-1})	MMR (mg O_2 kg^{-1} h^{-1})	AS (mg O_2 kg^{-1} h^{-1})
E1	Exposed	127.8 ± 2.1	42.5 ± 2.2	1.63 ± 0.03	3.34 ± 0.11	3.72 ± 0.18	430.0 ± 37.1	307 ± 32
E2	Exposed	127.8 ± 2.0	41.2 ± 1.9	1.61 ± 0.03	3.45 ± 0.16	4.06 ± 0.17	473.9 ± 42.7	336 ± 37
E3	Exposed	-	-	1.66 ± 0.02	-	-	-	-
S1	Sheltered	128.6 ± 2.4	40.0 ± 1.3	1.47 ± 0.05	2.25 ± 0.10	3.09 ± 0.16	344.8 ± 31.8	222 ± 42
S2	Sheltered	131.4 ± 1.8	42.3 ± 2.1	1.51 ± 0.07	2.42 ± 0.20	2.89 ± 0.23	341.9 ± 45.7	215 ± 94
S3	Sheltered	-	-	1.33 ± 0.06	-	-	-	-

Fig. 1 Map of Lizard Island with the location of the three sheltered (S1, S2, S3) and three exposed (E1, E2, E3) sites. Experimental swimming and respirometry trials were conducted on specimens collected from sites E1, E2, S1 and S2.

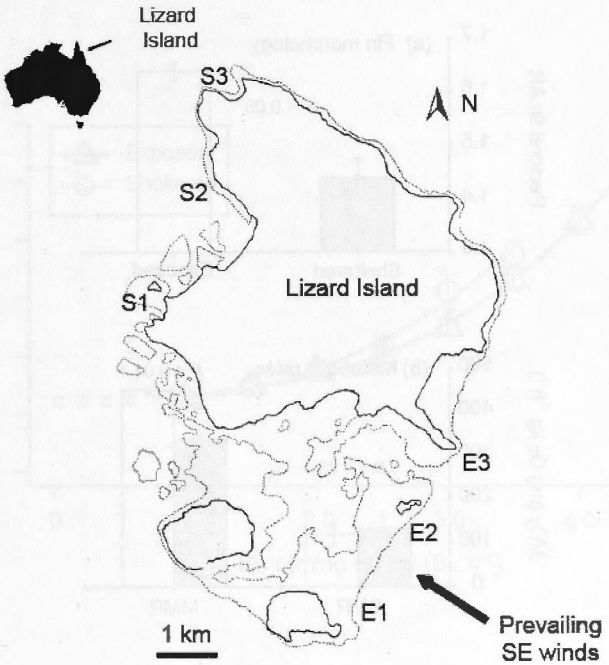


Fig. 2 Average (± SE) a) pectoral fin aspect ratio (AR) b) metabolic rates (standard metabolic rate = SMR; maximum metabolic rate = MMR), and c) experimental (U_{p-c}, U_{crit}) swimming speeds (U) for *A. polyacanthus* from sheltered (grey) and wave exposed (white) sites.

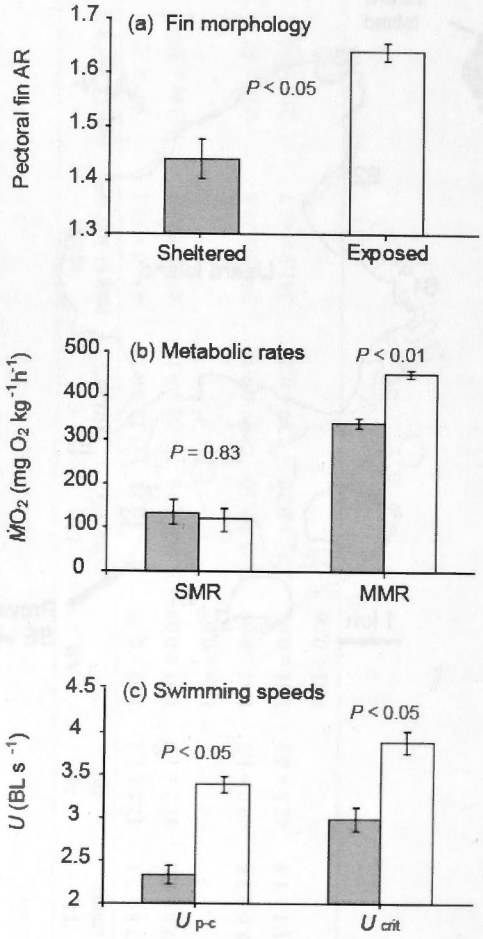
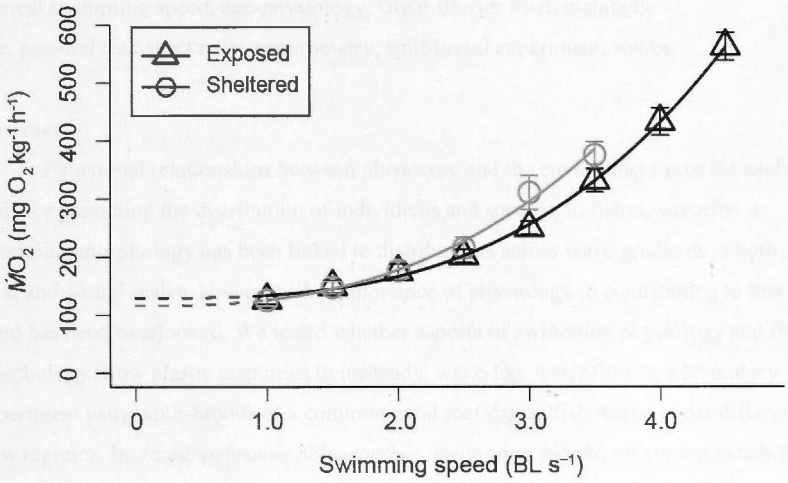


Fig. 3 Oxygen consumption rate ($\dot{M}O_2 \pm$ SE) as a function of swimming speed (U, body lengths second^{-1}) for exposed and sheltered fish from each of four sites sampled ($n = 10$ fish per exposure, black triangles = exposed; grey circles = sheltered). Oxygen consumption curves are as follows: $\dot{M}O_2 = 4.5U^{3.0} + 129.1$ for wave exposed fish; $\dot{M}O_2 = 9.93U^{2.63} + 115.8$ for sheltered fish.



APPENDIX - IV

Adaptive plasticity to water flow habitats in a coral reef fish

Binning SA, Ros A, Nusbaumer D and **Roche DG** (in revision) *Journal of Experimental Biology*

Keywords

Critical swimming speed, eco-physiology, Great Barrier Reef, metabolic rate, pectoral fin aspect ratio, respirometry, split-brood experiment, waves

Abstract

Functional relationships between phenotype and the environment provide useful tools for predicting the distribution of individuals and species. In fishes, variation in swimming morphology has been linked to distributions across wave gradients at both local and global scales. However, the importance of physiology in contributing to this trend has been overlooked. We tested whether aspects of swimming physiology and fin morphology show plastic responses to unsteady, wave-like water flow in a laboratory experiment using split-broods of a common coral reef damselfish reared under different flow regimes. In *Acanthochromis polyacanthus*, swimming speeds, maximum metabolic rates, aerobic scope and blood haematocrit were higher in fish reared in experimental tanks with unsteady (wave driven) water flow than in fish reared in tanks with low water flow. Surprisingly, fin shape did not differ between individuals reared in our experimental flow treatments and was not correlated with swimming speed performance in our fish. Our results suggest that exposure to water motion is sufficient to induce plastic physiological changes which increase swimming performance in *A. polyacanthus*. Despite pectoral fin shape (aspect ratio) being largely attributed to differences in swimming performance both within and among species, we found that physiological traits were better predictors of swimming ability in our study. These results suggest that the established ecomorphological relationships between fish assemblages and water motion should be re-evaluated to take differences in physiology into account. We suggest that a general ability to adapt physiologically to different flow environments is a likely explanation for the widespread distribution of this and other species across wave gradients on the Great Barrier Reef.

Introduction

The precise morphological and physiological traits that elevate survivorship depend on the local environment. Organisms must either possess adaptations that allow them to handle these physical challenges, or they must move to more amenable areas. Together these two processes help to determine a species' distribution. Species that have broad tolerances and/or show adaptive phenotypically plastic responses might benefit from natural environmental variability and be distributed across a more diverse array of habitats than species lacking these traits. Studying the mechanisms driving intraspecific differences in performance in widespread species can help us to understand key evolutionary processes such as local adaptation and speciation (West-Eberhard 2005).

Researchers are increasingly concerned with the impact of accelerating changes in weather patterns on organisms. Aquatic systems have received much attention given the ecological, social and economic importance of coastal and riparian habitats (e.g. Harley et al. 2006, Palmer et al. 2008, Brierley and Kingsford 2009). Climate change research in marine systems has largely focused on the effect of increased sea surface temperature and ocean acidification on coastal communities (Munday et al. 2012,Koch et al. 2013). However, the frequency and intensity of winds and extreme weather events are also increasing across ocean basins globally, which will likely impact the structure of aquatic species assemblages (e.g. Emanuel 2005, Webster et al. 2005). High winds increase unsteady, wave-driven water flow. Mobile animals living in variable or high water flow need to swim fast to avoid being swept away. Adaptations to these habitats include morpho-physiological traits that promote speed and high aerobic capacity. For labriform (pectoral-fin) swimming fishes, these traits include tapered fins, high maximum metabolic rate (MMR) and large aerobic scopes (AS) (Walker and Westneat 2002, Wainwright et al. 2002, Binning et al. 2013a). Fast-swimming fish might also benefit from an increased proportion of red blood cells (haematocrit, Hct), which is one component required for overall increased oxygen carrying capacity (Gallaugher and Farrell 1998).

Functional relationships between swimming morphology and wave-driven flow have been repeatedly documented in cross-species studies of freshwater and marine fishes (e.g. Bellwood et al. 2002, Fulton et al. 2005, Langerhans 2008). This

relationship provides a strong framework for predicting species distributions and the potential for range expansions based on changes in water flow and easy to measure morphologies such as body and fin shape. Recently, similar relationships in terms of fin shape differences were found within a single species of coral reef fish, *Acanthochromis polyacanthus* (Bleeker 1855), which is distributed across large gradients in water flow on the Great Barrier Reef (Binning et al. 2013a, Fulton et al. 2013). In addition to morphological variation, natural populations of *A. polyacanthus* were also found to vary physiologically in terms of their maximum metabolic rates and aerobic capacity suggesting that more than just fin shape is responsible for the performance differences observed (Binning et al. 2013a). This striking variation among populations in swimming performance provides a rare opportunity to test the importance of these underlying factors in driving these patterns, especially given the unique life-history of this species. *A. polyacanthus* lacks a pelagic larval stage, and broods can remain with their parents for several months (Doherty et al. 1994, Kavanagh 2000). This has important implications for gene flow. Population divergence occurs over spatial scales as little as 3 km when sites are separated by deep-water (> 10m) channels, providing a foundation for exploring variation in functional traits at small spatial scales (Bay et al. 2008, Miller-Sims et al. 2008). Do differences in swimming performance reflect local adaptation due to selection on genetic variation and/or are they due to adaptive phenotypically plastic responses to local flow conditions? Are these patterns similar for both morphological and physiological traits and which traits contribute more to overall swimming performance? Given that shallow marine environments are increasingly affected by changes in water flow (Harley et al. 2006), it is fundamental that we understand how species respond. Specifically, can short-term adaptive phenotypic plasticity help species persist in their local habitats?

We tested whether aspects of swimming physiology and fin morphology show plastic responses to unsteady, wave-like water flow in a laboratory experiment using split-broods reared under different flow regimes. We predicted that if swimming ability is an adaptively plastic trait, juvenile *A. polyacanthus* reared under constant oscillatory water-flow conditions will develop morphological (tapered fins) and physiological (high maximum metabolic rate, aerobic scope and blood haematocrit) traits that are associated with high-speed swimming in the wild.

Methods

Fish sampling and experimental design

We collected fish from 16 broods at Lizard Island, northern Great Barrier Reef, Australia (14° 40' S; 145° 28' E). Eight broods were on the predominantly windward (wave exposed) and eight on the leeward (sheltered) side of the island. Patterns of water motion (wave height and water flow velocity) differ more than 6-fold between these sites during windy conditions (Fulton and Bellwood 2005): average water flows of approximately 38 cm s^{-1} versus 6 cm s^{-1} at windward and leeward sites respectively. Broods were selected based on their developmental stage (stage 2 - 3, 4 to 6 weeks old) (Kavanagh 2000), distance apart (> 30 m between broods), and brood size (> 30 fry). Developmental stage was chosen to maximize the chance of survivorship in the aquarium setting and enable broods to be uniquely identified using Visible Implant Elastomer Tags (VIE tags, North-West Marine Technologies). Only broods with two parents and no other similar-stage families within 10 m were selected to increase the likelihood that all collected individuals from a brood were full-siblings. We collected fish using 10% Aqui-S solution and hand nets, and transported them in plastic bags to the aquarium facilities at Lizard Island Research Station within 1h of capture. Each brood was housed in separate aquaria (40.0W× 29.0L× 18.0H cm) with a flow-through water system directly from the reef. Fish were fed approximately 0.25g NRD 2/4 pellets (Primo aquaculture) per tank each morning and at dusk. After three days, we measured and injected 24 individuals from each brood (384 fish total, total length L_T= 3.3 ± 0.1 cm; mass M = 0.5 ± 0.1 g; means ± s.e.) with VIE tags using 1cc gauge syringes to uniquely colour-code siblings. After two days to recover, fish were assigned to treatment tanks (384 fish total).

