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THE INFLUENCE OF HABITAT ON THE STRESS AND CONDITION OF CORAL REEF FISHES

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Statement of Contribution of Others

Chapter 2: E.P.F, M.I.M., S.C.M and A.S.H. conceived the ideas and designed methodology; E.P.F, collected the data, analysed the data and wrote the initial draft of the manuscript; all authors contributed towards the final manuscript.

Chapter 3: E.P.F, A.D, M.I.M., and A.S.H. conceived the ideas and designed methodology; E.P.F, A.D., and C.R.H. collected the data; E.P.F, analysed the data and wrote the initial draft of the manuscript; all authors contributed towards the final manuscript and gave final approval for publication.

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Abstract

Coral reefs are one of the world's most biodiverse ecosystems, yet they are also one of the most threatened. Unprecedented global coral bleaching events, coupled with local stressors (e.g., severe storms, outbreaks of Crown-of-Thorns starfish, declining water quality) have caused regional-scale loss of corals along the Great Barrier Reef (GBR) and on reefs globally, with subsequent effects on reef fish populations. While populations of many reef fish species decline rapidly following coral loss, populations of some fish species (including some species that are considered coral dependent) persist. The potential sublethal effects (i.e., declines in fitness) of coral loss on these surviving individuals are unknown, and are likely to have important repercussions for population replenishment and community dynamics over longer timeframes. The objective of this thesis was to investigate the sublethal impact of habitat degradation on the fitness level of reef fishes. These sublethal impacts were primarily explored in relation to the effects of live coral cover and structural complexity of habitats on the stress response in coral reef damselfish.

To provide a better understanding of how and what habitat features influence the stress response and fitness metrics in reef fishes, I explored whether preferential habitat features related to cortisol levels and their influences on fitness traits, such as lipid content and Fulton's condition factor K (**Chapter 2**). At Lizard Island, in the northern GBR, the basal cortisol concentration, lipids storage, Fulton's K and habitat composition (percent cover of microhabitat features, structural complexity and depth) were quantified for three species of coral reef damselfish at a scale relevant to the individual. I identified particular habitat features that each species was associated with, and how the availability of those particular resources influenced cortisol concentration. I then explored the association between cortisol concentrations to lipid content and Fulton's K. I found that fish residing in area of lower amounts of preferred habitat features had higher cortisol levels and lower lipid content. Interestingly for the coral-dwelling species, the structural complexity (not coral cover) of their environment was found to have a direct relationship with cortisol concentration, while parts of the coral community (mounding coral) had strong associations with fitness metrics.

To directly test if living in degraded habitats elicits a stress response, I experimentally investigated the mechanistic influence of habitat degradation across primary, secondary, and tertiary levels of the stress response in three coral-dwelling species, each varying in their specialization of using live coral (**Chapter 3**). I reared settlement-age damselfishes for three

weeks in treatments consisting of live coral, dead coral covered with algae, and a mix of equal amounts of live and dead coral, measuring cortisol concentrations, metabolic performance, behaviours, and growth metrics. This experimental design removed the influence of structural complexity, focusing solely on the importance of live coral tissue but also removes any potential effect of predation and the risk of predation. Results showed no consistent impact of habitat treatment on metabolic performance, with only one species (*P. moluccensis*), exhibiting higher cortisol levels in degraded treatments. However, growth and behaviours of the species were impacted by habitat treatments in a gradient-like response, with the most specialized species exhibiting the greatest impact. In degraded treatments, fishes spent more time away from the sheltering habitat and tended to be smaller compared to those in treatments with live coral.

Survival is a key fitness metric and as coral reef fishes experience extremely high mortality shortly after settling to reef, I investigated the relative contribution of habitat features, local fish community and morphological characteristics on the survivorship of 5 species of newly settled damselfishes (**Chapter 4**). Naturally settling fishes were tagged, photographed and monitored daily for two-weeks to assess natural mortality rates. Overall mortality was high, with most events occurring within the first three days following settlement. Mortality rates did not differ among species; however, the driver of mortality differed among species. Broadly, species that associated more with benthic habitats (*P. moluccensis* and *P. amboinensis*) were more influenced by habitat features (mounding coral, sand, rugosity and algae). While more generalist species (*P. chrysurus* and *P. adelus*) were more influenced by morphological characteristics (body depth, ocellus size and rugosity). Overall, the strongest predictors of survivorship were found to be the structural complexity (in two species), body depth and ocellus size.

With knowledge of the importance of structural complexity on the survivorship of young fishes, I investigated how structural complexity affected predation risk and escape performance, and whether the mechanism underlying the response was stress-related (**Chapter 5**). Prey reared in variously complex environments for two-weeks were exposed to odours from predators, herbivores, or saltwater controls prior to a simulated predation strike. As expected, I found that predator odours elicited heightened escape responses compared to controls. Additionally, I found that the prey from higher complexity environments similarly performed heightened escape responses, suggesting that increased complexity increased the perception of risk. However, analyses of cortisol concentrations revealed an interaction between complexity

and risk odours, whereby prey fish had elevated cortisol concentrations when forewarned with predator odours, but only when complexity levels were low. My study suggests that as complexity is lost and risk increases, prey may be able to compensate by assessing predation risk more readily, likely as a result of receiving additional visual information.

The present research highlights the many ways that benthic habitats may influence the well-being of young coral reef damselfish. Live coral tissue was found to influence the lipid content (**Chapter 2**), growth rates and behaviour (**Chapter 3**), which may occur through non-stress related mechanisms. In contrast, structural complexity of habitats was found to influence the stress levels of fish (**Chapter 2 and 5**), the escape response (**Chapter 5**) and the overall survivorship (**Chapter 4**). Overall, the results of this thesis emphasize that the benthic habitats can influence the behaviour, condition, hormone levels, survivorship and ultimately the fitness of coral reef fishes. As a result, sublethal impacts of habitat degradation have the potential to alter population demographics in coral reef fishes. Together these findings may help explain the observed changes in abundance of particular species following habitat degradation events, which is prevalent in the literature. For instance, reduced growth rates or altered sheltering may increase predation rates and residing in low quality habitats can increase stress, reduce lipid stores, and potentially slow maturation time and relative contribution to future populations. This thesis provides new insights to the importance of both habitat features and structural complexity on coral reefs. It also provides a foundation of information on how and which species may be impacted as reef degradation continues in the future.

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Chapter 1: General Introduction

Unprecedented global coral bleaching events, combined with local stressors such as severe storms, outbreaks of Crown-of-Thorns starfish (COTS), and declining water quality, have led to the regional-scale loss of corals along the Great Barrier Reef and on coral reefs worldwide (Alvarez-Filip et al., 2009; Cheal et al., 2017; De'ath et al., 2012; Hughes et al., 2018a; Ortiz et al., 2018; Wilson et al., 2006). Following coral mortality, the dead coral skeletons are rapidly colonised by algae and other benthic taxa (Hughes, 1994; Tebbett et al., 2019), that compete with coral for benthic space (McCook, 2001) and have been shown to reduce the recruitment, growth, and survival of corals (Burgo and Hoey, 2024; Kuffner et al., 2006; Venera-Ponton et al., 2011). This can slow the recovery of coral populations (Hughes et al., 2007) and ultimately result in a less diverse and degraded benthic community (Burn et al., 2023; Hughes et al., 2018b). Such transitions in benthic community composition also have the potential to reduce the structural complexity of the habitat (Alvarez-Filip et al., 2009), a critical feature for maintaining biodiversity of coral reefs (Darling et al., 2017). Depending on the driver of degradation (e.g., cyclone vs. COTS and bleaching) the dead coral skeleton may remain for a few years, however, without the return and growth of hard corals, reefs will enter a state of net erosion, with dead coral skeletons being eroded to homogenous rubble (Garpe et al., 2006; Sheppard et al., 2002).

Declines in benthic diversity and structure towards a more degraded state have implications for reef-associated species, as many species have tight associations and even symbiotic relationships with corals (Norin et al., 2018; Pratchett et al., 2012; Rouzé et al., 2014). Given these close associations it is not surprising that decreases in the cover of live coral and increases in other benthic taxa lead to shifts in the composition of associated fish communities (Adam et al., 2011; Bellwood et al., 2006; Graham et al., 2006; Sheppard et al., 2002). Changes in fish communities associated with shifts in the composition and structure of habitats are often assumed to be related to increased mortality or migration. Importantly, predator-prey relationships have been shown to be sensitive to habitat change, with prey fishes often being more susceptible to predation, and hence the increased mortality, in degraded habitats. This has been attributed to many factors including reductions in habitat complexity (Almany, 2004; Beukers and Jones, 1997), the conspicuousness of fishes against either bleached or algae covered substrata (Coker et al., 2009; Marshall et al., 2019; McCormick, 2009), and reduced ability to escape predators in altered habitats (Boström-Einarsson et al., 2018; McCormick, 2009; McCormick and Allan, 2017). Habitat degradation may also promote the migration of some species to more preferable (i.e., less degraded) sites if locally available. While migration has been shown to be successful in larger individuals, smaller individuals were found to be less successful and disappeared more often, presumably being predated on (Pratchett et al., 2020). In the end, the process of migration can lead to increased competition on healthier sites and leave more fish vulnerable to predation (Coker et al., 2012b; McCormick, 2012).

While the declines in abundances of reef fishes following coral loss and habitat degradation are well documented (e.g., Bellwood et al., 2006; Cheal et al., 2017; Jones et al., 2004; Pratchett et al., 2008; Wilson et al., 2006), the potential sublethal effects of habitat degradation have received less attention. For corallivores, reef degradation results in a decline in availability of dietary resources, which together with reductions in population size (Graham et al., 2009; Pratchett et al., 2006) has been documented to reduce their body condition (Berumen et al., 2005; Pratchett et al., 2004), growth rates (Kokita and Nakazono, 2001), and behaviour (Keith et al., 2018; Thompson et al., 2019) likely impacting their overall fitness. However, many fishes associated with live corals use them not as a food source but as a structural habitat for refuge, and following the degradation of preferred habitat these coral-dwelling fishes may persist but would be constrained to living on less preferential habitat (Pratchett et al., 2012; Wismer et al., 2019). It is, however, largely unknown whether degradation due to coral mortality results in any sublethal effects on these surviving coral associated fishes, with likely repercussions for population replenishment and community dynamics.

Shelter is a fundamental resource for animals, providing protection from the natural environment and refugia from predators. The presence of shelter is known to produce many benefits, including reducing stress (Woodley and Peterson, 2003) and metabolic costs (Chrétien et al., 2021; Millidine et al., 2006; Woodley and Peterson, 2003), promoting growth (Finstad et al., 2007; Hossain et al., 1998) and increasing survival rates (Almany, 2004). While the physical structure of individual coral colonies may remain for years following coral mortality (Sheppard et al., 2002), evidence suggests that the presence of live tissue is required for many coral-associated fishes to exhibit shelter seeking behaviour. For example, Boström-Einarsson and colleagues (2018) found that following the death of host coral, fishes immediately stopped sheltering among the branches and switched to sheltering at the base of the coral or swimming away from the structure entirely when a predatory threat was artificially induced. This change in sheltering behaviour may explain why other studies have found fewer juvenile fishes surviving on dead colonies compared to live or bleached coral (Bonin et al., 2009a).

If the transition from live coral-dominated to a more degraded and algae covered habitat is perceived as an environmental stressor by coral-associated fishes, then the loss of live coral may reduce overall fitness of these fishes by inducing a stress response and altering their metabolism and growth. To evaluate condition and fitness in relation to habitat disturbances, ecologists are increasingly tracking stress levels across a diverse range of taxa (e.g. mammals, reptiles, birds and fishes; Graham et al., 2012; Josserand et al., 2017; King et al., 2016; Kitaysky et al., 1999; Martínez-Mota et al., 2007). Stress is typically measured as the production of glucocorticoids such as cortisol, a metabolic hormone that is an adaptive response to maintain homeostasis from a perceived threat by increasing plasma glucose to prepare an organism for a fight or flight response (Wingfield and Sapolsky, 2003). However, extended periods of elevated cortisol levels have negative consequences on fitness by altering metabolism and behaviour, suppressing growth and reproduction (Pankhurst, 2011), and reducing the quality and survival of offspring (Gagliano and McCormick, 2009; McCormick, 1998; McCormick and Gagliano, 2009). This theorized relationship is known as the 'Cortisol-Fitness Hypothesis' (Figure 1.1) and is based on two ideas: first, cortisol concentrations will rise during environmental challenges, resulting in elevated basal cortisol levels; second, these environmental challenges are linked to decreased fitness levels, as individuals divert resources to manage the stress (Bonier et al., 2009a). Together, these two principles form the core of the Cortisol-Fitness Hypothesis, which hypothesises a negative relationship between basal cortisol and fitness levels (Bonier et al., 2009a). In the context of coral reefs, coral associated fishes may have higher than average cortisol levels leading to a lowering of fitness following a degradation event of their habitat, which may aid in explaining the declines in abundance with important ramifications for population regulation.

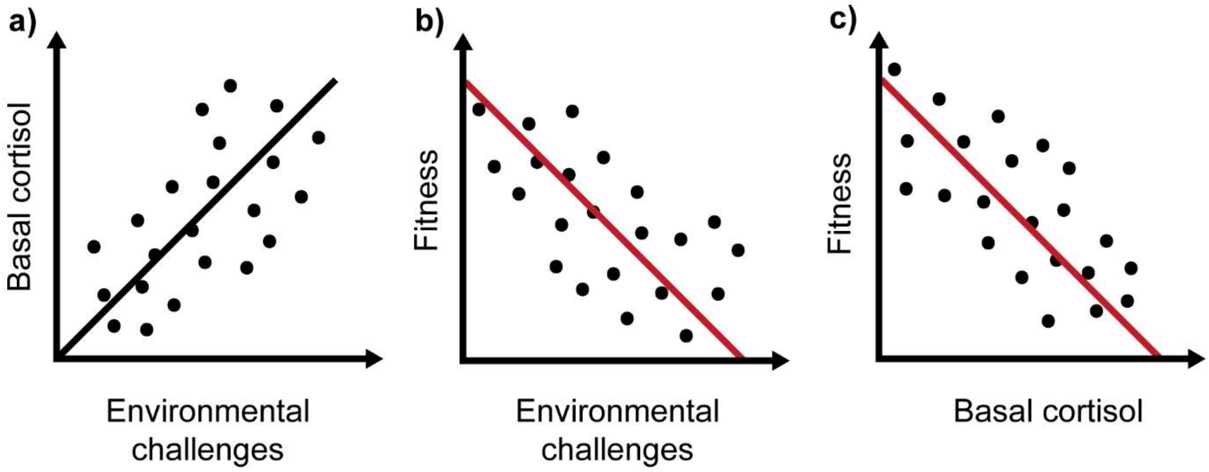

Figure 1.1 *The theoretical foundation of the Cortisol–Fitness Hypothesis is based on the following tenets: (a) Basal cortisol levels are predicted to increase with environmental challenges. (b) Increasing environmental challenges are associated with decreasing fitness because resources must be reallocated towards coping with these challenges at the expense of reproduction or self-maintenance. (c) Together, these tenets lead to the central prediction of the Cortisol–Fitness Hypothesis: a negative relationship between baseline cortisol and fitness* (See Bonier et al., 2009a).

The magnitude of impact that habitat degradation has on a species may directly relate to their reliance of, or specialization in using, that habitat. Species that are reliant on a limited suite of resources (i.e., specialists) are sensitive to changes in the availability of their preferred resources, and as such tend to be more vulnerable to habitat degradation than those species which can access a wider range of resources (i.e., resource generalists; Berumen and Pratchett, 2008; Vázquez and Simberloff, 2002). While coral-dependent fishes are generally more vulnerable to degradation than non-coral dependent reef fishes, there is considerable variation within coral-associated fishes in terms of their reliance on coral species and vulnerability to disturbance. For example, highly specialized species that rely on a few or even a single species of coral for food or habitat (Munday, 2004; Pratchett, 2007) are extremely susceptible to reductions in the availability of their preferred coral. Although evidence shows that some highly specialized coral-dependent fishes are unsuited to use alternate corals (Berumen and Pratchett, 2008; Munday, 2004), other less specialized fishes may persist by using less preferential corals or habitats (Wismer et al., 2019), suggests that responses may be species-specific but in relation to their degree of specialization. However, living in a suboptimal habitat is likely to have repercussions on the fitness and survival of individuals (Berumen et al., 2005; Garcia-Herrera et al., 2017; Pratchett et al., 2004).

With predicted increases in the frequency and/or intensity of disturbances impacting coral reefs (Emslie et al., 2024), a deeper understanding of the physiological impacts and species' potential resilience to coral loss and associated habitat degradation is needed. Measuring the cortisol production and the subsequent metabolic performance of fishes persisting in degraded habitats will provide a better understanding of the long-term physiological consequences of habitat change on the population regulation of coral reef fishes.

Thesis outline

The overall objective of this thesis was to investigate the sublethal impact of habitat degradation on the fitness of reef fishes, primarily focusing on the effects of live coral cover and structural complexity of habitats on the stress response in coral reef damselfish. In my first data chapter (**Chapter 2**), I aimed to identify which characteristics of habitat degradation influence stress and fitness. To do this I explored the Cortisol-Fitness hypothesis by correlating benthic features (including coral cover and complexity) to basal cortisol levels, and in turn cortisol levels to multiple fitness metrics. The aim of **Chapter 3** was to examine if living in degraded algae covered habitats caused stress. By experimentally manipulating the amount of live coral, compared to dead coral covered with algae, I investigated if residing in degraded habitats induced a stress response and if metabolic performance, behaviour, or growth were affected. In **Chapter 4** I aimed to explore how important habitat features (including coral cover and complexity) were for the survival of newly recruited fishes. Here I compared the habitat features of settlement sites to the local fish communities and individual characteristics on survivorship provided a comparative exploration. Lastly, I explored how levels of structural complexity influenced anti-predation behaviours and stress levels (**Chapter 5**) as a means of explaining the patterns found in previous chapters. This thesis broadly aims to link variations in live coral cover and structural complexity to changes in cortisol concentrations, exploring how these changes may lead to sublethal consequences, altered behaviours, and body condition in fish, thereby helping to explain changes in abundance following degradation events. This was explored across multiple damselfish species that vary in their reliance on live coral.