Four identical rectangular aquaria (50.0W×100.0L× 35.0H cm, 175 litres) were fitted with two Vortec MP40 (Ecotech Marine) propeller pumps (one on each end, lengthwise across the tank) programmed to operate alternately and create unsteady flow conditions simulating a wavy, reef crest-like habitat (period = 2 sec, wave amplitude = 6 cm). Pumps were initially fitted with protective cages (13.0W×16.0L× 12.0H cm) covered with 10 mm stretch monofilament mesh to prevent access by small fish. These cages were removed after four months of fish growth. Flow velocity in the tanks was measured with passive particles at various depths and locations within each tank to ensure similar flow conditions across all four tanks. Flow ranged between 0.0 - 27.5 cm

s^{-1} (average flow 19.5 ± 0.15 SD cm s^{-1}) when cages were present, and 0 – 48.7 cm s^{-1} (average flow 35.2 ± 3.4 SD cm s^{-1}) when cages were removed. Four additional aquaria were assigned to the calm treatment and supplied with bubblers (water flow < 4.0 cm s^{-1}) to simulate calm lagoonal conditions with low water flow. Mesh covers prevented predation by birds. The tanks experienced natural light and temperature regimes throughout the experiment (February- October 2012). All tanks contained three cylindrical PVC pipe shelters (large; 15.0L×11.0H, medium; 9.0L×5.5H, small; 7.0L×11.0H) affixed to the bottom with silicone to provide shelter and the opportunity for fish to flow-refuge, which is a commonly observed behaviour in wild coral reef fishes (Johansen et al. 2007).

Three individuals from each brood were randomly assigned to each tank (48 individuals per 175 L tank, 3.6 L of water per fish). Fish in each tank were fed 0.75g NRD 2/4 pellets (Primo Aquaculture, Australia) each morning and dusk for six weeks, then switched to 0.25g NRD 2/4 pellets and 0.75g NRD 5/8 pellets twice a day. After four months, feeding rations were changed to 0.5g NRD 5/8 pellets and 0.5g NRD G12 pellets per tank twice a day. After seven months, feeding rations were changed to 1.0g NRD G12 pellets per tank twice a day for the remaining 6 weeks of the experiment. Fish were retagged after six weeks and four months to ensure the VIE tags remained visible. Organic material deposited in the tanks was siphoned out weekly to promote water quality. An infection in one of the unsteady flow tanks caused the mortality of all 48 individuals after 3.5 months. Mortality in the remaining tanks during the course of the experiment was low (< 5.0 %). All healthy individuals were released at their site of capture after the completion of the experiments.

Fin morphology

We measured pectoral fin morphology four and eight months after the start of the experiment as per Binning and Fulton (2011): fish were sedated in a water bath containing 10% Aqui-S solution, and their extended fin was photographed on gridded waterproof paper with a digital camera (Panasonic Lumix DX3). We measured the length of the leading edge and total fin area using ImageJ software (V 1.43). Fin AR was then calculated as the length of the leading edge squared divided by the total fin area. Only undamaged and fully spread fins were analysed. Only one fish per brood from each tank was randomly selected and measured after four months to minimize

stress and potential damage to the fins. Fin AR measurements were averaged for siblings in the same experimental tank for the eight month measurement. In total, we measured 112 individuals after 4 months (L_T = 7.8 ± 0.0 cm; M = 3.9 ± 0.0 g) and 188 individuals after 8 months (L_T = 10.0 ± 0.1 cm; M = 17.7 ± 0.2 g).

Respirometry and swimming performance

We measured gait transition speed ($U_{p\text{-}c}$), critical swimming speed (U_{crit}), and oxygen consumption rates ($\dot{M}O_2$: mg $O_2 kg^{-1} h^{-1}$) in September and October 2012 after fish had spent a minimum of seven months in the experimental treatments. Due to time and equipment constraints, we were unable to run a balanced design including tank as a factor in the analysis. To control for tank effects, we instead selected fish from tanks at random until one individual from each of the 16 broods had been tested from each treatment (32 fish total, L_T = 10.4 ± 0.1 cm; M = 19.0 ± 0.4 g). Fish were fasted for 24h prior to the swimming trials to standardize a post-absorptive state that maximizes energy availability for swimming (Johansen and Jones 2011). We obtained length measures for individual live fish before swimming trials by holding each fish in a plastic bag half-filled with water and measuring total length (TL), body width (BW) and body depth (BD) with handheld callipers. Mass was measured directly on a scale. These measures were inputted into the respirometry software and used to calculate the flow rate of water in total lengths per second (herein body lengths per second, BL s^{-1}). We measured $\dot{M}O_2$ as a function of swimming speed (U) following a U_{crit} protocol (reviewed in Plaut 2001). Swimming trials were carried out in a 4.8 L custom-built Steffensen-type swim tunnel respirometer using intermittent-flow respirometry (Brett 1964, Steffensen, et al. 1984). The swim chamber dimensions were 30.0 L × 7.0 W × 7.0 H cm. Temperature was maintained at a constant 26 ± 0.1°C using a TMP-REG temperature analyser and regulator system (Loligo Systems, Denmark). Oxygen levels in the respirometer were recorded using a fibre optic oxygen meter (Presense Fibox 3) online feed into the AutoResp 1 Software (Loligo Systems, Denmark). The flow in the working section of the respirometer was calibrated using a digital TAD W30 flow-meter (Höntzsch, Germany). Solid blocking effects of the fish in the working section of the respirometer were corrected by the respirometry software (AutoResp, Loligo Systems) following Bell & Terhune (1970). The cross-sectional area of fish was always less than 9 % of the chamber cross-sectional area corresponding to approximately 4 % greater effective water velocity around fish compared to the water velocity in the empty swim

chamber (Webb 1975). We used ten minute loops with a 240 s flush, 60 s wait and 300 s measure cycle. Once an individual's length and mass measures were inputted into the respirometry software, three loops were run without a fish to measure initial background rates of respiration due to bacterial load in the test chamber. The fish was then placed in the respirometer and left to acclimate for five to eight hours at a swimming speed of 1.0 BL s^{-1} until its oxygen consumption rate stabilized. This speed corresponded to the lowest water flow necessary to ensure constant swimming and minimize spontaneous activity in *A. polyacanthus* (Binning pers. obs.). We measured oxygen consumption rate at 1.0 BL s^{-1} by averaging the three $\dot{M}O_2$ measurements immediately prior to the onset of the first trial (Binning et al. 2013b, Roche, et al. 2013a). Flow speed was then incrementally increased by 0.5 BL s^{-1} every three 10-minute loops (i.e. every 30 minutes) for the duration of the experiment. Fish were continually monitored for a change in swimming mode (gait transition) from pectoral-fin only to a pectoral-caudle fin assisted mode (Drucker and Jensen 1996) for more than five seconds continuously ($U_{p\text{-}c}$), at which point the flow speed and time into the interval was recorded (Johansen and Jones 2011). We also noted the gate transition from pectoral-caudle assisted to burst and coast swimming. For labriform-swimming fishes, this transition generally marks the recruitment of anaerobically-powered energy metabolism (Svendsen et al. 2010). Burst and coast swimming was defined as an event that included caudal fin beats (typically 1, 2 or 3 beats) and a subsequent forward glide motion >5 cm without the use of pectoral fins (see Svendsen et al. 2010). The trial stopped when the fish could no longer swim unassisted or was forced to rest on the back of the flow chamber (U_{crit}) for five or more seconds (Johansen and Jones 2011, Binning et al. 2013b). The time and water flow speed was recorded and the water flow was reduced back to 1.0 BL s^{-1} for at least 10 minutes to allow partial recovery from anaerobic burst and coast swimming. The fish was then removed from the test chamber and transferred to a separate holding tank. Three additional cycles were run without a fish to measure background $\dot{M}O_2$ in the empty chamber. Background oxygen consumption rates were determined from the slope of the linear regression between initial and final background oxygen consumption rates, and were subtracted from each $\dot{M}O_2$ estimate. The respirometer was drained and rinsed in freshwater every four fish to ensure that background consumption rates due to bacteria did not exceed 10 % of the oxygen consumption rates of the fish at 1.0 BL s^{-1}. Fin AR was measured on fish one day following their swim in the respirometer

following Binning and Fulton (2011) and methods described above. All healthy fish were released at their site of capture within one week following their swim.

We calculated a fish's gait transition speed ($U_{p\text{-}c}$) and critical swimming speed (U_{crit}) following the equation in Brett (Brett 1964):

$$U_{p\text{-}c} \text{ or } U_{crit} = U + U_i \text{ x } (t/t_i)$$

where U is the penultimate swimming speed before the fish changed gait ($U_{p\text{-}c}$) or fatigued and stopped swimming (U_{crit}); U_i is the swimming speed at which the fish changed swimming gait or was unable to continue swimming (i.e., swimming speed at increment i); t is the length of time the fish swam at the final swimming speed where fatigue or gait change occurred; t_i is the amount of time fish were swam at each speed interval in the trial (30 min).

We used a hydrodynamics-based power function to describe the relationship between $\dot{M}O_2$ and swimming speed (U) for each fish (Binning et al. 2013a, Roche et al. 2013a):

$$\dot{M}O_2 = a + bU^c$$

where a is $\dot{M}O_2$ at zero speed ($\dot{M}O_{2,\,min}$), which is an estimate of standard metabolic rate (SMR). $\dot{M}O_{2,\,max}$ or maximum metabolic rate (MMR) was estimated at a fish's maximum swimming speed during prolonged swimming (Roche et al. 2013). We calculated the aerobic scope (AS) as MMR minus SMR (Clark et al. 2013). We repeated this calculation using factorial aerobic scope, calculated as the ratio between MMR and SMR (Clark et al. 2013); this approach led to qualitatively similar results as those obtained with AS, therefore we only present AS.

Blood parameters

We sampled blood from one randomly selected individual per brood for each treatment (32 fish total, L_T= 10.1 ± 0.8 cm; M = 18.2 ± 0.4 g). Individuals were sedated using 10% Aqui-S solution until equilibrium was lost (< 20 sec). A small blood sample (<0.1 ul) was drawn from the caudal vasculature using a 25 gauge needle in a 1 ml syringe. Fish were then placed in a bucket of aerated seawater to recover. Blood was

immediately transferred to micro-haematocrit capillary tubes and centrifuged in a fixed speed haematocrit centrifuge (10 000 rpm) for 5 min. The proportion Hct was calculated at the ratio of red blood cells to total blood volume. Short-term changes in Hct levels caused by red blood cell swelling and splenic release can be induced by stress in fish (Gallaugher and Farrell 1998). Therefore, we took all efforts to minimize the time taken from capture to sampling (< 3 min per fish). We also tested for differences in blood cortisol levels between the two water flow treatments groups. Cortisol has previously been used to assess both background stress levels as well as responses to acute stressors in coral reef fishes (Soares et al. 2011). We used a species-independent commercial cortisol enzyme immunoassay kit (DetectX, Arbor Assays, Ann Arbor, Michigan USA). To avoid matrix effects, 10ul of plasma was extracted twice with 3ml of diethyl ether (recovers 95% of steroids). Diethyl ether was evaporated in a speedvac and the non-polar fraction was resuspended in 1ml of assay buffer. Immunoassays were carried out following the manual of the kit. Intra-assay precision was 10.6 %CV. Cross reactivities reported by the kit are: 1.2% for cortisone and <0.1% for progesterone.

Statistical analyses

All analyses were performed in R v2.15.2 (R Development Core Team 2012). The assumptions of the models were assessed with diagnostic plots and Shapiro-Wilks tests, and transformations were used where necessary. We used general linear mixed models (LMMs; lme function in R) to compare values of metabolic rate (MMR, SMR), swimming speeds ($U_{p\text{-}c}$, U_{crit}) and aerobic scope (AS) between collection sites (leeward or windward; fixed factor) and flow treatments (unsteady or calm; fixed factors) while controlling for brood identity (random factor). We used Pearson correlations to test for relationships between swimming speed ($U_{p\text{-}c}$, U_{crit}) and fin shape (AR). Four-month fin aspect ratio measures were $\log_{10}$ transformed to meet the assumptions of normality. We ran two LMMs to compare values of fin AR (at four and eight months), specifying collection site and water flow treatment as fixed factors and brood and tank identity as random factors. We used a generalized linear mixed model (GLMM; lmer function in R) with a binomial error term to compare Hct between collection sites and water flow treatments (fixed factors), controlling for brood identity (random factor). We tested for differences in plasma cortisol level ($\log_{10}$ transformed) with a LMM (collection sites and water flow treatments as fixed factors, brood identity as a random factor).

Results

Acanthochromis polyacanthus juveniles reared in experimental tanks with unsteady water flow outswam their calm tank-reared conspecifics during swimming trials. Flow-reared fish had a higher gait transition speed ($U_{p\text{-}c}$, $F_{1,14} = 84.22$, $P < 0.001$, $d = 2.59$), faster maximum speeds (U_{crit}, $F_{1,14} = 110.3$, $P < 0.001$, $d = 2.96$), higher maximum metabolic rates ($F_{1,14} = 43.71$, $P < 0.001$, $d = 1.76$) and greater aerobic scope ($F_{1,14} = 50.01$, $P < 0.001$, $d = 2.15$), than calm-reared fish (Fig. 1a-c; Table 1). Standard metabolic rate did not differ between flow treatments ($F_{1,14} = 2.98$, $P = 0.11$, $d = 0.09$) or site of origin ($F_{1,14} = 0.94$, $P = 0.35$, $d = 0.89$). Therefore, the observed differences in aerobic scope were a result of increased maximum metabolic rates in flow-reared fish. For all the traits examined there was no effect of site of origin (all $P > 0.30$) or any interaction between site of origin and experimental treatment (all $P > 0.15$). This indicated that there were no detectable genetic differences for the focal traits when fish from different sites were reared in a 'common garden' setting.

Fin shape did not differ between treatments after either four or eight months ($F_{1,5} = 0.06$, $P = 0.82$, $d = 1.1$; and $F_{1,5} = 3.29$, $P = 0.13$, $d = 1.31$) and there was no effect of site of origin ($F_{1,98} = 0.22$, $P = 0.64$, $d = 0.14$ and $F_{1,91} = 0.49$, $P = 0.49$, $d = 0.15$) (Fig. 2). A range of fin shapes was present within most broods (aspect ratio range: 1.09 – 1.57 across all fish; Table 2), but differences in fin shape did not explain differences in swimming speed performance among fish (Pearson correlation; $U_{p\text{-}c}$: r = - 0.35, n = 28, $P = 0.07$; U_{crit}: r = -0.12, n = 29, $P = 0.52$, Fig. 3).