Chapter 2: Examination of the Cortisol-Fitness Hypothesis in juvenile reef fish: effect of habitat association on cortisol levels and fitness

2.1 Introduction

Fitness refers to an organism's ability to survive, reproduce, and pass on its genes to the next generation. This concept is central to evolutionary biology because it directly influences natural selection (Brommer, 2000; Doulcier et al., 2021). By studying fitness, scientists have aimed to predict the success and adaptability of organisms within their habitats, providing insights into population dynamics, species interactions, and ecosystem health (Alif et al., 2022; Breuner et al., 2008; Doulcier et al., 2021). Understanding fitness helps in predicting how populations might respond to environmental changes and in formulating conservation strategies to support biodiversity (Jimeno et al., 2020; Lattin et al., 2016; Turner et al., 2003).

Fitness is measured using various metrics that represent different facets of an organism's life. For instance, body condition indices (e.g., Fulton's K) provide a proxy of an individual's health at a point in time by relating its weight to its length, indicating the relative bulk of an individual (Robinson et al., 2008), and have been used to provide insights into the reproductive potential of a population (Baker et al., 2013; Waye and Mason, 2008). Direct measurements of lipid content can also be a useful measure by serving as a precise indicator of an animal's energy reserves (Weatherhead and Brown, 1996). In recent decades, there has been a growing trend in assessing stress (cortisol concentrations) in freely roaming populations as a means to assess their physical well-being or condition (Bonier et al., 2009b; Creel et al., 2002; Turner et al., 2003). This is primarily because the stress hormone cortisol plays a crucial role in modifying metabolic processes, enabling individuals to meet heightened energy requirements when confronted with environmental disturbances or obstacles (Sapolsky et al., 2000). For example in various ecosystems, the degradation or fragmentation of habitats has been observed to trigger a stress response and elevate cortisol production in organisms (Creel et al., 2002; King et al., 2016; Martínez-Mota et al., 2007; Mills et al., 2020; Turner et al., 2003). Consequently, baseline cortisol concentrations have proven to be a valuable physiological indicator and monitoring tool (Baker et al., 2013).

In general, individuals or populations with elevated basal cortisol levels are presumed to be in poorer condition, exhibiting reduced fitness. This presumption forms the basis of the

Cortisol-Fitness Hypothesis (hereafter CFH), which proposes two key ideas: firstly, during environmental challenges, cortisol concentrations will increase leading to elevated baseline cortisol levels; secondly, environmental challenges are associated with decreasing fitness levels, as individuals allocate resources to cope with such challenges (Bonier et al., 2009a; Breuner et al., 2008). In combination, these two principles lead to the central prediction of the CFH, which postulates a negative relationship between baseline cortisol concentrations and fitness levels (Bonier et al., 2009a). This hypothesis suggests a direct link between stress-induced hormonal changes and overall fitness, providing a framework to study how organisms manage energy allocation during stressful conditions and the subsequent effects on their health and reproductive success.

Cortisol is a hormone secreted by all vertebrates, produced via the activation of the hypothalamic-pituitary-adrenal (HPA) axis in mammals and the hypothalamic-pituitary-interrenal (HPI) axis in fishes, amphibians, and reptiles (Smith and Vale, 2006; Sopinka et al., 2015). Cortisol mediates physiological and behavioural responses, serving a multitude of functions that are essential for survival (Barton, 2002; Sapolsky et al., 2000). In broad terms, cortisol can be categorized as a metabolic hormone, as it tends to increase in response to the body’s energetic requirements when encountering challenges like unfavourable climatic conditions or limited resources (Bonier et al., 2009a). To meet heightened energy demands during stress, cortisol triggers metabolic pathways such as glycolysis and gluconeogenesis, mobilizing energy reserves (Mommsen et al., 1999; Sadoul and Vijayan, 2016). Although these changes may be crucial for survival in the short-term, chronic elevation of cortisol can have negative impacts, including suppressed immune function, reduced reproductive output, and impaired growth, ultimately affecting an individual’s long-term fitness (Barton, 2002; Pankhurst, 2011; Sadoul and Geffroy, 2019).

Despite the expected relationship between cortisol concentrations and fitness levels, there is a lack of consistent results from studies examining the CFH (Breuner and Berk, 2019; El Moustaid et al., 2019). While elevated cortisol levels are often presumed to indicate poor condition and reduced fitness, the evidence for such relationships is equivocal with some studies reporting that high baseline cortisol levels correlate with lower fitness, while others do not find such clear relationships (reviewed by Bonier et al., 2009a). Breuner and Berk (2019) propose that within the CFH, an individual’s performance must be considered within the context of resource allocation and acquisition (See van Noordwijk and de Jong, 1986). They argue that the availability and distribution of resources may play a critical role in mediating the observed relationship between cortisol and fitness. This framework suggests that cortisol's impact on fitness is not straightforward but depends on how effectively individuals can access and utilize resources in their environment.

Access to preferred resources can significantly influence individual fitness (Johnson, 2007; Norris and Marra, 2007). Resources such as food, shelter, and mates are critical for survival and reproduction. When these resources are abundant, individuals can allocate energy towards growth, reproduction, and maintaining immune function, thereby enhancing their fitness. However, when resources are limited or heavily competed for, fitness can be adversely affected. For example, in environments where food or water is scarce, individuals may experience increased stress and elevated cortisol levels as they struggle to meet their energetic needs (Marra and Holberton, 1998). Additionally, competition for limited resources can exacerbate stress, further influencing cortisol levels and fitness. Understanding how resource availability and competition affect cortisol levels and consequently fitness is essential for comprehending organismal responses to environmental changes. This knowledge is particularly relevant in the context of habitat degradation and climate change, where resource availability can be unpredictable and unevenly distributed.

Coral reefs are one of the world's most biodiverse ecosystems, yet a range of disturbances (namely thermally-induced bleaching, population explosions of coral predators, severe storms, declining water quality, and fishing; De'ath et al., 2012) are reducing the availability of habitats and associated resources. Along with declines in live coral, the structural complexity of coral reefs, which provides numerous niches and refuges is declining, affecting organisms that depend on these habitats. Fishes that are site-attached and have a close association with the benthos, therefore, make ideal case study species for examining the cortisol-fitness relationship, especially in the context of changing resources (Hughes et al., 2017; Ortiz et al., 2018). As coral reefs degrade, fish species that are closely associated with specific habitat characteristics may experience increased stress and elevated cortisol levels due to reduced habitat quality and availability. These changes may impact their lipid reserves or Fulton's K, providing a natural experiment to study the effects of environmental stressors on fitness. The varying degrees of habitat dependence among different species of reef fish also allow for comparative studies on how specific habitat changes influence cortisol levels and fitness outcomes.

In the current study, we tested the CFH on three congeneric damselfish species (f. Pomacentridae) with varying habitat associations. We examined whether basal cortisol levels correlate with habitat characteristics, such as the percentage of preferred benthic cover or habitat complexity, and if fitness traits (total lipids and Fulton's K) are mediated by these characteristics through cortisol concentrations. We hypothesized that increased environmental challenges, such as reductions in preferred resources, would elevate basal cortisol levels and inversely affect fitness traits. We focus on juvenile fishes to avoid potential confounding effects of reproductive activity on cortisol levels, as elevations in cortisol are known to be an intrinsic part of breeding for some species (Crespi et al., 2013; Lattin et al., 2016). Whole-body cortisol assays were employed as they are recognized as a good proxy for HPI axis activity during basal conditions (Pavlidis et al., 2013; Sadoul and Geffroy, 2019) and are ideal for smaller fish where blood sampling is challenging (Aerts et al., 2018).

2.2 Methodology

Study species

Three common site-attached congeneric damselfish species that differ in their habitat use were selected for the study (Chapter 4; Pratchett et al., 2016): (1) the lemon damsel, *P. moluccensis*, that is an obligate coral-dwelling damselfish often found on colonies of fine branching coral; (2) the Ambon damselfish, *P. amboinensis*, that is a facultative coral-dwelling species associated with a variety of substrata including sand, live and dead coral; (3) the white-tailed damselfish, *P. chrysurus*, that commonly inhabit algal-covered rubble sandy areas.

Field protocol

To investigate the effect of habitat on the basal cortisol levels and fitness (total lipids and Fulton's K), individuals of the three damselfish species were collected during November and December 2021 from the reefs around Lizard Island (14.6680˚ S, 145.4638˚ E). Fish were collected from four sites on the leeward (i.e., western) side of Lizard Island. Each site was 15x25m and was marked with flagging tape in 5x5m cells. Upon locating a juvenile of each of the target species (mean ± SD standard length: 3.11 ± 0.39 cm), the focal fish was observed for at least 30 s to estimate the centre of its home range. These species resides within small and well-defined home ranges; for example it has been shown that *P. moluccensis* possesses a high site fidelity (0.42 m^2, 50% KUD) (Streit et al., 2021). Fish were captured using clove oil and hand nets, transferred to a plastic bag and immediately euthanized in ice seawater slurry. Each fish was then placed in a labelled plastic bag and stored on ice. A stopwatch was used to collect capture time until the euthanasia. Only one dive per day was conducted at each site and only one fish was collected in each 5x5m cell as to reduce potential diver disturbances.

After each fish was collected, benthic composition was quantified by placing a 0.75 x 0.75m quadrat (0.56 m^2) over the centre of the estimated home range and a photograph was taken from a height of ~1m above the substratum. The photoquadrats were analysed using PhotoQuad (Trygonis and Sini, 2012) recording the substratum under 100 stratified random points within each quadrat. The substratum was categorized as pavement (smoothed reef structure), rubble, sand, macroalgae, hard coral, or soft coral. Hard and soft corals were identified to genus and later grouped into broad growth forms (i.e., coarse branching, fine branching, mounding and soft coral) for analysis (Pratchett et al., 2015). The rugosity (i.e. structural complexity) of the substratum within the quadrat was estimated as the ratio of a chain (link size 1.5 cm) that followed the reef contour to the linear distance across the centre of the quadrat (0.75 m) and parallel to two sides of the quadrat (McCormick, 1994). A second measurement was conducted using the same chain perpendicular to the first measure, and the mean of the two measurements calculated and used to represent rugosity. The time and water depth of each quadrat were recorded and depth standardised using tide charts. To estimate baseline benthic composition and compare across species settlement association, an additional 35 control quadrats were haphazardly placed throughout each site (140 in total), photographed and benthic characteristics quantified as described above (see Table S1).

Fitness measures

Collected fish were returned to the lab within 120 mins of capture where they were photographed laterally against a grid sheet, their blotted wet weight was recorded using a digital balance, and their total length (TL) and standard length (SL) measured using callipers. Fulton's condition index was quantified as the ratio of wet weight (in kg) to the cube of standard length (in cm) (i.e., $K= weight/SL^3$). The head was removed, and the remaining body sample was stored in a labelled cryovial and immediately frozen in liquid nitrogen for storage.

For cortisol and lipid extraction the fish were freeze dried (Christ Alpha 1-2 LD plus, 0.2 mbar, 48 h, -50ºC) and weighed (Mettler Toledo UMX2 Ultra-Microbalance). Dried samples were ground using a pestle and mortar then homogenized in a 10 ml vial, with 2 ml of phosphate-buffered saline (PBS, Sigma-Aldrich) using metal beads and a bead beater (4 and 8 mm stainless-steel beads; Biospec Products, Mini Beadbeater TM) for 3 mins. The homogenate was then transferred to a glass vial (40 ml), rinsed with an additional 2 ml (4 ml total) of PBS and the solvent ethyl acetate was added at a 1:9 ratio (36 ml). The samples were vortexed (Lab-Line Instruments, Super-Mixer 1291, 3x speed), for 1 min before being centrifuged (Beckman Coutler, Allegra X-15R) at 2,000 rpm for 3 min at 4 °C. The supernatant was collected in a pre-weighed glass vial and dried under a constant stream of N_2 (Techne Dri-Block DB.3D Sample Concentrator, 40˚C). The extraction steps were performed two times, pooling the extraction steps. The difference between the pre and post weight of the glass vials were used to estimate total lipid content. Samples were then reconstituted within 48 hours using 1.5 ml of assay buffer and loaded into a commercially available cortisol ELISA kit (Cayman Chemical Item Number 500370). Each sample was analysed in triplicates with a spectrophotometer (SpectraMax Plus 384 Microplate Reader, Molecular Devices) and the average absorbance was calculated from readings between 405 and 420 nm.

Assay validations steps (parallelism, accuracy and precision) were performed, prior to measuring cortisol concentrations, following recommendations from Metcalfe et al., (2018). Parallelism was confirmed by an ANCOVA's homogeneity of slopes assumption by comparing dose–response curves of diluted samples for each fish against a standard curve (ANCOVA, P>0.05, n=3; Fig S1). In brief, the reconstituted samples (n=3) were diluted (1:1, 1:2, 1:3, 1:4, and 1:5) and compared against the cortisol standard curve (Cayman Chemical ELISA kit, 39.1–5000 pg/ml range). The optimal dilution for the samples fell between the dilutions 1:1 and 1:2; therefore, a sample dilution of 1:1.5 was chosen to achieve a 50% relative maximum binding and only sample dilutions falling within 20–80% B/B_0 relative maximum binding were accepted. The accuracy or recovery rate of the extraction method was assessed by spiking samples (n=4) with 800 pg of cortisol. For each of the four samples, two fish were homogenized, pooled and split into halves, with one half receiving the spike and the other the assay buffer. Both halves were then processed in the same way as all the other samples. The spike's recovery (percentage) was expressed as spiked sample result-unspiked sample result×100/known spike (800 pg ml^{-1}), and the mean recovery was 91.0%, (n=4). Intra-assay precision of triplicate samples was determined using the coefficient of variation (CV) and found to be 7.7 ± 4.9 (mean ± SD, n=114). Fig S1 summarizes results for dose–response curves, comparison between standard curve and recovery rate from method of extraction.

Statistical analyses

Settlement associations

The time required to capture and collect samples is known to influence cortisol concentrations over relatively short periods (3-8 mins; Müller et al., 2006). Therefore, the effect of capture time (time from the attempted capture until euthanasia) on the cortisol concentrations was examined using linear models. Time to capture was found to have no significant effect ($P > 0.05$) on the cortisol concentrations of the three species either collectively (Fig. 2.1), or independently (See Table S2) and was therefore not included in any further analysis.

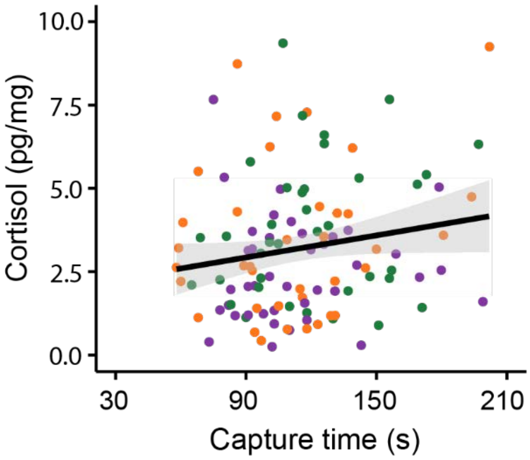

Figure 2.1 *Relationship between baseline cortisol concentrations from whole-body homogenates of pomacentrids and time of capture (time from attempted capture until euthanasia). P. amboinensis (orange), P. chrysurus (green), P. moluccensis (purple); n = 37, 37, 40 respectively. Solid line represents a linear regression trend line and shaded area the 95% confidence intervals. Cortisol levels were unrelated to capture time (See Table S2 for statistics).*

Habitat associations

To test whether the three damselfish species associated with particular habitat characteristics, differences in benthic composition between species and control quadrats were investigated, using permutational multivariate analyses of variance (PERMANOVA) based on Bray-Cutis distances (n = 10000 permutations). Following significance, planned contrasts between each species and control quadrats were conducted. The nature of differences found between species by each PERMANOVA were visually displayed using a canonical discriminant analysis (CDA; Seber, 1984). Species centroids and their 95% confidence intervals were plotted along the first two canonical axes, with habitat variables as vectors. The nature and strength of their association with each species are shown by their direction and length.

Structural equations models

Due to the possible complex network of interactions between habitat characteristics, cortisol concentrations and fitness traits, we carried out piecewise structural equation modelling (SEM; Lefcheck and Duffy, 2015). In a step-by-step fashion, piecewise SEM integrates insights from various individual models into a unified causal network (Lefcheck, 2016; Shipley, 2009). We constructed a single causal network per species, using knowledge from the habitat preferences and ecological theory of importance of structural rugosity to define the paths (Grace, 2006). Using SEMs we disentangled the relative contributions of preferred habitat characteristics and habitat complexity on basal cortisol responses and examined cortisol's downstream effects on fitness traits. Based on the CDA habitats characteristics with higher associations were considered preferred (Chapter 4) and were included in SEM's as habitat resources. *P. amboinensis* was found to associate with depth and sand while *P. chrysurus* associated with sand and rubble and lastly *P. moluccensis* associated with fine branching and mounding corals. Additionally rugosity, a measure of 3D structural environment, was included for all species as it is known to be an important feature which can regulate community dynamics (Almany, 2004; Morais and Bellwood, 2019; Rilov et al., 2007; Vicente et al., 2024) and may be relevant with positive and negative associations (Chapter 5).

For each species two types of models were used: one exploring the CFH and one including the access to important habitat features. We first modelled the effect of cortisol's concentration onto total lipids reserves, a fitness trait that is controlled by the metabolism and then explored the effect of lipids onto Fulton's K, a condition metric which is derived from a length to volume ratio. As resource allocation may be an important facet that is under explored in the CFH (Breuner and Berk, 2019) we modeled the relationship between the amount of preferred habitat feature and basal cortisol concentrations, and subsequently examined its effects on lipid reserves and Fulton's K.

We fit the component models as generalized linear mixed-effects models (GLMMs). Additional paths were included when test of separation identified significant missing paths (Taylor et al., 2020b) and are represented as hollow arrows. The overall fit of the SEMs were evaluated using Shipley's test of d-separation, which returns a Fisher's C statistic (Shipley, 2009). Coefficients in the text are standardized coefficients (β) which indicate the relative strength and direction of predictors' effects on the dependent variable by expressing changes in terms of standard deviations, allowing direct comparison across variables measured on different scales. We used the R package GLMMTMB for component models and the piecewiseSEM package to construct causal network (Lefcheck, 2016). For random factors, site was included in all GLMM's and temperature was included in component models predicting cortisol concentrations. Model validation and diagnostics (e.g., qq plots, homogeneity of variance) were checked using the DHARMa package (Hartig and Hartig, 2017). All linear models used a Gaussian distribution to accommodate their error structure. We calculated the estimated marginal means (EMMs) based on the GLMMs above and each model's marginal (R^2_m) and conditional (R^2_c) as an estimate of effect size (Length, 2023).

2.3 Results

Habitat associations

There was a significant difference in habitat associations among the damselfish species at settlement (PERMANOVA; $F_{3,250} = 7.09$, $P < 0.001$), with *P. moluccensis* being associated with more rugose microhabitats and a higher cover of fine-branching and mounding corals, while *P. amboinensis* was associated with deeper sites with a higher cover of sand and *P. chrysurus* was associated with a higher cover of rubble, sand, and pavement (Fig. 2.2). The habitat associations of all three species differed from that of control quadrats ($P < 0.05$), indicating that each species associated with particular microhabitat characteristics.

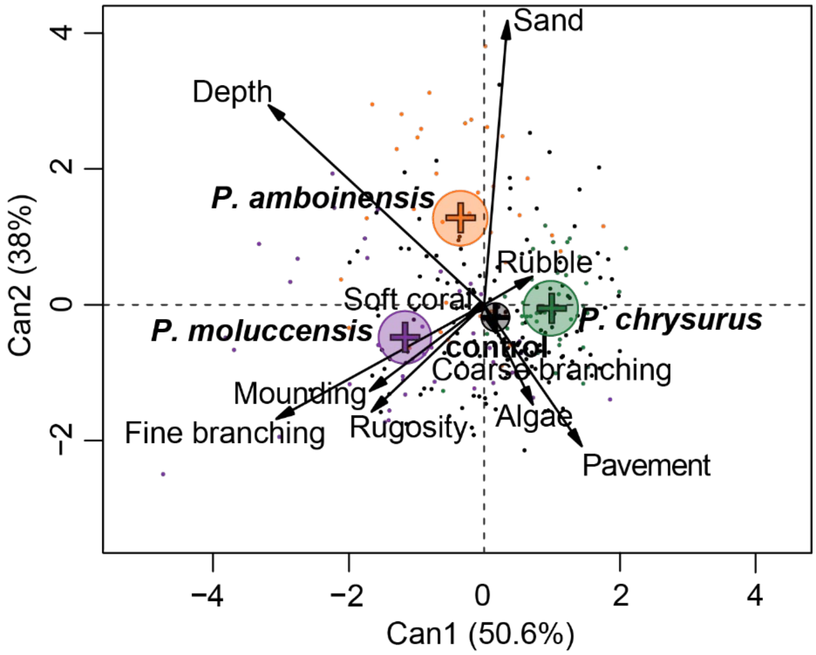

Figure 2.2 *Biplots of canonical discriminant analyses showing differences in the juvenile habitat associations of three congeneric damselfish species and control quadrats. Crosses represent species centroids, circles surrounding crosses represent 95% confidence intervals for species. Vectors represent the direction and strength of the original variables to discriminate among species. Pomacentrus amboinensis (orange), P. chrysurus (green), P. moluccensis (purple), and control quadrats (black).*

Structural equation models on CFH

The SEMs fitted the data moderately for *P. amboinensis* ($C_2 = 2.28$, $P = 0.32$) and less well for *P. chrysurus* ($C_2 = 6.00$, $P = 0.05$), however the model for *P. moluccensis* was fully saturated, with no remaining degrees of freedom to calculate the goodness-of-fit index and therefore could not be assessed. Whole-body cortisol concentrations significantly correlated with fitness traits in two of the three species (Fig. 2.3A-C). Increase in cortisol concentrations was found to significantly reduce lipid content in *P. moluccensis* ($\beta = -0.447$, $P = 0.002$) and *P. chrysurus* ($\beta = -0.369$, $P = 0.016$). Additionally, tests of direct separation revealed other unpredicted relationships (hollow arrows) with the Fulton's K of *P. moluccensis* increasing with cortisol concentration ($\beta = 0.404$, $P = 0.005$, Fig 2.3A). Cortisol concentrations were found to have no significant relationships with fitness traits in *P. amboinensis* (Fig 3B).

Using the R^2_m, cortisol explained 20%, 1% and 14% (Fig. 2.3A-C) of the variability in lipid content across the species. The large disparity between R^2_m and R^2_c (0.01, 0.37) for *P.*

amboinensis indicates an important influence of site (random factor) on the lipid content, but only for this species. In general, the models performed more poorly at explaining Fulton's K with R^2_m values of 36%, 10%, and 1%, except in *P. moluccensis* which had a direct relationship with cortisol.

Inclusion of habitat resources in CFH SEM's

Overall, the SEMs fitted the data well (*P. moluccensis*: $C_{10} = 6.12$, P = 0.81; *P. amboinensis*: $C_{10} = 7.22$, P = 0.70; and *P. chrysurus*: $C_{12} = 11.10$, P = 0.52) with habitat characteristics correlating significantly with whole-body cortisol concentrations for each of the three species (Fig. 2.3D-F). Increases in one preferred habitat characteristic was found to significantly reduce cortisol concentrations in each species: for *P. moluccensis* the relative influence of rugosity on cortisol was of key importance (β = -0.410, P = 0.006, Fig. 2.3), for *P. amboinensis* the percent cover of sand was important (β = -0.524, P = 0.002), while for *P. chrysurus* the amount of rubble significantly influenced whole body cortisol (β = -0.500, P = < 0.001). For *P. amboinensis*, increased depth was related to increased cortisol (β = 0.405, P = 0.016), but depth also had a high level of covariance with sand (β = 0.489, P = 0.002).

Tests of direct separation revealed other unpredicted relationships, which were included in final SEMs to improve fit (hollow arrows, Fig. 2.3D-F). This revealed that *P. moluccensis* residing in habitats with more mounding corals had higher lipid content (β = 0.299, P = 0.025) and similarly, *P. amboinensis* in areas with more structural rugosity had higher lipid content (β = 0.386, P = 0.015). Also *P. amboinensis* residing in shallower waters had higher Fulton's K (β = -0.329, P = 0.025) and similarly *P. chrysurus* residing in habitats with more rubble had higher Fulton's K (β = 0.366, P = 0.017). While not in the expected pathway, *P. moluccensis* with higher cortisol concentrations were found to have a higher Fulton's K (β = 0.404, P = 0.005).

The models' ability to explain cortisol concentrations was fairly consistent among species with R^2_m explaining 16%, 23% and 31% (Fig. 2.3D-F). All significant correlations between cortisol and fitness traits from initial models remained, including the negative correlation between cortisol concentration and lipid content in *P. moluccensis* (β = -0.422, P = 0.002) and *P. chrysurus* (β = -0.369, P = 0.016) and the lipid contents positive correlation to Fulton's K in *P. moluccensis* (β = 0.648, P = < 0.001). The models' overall ability to explain fitness traits varied between species but remained consistent within species. *P. moluccensis* models yielded R^2_m explaining 29% and 36% (lipids and Fulton's K, respectively), while models for *P. amboinensis* yielded 16% and 22%, and for *P. chrysurus* 14% and 14%. The inclusion of habitat traits improved the predictive ability of the models. Comparing the R^2_m values of models (Fig. 2.3A-C to D-F) the addition of habitat resources increased the variance explained in the lipid content of *P. moluccensis* and the Fulton's K in *P. amboinensis* and *P. chrysurus*. All coefficients and their associated P values for each SEMs are provided in Table S3 and S4.

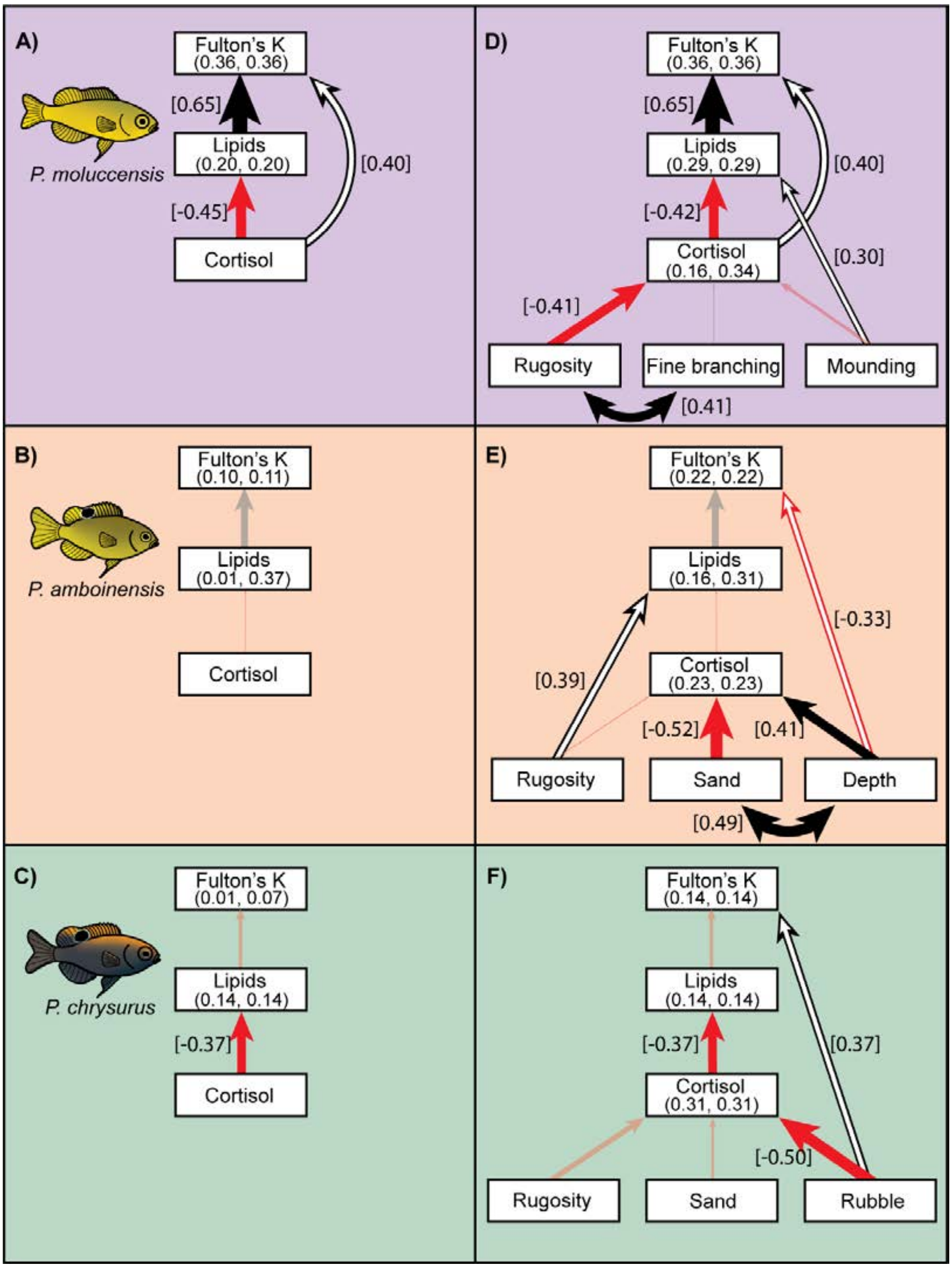

Figure 2.3 *Directed acyclic graphs display standardized regression coefficients for hypothesized pathways for habitats characteristics influence on cortisol and ultimately fitness traits (lipids and*

Fulton's K). Solid arrows indicate significance pathways ($P < 0.05$) and faded arrows represent non-significant pathways ($P > 0.05$) with line thickness reflecting coefficient values and significant coefficient values supplied in brackets. Colour denotes either a positive relationship (black) or negative relationship (red). Double-headed curved arrows specify the correlated error structure. Hollow arrows represent significant additional pathways omitted from expected pathways but detected by test of direct separation. The variance explained (R^2) for each endogenous factor is found below in parentheses with marginal (R^2_m) followed by conditional (R^2_c).

2.4 Discussion

Understanding the roles of cortisol levels and habitat in influencing the fitness metrics of coral reef fish, particularly small-bodied species closely associated with the benthos, is crucial for assessing their capacity to withstand future environmental challenges (Wilson et al., 2008a). The use of SEMs to explore both direct and indirect relationships in complex systems is becoming increasingly common (Casey et al., 2017; Desbiens et al., 2021; Morais and Bellwood, 2019; Skinner et al., 2024). In the current study, structural equation modelling (SEM) method provided a unique perspective on the cascading impacts of habitat features on individuals' cortisol concentrations and subsequently on their overall fitness levels. The final SEMs revealed significant relationships among the fitness metrics and cortisol, which were further improved by the inclusion of habitat features. This shows the interconnections between habitat quality, cortisol and fitness and also, that hormonal changes can scale up to affect higher organisational levels, therefore cumulatively having the potential to influence community dynamics.

To date, the evidence supporting the CFH is mixed. Some studies report the expected negative relationship between cortisol levels and fitness traits (Angelier and Wingfield, 2012; Buck et al., 2007), others find a positive relationship (Chastel et al., 2005; Meylan and Clobert, 2005) and some detect no relationship all together (Eeva et al., 2003; Husak et al., 2006). The results of our study indicate that cortisol can predict fitness levels, although the relationship varies among species. Specifically, we observed a negative relationship between cortisol and lipid content in two of the three species, thereby generally supporting the CFH. Notably, we also observed that cortisol in *P. amboinensis* showed no relationship with lipid content, suggesting that such relationships may sometimes be absent and/or influenced by other factors not considered in this study. Cortisol was more effective at predicting lipid content, which is a biochemical fitness metric more closely related, than Fulton's K, a morphometric measure of fitness.. The closeness of cortisol to lipids, compared to Fulton's K, also likely influences the speed at which these fitness metrics are impacted in response to stress. This highlights cortisol's closer link to lipid storage, as increased activation of the HPA/HPI and release of cortisol can modulate energy metabolism and storage, which are critical for maintaining homeostasis and overall fitness in stressful environments (Peckett et al., 2011). Also, this underscores the importance of selecting fitness traits which may be more sensitive to cortisol rather than broader condition indices such as Fulton's K (Waye and Mason, 2008). Nevertheless, the SEM for *P. moluccensis* revealed a strong and positive relationship between cortisol and Fulton's K. While cortisol may decrease lipid reserves directly, its positive relationship with Fulton's K suggests it may also play a role in determining size or maintaining overall body condition and performance during stressful conditions. These relationships highlight the intricate balance between stress responses, energy metabolism, and fitness outcomes in ecological contexts.

The inclusion of habitat traits to the SEMs enhanced our ability to predict fitness traits from basal cortisol levels. Although the improved habitat inclusive models had little to no changes in the regression coefficients, the variance explained (R^2) of at least one fitness trait was substantially higher with the inclusion of habitat traits of particular relevance for the species. For example, in *P. moluccensis* the marginal R^2_m of lipids was increased by nearly one third and the R^2_m of Fulton's K was greatly increased for both *P. amboinensis* (16x) and *P. chrysurus* (2x). In proposing the inclusion of resource availability to the CFH, Breuner and Berk (2019) highlighted its potential utility in reducing unexplained variability and fostering consensus regarding the CFH's application. In our study, including percent cover of various microhabitat features, structural rugosity, and depth reduced the variability in fitness estimates, but importantly, specific traits which species associated with were found to be particularly important. Therefore, the current study further advocates the inclusion of habitats which are of particular relevance to the species of interest.

Our models established significant indirect and direct pathways of influence of habitat features on basal cortisol and/or fitness traits. In some cases, cortisol was a mediator of the influence of the habitat traits on fitness levels. For example, in *P. moluccensis* rugosity was negatively correlated with cortisol, which in turn was negatively correlated with lipids. This indicates that changes in habitat features may elicit the stress response (Barton, 2002). In other occurrences, the habitat features related directly to the fitness levels, for example in *P. amboinensis* rugosity had no relationship with cortisol but rugosity levels were positively correlated with lipid content. This suggests that these habitat features influence fitness level via other, non-stress related pathways. In all, this reveals how habitat quality can have both direct and indirect impacts on fitness levels.

As expected, habitat associations were found to be consequential and to covary with cortisol concentrations and fitness traits. In each species, one preferred habitat feature had a negative correlation with basal cortisol levels. For example in *P. chrysurus* a known rubble specialist (Chapter 4; Öhman et al., 1998), and rubble had a strong negative correlation with cortisol concentration (-0.50) but a positive correlation with Fulton's K (0.37). This is unsurprising as *P. chrysurus* is a known algal farming species (Low, 1971; Pratchett et al., 2016), and increased surface area of rubble likely provides a larger farm area as well as shelter, therefore improving overall fitness. Similarly for *P. amboinensis*, which is known to settle at the base of reefs (McCormick and Hoey, 2004), areas that are typically sandier (Ticzon et al., 2012), we found a strong negative correlation between sand and cortisol concentration (-0.52). Unconsolidated substrates, including sand and rubble, can support higher densities of invertebrates (Wen et al., 2013a), an important nutrient source for *P. amboinensis* (Debenay et al., 2011). After accounting for the influence of sand, depth was the only trait which was found to be positively related to cortisol concentration (0.41) and negatively related to Fulton's K (-0.33) for *P. amboinensis*. Interestingly, similar patterns of reduced fitness, including Fulton's K, gonado- and hepato-somatic indices, have been observed with increasing depth in a confamilial species (*Chrysiptera rollandi*; Hoey et al., 2007), and may be associated with reduced food availability. The direct and significant relationships identified here highlight the importance of particular habitat features at the hormonal and organismal level.