Blood samples revealed that flow-reared fish had greater blood Hct than calm-reared conspecifics (treatment $Z = 2.33$, $P = 0.02$, $d = 1.24$; site of origin: $Z = 0.48$, $P = 0.63$; $d = 0.22$; Fig. 1d). Plasma cortisol levels did not differ between flow treatments ($F_{1,14} = 0.23$, $P = 0.64$, $d = 0.36$) or with site of origin ($F_{1,14} = 0.16$, $P = 0.70$, $d = 0.34$).

Discussion

Species can adapt to changes in their environment via phenotypic plasticity (West-Eberhard 2005). Likewise, plastic species can broaden their distribution, creating patterns of phenotypic variation within species across a habitat gradient. Natural populations of *A. polyacanthus* on the Great Barrier Reef differ greatly in their fin morphology, physiology and swimming performance (Binning et al. 2013a, Fulton et al.

2013). However, causal and mechanistic explanations for this natural pattern as well as the relative importance of physiological vs. morphological differences in driving performance differences have remained unclear. We show that in *A. polyacanthus*, experimental exposure to unsteady water flow throughout ontogeny increases gait transition speed by 16 % critical swimming speed by 20% and aerobic scope by 27%, regardless of birth site (Fig, 1 & 2a). In addition, haematocrit, one component of blood oxygen carrying capacity, increased by 25 % in trained individuals (Fig. 2b). These results are unlikely to be caused by a stress response as blood cortisol levels were the same across sites and water flow treatments. To our knowledge, these results represent the first empirical evidence of adaptive plasticity in coral reef fishes to unsteady water motion similar to waves experienced on exposed reef crest habitats, and conclusively attribute performance differences to physiological rather than morphological traits.

Homoeothermic animals, including humans, experience similar physiological changes during exercise training (e.g. Carter et al. 2000, Kemi et al. 2002). Increased metabolic rates and oxygen carrying capacity enhance the delivery of oxygen to the muscles and likely resulted in the increased swimming performances we observed. Some exercise training experiments in teleosts fishes have produced similar results (reviewed in Davison 1997, Kieffer 2010). However, training effects in lower vertebrates such as reptiles and fishes are often less than in mammals, leading to the belief that poikilotherms are less plastic in their responses to exercise (reviewed in Davison 1997). Additionally, most of this research is limited to a few species of commercially important temperate fishes (Kieffer 2010). Very little has been published on the exercise physiology of tropical marine fishes despite their growing presence in the aquaculture industry and susceptibility to environmental stressors (see review in Kieffer 2010). Our results suggest that developmental plasticity in physiological traits related to oxygen uptake in response to exercise is an important mechanism promoting the survivorship of widespread species across water flow habitats, and should be considered in future research. Studies exploring the response of adult coral reef fishes to exercise training are now needed to assess how labile these physiological adaptations are within a fishes' lifespan.

Natural populations of *A. polyacanthus* have distinct fin shapes across a 40 km cline in wave-driven water motion and between windward and leeward sites across a

typical mid-shelf island (Binning et al. 2013a, Fulton et al. 2013). However, fin shape in *A. polyacanthus* did not show plastic or genetic influences in our experiments (no treatment or site effect; Fig. 2). Furthermore, fin shape was not correlated with critical swimming speed (Fig. 3). These results are surprising as fin morphology is believed to be a fundamental trait necessary for persistence in wave-swept habitats (e.g. Bellwood et al. 2002, Fulton et al. 2005). One possible explanation is that our fish did not reach full adult size before they were tested; mean fin AR across all broods was less than means reported from fully-grown adult fish from mid-shelf islands on the GBR (Table 2; Fulton et al. 2013). It is possible that the magnitude of difference between high and low AR fins from sub-adult fish in our study was not sufficient to produce detectable differences in swimming speeds. Nevertheless, clear differences in swimming performance ($U_{p\text{-}c}$, U_{crit}) were observed between treatments, and are likely explained by differences in the physiological parameters measured. Although tapered fins may enhance swimming speeds, we found that changes in blood haematocrit, maximum metabolic rate and aerobic scope are also important predictors of swimming ability in this fish. The established functional relationships between reef fish swimming performance and water motion should consider differences in physiology in addition to morphology when exploring distributional patterns both among and within species.

Water flow strength varies across very small scales on coral reefs. Even in exposed habitats, flow velocity decreases dramatically with depth and distance from the reef crest (Fulton et al. 2005). Since some physiological aspects of swimming ability are plastic, dispersing individuals may select their microhabitat based on fin shape. Within-brood variance in fin AR was quite high in our study (Table 2). If individuals assort to sites based on fin shape, selection may favour diversity in fin shape within a brood. This bet-hedging strategy may increase the range of habitats and locations in which offspring can thrive (Starrfelt and Kokko 2012). Microhabitat segregation can, in turn, reduce the chances of inbreeding if siblings are less likely to share similar habitats. Mechanisms that promote outbreeding are important in this species given its unique life-history. Unlike other Pomacentrid fishes, *A. polyacanthus* is a larval brooder and has limited dispersal abilities (Doherty et al. 1994, Kavanagh 2000). Despite high levels of genetic relatedness within a sampling area, Miller-Sims et al. (2008) found no evidence of inbreeding in this species. Together, these results suggest that selection for morphological variation within a brood may indeed be adaptive by 1) promoting habitat

segregation within a brood to minimize inbreeding and competition among siblings and 2) increasing the range of habitats and/or conditions that a brood can tolerate to spread the mortality risk during environmental fluctuations. We need to further explore the scale over which these diverging patterns in fin phenotype occur and assess the ability of individuals to make decisions on where and how far to disperse based on morphology.

Intraspecific variation in locomotor traits can facilitate range expansions and promote speciation. For example, Caribbean *Anolis* lizards have speciated largely based on their ability to exploit novel habitats via phenotypic divergence in their limb lengths (Losos 1990 a,b). Similarly, the morphological variability and physiological plasticity displayed by *A. polyacanthus* may be a primary explanation for its widespread distribution throughout the Great Barrier Reef and Coral Sea. *Acanthochromis polyacanthus* lacks a pelagic larval stage, and is now severely limited in its capacity for long-distance dispersal across its range (Doherty et al. 1994; Bay et al. 2008). However, during the last glacial maximum 20 000 years ago, the GBR and Coral Sea were connected by contiguous areas of shallow water promoting long-distance dispersal in *A. polyacanthus* (Miller-Sims et al. 2008). Recent research on this species has found wide physiological tolerances to hypoxia, temperature and pH conditions in the laboratory (Nilsson et al. 2007, Donelson et al. 2011, Rummer et al. 2013). The ability to disperse combined with flexible phenotypes may have allowed *A. polyacanthus* to successfully colonize habitats across a broad range of thermal, oxygen, pH and wave environments. Once sea levels began to rise, populations diverged genetically over time. This process can eventually lead to speciation if plastic traits become fixed after reproductive isolation (West-Eberhard 2005). However, plasticity may still be adaptive within this system given that environmental conditions in coral reef habitats fluctuate dramatically over small scales.

Climate change studies largely focus on the impacts of stressors like temperature and pH on marine organisms (e.g. Munday et al. 2012; Koch et al. 2013). However, the frequency and intensity of severe storms and weather systems are increasing, and pose serious challenges for coastal marine systems (e.g. Emanuel 2005, Webster et al. 2005; Harley et al. 2006). Water motion has known effects on patterns of species assemblages (Depczynski and Bellwood 2005, Fulton et al. 2005). Mechanisms promoting

adaptations to water flow in widespread species are less understood. High-speed swimming is important in mobile species during severe weather events as individuals must swim fast to avoid being swept away. Studies in temperate systems suggest that water flow fluctuations are energetically costly for fishes (Enders et al. 2003, Roche et al. 2013b). We found that exposure to water motion throughout ontogeny enhances swimming performance by 20-30% compared to individuals with limited experience in flowing water. We did not directly test for adaptations to predicted storm surges. Nonetheless, faster swimming speeds and higher aerobic scope would surely be advantageous for individuals during severe weather events. Do other widespread species show similar adaptive abilities? Can coral reef fishes more generally respond plastically to water flow? These questions remain unanswered. Nevertheless, the ability of *A. polyacanthus* to adapt physiologically to changes in water flow suggests some reef species may be resilient to certain aspects of climate change, and may help managers prioritize and target species and habitats for monitoring and protection.

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Table 1. Physiological and morphological traits measured in *A. polyacanthus* collected from sites around Lizard Island (± SE): fish mass (M), total length (BL), mean pectoral fin aspect ratio after four (4 month AR) and eight months (8 month AR), gait transition speed ($U_{p\text{-}c}$), critical swimming speed (U_{crit}), maximum metabolic rate (MMR), aerobic scope for swimming (AS) and proportion of blood hematocrit (Hct), number of fish measured per site and treatment (N).

Birth site	Treatment	M (g)	BL (cm)	4 months AR	8 months AR	U_{pc} (BL s^{-1})	U_{crit} (BL s^{-1})	MMR (mg O_2 kg^{-1} h^{-1})	AS (mg O_2 kg^{-1} h^{-1})	Hct
N	-	8	8	27	25	8	8	8	8	8
Sheltered	Calm	18.4 ± 0.5	10.6 ± 0.1	1.26 ± 0.08	1.28 ± 0.07	3.05 ± 0.06	3.56 ± 0.06	446.83 ± 23.66	293.92 ± 19.93	0.30 ± 0.03
Sheltered	Water flow	18.4 ± 0.8	10.2 ± 0.2	1.24 ± 0.06	1.23 ± 0.07	3.51 ± 0.09	4.15 ± 0.05	523.56 ± 21.03	402.05 ± 23.60	0.39 ± 0.02
Exposed	Calm	20.8 ± 0.6	10.8 ± 0.1	1.25 ± 0.09	1.27 ± 0.07	3.02 ± 0.08	3.66 ± 0.09	436.73 ± 14.38	285.61 ± 11.36	0.31 ± 0.02
Exposed	Water flow	18.2 ± 0.9	10.1 ± 0.1	1.30 ± 0.09	1.28 ± 0.06	3.56 ± 0.70	4.16 ± 0.06	542.06 ± 14.13	393.16 ± 16.12	0.43 ± 0.04

Table 2. Pectoral fin shape metrics for all 16 *A. polyacanthus* broods collected from leeward (sheltered) and windward (exposed) sites surrounding Lizard Island after 8 months of experimental rearing. The number of fish fins measured (N), range of fin aspect ratios (Min-Max AR) and the mean aspect ratio with standard deviation (Mean AR ± SD) are presented.

Brood	N	Min-Max AR	Mean AR ± SD
Sheltered			
1	11	1.11 - 1.40	1.26 ± 0.11
2	9	1.10 - 1.39	1.25 ± 0.09
3	10	1.17 - 1.37	1.28 ± 0.07
4	15	1.11 - 1.43	1.25 ± 0.09
5	9	1.12 - 1.48	1.24 ± 0.13
6	12	1.18 - 1.43	1.31 ± 0.08
7	10	1.15 - 1.32	1.22 ± 0.06
8	16	1.09 - 1.57	1.25 ± 0.13
Exposed			
1	14	1.12 - 1.39	1.27 ± 0.08
2	14	1.11 - 1.41	1.25 ± 0.10
3	10	1.14 - 1.36	1.24 ± 0.08
4	7	1.12 - 1.33	1.23 ± 0.09
5	12	1.12 - 1.43	1.23 ± 0.09
6	17	1.12 - 1.45	1.25 ± 0.10
7	8	1.14 - 1.33	1.24 ± 0.07
8	14	1.10 - 1.37	1.23 ± 0.08
All fish	188	1.09 – 1.57	1.25 ± 0.02

Figure 1. Average (± SE) (A) gait-transition ($U_{p\text{-}c}$) and (B) critical (U_{crit}) swimming speeds, (C) aerobic scope (oxygen consumption rate; $\dot{M}O_2$), (D) oxygen carrying capacity (Haematocrit) for *Acanthochromis polyacanthus* from windward (exposed habitats; grey bars) and leeward (sheltered habitats; white bars) sites in wave and calm rearing treatments. Letters (a,b) indicate significant differences between groups.

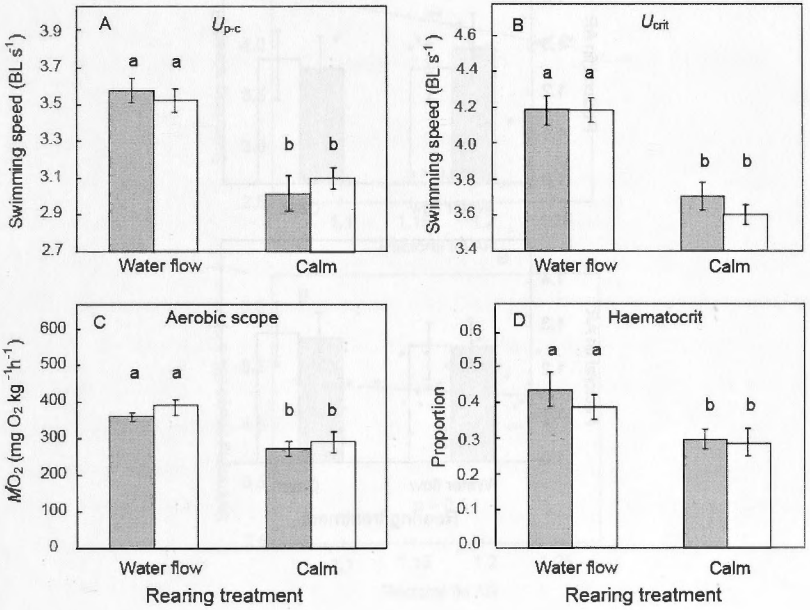


Figure 2. Average (± SE) pectoral fin aspect ratio (AR) of *A. polyacanthus* after (A) four and (B) eight months of rearing in either wave or calm treatment tanks. Grey bars represent fish captured from windward (exposed) sites, and white bars represent those captured from leeward (sheltered) sites.

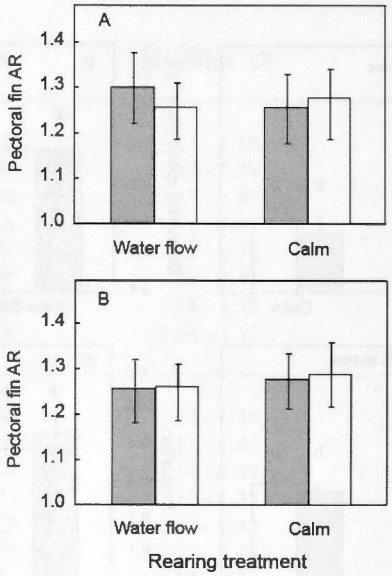
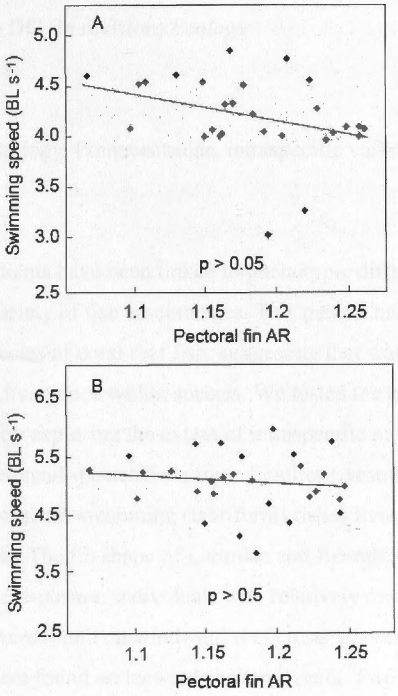


Figure 3. Correlation between pectoral fin aspect ratio (AR) and critical swimming speeds of *A. polyacanthus* after (a) four (U = -2.7 AR + 7.4; r = -0.35, $P > 0.05$) and (b) eight (U = -1.2 AR + 6.4; r = -0.12, $P > 0.5$) months.



APPENDIX - V

Intraspecific phenotypic diversity in fin shape across water flow gradients in labriform swimming fishes

Binning SA and **Roche DG** (in revision) *Ecology*

Keywords

Aspect ratio, eco-morphology, Pomacentridae, intraspecific variation, polymorphism

Abstract

Water flow gradients have been linked to phenotypic differences and swimming performance across a variety of fish assemblages. This pattern has also been described within a widespread species of coral reef fish, suggesting that water motion can shape patterns of phenotypic divergence within species. We tested the generality of this functional relationship by exploring the extent of intraspecific morphological divergence within widespread species from three families (Acanthuridae, Labridae, Pomacentridae) of pectoral-fin swimming (labriform) fishes living across localized wave exposure gradients. The fin shape of Labridae and Acanthuridae species was strongly related to wave exposure: individuals with relatively more tapered, higher aspect ratio (AR) fins were found on windward reef crests whereas individuals with rounder low AR fins were found on leeward sheltered reefs. Two species of Pomacentridae showed similar trends, but there was no family-level effect of wave exposure on fin shape even when species employing drag-based rowing were excluded. However, there was a significant overall trend in the Pomacentridae family when fin shapes of three species were compared across flow habitats at very small spatial scales (< 100 m) at different depths. Contrary to our predictions, patterns of species abundance were unrelated to trends in fin shape variation within species. These results suggest that functional relationships among swimming phenotype and water flow are at least partially responsible for shaping phenotypic divergence within widespread species of coral reef fishes at multiple spatial scales. However, some species may have behavioural mechanisms that enable their persistence across a range of flow habitats in the absence of morphological differences.

Introduction

Organisms must adapt their phenotype to challenges imposed by the environment. Widespread species face additional pressure as environmental conditions are variable through space and time, and morphological adaptations to one habitat may be maladaptive in another. This can lead to patterns of differentiation within a species if finding the right fit between phenotype and environment influences performance in a given habitat (e.g. Smith and Skulason 1996; Puebla 2009).

In shallow marine environments, wave-driven water flow is an important environmental stressor requiring specific adaptations to maximize fitness (Kaandorp 1999; Denny and Gaylord 2002). Fishes can use a variety of body regions and fin appendages for swimming, but most tend to use a small subset of these available locomotor modes for their daily activities (e.g. Fulton 2007). As a result, fishes can be broadly categroized into two distinct swimming modes: (1) body and caudal fin swimmers are powered by movement of the caudal fin and the posterior half of the body, (2) median-paired fin (MPF) swimmers generate thrust through movements of their pectoral (paired) or anal and dorsal (median) fins while maintaining the body and tail rigid (Webb 1994; Sfakiotakis et al. 1999; Blake 2004). For fishes using their pectoral fins to generate thrust (labriform MPF swimmers), the distribution of species on coral reefs is related to differences in fin shape across water-flow gradients (e.g. Bellwood and Wainwright 2001; Bellwood et al. 2002; Fulton et al. 2005). Species with tapered, high aspect-ratio (AR) pectoral fins tend to use lift-based thrust similar to flapping birds to efficiently sustain high swimming speeds and occupy high water flow habitats (Vogel 1994; Walker and Westneat 2000; Bellwood et al. 2002; Walker and Westneat 2002a). In contrast, species with rounded, low AR fins use a rowing motion to produce drag-based thrust, which promotes manoeuvrability (Walker and Westneat 2000). These species typically occupy sheltered, low flow habitats (Walker and Westneat 2002a). Recently, these functional rules were found to apply within a single species of coral reef fish, *Acanthochromis polyacanthus* (Binning et al. 2013; Fulton et al. 2013). This species displays pronounced differences in fin shape across a 40 km cline in wave-driven water flow across the Great Barrier Reef continental shelf (Fulton et al. 2013). The same intraspecific patterns in fin shape related also occur at an even smaller scale: fish collected from windward reef crests where wave action is high have tapered fins, swim faster, have a higher metabolic rates and a larger aerobic scope than

conspecifics from sheltered leeward sites less than 7 km away (Binning et al. 2013). The small spatial scale over which these patterns occur is remarkable, and suggests that fin shape could be a relatively flexible trait in coral reef fishes.

Wave energy can vary across very fine spatial scales, especially on coral reefs that are commonly exposed to wind-driven waves. As waves approach the shore and interact with the reef substrate, the majority of the flow velocity is attenuated within the first 50 m (Madin et al. 2006). Water motion also attenuates with depth. For example, reef slope habitats at 9 m depth experience dramatically lower flow regimes than shallow reef crests at 3 m (Fulton and Bellwood 2005). These physical properties of reef habitats create dramatic gradients in water flow between the hydrodynamically disturbed reef crests and the comparatively calm reef slope and lagoonal habitats only meters away (Fulton and Bellwood 2005; Madin et al. 2006). If water motion is the driving force behind intraspecific differences in fin shape and swimming performance, we would expect similar morphological variation within species that occupy different depths on the same reef. To date, these morphological patterns have not been investigated across depth gradients, and the only reports of intraspecific variation in fin shape in coral reef fishes are in a damselfish species that exhibits a peculiar life history, *A. polyacanthus*. This species is unusual among reef fishes in that it lacks a pelagic larval phase, and therefore has limited long-distance dispersal abilities (Doherty et al. 1994; Miller-Sims et al. 2008). As such, *A. polyacanthus* exhibits a high degree of population genetic structure at small scales (Doherty et al. 1994), making it difficult to generalize trends to other coral reef fishes with dramatically different life history strategies and dispersal capabilities.

A general trend for fin shape related to water motion may not be expected within all widespread species given the behavioural flexibility, importance of shelter use and different biomechanical swimming strategies of many reef fishes (e.g. Ménard et al. 2012; Heatwole and Fulton 2013). For example, flow refuging behind structures expands the swimming potential of some labriform fishes (Johansen et al. 2007; Johansen et al. 2008). Other slower-swimming species tend to remain close to the substratum, where flow velocities are attenuated due to boundary-layer effects. Rounded fins, which are better for manoeuvring, may also be more advantageous for navigating the complex reef habitat (e.g. Walker and Westneat 2000; Fulton et al. 2001; Gourlay

and Colleter 2005). For these species, increasing fin AR may not be advantageous since a more symmetrical fin shape is optimal for thrust generation when using a horizontal rowing stroke (Walker and Westneat 2000; Wainwright et al. 2002; Walker and Westneat 2002b). Some species can also recruit additional fins (e.g. pelvic, caudal) for stability and postural control when encountering high flow conditions (Lauder and Drucker 2004; Webb 2006; Heatwole and Fulton 2013). These strategies may circumvent the need for morphological adaptations that promote efficient swimming for exploiting high flow habitats. Whether morphological changes in fin shape are common intraspecific adaptations to different water flow regimes has not been tested across other species and families of labriform-swimming coral reef fishes with various life-history strategies.

Here, we evaluated the generality of intraspecific phenotypic divergence in widespread coral reef fishes by exploring patterns of fin shape divergence in 12 common species from three families of pectoral fin swimming (labriform) fishes on the Great Barrier Reef. We asked: (1) do most species display intraspecific morphological variation in fin shape across wave energy gradients; (2) is there evidence for morphological variation in fin shape across small (< 100 m) spatial scales; and (3) are patterns of species abundance across wave habitats related to intraspecific morphological variation? Our general prediction is that individuals from sites experiencing higher levels of incident water flow will display relatively higher AR pectoral fins than conspecifics from calmer habitats across all species and families. Given that different fin shapes are adaptive in different flow habitats, we also predicted that species displaying fin shape polymorphism should be found in equal abundance across flow regimes.

Materials and Methods

Study sites and species

This study was conducted between March 2010 and August 2013 at sites around Lizard Island, northern Great Barrier Reef, Australia (14° 40' S; 145° 28' E). For question (1), three sites located on reef crest habitats (2- 4 m depth) were chosen on the windward (wave exposed) and three sites on the leeward (sheltered) side of the island (Fig. 1). Patterns of water motion at these sites have been measured previously with wave height and water flow velocity differing more than 6-fold between sites of

different exposure during windy conditions (Fulton and Bellwood 2005; Heatwole and Fulton 2013): wave exposed sites experience average water flows of approximately 38 cm s^{-1}, whereas sheltered sites experience flows of about 6 cm s^{-1}. Windward and leeward sites around Lizard Island are relatively similar in terms of canopy cover, structural complexity, fish habitat use, and other (non-wave related) abiotic variables (Crossland and Barnes 1983; Goatley and Bellwood 2011; Heatwole and Fulton 2013). For question (2), two sites each on the reef slope (8 -10 m depth), crest (2 – 4 m depth) and back lagoon (1 – 2 m depth) were chosen on the windward side of the island. Similar gradients in water motion exist across this depth scale at these sites (Fulton and Bellwood 2005). Species were chosen based on their presence at all sampled sites at Lizard Island as well as their diversity in terms of life histories. The goal was to obtain a diverse and representative sample of widespread fishes rather than an exhaustive sampling of all potential species. In total, thirteen species were selected: Pomacentridae (7 species), Labridae (3 species) and Acanthuridae (2 species) (Table 1).

Fin morphology

Fishes for questions (1) and (2) were collected with hand spears or barrier nets then sedated with 10% Aqui-S solution and euthanized in ice slurry to measure pectoral fin morphology. Fish total length (± 1mm) was measured with a ruler. Pectoral fins were dissected at the base of the fin, spread out and pinned onto a foam sheet, fixed into position using a concentrated formalin solution (39% formaldehyde), and digitally photographed (Panasonic Lumix DX3) on gridded paper. The length of the leading edge and total fin surface area were measured using ImageJ software (v 1.43). Fin AR was then calculated as the length of the leading edge squared divided by the total fin area (Binning and Fulton 2011; Binning et al. 2013). In total, we measured fins from 806 adult individuals from 12 species (Table 1, 2).

Species abundance surveys

For questions (3), the abundance of adult individuals from all species of interest was recorded at all study sites by SCUBA divers using underwater belt transects (50 × 4 m). The sites surveyed encompassed a range of habitat types typical of mid-shelf reefs. Visual censuses were conducted on calm weather days (winds < 5 knots) on reef crests (1.5 – 4 m depth) for species in question (1) as well as on reef slopes (8-10 m depth) and back flat (1.5-3 m depth) for specied in question (2). All adult individuals

(displaying adult coloration and/or > 5 cm total length) were counted for each species in four replicate transects at each site covering 800 m^2 of habitat per site.

Statistical analysis

We used three linear mixed effect models (LMM: lme4 package in R) to compare fin morphology (AR) among: (a) wave exposure and fish family (fixed factors) controlling for fish species and site (random factors), (b) wave exposure and Pomacentridae species (fixed factors), controlling for site; and (c) reef habitat and Pomacentridae species (fixed factors) controlling for site. Model assumptions were verified with diagnostic plots of residuals vs. fitted values. All analyses were performed in R v2.15.2.

Results

Fin morphology

The effect of wave exposure on fin AR differed among fish family (LMM exposure × family: $\chi^2_{(2)} = 10.67$; $P < 0.01$). However, when rowing MPF swimmers (*P. amboinensis* and *P. moluccensis*) were removed from the analysis, this interaction was non-significant ($\chi^2_{(2)} = 5.27$, $P > 0.05$; Fig. 1, Table 1), and there was an overall trend for species with more tapered fins to be found in exposed habitats across all three labriform families (LMM: exposure $\chi^2_{(1)} = 12.05$, $P < 0.001$; family $\chi^2_{(2)} = 3.30$, $P > 0.10$).

Within the Pomacentridae family, the effect of wave exposure on fin AR differed among species (LMM exposure × species: $\chi^2_{(6)} = 53.69$, $P < 0.001$; Fig 2). Excluding rowers from this analysis did not change the results qualitatively.