Due to the pervasive importance of structural rugosity's influence on various levels of organisation on coral reefs including community composition (Darling et al., 2017; Richardson et al., 2017; S. K. Wilson et al., 2009), productivity (Graham and Nash, 2013; Rogers et al., 2018), recruitment (Coker et al., 2014; Webber et al., 2024), survival (Chapter 4; Fontoura et al., 2020), predation risk and stress (Chapter 3; Woodley and Peterson, 2003), rugosity was included in all models. Interestingly, for *P. moluccensis*, the coral specialist, structural rugosity rather than coral cover (i.e., fine branching or mounding), had the greatest influence on cortisol. Indeed, rugosity was found to have a strong negative correlation (-0.41) with cortisol concentration. This suggests that for this species, residing in low rugosity environments is potentially more stressful than living in environments with low coral cover and that potentially changes in rugosity may be a greater stressor than changes in coral cover. That said, the amount of mounding corals was found to have a positive correlation (0.30) with lipid content in *P.*

moluccensis. However, the lack of mediation through cortisol indicates that residing on high coverage of mounding corals may provide fitness benefits through non stress related pathways. This relationship with lipids may indicate a biological trade-off in *P. moluccensis*' association with mounding corals, which serve as an important settlement habitat but also result in higher mortality rates for newly recruited fish (Chapter 4). Survivors in this riskier environment may experience increased lipid storage and/or growth rates. Although the level of rugosity was not related to cortisol concentration in *P. amboinensis*, it was positively related to lipid content (0.39), which may provide greater access to food while not affecting stress. The level of rugosity had no relationship with either cortisol or lipid content in *P. chrysurus*. Species-specific responses to habitat traits generally align with the habitat characteristics they are associated with, as shown in the current study where species are linked to various specific microhabitat features. The inclusion of habitat features can improve our ability to predict fitness traits, especially when these features are ones that the organism preferentially associates with.

When putting these results in the context of the CFH, this study suggests that cortisol may actually be negatively related to fitness in coral reef fishes. However, it is important to remember this is the final tenant of the hypothesis and the precursor is that basal cortisol levels will rise, and fitness levels decline, with environmental challenges (Bonier et al., 2009a). In the context of this study the environmental challenge was the access to preferred habitat features, and we generally found that individuals collected from areas with lower cover of their preferred habitat had higher cortisol levels. Following a disturbance, coral cover tends to decline (De'ath et al., 2012; Wilson et al., 2006) and eventually structural rugosity with it (Alvarez-Filip et al., 2009; Sano et al., 1987) and the coral skeleton becomes covered in algae, increasing rubble, marking a shift in the availability of resources. Such changes would likely amplify some relationships while weakening others, For example, if rugosity were to decline further, its negative relationship with cortisol for *P. moluccensis* would likely be amplified. However, the increased availability of rubble may cause a breakdown of its relationship with *P. chrysurus* as access becomes unlimited. These large-scale degradation events are increasing in both intensity and frequency (Emslie et al., 2024), highlighting that assessment of fitness traits and the factors that can influence them will be of greater importance moving forward to predict which species will adapt to future conditions. However, tracking cortisol and fitness of individuals prior to, during and post-disturbance will be required to fully understand the impacts of disturbance.

As ecologists are increasingly interested in the assessment of cortisol as a physiological indicator and monitoring tool (Creel et al., 2002; Lattin et al., 2016; Martínez-Mota et al., 2007), using tissue cortisol extraction is proving to be a valuable method (Chapter 3; Chapter 4; King et al., 2016; Yeh et al., 2013). This technique enables scientists to explore multiple novel research questions about habitat features and their effects on small species or early life stages, where blood collection is challenging (Aerts et al., 2018; Yeh et al., 2013). This method increases the variety of fish sizes that can be included in studies like this one, enabling us to examine the CFH in juvenile damselfishes. Employing this approach would significantly broaden the range of habitats, life stages, and species that can be studied with physiological tools, thereby enhancing their contribution to conservation ecology overall.

In conclusion, this study found that in most cases basal cortisol concentration correlated with fitness traits, generally aligning with the CFH. This indicates that cortisol may be useful as a fitness metric itself and the employment of monitoring basal cortisol levels may be particularly informative during periods of environmental changes, such as mass bleaching events. While our initial models found that cortisol did help explain fitness traits, the inclusion of preferred habitat resources improved these models, highlighting the intimate connection reef fishes have with their benthic habitats. In all, this study provides evidence that future studies exploring the CFH could benefit from the inclusion of relevant resource availability for the specific species of interest.

Chapter 3: Habitat degradation has species-specific effects on the stress response of coral reef fishes

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3.1 Introduction

Coral reefs are one of the world's most biodiverse ecosystems, yet they are also one of the most threatened. Unprecedented global coral bleaching events, coupled with local stressors (e.g., severe storms, outbreaks of Crown-of-Thorns starfish, declining water quality), have caused regional-scale loss of corals along the Great Barrier Reef and on reefs globally (Emslie et al., 2024; Hughes et al., 2018a; Ortiz et al., 2018; Pandolfi et al., 2003). Following the mortality of corals, the dead coral skeletons become colonized by diverse benthic assemblages, in particular algae (Diaz-Pulido and McCook, 2002). Such changes in benthic communities (i.e., loss of live coral and increases in algal cover) have been shown to lead to shifts in the composition and function of reef fish assemblages (Jones et al., 2004; Richardson et al., 2018; Wilson et al., 2008b). While populations of many reef fish species decline rapidly following coral loss (Pratchett et al., 2008), populations of some fish species persist, including some species that are considered coral dependent (Wismer et al., 2019). However, the potential sub-lethal effects (i.e., those that lead to declines in fitness) of coral loss on these surviving individuals are unknown, and likely have important repercussions for population replenishment and community dynamics over longer timeframes.

Reef fish species exhibit various responses to the shifts from coral- to algal-dominated seascapes. For example, following disturbances, an increase in dietary resources may lead to increases in the abundance of herbivorous fishes (Adam et al., 2011; Santano et al., 2021) and an increase in their growth rates (Hart and Russ, 1996; Taylor et al., 2020a). Conversely, coral loss may result in a major decline of corallivores, particularly in species that are highly selective for the corals they consume (Pratchett et al., 2006). These changes in abundance are often, though not exclusively, attributed to shifts in dietary resources. Indeed, coral-dwelling species also experience significant declines (Jones et al., 2004; Pratchett et al., 2008; Wilson et al., 2006) and even local extinctions following coral loss (Munday, 2004). The magnitude of impact that habitat change has on species may directly relate to their specialization in using that habitat (MacNally, 1995). Highly specialized species, which rely on a few or even a single species of coral type, are extremely susceptible to reductions in the availability of their preferred habitat and have shown local extinctions following the loss of such coral (Bellwood et al., 2006; Munday, 2004). Other species with a lower degree of specialization can be more flexible in habitat use following the reductions in the abundance of their preferred habitat. For example, the damselfishes *Dascyllus reticulatus* and *Pomacentrus moluccensis* are both considered obligate coral-dwelling fishes, but have different levels of specialization, with *P. moluccensis* using a wider range of coral taxa than *D. reticulatus* across the same sites (Pratchett et al., 2012). Following widespread coral loss due to feeding by Crown-of Thorns starfish (*Acanthaster* cf. *solaris*), both damselfish species declined in abundance; however, the impact was greater on the more specialized *D. reticulatus* (Pratchett et al., 2012). Clearly, resource specialization (diet and/or habitat) may influence the resilience of a species to any disruption of a key resource.

At the individual level, coral dwelling fishes may be experiencing a stress response as habitat degrades towards more algal-dominated seascapes (Schrandt and Lema, 2011). These responses to stress are categorized into primary responses (neurological and hormonal changes like cortisol and adrenaline production), secondary responses (metabolic changes such as cardiovascular and respiratory activity), and tertiary responses (alterations in growth, body condition, behaviour, and survivorship; Barton, 2002). Responses to stress can alter all levels of biological organization from molecular to community levels and studies indicate that this may be occurring in reef fishes. For example, Feary et al., (2009) found the growth rates of two coral dwelling damselfishes (*D. melanurus* and *Chrysiptera parasema*) to be directly related to the percentage of live coral cover. Behaviourally, *P. moluccensis* have also been shown to spend more time between coral branches when associating with live coral, versus more time away from or around the base of dead corals (Boström-Einarsson et al., 2018). Some evidence suggests that the metabolism of coral dwellers may also be impacted when residing within the branches of dead corals. Garcia-Herrera et al. (2017) found that *D. marginatus* sheltering in dead coral skeletons had a resting metabolic rate which was 19% higher than when the fish had no shelter, indicating that higher respiration rates may be induced by increased stress related to the habitat. Altered behaviours, growth or metabolism may be a coping response to deal with the stress of changing environments.

Given the strong connection between an organism and its habitat attributes, the choice of habitat is crucial to determine an individual's ability to persist. A reduction of the availability or quality of an important resource may elicit a stress response and can have important physiological consequences, ultimately leading to a decline in fitness (Dubuc et al., 2024; Huey, 1991). While the exact cause of stress in the case of coral reef habitat degradation is unknown, prior research suggests degraded coral reef environments may promote cyanobacteria and/or diatoms, which can influence the olfactory landscapes (McCormick et al., 2017), potentially disrupting crucial olfactory cues involved in predator-prey interactions, feeding or reproduction (Boström-Einarsson et al., 2018; Chivers et al., 2019; McCormick et al., 2019b). Furthermore, degraded and algae-covered coral may also be considered a lower-quality shelter, affecting overall protection (Millidine et al., 2006). Degraded habitats can also make fishes more visually apparent, potentially increasing predation risk (Coker et al., 2009; Hemingson et al., 2022). Together, these changes can disrupt an individual's energetic budget and incur additional costs for homeostasis as organisms adjust their physiology and morphology in response to environmental stress (Dubuc et al., 2024; Sokolova, 2021). These changes can generally be detected through increased standard metabolic rate (SMR) and altered maximum metabolic rate (MMR; Mandic and Regan, 2018) with any constraint to an individual's metabolism leading to potential trade-offs in key fitness metrics, such as reduced growth and reproduction, changes in behaviour, and increased disease susceptibility (Mandic and Regan, 2018; Zambonino-Infante et al., 2017).

While declines in coral dwelling fishes are well documented following coral loss (Jones et al., 2004; Wilson et al., 2006), the mechanistic response leading to these declines is unknown. Also, the impact of living in degraded environments on the remaining individuals is not well known but evidence suggests that their stress response may be inducing changes to various organization levels (Barton, 2002; Petitjean et al., 2019). We hypothesized that coral-dwelling fishes living in degraded environments would experience increased basal cortisol concentrations when exposed to degraded environmental conditions which could induce metabolic, growth and behavioural changes. Therefore, the current study experimentally investigated the mechanistic influence of habitat degradation across primary, secondary and tertiary levels of the stress response in three coral dwelling species (*P. amboinensis*, *P. moluccensis* and *D. aruanus*), which vary in their specialization of using live coral (Pratchett et al., 2012).

3.2 Methodology

Study species & housing conditions

Three common damselfish species (Pomacentridae) that vary in their selectivity for live coral at settlement were used in the study: (1) the Ambon damselfish, *P. amboinensis*, is a habitat generalist with recently-settled and juvenile individuals usually associating with a variety of substrata at the base of reefs, including live coral (McCormick et al., 2010; Pratchett et al., 2016) (2); the Lemon damsel, *Pomacentrus moluccensis*, is an obligate coral-dwelling damselfish that settles to fine branching coral (Booth, 2000; Öhman et al., 1998; Streit et al., 2021); (3) the Humbug damsel, *Dascyllus aruanus*, is a highly selective obligate coral dweller which settles to and lives in dense aggregations on colonies of fine branching coral (Chase et al., 2018; Holbrook et al., 2000). Based on their proportional decline and their use of dead coral following a degradation event, *P. amboinensis* is known as the least coral associated, followed by *P. moluccensis*, and lastly *D. aruanus* as the most coral associated of the three species (See Pratchett et al., 2012).

Settlement-stage damselfish that had not been exposed to reef habitats were collected with light traps around Lizard Island (14°40'S, 145°28'E), northern Great Barrier Reef (GBR) in October and November of 2022. Light traps were moored in approximately 20 m of water and at least 50 m from the nearest reef. Light traps were deployed at dusk and collected at dawn the following morning, whereby the catch was immediately sorted to species and reared in 42L tanks (43 x 32 x 31cm) with no more than 30 individuals of conspecifics per tank. Each tank was supplied with fresh flow-through seawater and sections of PVC pipe as shelter. Fish were reared in these tanks for three days and were fed *ad libitum* with newly hatched *Artemia* twice daily. Each fish was then transferred to a plastic bag containing aerated seawater where it was laterally photographed against grid paper before being haphazardly placed in one of the three habitat treatments (healthy, degraded, or mixed) and reared for three weeks. The initial average standard length of *P. amboinensis*, *P. moluccensis* and *D. aruanus* were 14.0 mm (SE±0.1), 13.7 mm (±0.1) and 9.2 (±0.1), respectively. The three habitat treatments represented different levels of coral reef degradation that fish may be exposed to in the wild. The three habitat treatments (~12 x 12 x 12cm) consisted of; live coral, dead coral covered with algae, and mixed (equal amounts of live and dead coral): which were used as proxies for healthy, degraded, and mixed reef patches, respectively. The healthy treatment was made up of a fine branching live coral (*Pocillopora damicornis*); and the degraded habitat treatment consisted of a dead fine branching rubble covered with algae. The dead degraded rubble in both the mixed and degraded treatments were specifically collected to be dead rubble from *Po. damicornis* to ensure similar structural complexity as the live *Po. damicornis* treatment.

Treatment tanks consisted of a shallow sand base with a terracotta tile (11 x 11 x 1 cm) in the middle which supported the corresponding habitat structure and were continuously supplied with fresh flow-through seawater. All sides of the tanks were covered in black plastic to ensure fish could not see or interact with fish in adjacent tanks. Two sizes of treatment tanks were used during the study to account for the difference in gregariousness among species (i.e., *D. aruanus* is a naturally schooling species while *P. amboinensis* and *P. moluccensis* are generally solitary). Therefore, *P. amboinensis* and *P. moluccensis* individuals were reared alone in a ~3L tank (18 x 13 x 13 cm) while *D. aruanus* individuals were reared in groups of three fish in ~5.2L tank (23 x 15 x 15 cm). Fish were fed to satiation with newly hatched *Artemia* twice daily in treatment tanks for 3 weeks. Two batches of experimental fish were reared each for three weeks (Oct-Nov and Nov-Dec).

Morphometric measures

The initial and final (after 3-weeks) standard length (SL) and body depth (BD) were measured from the lateral photographs of each individual. Additionally, the final blotted wet mass was recorded and Fulton's condition factor (K) calculated as the ratio of wet mass to the standard length cubed (i.e., $K=[\text{wet mass, kg}]/[\text{SL, m}]^3$).

Behavioural assessments

After three days in habitat treatments space-use of the treatment fish was recorded using a small video camera (GoPro Hero 9) positioned 20 cm directly above each tank using a purpose-built stand. Cameras filmed for 15 mins and the first 10 mins was discarded to allow fish to acclimate to the presence of the camera. From the videos, space-use was measured for 3 minutes as the duration of time spent using three categories (1) away from habitat (>1.5 body lengths from the habitat), (2) edge of habitat (within 1.5 body lengths of the habitat), or (3) within habitat (either directly above or within the habitat). All videos were analysed in Solomon coder software (Solomon Coder).

Respirometry

Oxygen uptake rates ($\dot{M}O_2$) were measured using automated intermittent-flow respirometry, following best practices described by Svendsen et al. (2016). Three key metabolic variables were calculated to assess metabolic performance: standard metabolic rate

(SMR), maximum metabolic rate (MMR), and aerobic scope (AS). SMR represents the minimum maintenance metabolism of a resting and post-absorptive individual (Chabot et al., 2016; Dubuc et al., 2021). MMR corresponds to the maximum oxygen consumption rate (Norin and Clark, 2016) that was reached following a three-minute swimming challenge followed by one minute air exposure to induce exhaustion (Norin and Clark, 2016). AS was calculated as the difference between SMR and MMR, representing the range of aerobic energy available for activities such as swimming, digestion or growth (Rosewarne et al., 2016).

Eight custom-made chambers fitted with additional plunger to restrict swimming and reduce volume (~ 0.09 L, including tubbing) were placed in two temperature controlled (28.5 ± 0.5°C) holding tanks (~ 1000 L), each housing 4 chambers. Each chamber was connected to a recirculation and a flush pump and covered with a black plastic to limit visual stimuli. Timing of flush pumps was controlled by AquaResp (V3.0) to repeat a 9 min cycle, that consisted of a 6-min measure period (including a 2 min wait period), followed by a 3-min flush period. The oxygen level within the chambers was measured every 2 seconds using a calibrated oxygen-sensitive REDFLASH® dye sensors on contactless spots fitted to the recirculation loop. Each oxygen sensor was connected to a four channel Firesting Optical Oxygen Meter (Pyro Science e. K, Aachen, Germany) via fibre-optic cables. Oxygen probes were calibrated to 100% saturation using aerated seawater before each experiment. The entire system was bleached daily (>3 h) to minimise bacterial growth.

Prior to each trial, fish were fasted for 24 h to ensure a post-absorptive state (Chabot et al., 2016; Zambonino-Infante et al., 2017). Between 07:00-08:00, up to eight fish which had undergone 21 days in treatments, were haphazardly selected. Fish were blotted, weighed and then transferred to a circular magnetic stirrer based swimming chamber (15 cm diameter and ~1.5L; for design see Nilsson et al., 2007) and acclimated for 1 min, then swam for 3 min. Flow rate was increased until fish could no longer match the current speed and adjusted as needed to ensure maximum swimming. Following the swim challenge, fish were exposed to air for 1 min and then immediately transferred to a respirometry chamber. This allowed for MMR to be measured immediately following exercise. Fish remained undisturbed in chambers for ~ 8 h, which was sufficient to reach a plateau corresponding to SMR (typically fish recovered within 1 h). Following each trial, fish were removed from chambers, and photographed following the previously described procedure.

To calculate MMR, the first 3 slopes obtained for each individual fish (9 min each) were broken up into 1 min increments from which $\dot{M}O_2$ was calculated using the commercial software LabChart v. 8.1.10 (ADInstruments, Sydney, NSW, Australia). Among these values, MMR was taken as the average of the two highest $\dot{M}O_2$ values. SMR was calculated by taking the mean of the 10 lowest $\dot{M}O_2$ values after removing the 2% lowest values using the calcSMR function in fish MO2 R package (Claireaux and Chabot, 2016). Metabolic variables were then allometrically scaled to the average mass of each species. The mass scaling exponents were determined for each species by plotting separately the raw SMR and MMR values (mg h^{-1}) as a function of mass (g) on log-scaled transformed axis (Rosewarne et al., 2016). SMR and MMR values were then allometrically mass-adjusted as followed:

$$\dot{M}O_{2\text{scaled}} = \dot{M}O_{2\text{measured}} \text{ x } (M_{\text{measured}}/M_{\text{standard}})^{(1\text{-}b)}$$

where $\dot{M}O_{2\text{scaled}}$ is the $\dot{M}O_2$ allometrically mass-adjusted to the average weight, $\dot{M}O_{2\text{measured}}$ is the mass-adjusted $\dot{M}O_2$, M_{measured} is the weight of the individual, M_{standard} is the average weight, b is the mass-scaling exponent. AS was calculated as the difference between allometrically mass-adjusted MMR and SMR.

Cortisol analysis

A subset of fishes which did not undergo respirometry trials were quickly caught, euthanized through cold shock using an ice slurry, and then snap frozen in liquid nitrogen to assess cortisol concentrations. Whole-body cortisol was extracted using a method described by Allan et al., (2020) and was measured with a commercially available cortisol ELISA kit (Cayman Chemical Item Number 500370). Prior to quantifying cortisol concentrations, assay validations steps (parallelism, accuracy and precision) were performed for the cortisol ELISA kit, following recommendations by Metcalfe et al., (2018); see supplemental materials for details. Fish were individually freeze-dried (Christ Alpha 1-2 LDplus, 0.2 mbar, >48 h, -50 ºC) and weighed (Mettler Toledo UMX2 Ultra-Microbalance, 0.1 µg readability). The freeze-dried samples were then placed in an Eppendorf vial (2 ml), with phosphate-buffered saline PBS (0.5 ml) and homogenized using glass beads in a shaking mill (MP Biomedical FastPrep24) for 3 min. The whole-body homogenate was then transferred to a 10 ml glass vial and rinsed with an additional 0.4 ml of PBS. The solvent (ethyl acetate) was then added at a 1:9 ratio and the samples were vortexed for 1 min before being centrifuged (Eppendorf 5810 R) at 3,500 rpm for 5 min at 4 °C after which the supernatant was collected in a glass vial. The extraction steps were performed three times, pooling each extraction step. The ethyl acetate was dried off in a centrifugal vacuum concentrator (Thermo Savant SpeedVac SC110A, 43°C) and samples were reconstituted within 48 hours using 1 ml assay buffer and analysed in triplicate with a spectrophotometer (SpectraMax Plus 384 Microplate Reader, Molecular Devices). The average absorbance was calculated from readings between 405 and 420 nm.

Statistical analyses

Differences between cortisol concentration, metabolic variables (SMR, MMR and AS), and morphometric measures (mass, SL, BD and Fulton's K) were analysed using generalized linear mixed-effects models (GLMM; glmmTMB) for each species separately. Habitat treatment (live, dead, and mixed) was included a fixed factor, and Tank ID as a random factor in all models. Additionally, assay plate ID was included in cortisol concentration models to account for variability across plates. Pre-treatment SL and BD, derived from photographs taken of individual fish prior to entering rearing treatments, were included as covariates in their respective models. Specifically, pre-treatment SL was included in the SL models, and pre-treatment BD was included in the BD models for *P. amboinensis* and *P. moluccensis*. Because *D. aruanus* were reared in groups of three, individuals could not be tracked, and no covariates were used for *D. aruanus*. To estimate the potential effect of habitat treatment and random factors, we calculated the estimated marginal means (EMMs) based on the GLMM's above and each model's marginal and conditional R^2 as an estimate of effect size (Length, 2023). Model validation and diagnostics (e.g., qq plots, homogeneity of variance) were checked using the DHARMa package (Hartig and Hartig, 2017). All linear models used a Gaussian distribution to accommodate their error structure. Pearson's Chi-square goodness-of-fit tests (χ^2) were used separately for each damselfish species and treatment to determine non-random variation in space-use around habitat in tanks (Chase et al., 2020). All analysis was performed in R, version 4.3.1(R Core Team, 2023).

3.3 Results

Primary response

A high level of variability in cortisol concentration was observed between the species (Fig. 3.1). Habitat treatment had no effect on the basal cortisol concentration of either *P. amboinensis* or *D. aruanus* (GLMM: $p > 0.05$), however habitat treatment did affect the basal cortisol concentration of *P. moluccensis* (GLMM: $p = 0.013$, $R^2_m = 0.14$; Fig. 3.1, Table S1).

P. moluccensis from the live coral treatment had significantly lower cortisol concentrations (25.9 pg mg^{-1} ±17.3) than the degraded treatment (46.2 pg mg^{-1} ±17.9; Table S2).

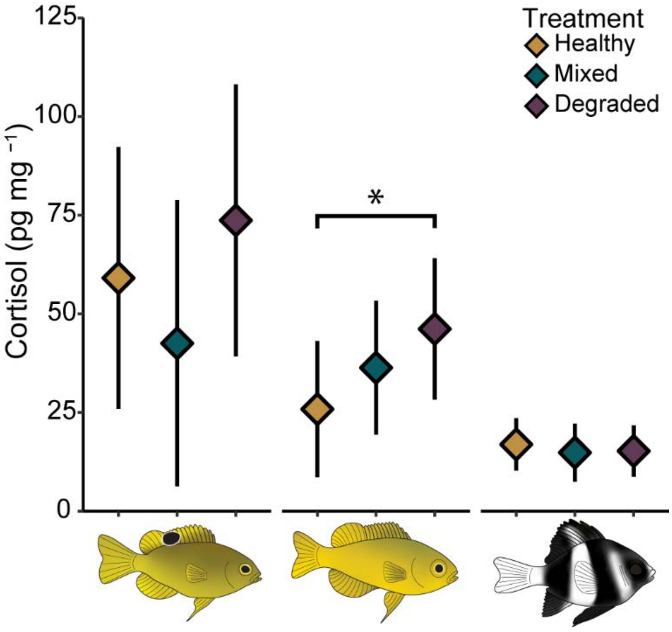

Figure 3.1 *The effect of habitat rearing treatments (live coral, mixed and degraded habitat) on cortisol concentration in P. amboinensis, P. moluccensis and D. aruanus. Estimated marginal means are provided (± 95% CI) and significance is noted as follows (* < 0.05). Sample size from left to right (7, 6, 7, 11, 9, 10, 6, 6, 6).*

Secondary response

There was no consistent effect of habitat treatment on the metabolic performance of the three damselfish species. Habitat treatment was found to have no effect on the SMR of any species (GLMM: $p > 0.05$; Fig. 3.2A-C; Table S3). Habitat treatment also had no impact on the MMR of *P. amboinensis* (GLMM: $p > 0.05$; Fig. 3.2D). However the MMR of *P. moluccensis* was influenced by habitat treatment (GLMM: $p = 0.033$ $R^2_m = 0.12$; Fig. 3.2E), with fish from the mixed treatment (2568.6 mg O_2 kg^{-1} h^{-1} ±262.0) having a significantly higher MMR compared to fish from the degraded treatment (2102.4 mg O_2 kg^{-1} h^{-1} ±246.1); the mean MMR for *P. moluccensis* from the live coral habitat were intermediate (2312.0 mg O_2 kg^{-1} h^{-1} ±232.8; Table S4). Similarly, the MMR of *D. aruanus* was influenced by habitat treatment (GLMM: $p = 0.019$ $R^2_m = 0.10$; Fig. 3.2F), with fish from the live coral (2414.0 mg O_2 kg^{-1} h^{-}

^{1} ±187.0) having a significantly higher MMR compared to fish from the degraded treatment (2056.9 mg O_2 kg^{-1} h^{-1} ±191.0). Lastly, habitat treatment no impact on the AS of *P. moluccensis* or *D. aruanus* (GLMM: $p > 0.05$; Fig. 3.2H-I) but did influence the AS in *P. amboinensis* (GLMM: $p < 0.031$ $R^2_m = 0.14$; Fig. 3.2G). *P. amboinensis* from the live coral had a higher AS (1246.1 mg O_2 kg^{-1} h^{-1} ±198.0) compared against the mixed treatment (915.5 mg O_2 kg^{-1} h^{-1} ±187.6) but not the degraded treatment (961.5 mg O_2 kg^{-1} h^{-1} ±178.9).

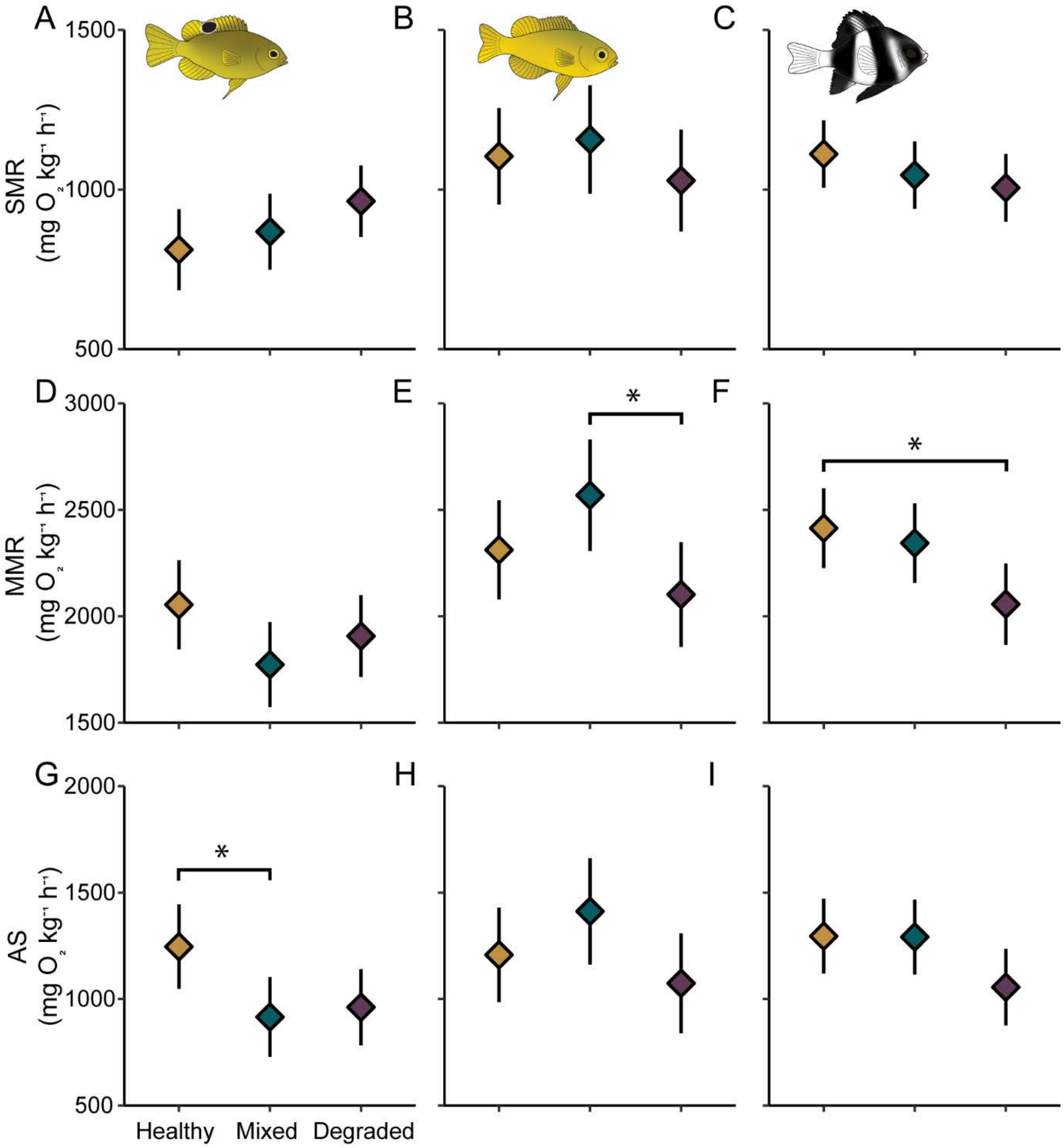

Figure 3.2 *The effect of habitat rearing treatments on metabolic variables for P. amboinensis, P. moluccensis and D. aruanus. Estimated marginal means are provided (± 95% CI) for standard metabolic rate (A-C), maximum metabolic rate (D-F) and aerobic scope (G-I). Asterisks denote significance is noted as follows (* < 0.05). Sample size from left to right (n =14, 16, 18, 19, 15, 17, 8, 8, 8).*

Tertiary response

Behaviour

Within each species the space-use differed among the habitat treatments, indicating non-random use (Fig. 3.3). Across all treatments, *P. amboinensis* spent significantly less time away from habitat than within or on the edge ($\chi^2_2 = 16.6$, $p < 0.001$; $\chi^2_2 = 39.9$, $p < 0.001$; $\chi^2_2 = 9.1$, $p = 0.01$). *P. moluccensis* spent significantly more time within the habitat than far away for the healthy and mixed treatments ($\chi^2_2 = 87.5$, $p < 0.001$; $\chi^2_2 = 65.6$, $p < 0.001$), but in the degraded treatment was found to spend nearly equal time in all zones ($\chi^2_2 = 0.45$, $p = 0.80$). Comparatively, *P. moluccensis* from the degraded treatment spent ~21% more time away from habitat than in the healthy treatment. Similarly, *D. aruanus* from the healthy and mixed treatments spent nearly all their time within or on the edge of their habitat ($\chi^2_2 = 295.1$, $p < 0.001$; $\chi^2_2 = 178.8$, $p < 0.001$) while in the degraded treatment they spent most of their time away from the habitat ($\chi^2_2 = 60.5$, $p < 0.001$). Comparatively, *D. aruanus* from the degraded treatment spent ~58% more time away from habitat than in the healthy treatment.

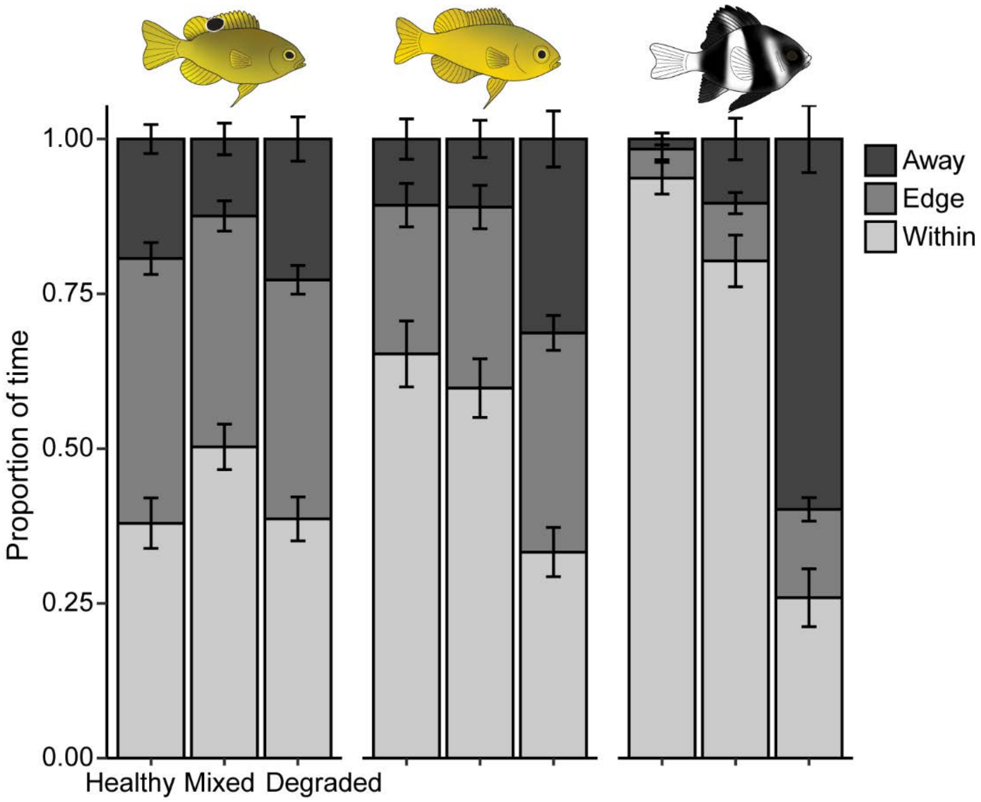

Figure 3.3 *Average space-use position (proportion ±SE) of P. amboinensis, P. moluccensis and D. aruanus over three minutes in various habitat treatments (healthy, mixed, and degraded). Spatial position was tracked and grouped into either within the habitat, on the edge of habitat (≤ 1.5 body length) or away from habitat (> 1.5 body length). Sample size from left to right (n = 25, 27, 26, 30, 27, 29, 42, 42, 42).*

Morphometric

P. amboinensis reared in the different habitat treatments showed no detectable differences in mass, standard length, body depth or Fulton's K (GLMM: $p > 0.05$; Fig. 3.4A-D; Table S5) after three weeks. *P. moluccensis* from the live coral treatment tended to have a higher mass, standard length and body depth than conspecifics from the mixed and degraded treatments, however these differences were not significant (GLMM: $p > 0.05$; Fig. 3.4A-C). There was no difference in the Fulton's K of *P. moluccensis* among habitat treatments (Fig. 3.4D). The mass of *D. aruanus* was significantly impacted treatment they were reared in (GLMM: $p < 0.007$, $R^2_m = 0.07$; Fig. 3.4A, Table S6). Their mean mass (± CI) of *D. aruanus* reared for 3-weeks in the live coral treatment was significantly higher (88.5 mg ± 5.1) than from those in the degraded habitat (77.1 mg ± 5.1), but not different the mixed treatment (82.4

mg ± 5.1; See Table S3). Similarly, *D. aruanus* from the live coral treatment tended to have a higher standard length, body depth and Fulton's K, however these differences were not significant (GLMM: p > 0.05; Fig. 3.4B-D).