For Pomacentridae sampled across different depths at windward sites, the effect of depth differed among species (LMM depth × species: $\chi^2_{(4)} = 33.05$, $P < 0.001$; Fig. 3, Table 2). However, this pattern was driven by large differences in fin AR among the species sampled (LMM species $\chi^2_{(2)} = 1361.01$, $P < 0.001$; Fig. 3). Overall, the intraspecific trend in fin shape variation was similar across all species: higher AR fins were found in crest habitats whereas lower AR fins were found in slope and lagoonal habitats (LMM: depth $\chi^2_{(2)} = 39.04$, $P < 0.001$; Fig 3).

Species abundance surveys

Fish abundance varied across species and sites. Some species were more abundant in exposed, windward habitats (*Acanthurus nigrofuscus, Ctenochaetus striatus, Thalassoma jansenii, Pomacentrus lepidogynes Chromis atripectoralis*); others were more abundant in sheltered leeward habitats (*Amblyglyphidodon curacao, Pomacentrus moluccensis, Halichoeres melanurus*); the remaining species were equally abundant across both windward and leeward sites (*Abudefduf whitleyi, Acanthochromis polyacanthus, Pomacentrus amboinensis, Hemigymnus melapterus;* Fig. 4a,b). Even at windward sites, the abundance of damselfish varied across slope, crest and lagoonal habitats (Fig. 4c). The patterns of fish species abundance across flow habitats did not correspond to patterns of intraspecific variation in fin shape (Table 1,2; Fig. 4).

Discussion

Water flow has well-established community-structuring effects in freshwater (e.g. Langerhans 2008; Niu et al. 2012; Belmar et al. 2013) and shallow marine (e.g. Leonard et al. 1998; Denny and Wethey 2001; Depczynski and Bellwood 2005; Fulton and Bellwood 2005; Madin and Connolly 2006; Bird 2011) habitats (but see Denny 2005). There is growing evidence also suggesting that water flow influences patterns of intraspecific phenotypic variation in freshwater fishes and marine invertebrates (e.g. Langerhans et al. 2003; Peres-Neto and Magnan 2004; Langerhans 2008; Todd 2008; Rouleau et al. 2010; Hayne and Palmer 2013). Recent studies have shown phenotypic divergence in pectoral fin morphology within one widespread species of damselfish across water flow gradients at both regional and local scales (Binning et al. 2013; Fulton et al. 2013). Here, we show that close functional relationships among flow habitat and swimming morphology can be generalized to include other widespread species of labriform swimming fishes. We found that, in general, individuals collected from high flow areas had more tapered fins whereas individuals in sheltered habitats tended to have rounder fins. This was true even for individuals collected across different depths on the same continuous reef. Nevertheless, this trend was weaker in the Pomacentrid family, and fin shape variation was a poor predictor of species abundances across sites suggesting that other mechanisms are responsible for enabling the persistence of some species across a range of flow habitats.

Labriform propulsion is the primary swimming mode for a wide diversity of marine and freshwater fishes (Westneat 1996; Drucker et al. 2005). Within this mode, however, a range of morphological, structural and musculoskeletal differences exist between species (Wainwright et al. 2002; Drucker et al. 2005; Thorsen and Westneat 2005). In general, fishes with high AR fins produce lift-based thrust to power swimming, similar to the wing flapping motion of birds (Vogel 1994; Walker and Westneat 2002a). This form of swimming is advantageous in fast-moving water since high AR fins increase the lift-to-drag ratio on the fin's surface and enhance forward thrust production when flapping (Vogel 1994). Conversely, flapping fins are presumably less useful than drag-based rowing in low-flow habitats since generating thrust through lift is less effective at low speeds (Vogel 1994). Tapered, flapping fins are a derived trait that has arisen independently multiple times in at least two perciform lineages, the Acanthuuroidei and Labroidei (Drucker and Lauder 2002; Quenouille et al. 2004; Thorsen and Westneat 2005). It is possible that tapered pectoral fins morphology adapted for high wave energy environments, having arisen later in evolutionary time, are more adaptable to changes in the environment than previously thought.

Within-species differences in fin AR observed in this study likely enhance the swimming performance of individuals in their chosen flow habitats. These patterns are consistent with trends documented across species (Bellwood and Wainwright 2001; Bellwood et al. 2002; Fulton et al. 2005): in flapping species, individuals with higher AR fins were generally associated with high-flow, windward habitats whereas individuals with relatively lower AR fins were associated with low-flow, leeward habitats. These results suggest that fin shape variation is an adaptation to wave habitat in widespread species from these families. Interestingly, transect data did not support our initial prediction regarding species abundances. Since having the right fin shape for a given wave environment should be advantageous, we predicted that species displaying fin shape variation across habitats should be equally abundant in both sheltered and exposed habitats. Conversely, species without fin shape variation should be relatively more abundant in the habitat for which they are morphologically better suited. These predictions were true of some species. For example, *A. polyacanthus* does have distinct fin morphologies and is equally abundant across windward and leeward sites (Fig. 1a, 3) (Binning et al. 2013). Similarly, *P. moluccensis* has rounded fins in all habitats, and is more abundant at sheltered, leeward sites (Fig. 1a, 3). However, for most species,

abundance was unrelated to patterns of fin shape variation (Fig. 1). Factors other than fin shape are clearly important for species persistence across habitats.

A general trend between morphology and habitat was not observed in Pomacentridae despite variation in the fin shapes of two species, *Acanthochromis polyacanthus* and *Pomacentrus lepidogynes*, as predicted by their wave habitat (Fig. 2). This was the case even when the two rowing species, *P. amboinensis* and *P. moluccensis*, were removed from the analysis. These species produce drag-based thrust by rowing their large, rounded fins, even in wavy crest habitats despite the disadvantage of this swimming style in high-flow conditions. A variety of behavioural strategies may help explain the ability of Pomaentridae to colonize a wide range of flow habitats despite an apparent lack of morphological flexibility. Some damselfishes commonly refuge in shelters during the day and at night (Aguilar-Medrano et al. 2012; Ménard et al. 2012). This behaviour significantly reduces the flow experienced by individuals, and allows fish to inhabit high flow areas well beyond their maximum sustained swimming abilities (Johansen et al. 2007; Johansen et al. 2008). The extent of flow refuging depends on swimming ability, with slower species refuging more frequently than faster ones (Johansen et al. 2008). Similarly, pectoral fin morphology in damselfishes is highly related to species-specific behaviours such as territoriality and foraging (Aguilar-Medrano et al. 2012). Given the ability of some species to exploit flow refuges when needed, there may be less selection pressure on demersal damselfishes for morphological or physiological adaptations that promote sustained swimming in high-flow habitats. Moreover, high-speed bursts and manoeuvring within the complex reef habitat when chasing intruders or escaping attacks may make rowing in round-fined, territorial species such as *Pomacentrus amboinensis* and *Pomacentrus moluccensis* useful regardless of the overall flow structure of the habitat. Conversely, planktivorous species such as *Abudefduf whitleyi, Amblyglyphidodon curacao* and *Chromis atripectoralis* are lift-based flappers and have relatively tapered fins in all habitats, which is likely advantageous for occupying positions high in the water column, where speed, either to hold station or move throughout the environment, is favoured over manoeuvrability (Aguilar-Medrano et al. 2012). Additionally, the schooling behaviour common in *A. whitleyi* and *C. atripectoralis* may also reduce energy consumption in high flow habitats, again expanding the swimming potential of these species (Johansen et al. 2010).

Although *A. curacao* and *P. amboinensis* did not exhibit differences in fin shape across leeward and windward sites, predicted patterns of fin shape relative to water flow were observed across small spatial scales in all three damselfish species tested. These differences were less pronounced in both *P. amboinensis* (13 % difference between extremes) and *A. curacao* (18 % difference) than in *A. polyacanthus* (29 % difference). Nonetheless, these results suggest that individuals may be able to select their wave energy habitat based on fin shape when sites experiencing vastly different hydrodynamic environments are contiguous: individuals with more tapered fins can settle on the wavy reef crest whereas individuals with less tapered fins may be able to colonize low-flow habitats simply by moving deeper or further away from the reef crest. Conversely, disruptive selection on fin shape may lead to different mean trait distributions across wave environments as we observed. In *A. polyacanthus,* parents produce juveniles with a range of fin shapes within a single brood regardless of their flow habitat (Binning, unpublished data). It is unknown to what extend fin shape is heritable in species with pelagic larvae, but differences may reflect diverse strategies related to pelagic larval duration and/r recruitment to natal vs. foreign reefs. Selection for morphological variation within a brood may be adaptive for species with low levels of self-recruitment. This variation could increase the range of habitats and/or conditions that a brood can tolerate to spread the mortality risk during environmental fluctuations (e.g. Morrongiello et al. 2012). In contrast, we may also expect high levels of heritability in fin shape in species that commonly self-recruit and where a range of wave habitats is not available. These hypotheses remain to be tested as does the ability of individuals to make decisions about where and how far to disperse based on their morphology.

Shallow marine environments are threatened by changes in a range of abiotic conditions as a result of climate change (e.g. Harley et al. 2006). In addition to stressors such as changes in temperature and water pH, fishes are experiencing fluctuations in their physical flow environment, with the frequency and intensity of severe storms and weather events increasing across ocean basing globally (see Harley et al. 2006). Swimming in oscillating water flow is energetically costly for both BCF and MPF fishes when holding station (Enders et al. 2003; Roche et al. 2013). Tapered fins promote efficient swimming across a wide range of swimming speeds and are

particularly well suited for survival in high flow habitats (Bellwood and Wainwright 2001; Walker and Westneat 2002b; Fulton et al. 2005). Many studies have demonstrated that wave-driven water flow is a major structuring force on marine organisms in shallow habitats, and the ability to withstand these forces strongly influences species distributions. We show that these functional rules also apply to populations of widespread species inhabiting distinct flow habitats across multiple scales. Importantly, we demonstrate the potential for a range of species to finely partition their microhabitats based on flow structure and fin shape. The extent to which fin shape is heritable, or plastic within species remains to be tested, but would shed further light on the importance of water-motion in shaping community and population structure, and the potential for species to rapidly modify their phenotype to climate-induced changes in water motion.

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Figure 1. Bar charts representing mean (± 95% CI) pectoral fin aspect ratios pooled across species from three families of fishes sampled at three windward (dark bars) and three leeward (light bars) sites around Lizard Island. Bars that do not overlap represent statistically significant differences. Illustrations are of a representative species from each family (Acanthuridae: *Acanthurus nigrofuscus*; Labridae: *Thalassoma jansenii*; Pomacentridae: *Chromis atripectoralis*)

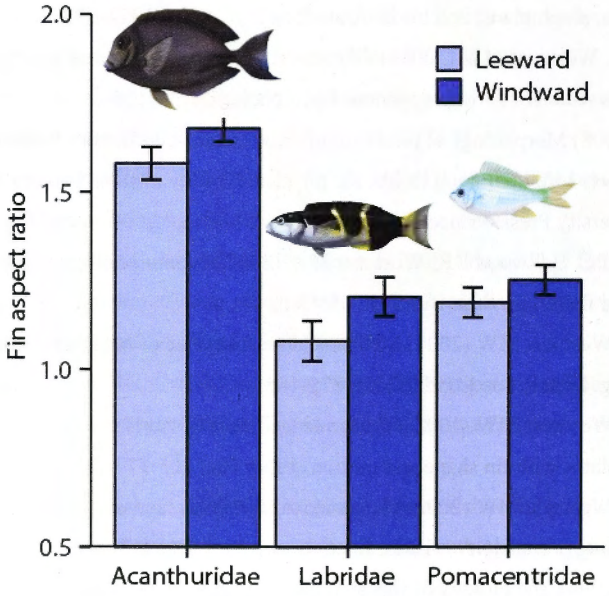

Figure 2. Bar charts representing mean (± 95% CI) pectoral fin aspect ratio of all species of Pomacentridae sampled from exposed windward (dark bars) and sheltered leeward (light bars) reef crest habitats. Dashed line separates species using life-based flapping (Flappers) from those using drag-based rowing (Rowers) forms of labriform locomotion. Bars that do not overlap represent statistically significant differences.

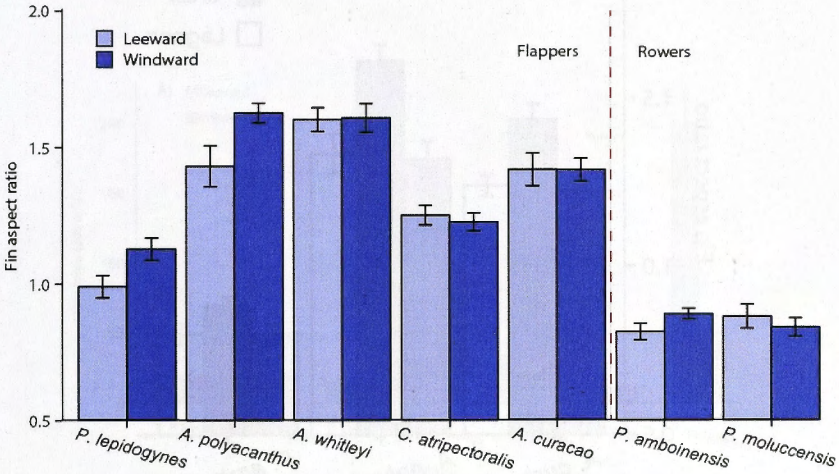

Figure 3. Bar charts representing mean (± 95% CI) pectoral fin aspect ratio of three species of Pomacentridae sampled from windward sites in three distinct habitats: exposed reef crests (dark bars), deeper reef slopes (light bars) and sheltered reef lagoons (white bars). Bars that do not overlap represent statistically significant differences.