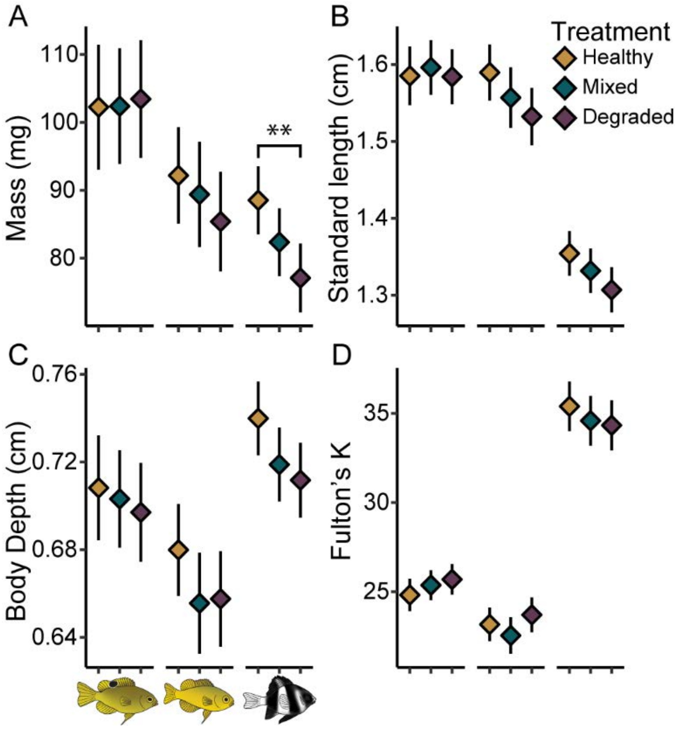

Figure 3.4 *The effect of habitat rearing treatments (live coral, mixed and degraded habitat) on the morphometric variables A) mass, B) standard length, C) body depth and D) Fulton's K of early post-settlement fishes P. amboinensis, P. moluccensis and D. aruanus. Estimated marginal means are provided (± 95% CI) and significance is noted as follows (* < 0.05 and ** < 0.01). Sample size from left to right (n = 24, 28, 27, 31, 26, 29, 14, 14, 14).*

3.4 Discussion

Numerous studies have shown that habitat degradation has negative effects on populations of reef fish (Cheal et al., 2017; Pratchett et al., 2008; Wilson et al., 2006), however, few have examined if the individuals that persist are experiencing sublethal effects. The present study demonstrates that settling to degraded environments has the potential to influence fitness characteristics such as growth and metabolism in juvenile damselfishes, with adverse effects potentially mediated through behavioural adjustments. Although our study suggests some general responses among species, most observed changes were species-specific with the two species that have a greater reliance on live corals at settlement (i.e., *P. moluccensis* and *D.*

aruanus) being more impacted than the species with less reliance on live corals (i.e., *P. amboinensis*). Additionally, there was substantial within-species variability in the different metrics, highlighting that individual phenotypic plasticity may be critically important in shaping an individual's response to environmental degradation.

Is degradation a stressor and does it induce a metabolic cost?

Habitat degradation has been shown to trigger stress responses in associated species across a range of ecosystems (King et al., 2016; Martínez-Mota et al., 2007), with evidence suggesting this is also the case for coral reef fishes (Garcia-Herrera et al., 2017). For instance, increases in the physical structure of the environment has been shown to alleviate the cortisol concentrations when threats and predators are present (Fakan et al., 2023; Woodley and Peterson, 2003). Additionally, agriculturally induced degradation of freshwater streams has been shown to increase the basal cortisol concentration in the largemouth bass (*Micropterus salmoides*) and reduced the responsiveness (i.e., increases in cortisol production following a stressor) in the brown bullhead (*Ameiurus nebulosus*) (King et al., 2016). Therefore, we hypothesized that coral-dwelling fishes living in degraded environments would experience increased basal cortisol concentrations when exposed to degraded environmental conditions. Contrary to expectations, we found no consistent effect of habitat degradation on basal cortisol levels of juveniles of three species of damselfish. While we did observe higher cortisol concentrations in juvenile *P. moluccensis* reared in degraded environments for 3-weeks, we found no influence of habitat type on *P. amboinensis* or *D. aruanus*. Although only evident in one of the three species examined, the elevated cortisol levels in juvenile *P. moluccensis* provides the first evidence, to our knowledge, that a coral-associated fish may perceive a degraded environment as stressful (See Schrandt and Lema, 2011).

Interestingly, *D. aruanus*, the species with the strongest association to coral (Pratchett et al., 2012), had cortisol levels that were low and consistent across the three habitat treatments. This suggests that living in degraded habitats is either not perceived as a stressor or alternatively, that the effects were alleviated by other coping mechanisms. Behaviourally, this species exhibited the strongest avoidance of degraded habitats, thereby reducing its exposure to these conditions and potentially alleviating some of the adverse effects. As behaviour is a common mechanism used to alleviate exposure to stressors (Dubuc et al., 2019; Martínez-Mota et al., 2007), such behavioural adjustment may explain why this more coral associated species showed less impact when compared to *P. moluccensis*. Additionally, it is possible that *D.*

aruanus experienced an initial acute stress response, but the fish may have acclimated by the end of the experimental period, leading to a lack of variation in cortisol concentrations among treatments but realised effects on other traits (e.g., reduced growth in the present study). The attenuation of stress responses following acclimation to chronic stressors has been observed in other species, such as the brown bullhead (*Ameiurus nebulosus*), that exhibited a lack of a stress response to a physical stressor when residing in degraded habitats (King et al., 2016), which can indicate the inability of a species to deal with environmental stressors (Barton, 2002; Romero, 2004; Schrandt and Lema, 2011).

In the current study, we also found that regardless of the habitat treatment, cortisol concentrations were considerably higher for *P. moluccensis* and *P. amboinensis* than for *D. aruanus*. While this could be an artefact of the experiment, such as rearing in smaller tanks (Ramsay et al., 2006), it is unlikely as other studies examining whole-body homogenates in young juvenile pomacentrids have reported similar cortisol levels to those found in the current study for *P. moluccensis* and *P. amboinensis* (See Allan et al., 2020; Fakan et al., 2023). Together, our results show that different species residing in identical habitats can respond to habitat quality in different ways, highlighting the species-specific stress responses to habitat degradation.

Shelter is vital for animal survival (Steele, 1999) as it is central to predator-prey interactions, determines survival strategies such as camouflage or vigilance (Hemingson et al., 2022; Lima and Dill, 1990; Lind and Cresswell, 2005) and aids in energy savings (Reidenbach et al., 2006). Studies indicate that the absence of refuge leads to increased metabolism in fishes (Chrétien et al., 2021; Millidine et al., 2006). Furthermore, changes in shelter quality, not just its absence, can also impact metabolism (Garcia-Herrera et al., 2017). Therefore, in our study, we hypothesised that a shift between live tissue to algae-covered corals in shelter habitat would result in changes in metabolism. While some differences in the metabolic variables were detected, they were not nearly as pronounced as initially expected. The lack of metabolic response to habitat degradation in the present study may be related to the rearing environment, provided relatively “stable” conditions, without any predator or influence of external environmental factors such as current or UV. Therefore, extra energy was not required by individuals raised in degraded shelters. In contrast, previous research on *D. marginatus* found a 19% increase in SMR when individuals sheltered in clean dead coral, highlighting how shelter quality alone can influence metabolic rates (Garcia-Herrera et al., 2017). Methodological differences, such as using young juveniles in our study versus adults in theirs, may contribute to these contrasting findings, although *Dascyllus spp*. exhibit strong preference for live coral habitats throughout their life stages (Berenshtein et al., 2015; Liberman et al., 1995). Additionally, while our respirometry trials were conducted with only the fish in the chambers, their trials also included the habitat, potentially influencing oxygen consumption rates due to bacterial respiration on dead coral surfaces. Overall, our study suggests long-term impacts of degraded environments, whereas Garcia-Herrera and colleagues (2017) likely captured short-term effects of sheltering in non-preferred habitats, possibly explaining their higher SMR results.

Can sublethal effects influence communities?

Our behavioural space-use results displayed a gradient-like response: species with a higher degree of specialization for live coral tended to spend a greater proportion of time away from degraded habitats compared to healthy and mixed habitats. For example, *D. aruanus* in the live coral treatment spent less than 2% of their time away from the habitat, while in the algae covered treatment, they spent almost 60% of their time away from the habitat. Similarly, *P. moluccensis* in the live coral treatment spent 11% of their time away from the habitat, which was increased to almost 31% of their time away from the habitat when only dead algae covered habitat was available. Lastly, *P. amboinensis* exhibited the least change in time away between the habitat treatments; 19% in live coral and 23% algae covered treatments. This suggests *P. amboinensis* may have a lower association with live coral (Fakan et al., 2024; Ticzon et al., 2012) than previously thought (McCormick and Hoey, 2006; Pratchett et al., 2012). Overall, these behavioural modifications highlight that species are impacted differentially by coral degradation depending on their degree of association with live coral, with potentially serious consequences for those highly associated with corals, as they tend to avoid degraded structural habitats.

The condition of the host coral is known to affect how coral dwelling fishes view and use coral habitats. The tendency for *D. aruanus* and *P. moluccensis* to spend a greater proportion of time dwelling within live coral as opposed to algae covered is consistent with previous studies. For example, Boström-Einarsson et al. (2018) showed that *P. moluccensis* spent over 80% of their time sheltering between coral branches, but following coral mortality and algal growth, fish spent more than 80% of their time outside their coral colony. Although the primary driver for fish avoiding dead coral colonies remains unclear, our experiment indicates that this degraded state may interfere with the colonies' ability to provide refuge for coral dwelling fishes. Some work suggests that fish may avoid degraded habitats due to changes in the olfactory perception of the habitat, rendering it unsuitable for shelter (See Boström-Einarsson et al., 2018). Indeed, coral reef fish behaviour such as space-use, feeding and microhabitat selection is well known to be modulated by olfactory information emitted from reef substrates (Chivers et al., 2019; Coppock et al., 2013; McCormick et al., 2019b) and certain components (i.e., cyanobacteria and diatoms) of the degraded habitats may be responsible for altering fishes' response to this information (McCormick et al., 2017). Our study provides additional evidence that degraded habitats may be responsible for altered sheltering behaviour in coral reef fishes, which may be associated with changes in the olfactory information. Such variations in habitat use are likely to have important consequences for fitness as they are intrinsically linked to resource allocation and predator avoidance. Indeed, similar behavioural metrics to those measured in this study were found to be important drivers of survivorship in juvenile *P. amboinensis* in the field (McCormick et al., 2018). Spending more time away or migrating to nearby healthy coral would increase predation risk. Also as fish density on coral habitats increase and availability of suitable shelter decreases, other subordinates will likely be forced to use riskier or suboptimal habitats (Coker et al., 2012b; McCormick, 2009). As a result, behavioural mediation in response to degrading environments may leave fish more vulnerable to predation.

Factors influencing growth rates can have profound effects on the individual and ultimately on population dynamics. In the current study we did find that *D. aruanus* had significantly less mass when living in degraded environments and a similar, albeit non-significant, pattern was observed in *P. moluccensis*. Additionally, the standard length and body depth in these two species tended to be smaller in the degraded treatments. These results align with previous work on coral-dwelling fishes, which found that juvenile and adult yellow-tail blue damselfish (*C. parasema*) had significantly reduced growth rates and the humbug damselfish (*D. melanurus*) tended to grow slower when coral cover was lost, over a 29 day period (Feary et al., 2009). Reduced growth rates, such as those observed in the current study, can increase the time individuals remain small enough to be consumed by many predators and delayed introduction of individuals to breeding populations (Booth, 1995; Goatley and Bellwood, 2016). For recently settled fishes, mortality is not only high but often selective (Hoey and McCormick, 2004), and being smaller for longer would substantially increase the risk of predation on these individuals (Goatley and Bellwood, 2016). Indeed, having smaller body depth has recently been shown to be a main predictor of survivorship in newly settled *P.*

chrysurus (Fakan et al., 2024), likely restricting predation via gape sizes (Mihalitsis and Bellwood, 2017). As a result, sublethal impacts of habitat degradation have the potential to alter population demographics in coral reef fish by changing their behaviours and growth rates. Together these may help explain the observed changes in particular species following habitat degradation events (Bellwood et al., 2006; Cheal et al., 2002; Pratchett et al., 2008; Wilson et al., 2006).

This laboratory-based study allowed us to control for many factors but did not include some potentially important ecological interactions and feedbacks, such as predation and competition. For example, fishes were readily supplied with food twice per day, but on the reef the availability of food could vary depending on the location or habitat which would have various ramifications. Additionally, fishes reared in tanks did not have to compete with adult fish for space or food and these were not exposed to any predator. However, the non-consumptive effects of predation are known to alter various traits including morphology and stress responses (Breves and Specker, 2005; Ferrari et al., 2020; McCormick et al., 2019a; Mitchell and Harborne, 2020). Although these factors may negatively interact with habitat degradations impact on newly settled fishes, some caution should be taken when extrapolating this laboratory-based study.

In conclusion, this study provides evidence, albeit mixed, that habitat degradation induces stress responses across the primary, secondary and tertiary levels in coral dwelling fishes, mainly depending on their degree of live coral association. *P. moluccensis* was the only species to exhibit a primary stress response, confirmed by elevated cortisol concentration, suggesting that this species recognises habitat degradation as a stressor. The lack of primary stress response in the other study species may either indicate that lower habitat quality is, in fact, not stressful, or that some level of acclimation has occurred over time leading to muted stress responses. Secondary stress responses differed between species but were less pronounced than expected. This may be partially due to tertiary responses as *P. moluccensis* and *D. aruanus* showed strong behavioural avoidance of degraded habitats, although leading to subsequent morphological changes with individuals being smaller in these habitats. Changes at the individual level can translate to changes in local populations, communities and ecosystems. Therefore, it is crucial to gain a better mechanistic understanding across lower levels of biological organisation which will allow us to better predict how coral reef communities will fare in the face of continued habitat degradation.

Chapter 4: Habitat and morphological characteristics affect juvenile mortality in five coral reef damselfishes

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4.1 Introduction

Settlement from the planktonic environment ends the larval stage for most marine fishes and establishes them into their juvenile habitats. Ontogenetic transitions to new habitats are a window of vulnerability, as newly settled juveniles are exposed to a wide range of predators for the first time (Caley et al., 1996; Holbrook and Schmitt, 2003). This period is particularly important for coral reef fishes, where settlement is followed by high levels of juvenile mortality, creating a life-history bottleneck that can shape reef populations and communities (Almany and Webster, 2006; Doherty et al., 2004). However, the outcomes of predator-prey interactions can depend on a myriad of factors including prey boldness and escape performance (Fuiman et al., 2010; McCormick et al., 2018), size and condition of larvae (Booth and Beretta, 2004; Hoey and McCormick, 2004), camouflage (Mihalitsis et al., 2024), competition for refuge (Bonin et al., 2009b; Holbrook and Schmitt, 2002), predator density (Holbrook and Schmitt, 2003; Webster, 2002) as well as environmental factors including the availability of live coral or preferred habitat (Coker et al., 2012a; Majoris et al., 2018), rugosity and structure of coral (Beukers and Jones, 1997; Fakan et al., 2023; Fontoura et al., 2020). The relative importance of these factors vary among fish species, depending on their habitat and species associations at settlement (Webster, 2002). As settlement rates and early post-settlement survival are considered to be primary factors regulating population structures for coral reef fishes (Doherty et al., 2004; Doherty and Fowler, 1994; Jones, 1990), choices made by individuals at settlement can have population level consequences (Komyakova et al., 2019).

Coral reefs are biologically and structurally heterogeneous environments composed of a patchwork of microhabitats and habitat selection at settlement is well-established for reef fishes (Booth and Wellington, 1998; Caley et al., 1996; Sale et al., 1984; Tolimieri, 1995). With settlement occurring during the night, larval fishes primarily use audio cues to navigate and locate reefs (Kingsford et al., 2002) and olfactory cues of both local fishes and substrate for microhabitat selection (Coppock et al., 2020; Lecchini et al., 2005). This microhabitat selection process can differ markedly among species. For example, in cafeteria choice experiments settlement-stage *Pomacentrus moluccensis* consistently selected to settle on live coral and habitats containing conspecifics (either juveniles or adults), while *P. chrysurus* non-selectively settled onto rubble, live or dead coral and avoided habitats with conspecifics (Öhman et al., 1998). Furthermore, naturally settling pomacentrids have been shown to avoid experimental patches emanating olfactory cues of resident predators, suggesting a strong and innate olfactory recognition of predators (Vail and McCormick, 2011). In natural settings, however, the optimal habitats may not always be available with settlement patterns likely representing a compromise between locally available resources, selection, and avoidance preferences.