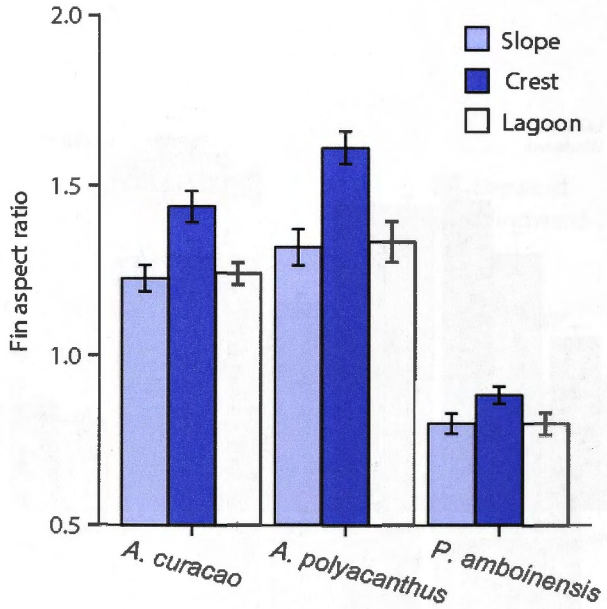

Figure 4. Bar charts of mean species abundance (± SE) recorded on transects around Lizard Island from reef crests (2 - 4 m) at leeward (light bars) and windward (dark bars) sites, or windward sites on the reef slope (8 – 10 m; light bars), reef crest (2 – 4 m; dark bars) and back lagoon (1 – 2 m; white bars) A) Pomacentrids were separated into species using lift-based flapping (Flappers) and drag-based rowing (Rowers; separated by dashed line) B) Labrid and Acanthurid species (separated by dashed line). C) Additional Pomacentrids sampled across distinct reef habitats at windward sites.

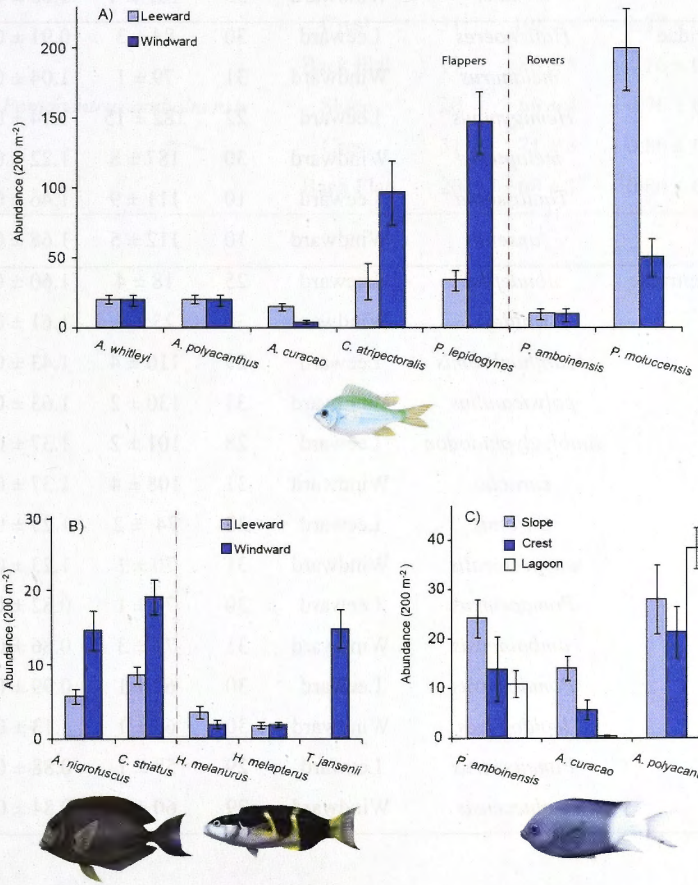

Table 1. Sample size (N) and average (± s.e.m.) fish total length (TL) and pectoral fin aspect ratio (AR) for 12 species from three families of widespread coral reef fishes from high flow windward and low flow leeward sites around Lizard Island.

Family	Species	Site	N	TL (mm)	AR
Acanthuridae	*Acanthurus*	Leeward	31	143 ± 3	1.69 ± 0.04
	nigrofuscus	Windward	31	163 ± 2	1.75 ± 0.02
	Ctenochaetus	Leeward	32	177 ± 5	1.49 ± 0.02
	striatus	Windward	33	169 ± 4	1.60 ± 0.02
Labridae	*Halichoeres*	Leeward	30	84 ± 3	0.91 ± 0.02
	melanurus	Windward	31	79 ± 1	1.04 ± 0.02
	Hemigymnus	Leeward	22	182 ± 15	1.14 ± 0.02
	melapterus	Windward	30	187 ± 8	1.22 ± 0.01
	Thalassoma	Leeward	10	111 ± 9	1.46 ± 0.05
	jansenii	Windward	10	112 ± 5	1.68 ± 0.05
Pomacentridae	*Abudefduf*	Leeward	25	18 ± 4	1.60 ± 0.02
	whitleyi	Windward	30	23 ± 4	1.61 ± 0.03
	Acanthochromis	Leeward	29	110 ± 4	1.43 ± 0.03
	polyacanthus	Windward	33	130 ± 2	1.63 ± 0.02
	Amblyglyphidodon	Leeward	28	101 ± 2	1.37 ± 0.03
	curacao	Windward	31	108 ± 4	1.37 ± 0.05
	Chromis	Leeward	32	74 ± 2	1.25 ± 0.02
	atripectoralis	Windward	31	70 ± 1	1.23 ± 0.02
	Pomacentrus	Leeward	30	71 ± 1	0.82 ± 0.01
	amboinensis	Windward	31	71 ± 3	0.86 ± 0.03
	Pomacentrus	Leeward	30	62 ± 1	0.99 ± 0.02
	lepidogynes	Windward	30	63 ± 1	1.13 ± 0.02
	Pomacentrus	Leeward	29	59 ± 1	0.88 ± 0.02
	moluccensis	Windward	29	60 ± 1	0.84 ± 0.02

Table 2. Sample size (N) and average (± s.e.m.) fish total length (TL) and pectoral fin aspect ratio (AR) for three Pomacentrid fishes collected on the reef slope (8-10 m), crest (3-5 m) and back flat (1-2 m) at windward sites at Lizard Island.

Species	Site	N	TL	AR
Acanthochromis polyacanthus	Slope	18	134 ± 8	1.26 ± 0.03
	Crest	33	130 ± 2	1.63 ± 0.02
	Back Flat	27	100 ± 5	1.27 ± 0.03
Amblyglyphidodon curacao	Slope	22	114 ± 6	1.19 ± 0.06
	Crest	31	108 ± 4	1.37 ± 0.05
	Back Flat	19	86 ± 5	1.16 ± 0.07
Pomacentrus amboinensis	Slope	20	66 ± 4	0.76 ± 0.04
	Crest	31	71 ± 3	0.86 ± 0.03
	Back Flat	20	68 ± 1	0.80 ± 0.01

APPENDIX - VI

Ectoparasites modify escape behaviour, but not performance, in a coral reef fish

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Keywords

Escape kinematics, Great Barrier Reef, parasite infection, predation risk, reaction distance, risk aversion behaviour

Abstract

Survival depends on escape responses and when to flee a predator. As a result, factors affecting the escape performance of prey species, including parasite infection, may profoundly influence the outcome of predator-prey encounters. Several hypotheses predict the responses of prey to simulated predator attacks based on intrinsic characteristics such as individual reproductive value and flight costs: as predation risk and reproductive value increase, so should the distance at which an organism begins to flee an escaping predator (flight initiation distance; FID). Conversely, FID should decrease if the costs of fleeing are high. Despite providing testable hypotheses, rarely have these theories been used to predict the escape behaviour of parasitized individuals. The bridled monocle bream (*Scolopsis bilineata*) is parasitized by a large Cymothoid isopod (*Anilocra nemipteri*), which attaches above the eye. In this species, ectoparasite infection is associated with increased energy costs and decreased endurance. We investigated the effects of infection on escape performance and FID. Maximum velocity, maximum acceleration, cumulative distance travelled and response latency did not differ among parasitized fish, unparasitized fish and fish that had their parasite experimentally removed. Parasitized fish were smaller, on average, than unparasitized individuals. Smaller, parasitized individuals allowed a threat to approach closer before fleeing (shorter FID) than larger parasitized or uninfected individuals. Since parasite infection has known effects on host growth and metabolism, we suggest that parasitism alters fish escape behaviour as predicted by two non-exclusive hypotheses: 1) by decreasing reproductive value (the asset-protection hypothesis) and 2) by increasing the relative costs of fleeing (the economic hypothesis) compared with uninfected and large

parasitized fish. The relative importance of each hypothesis in driving the trends observed remains to be tested.

Introduction

When to escape from a predator is a key behaviour influencing the fitness of mobile species. This decision must consider the time, energy and lost opportunity costs associated with fleeing as well as intrinsic traits such as size, previous experience and measures of kinematic performance such as maximum achievable speed and acceleration (Domenici, 2010; Januchowski-Hartley, Graham, Feary, Morove, & Cinner, 2011, Lagos et al., 2009; Lima & Dill, 1990; Møller, Grim, Ibanez-Alamo, Marko, & Tryjanowski, 2013; Stankowich & Blumstein, 2005). Extrinsic factors (e.g. predator approach speed, ambient temperature, habitat complexity, distance to shelter) can also influence an individual's decision-making when evaluating whether an approaching organism constitutes a threat (Bonenfant & Kramer, 1996; Cooper, 2006; Dill & Houtman, 1989; Domenici, Claireaux, & McKenzie, 2007; Domenici, 2010; Møller et al., 2013; Stankowich & Blumstein, 2005). As a result, escape behaviours involve the complex integration of biotic, abiotic and locomotor variables that should optimize the ratio of benefits to costs of remaining versus fleeing (Cooper & Frederick, 2007; Cooper & Frederick, 2010; Domenici, 2010; Lima & Dill, 1990; Stankowich & Blumstein, 2005; Ydenberg & Dill, 1986). Consequently, even slight changes in an organism's ability to react to, evade, or out-run a predator can alter individual risk perception and decisions about when to flee an approaching threat.

Parasitic infection can dramatically affect host behaviour and physiology (Barber, Hoare, & Krause, 2000). Several studies have linked impaired escape responses (decreased reactivity or locomotor abilities) to parasitism in a range of animals (e.g. Barber, Walker & Svensson, 2004; Goodman & Johnson, 2011; Libersat & Moore, 2000; Møller, 2008; Perrot-Minnot, Kaldonski, & Cézilly, 2007; Seppälä, Karvonen, & Tellervo Valtonen, 2004). However, these studies generally focus on the effects of endoparasites, many of which have complex life cycles and rely on transmission from prey to predator for their own success (Barber et al., 2000; Poulin, 2010). Consequently, there is a conflict of interest between hosts and parasites regarding predation-relevant behaviours (i.e. parasite increased trophic transmission hypothesis; Barber et al. 2000; Lafferty, 1999; Poulin, 2010, 2013). Conversely, the

fitness of directly transmitted parasites, including many externally attached ectoparasites, is enhanced if the host can successfully flee from a predator. As a result, these parasites should be selected to minimize any negative effects on host predation risk (Barber et al., 2000), and hosts should make decisions that will optimize their energy expenditure and chance of escape during a predator encounter. However, ectoparasites may impose additional costs on hosts due to their relatively large size (e.g. Fogelman, Kuris, & Grutter, 2009; Grutter et al., 2011). This may be particularly true in aquatic species given the challenges of moving through relatively dense water (Vogel, 1994). Various studies have found that ectoparasites negatively affect the swimming performance of fish in part by increasing drag, suggesting that the ability to escape an approaching predator may be severally impaired (Grutter et al., 2011; Ostlund-Nilsson, Curis, Nilsson, & Grutter, 2005; Wagner, McKinley, Bjorn, & Finstad, 2003).

The predicted response of parasitized hosts to predator attacks is not necessarily obvious. Flight initiation distance (FID) is the distance at which an organism begins to flee an approaching predator and provides a reliable estimate of an animals' perception of fear or risk (Stankowich & Blumstein, 2005). On the one hand, parasitized fish may be slower than uninfected fish, and thus more vulnerable to predation. One prediction of the economic hypothesis proposed by Ydenberg and Dill (1986) is that FID should increase with higher predation risk. If parasitized fish are less able to outswim a predator once a chase is initiated, we might predict that parasitized individuals will initiate their escape response earlier in an attempt to put more distance between themselves and a predator. On the other hand, the physiological burden imposed by parasites on their hosts mean that the energetic and lost opportunity costs of initiating an escape are much greater relative to unparasitized individuals (Binning, Roche, & Layton, 2013, Godin & Sproul, 1988; Ostlund-Nilsson et al., 2005). The economic hypothesis also predicts that FID should decrease when the costs of fleeing are high (Ydenberg & Dill, 1986). As such, parasitized individuals should only engage in costly flight when a predator approaches close and the threat is high (see Godin & Sproul, 1988; Møller, 2008; Ydenberg & Dill, 1986).

Parasitized or heavily-parasitized fish are often smaller than similarly-aged uninfected or moderately infected hosts, and previous studies on FID in fishes suggest that size is an important predictor of FID, with small fish generally fleeing at a closer

distance to a threat (smaller FID) than larger individuals (Gotanda, Turgeon, & Kramer, 2009; Januchowski-Hartley et al., 2011; Miller et al., 2011). This phenomenon has been largely attributed to the asset-protection hypothesis, which predicts that as reproductive value increases, individuals should engage in less risky behaviours in order to protect their reproductive assets (Clark, 1994; Cooper & Frederick, 2007). In fishes, reproductive value typically increases with size (Reinhardt, 2002, Rogers & Sargent, 2001). Thus large fish should increase their FID compared to smaller, less fertile individuals. Consequently, parasites may indirectly decrease host FID through interactions with fish size. Parasites can also directly decrease host reproductive value independent of size by physically castrating hosts (e.g. Fogelman et al., 2009; Lafferty & Kuris, 2009). Based on this logic, parasitized individuals should wait longer before fleeing from a threat.

On the Great Barrier Reef, the cymothoid isopod *Anilocra nemipteri* parasitizes the bridled monocle bream, *Scolopsis bilineata*, with up to 30% of fish infected at some sites (Grutter, 1994; Roche, Strong, & Binning, 2013). This species is directly transmitted to its host, although mancae of some *Anilocra* species may use optional intermediate hosts before settling on a definitive host where they grow into adults (Fogelman & Grutter, 2008). A single isopod typically attaches to a fixed location on one side of the host's head where it breeds repeatedly and can live for several years (Brusca, 1981; Roche, Strong, et al., 2013; Fig. 1). Parasites can grow to 16% of the fish's total length and reduce host growth (Roche, Strong, et al., 2013). *Anilocra nemipteri* does not exhibit any side bias in attachment preference on either the left or right side of the host's body (Roche, Strong, et al., 2013). However, parasitized fish are more highly lateralized (i.e. have a stronger side preference) than unparasitized individuals (Roche, Binning, Strong, Davies, & Jennions, 2013), a behaviour which may enhance escape responses by decreasing reaction time (Dadda, Koolhaas, & Domenici, 2010). These parasites also impair the swimming ability of *S. bilineata*, mostly by increasing drag at high speeds (Binning et al., 2013). In addition, parasite infection increases energetic maintenance costs and decreases the overall aerobic performance of infected fish (Binning et al., 2013).

We measured the effects of *A. nemipteri*, on the fast-start escape performance and risk-taking behaviour of the bridled monocle bream, *S. bilineata*. In a laboratory

set-up, we filmed escape responses and measured escape performance in fish from three treatment groups: 1) parasitized fish, 2) unparasitized fish and 3) fish with their parasite experimentally removed (test for physiological effects of parasite in the absence of drag). In a field experiment, we then assessed risk-taking behaviour by measuring individual FID elicited by an approaching snorkeler in parasitized and unparasitized fish. Based on escape theory, we predict that parasitized fish should flee earlier than unparasitized individuals if the presence of the ectoparasite impairs host escape. However, if the parasite does not affect host escape performance, parasitised fish should conserve energy by engaging in more risk-prone behaviour than unparasitized individuals and allow an approaching threat to come closer before fleeing.

Materials and methods

Study site and animal collection

Parasitized ($N = 28$) and unparasitized ($N = 12$) *Scolopsis bilineata* were collected in February and March 2012 using monofilament barrier nets (10 mm stretched mesh) and silicone hand nets from sites around Lizard Island, Northern Great Barrier Reef, Australia (14° 40' S; 145° 28' E). Fish were transported in aerated 20 l Handy Pail buckets (maximum 4 fish per bucket) to the Lizard Island Research Station within 1 h of capture, and transferred with silicone nets to individual holding aquaria (40.0W × 29.0L × 18.0H cm) with a flow-through water system transported directly from the reef. Tanks were kept under a natural light and temperature regime (28 ± 1 °C). All fish were provided with a round PVC shelter for refuge. Fish were fed to satiation once a day with raw prawn and fasted for 24h prior to the experiments to ensure fish were in a standardized (post-absorptive) state that maximizes energy availability for swimming (e.g. Marras, Killen, Claireaux, Domenici, & McKenzie, 2011). Holding tanks were siphoned out daily to maintain high water quality. All animals were kept in aquaria for a minimum of three days before performing swim trials to ensure all fish were healthy. Animals were collected and cared for under Marine Parks Permit # G12/34805.1 issued by the Australian Government Great Barrier Reef Marine Park Authority and the Queensland Government with approval from the Australian National University Animal Experimentation Ethics Committee (permit #: A2012/02) according to the Australian Code of Practice for the Care and Use of Animals for Scientific Purposes, 7th edition 2007.

Fast-start experiments

We tested three groups of fish with different infection statuses: unparasitized (fish total body length BL; 13.2 ± 0.8 cm; mean ± SD, $N = 12$), parasitized (BL; 11.8 ± 1.1 cm; mean ± SD, $N = 16$) and parasite-removed (BL; 12.7 ± 1.3 cm; mean ± SD, $N = 12$). Parasites were removed using forceps 24h before the start of the fast-start experiments by holding the fish in a shallow water bath and gently unhooking the isopod (Binning et al., 2013). This procedure took approximately 45s. Fish resumed normal behaviour within 2h of being returned to their holding tanks. Experiments were conducted in rectangular acrylic aquaria (70.0 L × 60W × 35H cm) supported by a wooden stand. Water height was maintained at 12 cm for all trials. This height limited movement of the fish in the vertical plane (Langerhans, 2009). A mirror was inclined at a 45 ° angle below the aquarium to allow filming of the escape responses in 3 dimensions within the same camera frame. Videos where vertical movement occurred were excluded from the analyses (< 2 % of videos). The tank was illuminated by three 150 W spotlights positioned 70 cm above the water. A continuous flow of seawater maintained the water temperature at a constant 28 °C.

Prior to escape response trials, fish were transferred from their holding tanks to the experimental arena using silicone nets to prevent mucus loss and fin damage, and left undisturbed for 30 min to acclimate to the arena. Numerous aerial predators, including raptors and pelagic seabirds, are commonly seen feeding on fishes around Lizard Island. Thus we used a mechano-acoustic stimulus, which simulates an aerial attack, to induce escape responses in *S. bilineata* (Marras et al., 2011). We attached a 50 ml PVC container filled with lead weights with a string to a platform 30 cm above the water surface. To ensure that the escape response was not initiated prior to the stimulus' contact with the water, the stimulus fell inside an opaque 15 cm wide PVC tube positioned 1 cm above the water surface (Dadda et al., 2010; Marras et al., 2011). The time of contact of the stimulus with the water surface was clearly visible in the mirror from below. We filmed the responses at 240 Hz with a high speed digital camera (Exilim EX-FH100, Casio, USA) mounted on a tripod directly facing the aquarium and the mirror. Individuals were stimulated up to three times at 30 minute intervals (Marras et al., 2011). The stimulus was dropped when the fish was facing the stimulus at a distance of no more than 10 cm from the bottom of the PVC tube. After the trials, individuals were returned to their holding tanks. In total, we filmed 127 escape

responses in 40 fish. Two fish developed a bacterial infection several days following experimentation. These individuals were anesthetized with an overdose of Aqui-S solution and then euthanized in an ice-slurry. No other individuals showed any signs of sickness, and care was taken to prevent the spread of disease by rinsing nets in a freshwater bath prior to handling different individuals. All healthy fish (38 individuals) were released back to their site of collection within 1 week of trials.

We used the MtrackJ plugin in the ImageJ v. 1.43 software (Meijering, Dzyubachyk, & Smal, 2012) to analyse the escape sequences. We tracked the two-dimensional coordinates of the fish's centre of mass (CoM) every 4.2 ms starting 21 ms (5 frames) before and ending 84 ms (20 frames) after the onset of the fish's first movement. The CoM was visually estimated at a proportional distance from the tip of the head corresponding to approximately 29% of an individual's total length. The following escape performance metrics were measured (Domenici & Blake, 1997; Marras et al., 2011): response latency (time between stimulus onset and fish response in ms), size- adjusted cumulative distance travelled (D in fish total body lengths; BL), size-adjusted maximum escape speed (U_{max} in BL s^{-1}) and maximum acceleration (A_{max} in m s^{-2}). Distance–time variables (D, U_{max}, A_{max}) were evaluated within a fixed time period of 58 ms (14 frames), corresponding to the approximate mean duration of stages 1 and 2 of the escape response across all treatments (Dadda et al., 2010; Marras et al., 2011). A five-point moving quadratic polynomial regression (Lanczos, 1956) was used to obtain smoothed values of speed and acceleration, the first and second derivatives of distance (Marras et al., 2011). For each fish, the best value (e.g., highest U_{max} or shortest latency) of each escape performance variable across the three stimulus presentations were chosen for analysis (see Domenici, 2011; Marras et al., 2011).

Flight initiation distance

We estimated FID in *S. bilineata* from the lagoon and adjacent reefs in front of the Lizard Island Research Station on calm weather days from July-August 2013. Water depth varied between 2 and 3 m at these sites with a visibility of approximately 15 m. We used snorkelers as the stimulus for flight initiation. Many studies of FID in terrestrial and aquatic systems have used humans as a stimulus to elicit flight (e.g. Bonenfant & Kramer, 1996; Carter, Goldizen, & Heinsohn, 2012; Gotanda et al., 2009; Januchowski-Hartley, Nash, & Lawton, 2012; Lagos et al., 2009; Møller,

Nielsen, & Garamszegi, 2008; Perez-Cembranos, Perez-Mellado, & Cooper, 2013). Recent studies found FID estimates to be relatively robust to variation among observers regardless of experience (Guay et al., 2013) or, in aquatic systems, whether observations were made on snorkel or SCUBA (Januchowski-Hartley et al., 2012).

Two snorkelers swam around the reef in search of *S. bilineata*. Only solitary, adult individuals that were foraging or moving slowly over the reef in an open area where they could be approached directly were targeted. Individuals less than 1.5 m from branching corals or other shelter were not approached to avoid the confounding effects of distance to a refuge on FID (see Miller et al., 2011). Similarly, trials were abandoned if individuals began swimming in any direction at a consistent speed before the observer initiated their approach. Once a suitable individual was spotted, we recorded fish infection status (parasitized or unparasitized, hereafter referred to as treatment) and total length (± 1 cm; actual error). Before data collection, all observers practiced estimating fish length underwater using model fish and objects of various sizes until they reached a precision of ± 1 cm. One snorkeler positioned themselves in direct line of view of the fish at a distance of approximately 5 m. The other snorkeler positioned themselves off to the side to avoid obstructing the trial. The first snorkeler duck-dived under the water until they were close to the substrate (within 1 m), and visually relocated the individual. The snorkeler then approached the focal fish at a quick but steady swimming speed, holding two weights marked with flagging tape beside their head, which was assumed to be the onset of the stimulus. When the fish began to flee (i.e. first began to turn away from the approaching snorkeler), the snorkeler dropped one weight where they were, and took a visual landmark of where the fish had been, which was marked with the second weight. The two snorkelers then measured the horizontal distance between the two landmarks with a tape measure to the nearest 1 cm (FID). The observers also scored the strength of an individual's reaction, or flight intensity, on a scale from 0 – 4 as follows: 0: no response (i.e. fish did not move in response to the snorkeler), 1: fish ceased previous activities (i.e. foraging) and moved a short distance away, but did not leave the immediate area, 2: fish changed directions and began a slow displacement away from the area, 3: fish changed directions and fled the area at a fast, but constant speed, 4: fish initiated an escape response characterized by a "C-start" unsteady burst behaviour (Domenici & Blake, 1997). Increasing intensity was assumed to be related to more energetically costly forms of locomotion, and therefore provided an estimate of

the costs of flight. Recent studies suggest that *S. bilineata* are strongly site attached and rarely travel far from their small territories during the day (Boaden & Kingsford, 2013, 2012). Therefore, to avoid pseudoreplication, we never sampled two similarly sized fish with the same infection status within 25 m. In total, we measured FID from 104 adult fish ($N = 50$ parasitized; 12 ± 3 cm; mean BL ± SD, $N = 54$ unparasitized; 14 ± 3 cm; mean BL ± SD).

Statistical analysis

All analyses were performed in R v2.15.2 (R Development Core Team, 2012). Assumptions of the models were assessed with diagnostic plots and Shapiro-Wilks tests for both univariate and multivariate tests. Distance-time variables were normally distributed. Since D and U_{max} are size adjusted (fish BL), and fast-start acceleration is size-independent (see Domenici & Blake, 1997), we used a multivariate analysis of variance (MANOVA using Pillai's trace) to test for an overall difference in distance-time variables (D, U_{max}, A_{max}) among treatments (parasitized, unparasitized and parasite-removed) in the fast start experiment. Fast-start escape latency violated parametric model assumptions. Therefore we used a non-parametric Kruskal-Wallis rank sum test to assess differences in escape latency among treatments.

Flight initiation distance was square-root transformed to meet model assumptions. Fish body size and treatment were not independent (t-test: $t_{101} = -3.97$; $P < 0.001$). Therefore, we used an analysis of covariance (ANCOVA) with centred fish body size as a covariate to test for the effect of parasitism on FID while controlling for body size (Schielzeth, 2010). Flight intensity did not meet parametric model assumptions. Thus, we used non-parametric Wilcoxon signed-rank tests to examine the relationship between flight intensity and FID in parasitized and unparasitized fish. We also used non-parametric Spearman correlation to test for a relationship between flight intensity and fish length.

Results

Fast-start experiments

Twelve escape response trials did not elicit a response in fish, and were not included in the analysis (9.4 % of trials; 3 unparasitized, 4 parasitized, 4 parasite-removed). Fish infection status (treatment) did not affect overall escape performance in

our experiments (MANOVA: $F_{2,72} = 1.79$, $P = 0.11$; Table 1). There was also no difference in escape latency among fish from the three treatment groups (Kruskall-Wallis test: $\chi^2_2 = 3.07$, $P = 0.22$) (Table 1).

Flight initiation distance

Fish body size estimates from field observations differed between treatments: parasitized fish were significantly smaller than unparasitized fish (*t*-test: $t_{101} = -3.97$, $P < 0.001$, $r = 0.37$). There was an overall effect of treatment and fish body size on FID (ANCOVA: $F_{3,100} = 4.31$, $P = 0.01$, $r^2 = 0.33$). When comparing fish of the same average size, there was a marginally non-significant effect of treatment on FID, with parasitized fish tending to have smaller FIDs than unparasitized fish (treatment: $t_{100} = 1.9$, $P = 0.06$; Fig. 2). There was also a marginally non-significant interaction between fish size and treatment on FID (interaction: $t_{100} = -1.916$, $P = 0.06$).

There was no relationship between FID and flight intensity for either parasitized (Kruskall-Wallis test: test: $\chi^2_{41} = 44.1$, $P = 0.34$; Fig. 3a) or unparasitized fish (Kruskall-Wallis: $\chi^2_{42} = 49.8$, $P = 0.19$; Fig. 3b). Similarly, there was no difference in flight intensity between parasitized and unparasitized fish (Wilcoxon test: $N = 104$, W = 1283, $P = 0.63$, $r = 0.05$). There was no significant correlation between flight intensity and fish total length (Spearman correlation: $N = 104$, $P = 0.224$, $r = 0.12$).