Selecting for a specific habitat or narrow scope of habitats is an ecological strategy (MacNally, 1995) that provides these specialist species improved fitness or survivorship and therefore a competitive edge over more generalist species when using their preferred habitats (Caley and Munday, 2003; MacNally, 1995). For example, the neon goby (*Elacatinus lori*) ends their larval phase by selectively settling on the outer wall of sponges before recruiting into the sponge tube where they spend much of their adult life (D'Aloia et al., 2011; Majoris et al., 2018). While *E. lori* are known to settle on various sponges, they preferentially settle on yellow sponges (*Aplysina fistularis*) over brown sponges (*Agelas conifera*) and this selection preference has been shown to significantly increase their survivorship in their host sponge (Majoris et al., 2018). Conversely, habitat generalist species have the ability to adapt to a wide range of environmental conditions and resources thus they can thrive in diverse habitats (MacNally, 1995). As habitat generalists, their associations with benthic characteristics at settlement should be broad and less focused (Wilson et al., 2008a), and the factors potentially affecting their survivorship are likely to be more varied. Clearly, for species that have strong associations with particular habitat at settlement, the availability of the required habitat will be a major determinant of their subsequent distribution patterns (Holbrook et al., 2000), while this will not necessarily be the case for less specialized species. Settlement into suboptimal habitats can be detrimental to growth and physical condition (Feary et al., 2009), yet much less in known about how preferences during settlement may affect survivorship (Booth and Beretta, 1994). Therefore, a better understanding of the environmental factors which promote survivorship is needed as these choices may strongly influence future population structures.

The capacity to perceive social contexts and subsequently make decisions about whether to settle within an existing assemblage of residents is crucial for both fitness and survival. For habitat specialists, olfactory cues of conspecifics can presumably indicate that the habitat is fitting (Lecchini et al., 2005), however for generalists, these cues may suggest higher density and/or competition and therefore may avoid such cues (Coppock et al., 2016). Joining aggregations of conspecifics may be costly, particularly for subordinates as adults aggression can influence settlement sites (Ben-Tzvi et al., 2009) and higher densities can increasing the likelihood of density-dependent mortality (Shima, 2001). Therefore, deciding to settle with or avoid conspecifics may be critical determinate of survivorship.

For species showing strong settlement preferences for particular environmental characteristics (Bonin, 2012; Coker et al., 2015; e.g., Holbrook et al., 2000; Pratchett et al., 2012), it is reasonable to expect that habitat selection would be beneficial. However, little is known about how habitat associations during settlement may affect the survivorship of reef fishes. To address this gap, we conducted an extensive field survey to determine whether the biological and physical factors of microhabitats used at settlement promote survival. Firstly, we quantified a suite of environmental and habitat factors of microhabitats on which we observed newly-settled damselfish individuals, including rugosity, depth, microhabitat composition and local fish community. We also measured for each fish different morphological traits recognized as important drivers of survival, namely standard length, body depth, and ocellus size. We then followed individuals through time to estimate natural mortality rates. This allowed us to establish whether the microhabitats on which fish settled were also associated with differential survivorship. We selected five common congeneric damselfish species that are known to vary in habitat use (Hata and Ceccarelli, 2016; Öhman et al., 1998; Pratchett et al., 2016): *P. adelus*, *P. amboinensis*, *P. chrysurus*, *P. moluccensis* and *P. wardi*. We hypothesize that some species would show stronger associations for particular habitat characteristics and for this selection to provide improved survivorship, while other species would have weaker habitat associations, and that the survivorship of these habitat generalists would be more influenced by non-habitat related traits.

4.2 Methodology

Study species

Five common site-attached congeneric damselfish species (*Pomacentrus*, Pomacentridae) that differ in their habitat use were selected for the study (Pratchett et al., 2016): (1) the lemon damsel, *P. moluccensis*, is an obligate coral-dwelling damselfish often found on colonies of branching coral; (2) the Ambon damselfish, *P. amboinensis*, is a facultative coral-dwelling species associated with a variety of substrata including live and dead coral; (3) the obscure damselfish, *P. adelus*, often associate with dead coral and rubble and pavement; (4) the white-tailed damselfish, *P. chrysurus*, commonly inhabit algal-covered rubble areas; (5) the Ward's damselfish, *P. wardi*, inhabit the reef edge or reef top associated with pavement, rubble and soft coral.

Field protocol

Habitat associations at settlement and early post-settlement survivorship of the five species were quantified across two shallow sites on the leeward (i.e., western) aspect of Lizard Island (14.6680° S, 145.4638° E), a high continental island in the northern Great Barrier Reef (GBR). Each site was 15x25 m, and flagging tape was attached to the substrata at 5 m intervals to create fifteen 5x5 m cells. Using these cells, key benthic features were mapped. Starting seven days prior to the new moon in October and November 2021, which coincides with the period of peak settlement for reef fishes on the northern GBR (Meekan et al., 1993), each site was systematically searched daily (08:00-13:00) for any newly-settled fishes of the five target species. Each newly-settled individual was captured using a dilute clove oil solution and hand-nets, transferred to a small plastic bag where it was laterally photographed against grid paper (0.5 cm grid) and tagged with an individually identifiable fluorescent elastomer tag (Northwest Marine Industries Inc.) using a 27-gauge needle (see Fig S1). When found in groups or near previously tagged individuals, each fish was uniquely tagged. Elastomer tags have been shown to not impact growth or survivorship of newly-settled pomacentrids (Hoey and McCormick, 2006). Fish were left in the plastic bag at the exact site of capture for 10 min (to allow full recovery), after which they were released at the site of capture. All tagged fish were observed to begin feeding within 30s of release. The site of capture was recorded on a site map, marked with a numbered tag and small piece of flagging tape attached to the substratum (see Fig S1). Morphological characteristics (i.e., standard length, body depth, and ocellus area) of each individual were subsequently measured using ImageJ from the lateral photographs of each fish. Standard length and body depth were highly correlated ($R^2 = 0.83$), therefore body depth was used in all further analysis, as it is considered a major morphological constraint which may restrict prey consumption (Mihalitsis and Bellwood, 2017).

While an individual fish was recovering from the tagging process, a 0.75 x 0.75 m quadrat centered on the exact site of capture was placed on the substratum and a photograph taken from a height of ~1 m above the substratum. The photo-quadrats were analysed using photoquad (Trygonis and Sini, 2012), recording the substratum under 100 stratified random points within each quadrat. The substratum was categorized as pavement (smoothed reef structure), rubble, sand, macroalgae, hard coral, soft coral, or other (e.g., clams, sponge). Macroalgae were identified to genus and hard and soft corals identified to genus and later grouped into broad growth forms (i.e., coarse branching, fine branching, mounding and soft coral) for analysis. The rugosity of the substratum within the quadrat was estimated as the ratio of a chain (link size 1.5 cm) that followed the reef contour to the linear distance across the centre of the quadrat (0.75 m) and again perpendicular to the first measure (McCormick, 1994), then the mean of both measures were used. The time and water depth of each quadrat was recorded, and depth standardised using tide charts. To estimate baseline benthic composition and compare across species settlement association, an additional 70 control quadrats were haphazardly photographed, and benthic characteristics measured in the same manner (see Table S1). All non-cryptic fishes in the immediate vicinity (within a 1 m radius) of the quadrat were identified to species, their total length (TL) estimated to the nearest cm. Surveys were conducted at each quadrat after the tagging of the focal recruit and each survey was conducted only once. From these surveys, individual species and life stages were pooled to create fish community variables (i.e., potential predators, conspecific juveniles and adults, congeneric juveniles and adults, and other territorial damselfishes). The potential predator group consisted of the non-transient predators *Pseudochromis fucus*, *Thalassoma lunare*, *T. hardwicke*, *T. nigrofasciatum*, and *Synodus dermatogenys*. Conspecific juveniles were defined as being ≤ 2 cm total length (TL), while adults were ≥ 3 cm TL. Territorial adults consisted of other noticeably aggressive pomacentrid species from the genera *Stegastes*, and *Dischistodus*. Together, these physical and biological microhabitat characteristics are known to correlate with distribution patterns of newly-settled reef fish (McCormick and Hoey, 2006).

To quantify survivorship, each site was thoroughly searched daily (15:00-17:00) for 14 days, and the presence or absence of tagged fish was recorded. When an individual appeared to be missing, a thorough search was conducted across an area of ~2 m radius from where the fish was initially tagged. If the tagged individual was not found over three consecutive days, it was classified as deceased.

Statistical analyses

Settlement associations

The differences among species in their association at settlement with (a) habitats characteristics or (b) fish community characteristics were explored separately using one-way permutational multivariate analyses of variance (PERMANOVA) based on Bray-Cutis distances (n = 10000 permutations). Bonferroni corrected pairwise comparisons were conducted. Control quadrats were included with habitats characteristics (a), to investigate how specialized species are and for which habitat characteristics. However, control quadrats could not be included in fish community characteristics (b) due to the grouping of conspecific and congeneric species. The nature of differences found between species by each PERMANOVA were visually displayed using a canonical discriminant analysis (CDA) (Seber, 1984). Species centroids and their 95% confidence intervals were plotted along the first two canonical axes, with habitat variables as vectors. The nature and strength of their association with each species are shown by their direction and length.

Survivorship

Differences in the survivorship among pomacentrid species were compared using a Cox's proportional hazard regression analysis (Cox PH) and trends were displayed using a Kaplan-Meier plot. This semi-parametric model describes the relation between the event (mortality), as expressed by the hazard function and a set of covariates (Bradbury et al., 2003). The Cox PH model assumption of propotional hazards between species were visually explored using Schoenfeld residuals and were satisfied. Next, the impact of morphological, environmental and local communities on the survivorship of each species were explored separately by Cox PH regressions. Initially, models were fitted using a maximum of six covariates, using the dredge function in the MuMIn package (Barton, 2009) in R (version 4.3.1). Then model averaging of non-significantly different weighted models ($wi \leq 0.95\%$), were used to construct the most likely model including significant covariates ($P = 0.1$). Again, the Cox PH model assumptions of proportional hazards for all significant variables within each species were explored and satisfied using Schoenfeld residuals. For *P. adelus*, ocellus size exhibited strong but highly variable influence on the model and was therefore included as a categorical predictor (< or ≥ median ocellus size) in the final model. Hazard ratios of the variables selected from the most likely model were visually explored using forest plots, which display the mean hazard ratio (HR) and 95% confidence intervals (CI). HR's above 1 indicates the covariate is positively associated with the probability of mortality occuring. The effect size, determined by 1-HR (see Bradbury et al., 2003), is denoted as odds ratios (OR). The OR can be interpreted as a percentage of their association with affecting the risk of mortality. For example, a HR of 0.16 is associated with reducing the risk of mortality by 84% (1-HR).

4.3 Results

Settlement associations

There was a significant difference in habitat associations among the five damselfish species at settlement (PERMANOVA; $F_{5,384} = 20.45$, $P < 0.001$), with *P. moluccensis* being associated with more rugose microhabitats and a higher cover of fine-branching corals, while *P. amboinensis* was associated with deeper and sandier sites and *P. chrysurus* being associated with a higher cover of rubble (Fig. 4.1a). In contrast, *P. adelus* and *P. wardi* exhibited more generalized associations with rubble, pavement and the macroalga *Turbinaria* and had a higher degree of spread of habitat use. Pairwise comparisons showed that all species significantly differed ($p < 0.05$) from each other and controls except, *P. adelus* and control quadrats and also *P. adelus* and *P. wardi* (see Table S2).

The five damselfish species also differed in their associations with fish community composition (PERMANOVA; $F_{4,315} = 7.62$, $P < 0.001$; Fig. 4.1b). Pairwise comparisons between all 5 species showed that most species associated with different fish assemblages at settlement ($p < 0.05$); the only exception being *P. amboinensis,* that was not significantly different to *P. moluccensis* or *P. chrysurus* (Table S3). Relatively low R^2 values for most pairwise comparisons of fish community characteristics suggests overlaps in associations of fish community variables, which is likely a response due to the coarse classifications of fish community traits (See Table S3). *P. adelus* associated with congeneric juveniles and *P. wardi* with congeneric adults and other territorial adults. *P. chrysurus* was found to associate with conspecific adults and predators. Both *P. moluccensis* and *P. amboinensis* tended to have a higher association with conspecific adults and juveniles than the other species, however, these associations were generally more variable, as indicated by the wider spread of habitat data.

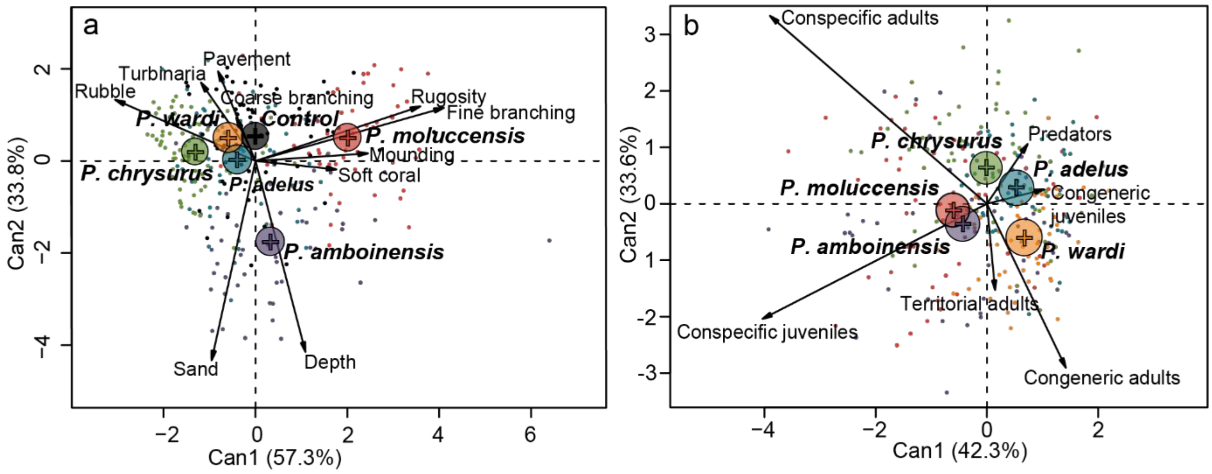


Figure 4.1 *Differences among five newly-settled pomacentrid species in their association at settlement with a) habitat characteristics and b) fish community characteristics. Biplots of canonical discriminant analyses show the first two canonical axes with loading scores. Crosses represent species centroids, circles surrounding crosses represent 95% confidence intervals for species. Vectors represent the direction and strength of the original variables to discriminate among species. Pomacentrus adelus (blue), P. amboinensis (purple), P. chrysurus (green), P. moluccensis (red), and P. wardi (orange) and control quadrats (black).*

Survivorship

The survival trajectories did not differ among the five pomacentrid species, with 33-52% of individuals remaining after 14 days post-settlement ($\chi^2_4 = 6.87$, P = 0.143; Fig. 4.2). Overall survival curves were steepest in the first four days, with 75.4% (*P. wardi)* to 58.7% (*P. amboinensis)* of individuals remaining after four days.

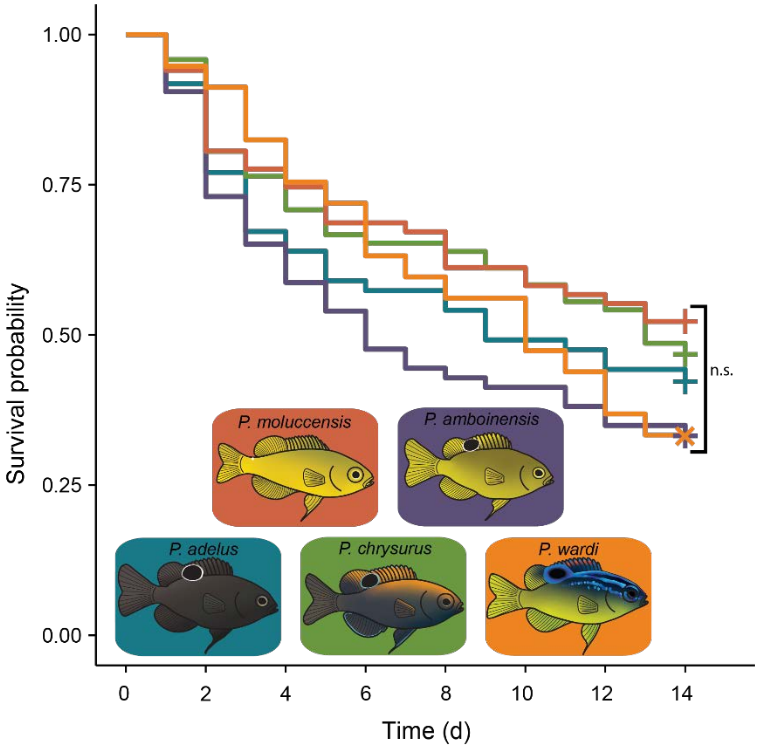


Figure 4.2 *Survival curves of five pomacentrid species for 14 days post-settlement at Lizard Island, northern Great Barrier Reef. Red: Pomacentrus moluccensis (n=67); purple: P. amboinensis (n=63); blue: P. adelus (n=61); green: P. chrysurus (n=72); orange: P. wardi (n=57).*

Influence of environmental associations on survivorship

P. moluccensis

The Cox PH regression analysis for *P. moluccensis* shows the presence and abundance of congeneric juveniles and the cover of mounding corals and sand significantly improved the fit of the model ($\chi^2_3 = 13.48$, P = 0.004; Figs 4.3a, 4.4). The presence and abundance of local congeneric juveniles (≤ 2 cm) were related with a reduced risk of mortality in *P. moluccensis* (HR = 0.69, P = 0.008) by 31% (i.e., 1-HR). In contrast, the cover of mounding coral was significantly positively associated with increased mortality risk (HR = 1.03, P = 0.040). Sand tended to increase the mortality risk but was found to be non-significant (HR = 1.04, P = 0.06).