Discussion

Escape behaviour is fundamental to the survival of mobile organisms when faced with a predator. Despite recent studies reporting decreased sustained swimming ability in parasitized fish (e.g. Binning et al., 2013; Grutter et al., 2011, Ostlund-Nilsson et al., 2005), we found that infection by large cymothoid ectoparasites did not impair the escape performance of *Scolopsis bilineata* in our experimental trials. During a simulated attack, parasitized fish were as likely to perform a characteristic fast-start response, and responded as quickly, as fast and travelled as far from the stimulus as healthy, unparasitized individuals. Similarly, when we removed the ectoparasite from infected individuals, performance did not differ from that of healthy fish. Binning et al. (2013) found that parasite removal restored overall prolonged swimming performance and aerobic capacity in *S. bilineata*, suggesting that the physiological effects of the parasite on its host are rapidly overcome. Our results support this conclusion, but also suggest

that large ectoparasites have no discernible impact on individual escape performance metrics. Fast starts are anaerobically powered, rapid accelerations followed by a change in direction, and tend to be mediated by large Mauthner-cell neurons in the brain (Domenici, Blagburn, & Bacon, 2011). This stereotyped reaction has implications for individual performance. Both locomotor and non-locomotor performance in fast-start behaviours tend to be repeatable through time, suggesting that escape performance is an intrinsic characteristic of an individual (Marras et al., 2011). Our results suggest that parasitic infection does not alter this intrinsic characteristic during a single attack, perhaps because single bursts are short behaviours that even sick fish can engage in effectively. When accelerating from rest, most energy is used to counteract the forces of inertia rather than drag, which is more important during prolonged swimming. Inertial forces should be similar for both parasitized and unparasitized individuals during burst behaviours, which may partly explain why no differences in maximum performance were found among treatments. However, recovery from energetically-costly burst activity depends on a fish's overall aerobic capacity, or aerobic scope. Parasitized *S. bilineata* have a smaller aerobic scope than unparasitized individuals (Binning et al., 2013), which suggests that repeated escapes or the ability to evade a predator during a sustained chase may be greatly impaired even if an individual's maximum kinematic performance abilities are not.

Parasitized fish, being more highly lateralized, should respond faster to a threat than unparasitized individuals (Roche, Binning et al., 2013). However, we did not find any advantage imparted by ectoparasites on escape latency. Our mechanical stimulus simulated an aerial attack by an avian predator. As *Anilocra nemipteri* attaches above the eye (Fig. 1), it may decrease the visual range or acuity of its host especially in response to an aerial stimulus. As a result, a strong side-bias may still elicit a more rapid response in parasitized individuals when facing a sudden attack in the same horizontal plane. The relative importance of lateralization in improving performance during aerial vs. aquatic attacks remains to be tested.

The effect of parasite infection on escape behaviour in the field was highly influenced by fish size. Overall, we found that large fish fled at a greater distance than small individuals, although this trend was much less apparent for unparasitised than parasitised fish (marginally non-significant interaction; see Fig. 2). This result is

generally consistent with previous studies on a range of coral reef fishes, which have also found increases in FID with fish total length (Gotanda et al., 2009; Januchowski-Hartley et al., 2011; Miller et al., 2011). The asset-protection hypothesis predicts that individuals with higher reproductive value should be more risk adverse, and therefore increase their FID (Clark, 1994; Cooper & Frederick, 2007). In fishes, fecundity, and thus reproductive value, increases dramatically with size (Reinhardt, 2002; Rogers & Sargent, 2001). Thus, we would expect to see greater risk-adverse behaviour (higher FID) in larger individuals. Interestingly, host growth is often adversely affected by parasite infection. The isopod *Anilocra apogonae*, infects the cardinalfish *Cheilodipterus quinquelineatus*, and effectively castrates its host by reducing gonad size, body length and weight compared to similarly-aged uninfected fish (Fogelman et al., 2009). At sites around Lizard Island, parasitized *S. bilineata* are on average 25 % smaller than unparasitized individuals, suggesting that *A. nemipteri* also stunts fish growth and reduces the reproductive value its host (Roche, Strong, et al., 2013). As a result, parasite infection may influence FID partially through its effect on fish size.

We predicted that, if there is no cost of parasitism on escape latency or distance derived kinematic traits, parasitized fish should wait until a predator has approached closer before fleeing. This prediction was based on the economic hypothesis and the assumed higher costs of flight in infected versus uninfected fish: as costs increase, FID should decrease (Ydenberg & Dill, 1986). For an average fish body size, there was a marginally non-significant effect of parasite infection on FID suggesting that the direct effect of parasites on FID is weak. However, the effects of parasitism on FID were particularly apparent for small fish, and less so for large fish. Small parasitized fish took more risks and fled at shorter FID than larger parasitized fish, which behaved more similarly to risk-averse uninfected fish of all sizes (Fig. 2). Metabolic costs are higher when fish are infected with ectoparasites (Binning et al., 2013; Grutter et al., 2011; Ostlund-Nilsson et al., 2005). This suggests that the observed relationships among size, parasite infection and FID might also be explained by flight and opportunity costs, which may be particularly important for small, parasitized individuals. As a result, parasite infection may alter risk-aversion behaviour in smaller parasitized individuals in two complementary ways: 1) by decreasing reproductive value and 2) by increasing the relative costs of fleeing compared to larger parasitized and/or unparasitized fish. Once flight was initiated, the intensity of the response was similar regardless of size or

infection status. We quantified flight intensity as the strength of the reaction, with higher intensities presumed to be more energetically costly than lower intensity reactions. As a result, it appears that fish trade-off the timing of the response rather than the intensity as way of modulating their escape behaviour. By reacting to only the most threatening stimuli, the risk-prone behaviour of small, infected fish is likely an energy-saving mechanism in the long run.

Although we have interpreted our results based on predictions derived from evolutionary theory, non-adaptive explanations for the patterns are also possible. Parasites may obstruct fish vision, and reduced FID may simply be a consequence of a decreased detection distance in smaller, infected fish. Visual acuity is also expected to increase with eye size, and thus fish size (McGill & Mittelbach, 2006). Therefore, it is possible that larger fish are able to detect an approaching predator and react earlier than smaller individuals. Isopods may also reduce host condition (e.g. Adlard & Lester, 1994; Fogelman et al., 2009), which may cause individuals to behave in non-adaptive ways.

Parasitism, by definition, imposes a cost on hosts. Consequently, infected individuals must develop strategies that minimize these costs while ensuring their own survival. We found that kinematic escape performance was unaffected by parasitism. However, small, parasitized *S. bilineata* behave differently than larger parasitized fish and unparasitized conspecifics when facing a threat in the field. Small, infected fish engage in riskier behaviour, probably as a result of their increased costs of fleeing and/or lower reproductive value compared to larger fish. This risky behaviour could be adaptive for both hosts and parasites by reducing the energetic and opportunity costs of flight except in the most threatening of circumstances.

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Table 1. Mean (± s.e.m.) escape response performance values for unparasitized fish, parasitized fish after removal of their parasite (parasite-removed), and parasitized fish. Variables measured are fish body lengths (total length) in centimetres, size-adjusted maximum escape speed (U_{max} in fish body lengths per second), maximum acceleration (A_{max}), size-adjusted cumulative distance travelled (D in fish total body lengths, BL), and escape latency (milliseconds).

Treatment	N	Total length (cm)	U_{max} (BL s^{-1})	A_{max} (cm s^{-2})	D (BL)	Latency (ms)
Unparasitized	12	13.2 ± 0.2	14.9 ± 1.4	7.0 ± 0.7	0.5 ± 0.1	11.8 ± 2.9
Parasite-removed	12	12.7 ± 0.4	14.1 ± 1.1	6.9 ± 0.5	0.5 ± 0.0	8.3 ± 7.8
Parasitized	16	11.8 ± 0.3	15.8 ± 1.0	7.1 ± 0.4	0.6 ± 0.0	14.6 ± 2.5

Figure 1. Bridled monocle bream (*Scolopsis bilineata*) with a Cymothoid ectoparasite (*Anilocra nemipteri*) attached above its eye. Parasites can attach on either the A) left or B) right side of its fish host. Photo credits: A) D.G. Roche and B) S. Gingins.

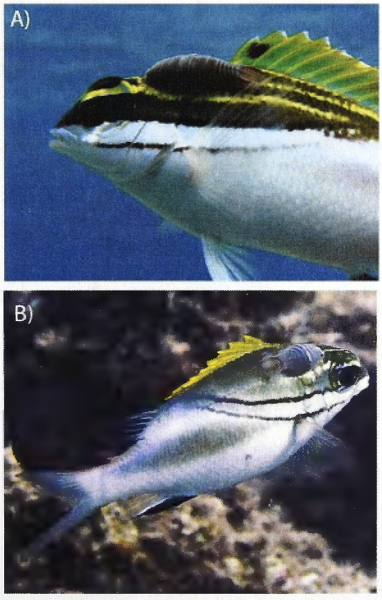


Figure 2. Fish body size (total length; cm) as a function of flight initiation distance (FID; cm) for parasitized (open circles) and unparasitized (filled circles) fish at Lizard Island. Lines represent the best fit linear regression for parasitized (long dashed line), unparasitized (small dashed line), and all fish (solid line).

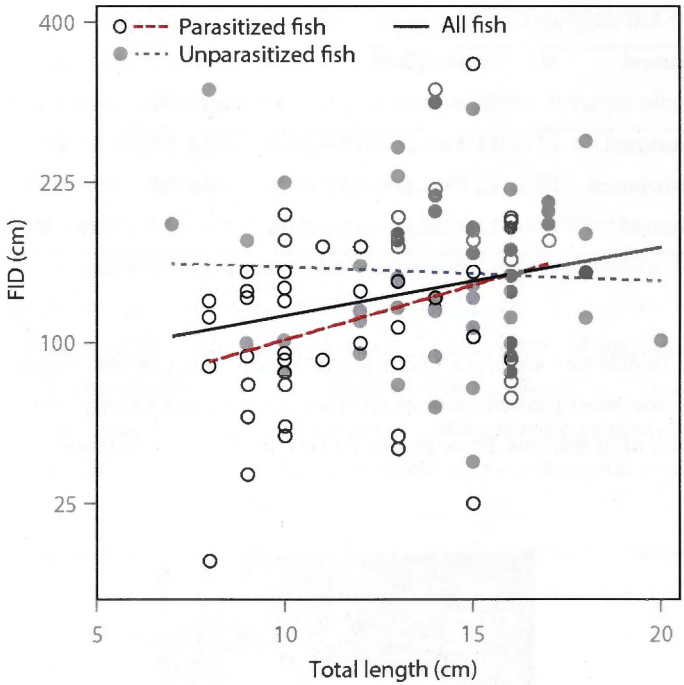
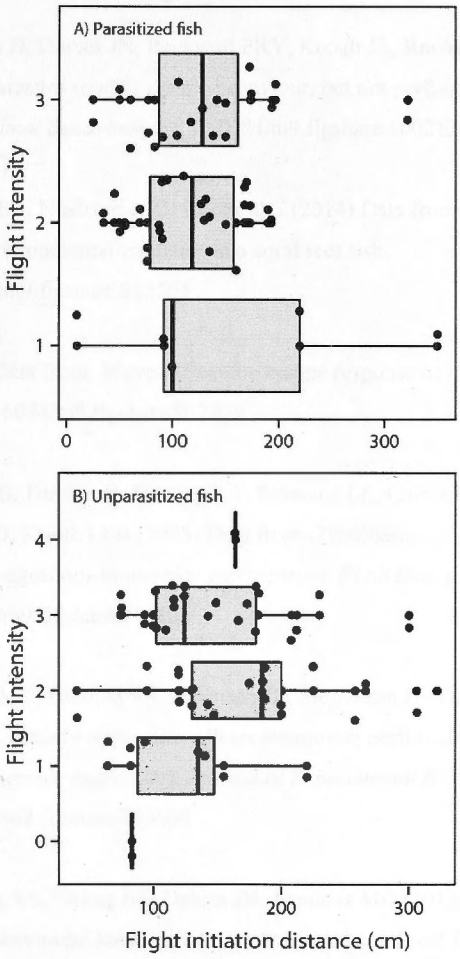


Figure 3. Inverted box plots showing the relationship between flight initiation distance and flight intensity for (a) parasitized and (b) unparasitized fish. Individual flight initiation distance (FID; cm) is plotted overtop of boxes to illustrate the distribution of the data.



APPENDIX - VII

Publicly archived datasets

Binning SA, **Roche DG** (2014) Data from: Water flow and fin shape polymorphism in coral reef fishes. doi:10.6084/m9.figshare.924800

Binning SA, Barnes JI, Davies JN, Backwell PRY, Keogh JS, **Roche DG** (2014) Data from: Ectoparasites modify escape behaviour, but not performance, in a coral reef fish. *Animal Behaviour* doi:10.6084/m9.figshare.1002130.

Binning SA, Ros AFH, Nusbaumer D, **Roche DG** (2014) Data from: Adaptive plasticity to natural environmental gradients in a coral reef fish. doi:10.6084/m9.figshare.923561

Roche DG (2013) Data from: Waves affect the escape response of juvenile coral reef fish. doi: 10.6084/m9.figshare.862938

Haff TM, **Roche DG**, Lanfear R, Binning SA, Schwanz LE, Cain KE, Kokko H, Jennions MD, Kruuk LEB (2013) Data from: Troubleshooting public data archiving: suggestions to increase participation. *PLoS Biology* doi:10.6084/m9.figshare.811801

Roche DG, Taylor MK, Binning SA, Johansen JL, Steffensen JF, Domenici P (2013) Data from: Unsteady water flow affects swimming performance in a labriform fish (*Cymatogaster aggregata*). *Journal of Experimental Biology* doi:10.6084/m9.figshare.789064

Roche DG, Binning SA, Strong LE, Davies JN, Jennions MD (2013) Data from: Increased behavioural lateralization in parasitized coral reef fish. *Behavioral Ecology and Sociobiology* doi:10.5061/dryad.jd25q

Binning SA, **Roche DG** and Layton C (2012) Data from: Ectoparasites increase swimming costs in a coral reef fish. *Biology Letters* doi:10.5061/dryad.r73v3