P. amboinensis

The addition of rugosity, congeneric adults and *Turbinaria* coverage significantly improved the final model ($\chi^2_3 = 12.57$, P = 0.005) for *P. amboinensis* (Fig. 4.3b, 4.4). Higher rugosity significantly reduced risk of mortality by 85% (HR = 0.15, P = 0.010). The abundance of local congeneric adults and *Turbinaria* were both associated with increasing the probability of mortality by 24 and 27% respectively (HR = 1.24, P = 0.010; HR = 1.27, P = 0.040).

P. adelus

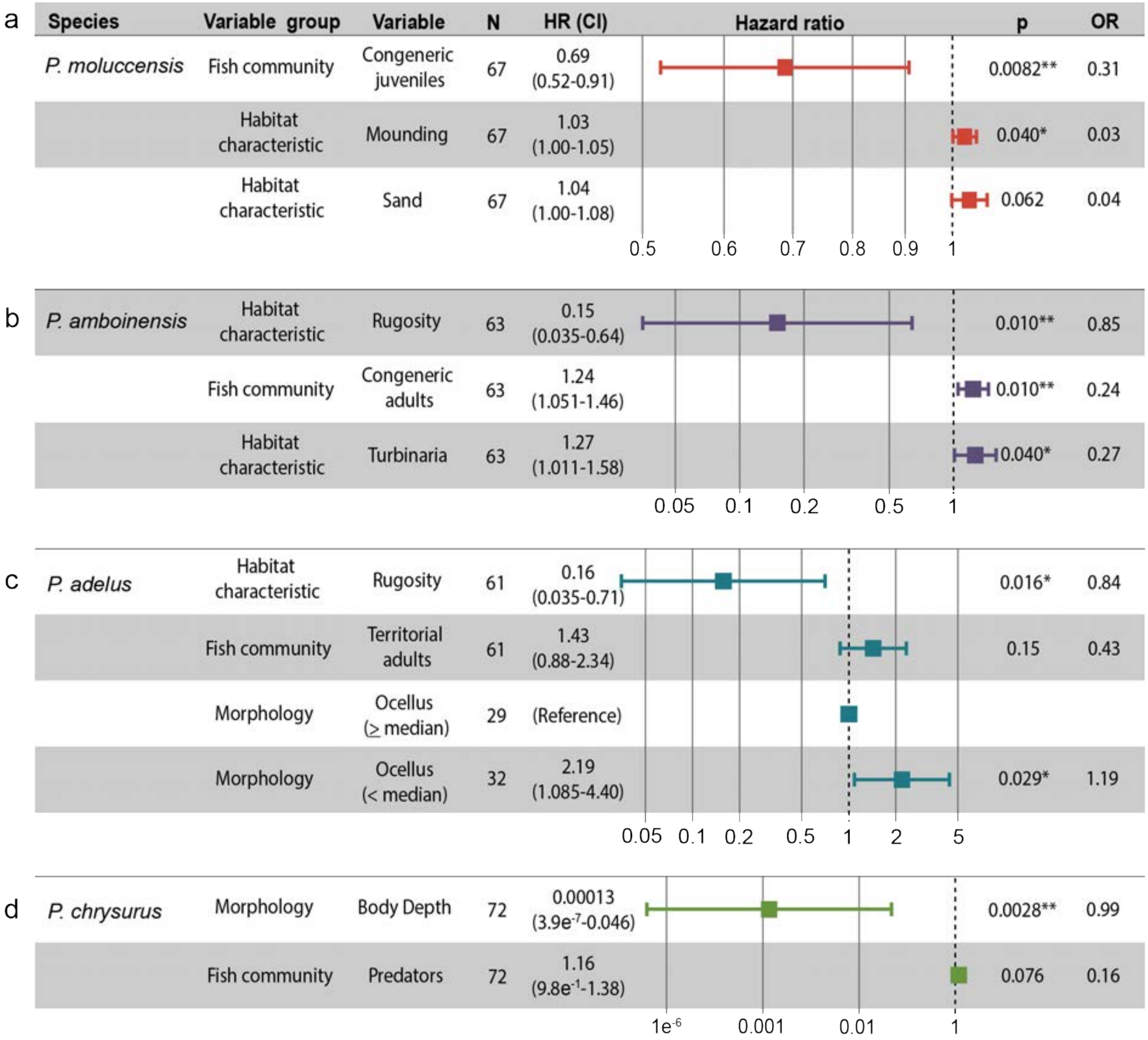
Rugosity, territorial adults and ocellus size (< median) significantly improve the survivorship model (χ^2_3 = 14.64, P = 0.002) for *P. adelus* (Fig. 4.3c, 4.4). Rugosity was negatively associated with mortality (HR = 0.16, P = 0.016), and improved the model fit by 84%. Having an ocellus smaller than the median (0.025mm^2) significantly increased the risk of mortality (HR = 2.19, P = 0.029) by 119% compared to individuals with an ocellus greater than median (Fig. 4.4). Territorial adults tended to increase the mortality risk but was found to be nonsignificant (HR = 1.43, P = 0.15).

P. chrysurus

Only body depth and predator abundance improved the survival model for *P. chrysurus* (χ^2_2 = 13.26, P= 0.0013; Fig. 4.3d). Having a shallower body depth was found to significantly increase mortality risk (HR = 0.0013, P = 0.003) by 99%. Increases in the abundance of local predators tended to increase mortality for *P. chrysurus*, however this was found to be non-significant (HR = 1.16, P = 0.076) (Fig. 4.4).

P. wardi

For *P. wardi*, no variables were found to significantly improve the survival model. The relative importance of the measured variable’s ability to explain the time-dependent mortality rate of *P. wardi* was considerably lower than the other *Pomacentrus* species examined. The relative importance of the best predictor (congeneric adults) was ~40%, less than half of other species’ best predictors (See Supplementary Fig. S2).



Species	Variable group	Variable	N	HR (CI)	p	OR
P. moluccensis	Fish community	Congeneric juveniles	67	0.69 (0.52-0.91)	0.0082**	0.31
	Habitat characteristic	Mounding	67	1.03 (1.00-1.05)	0.040*	0.03
	Habitat characteristic	Sand	67	1.04 (1.00-1.08)	0.062	0.04
P. amboinensis	Habitat characteristic	Rugosity	63	0.15 (0.035-0.64)	0.010**	0.85
	Fish community	Congeneric adults	63	1.24 (1.051-1.46)	0.010**	0.24
	Habitat characteristic	Turbinaria	63	1.27 (1.011-1.58)	0.040*	0.27
P. adelus	Habitat characteristic	Rugosity	61	0.16 (0.035-0.71)	0.016*	0.84
	Fish community	Territorial adults	61	1.43 (0.88-2.34)	0.15	0.43
	Morphology	Ocellus (≥ median)	29	(Reference)		
	Morphology	Ocellus (< median)	32	2.19 (1.085-4.40)	0.029*	1.19
P. chrysurus	Morphology	Body Depth	72	0.00013 ($3.9e^{-7}$-0.046)	0.0028**	0.99
	Fish community	Predators	72	1.16 ($9.8e^{-1}$-1.38)	0.076	0.16

Figure 4.3 *Forest plots of time-dependent Cox proportional-hazard regression analysis for Pomacentrus moluccensis, P. amboinensis, P. adelus, P. chrysurus (a-d). Each cube in the forest plot represents the hazard ratio surrounded by its 95% confidence interval and hazard ratios above 1 indicate that the covariate is positively associated with the probability of mortality occuring. Effect size calculated as 1-HR, is denoted (OR). Note: none of the variables considered were related to the survivorship of Pomacentrus wardi.*

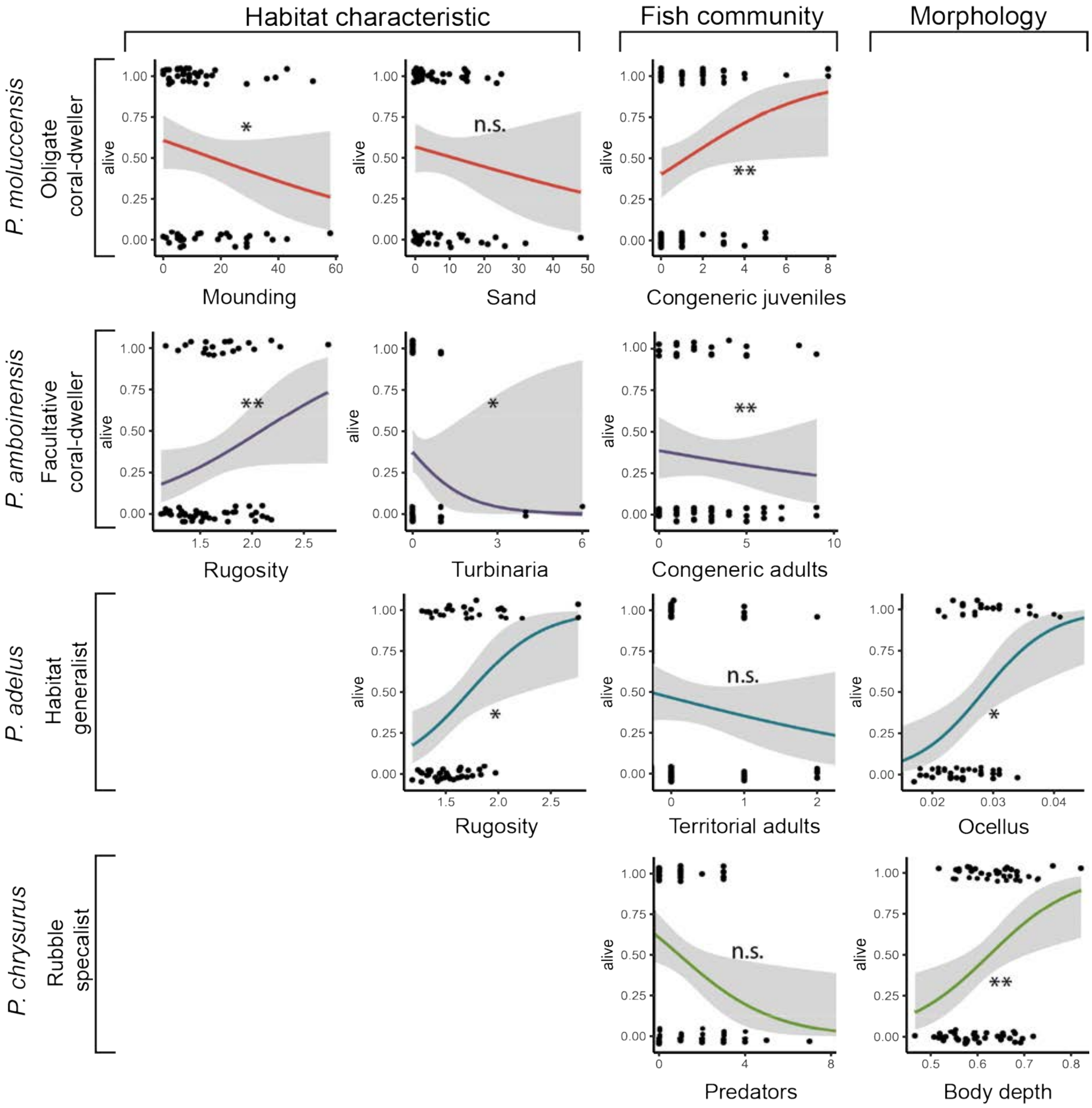


Figure 4.4 *Logistic regressions showing relationships of habitat, fish community and morphological variables selected by the Cox proportional hazard regression models that were found to influence the survivorship of four pomacentrid species: Pomacentrus moluccensis, P. amboinensis, P. adelus, and P. chrysurus. Note: significance notation derived from the Cox proportional hazard regression models and none of the variables considered were related to the survivorship of Pomacentrus wardi.*

4.4 Discussion

Overview

Identifying the drivers of survival following settlement is critical to understand and predict population structures for coral reef fishes (Booth and Beretta, 2021, 1994; Doherty and Fowler, 1994). Here we provide a detailed assessment the factors that affect early post-settlement survival of five congeneric coral reef damselfishes. Our study confirms that the drivers of mortality in juvenile coral reef fishes are species-specific and include a range of habitat, fish community and morphological characteristics. Contrary to expectation, we found only a weak relationship between the habitat associations at settlement and the factors that influenced subsequent survival. For example, *P. moluccensis* associated with areas of increased rugosity and cover of fine branching coral at settlement, *P. amboinensis* associated with deeper sites with higher cover of sand, and *P. chrysurus* associated with areas of higher rubble cover, yet none of these characteristics were important in predicting the survivorship in the respective species over the following 14 days. Interestingly, most of the habitat characteristics which were identified as important for survivorship were characteristics which showed a negative association at settlement. For example, relative to other species *P. amboinensis* had a negative association with the macroalga *Turbinaria* at settlement and those individuals which settled into areas with higher cover of *Turbinaria* had increased mortality. Similarly, *P. moluccensis* had a negative association with sand at settlement, and increasing levels of sand tended to increase mortality, albeit non-significantly. The one exception was that *P. moluccensis* associated with mounding corals at settlement, which was found to increase their risk of mortality over the next 14-days.

Our hypothesis that survivorship in generalist species would be broadly impacted by a range of habitat, fish communities and morphological characteristics was generally supported. Survivorship models for *P. adelus* selected three variables with each belonging to a habitat characteristic, fish community feature or morphological trait. While no variables were found to significantly improve the survival model for *P. wardi*, the six most important traits included three habitat characteristics, two fish community traits and one morphological (See Fig S2), broadly supporting that survivorship would be impacted by various traits. The lack of determination and clarity in this model may indicate that survival for this generalist species was particularly context dependent.

Habitat associations and their implications on survivorship

Coral reefs are heterogeneous environments composed of various microhabitat characteristics that may determine which species can thrive in that microhabitat. The current study illustrates clear differences in settlement associations among the five pomacentrid species examined, with three species (*P. moluccensis, P. amboinensis,* and *P. chrysurus*) associating for particular habitat characteristics, while *P. adelus* and *P. wardi* were more variable in their habitat associations (i.e., generalists). Our results of habitat associations align with previous works suggesting that *P. moluccensis* is associated with various types of hard coral at settlement (Pratchett et al., 2016; Ticzon et al., 2012) and *P. chrysurus* are predominantly found on rubble habitat (Low, 1971). In contrast, *P. amboinensis* is considered a facultative coral specialist (Pratchett et al., 2012), however its association with various types of hard coral were similar to the average coverage of the corals found in the control quadrats (see Table S1), suggesting little to no association with hard corals. While *P. amboinensis* was observed to associate with live coral, our data suggests that settling at the base of the reef in areas with higher cover of sand may be the primary habitat consideration for this species. Consistent with this hypothesis, Ticzon et al. (2012) found that juvenile *P. amboinensis* predominately associated with sand, using it more than any other species they investigated.

As mortality is a strong selective pressure on recently-settled reef fish, it is expected that selection for a particular microhabitat, would confer some fitness advantage (Majoris et al., 2018; Tolimieri, 1995). However, our results do not necessarily support this hypothesis, as most analyses did not identify the habitat characteristics chosen at settlement as important drivers of mortality. High rugosity or relatively high cover of fine branching coral were positively associated with settlement of *P. moluccensis,* however, neither were identified as important predictors of survivorship. In an earlier study using experimental mesocosms, the survivorship of *P. moluccensis* has been shown to increase when sheltering in coral with more rugosity (Beukers and Jones, 1997). It may be that the relationship between rugosity and survival follows a logistic-like curve, such that having a certain level of rugosity provides a benefit, but beyond a specific threshold, this benefit reaches a plateau. In the current study *P. moluccensis* may already be selecting for rugosity beyond the point of receiving a benefit, while *P. amboinensis* and *P. adelus* appear to be settling in the middle of their curve, such that those with above average rugosity benefited while those with below average rugosity suffer increased mortality. Conversely, *P. chrysurus* selectively settled in relatively lower rugosity microhabitats which was found not to relate to their survivorship, suggesting the importance of rugosity or level required is likely species specific, depending on the species ecological needs. As in some cases, increased rugosity may not always benefit prey (Fakan et al., 2023; Rilov et al., 2007).

Though the overall rugosity was not important for *P. moluccensis*, it may be that the finer structural components of coral are important. For example, we found that the cover of mounding corals in the immediate vicinity of newly-settled *P. moluccensis* was positively related to the probability of mortality. The lack of holes and reduced sheltering capacity of mounding coral (Aston et al., 2022) likely increases the exposure of small-bodied and newly-settled fish to predation (Almany, 2004). These areas with high cover of mounding corals did not necessarily translate to areas of lower rugosity, and may be related to the scale and nature of the rugosity provided (e.g., fine-scale structure within the branches of branching corals versus the vertical relief provided by mounding corals). While these likely underestimated the true rugosity, our results suggest that the finer scale refuge is important for survival. Consistent with this conclusion, Hixon and Beets (1993) found that *Chromis cyanea* had improved early post-settlement survival on experimental reef patches with small holes compared to large holes or no holes.

The habitat preferences of coral reef fish vary, and due to the high heterogeneity of reefs, some individuals inevitably settle in suboptimal habitats. Our data reveals that when assessing the relative influence of microhabitat characteristics on survival, insights from negatively associated habitat characteristics outweigh those from associated habitat characteristics. As fish traverse their home ranges, they are likely to use these suboptimal habitats. Thus, it is essential to consider all habitat characteristics surrounding their settlement site at a scale relevant to their home ranges. While data on the home ranges and space use of coral reef fishes, especially recently recruited juveniles, is limited, some studies suggest that adult *P. moluccensis* have home ranges of $< 2m^2$ (Streit et al., 2021), with juveniles expected to have even smaller ranges (Welsh et al., 2013). Anecdotal evidence by Booth and Beretta (1994) reports observing nine uniquely recognizable recruits which were never found more than 30 cm away from their initial location during a 30-day census period. This suggesting that home ranges of recruits may be relatively small and comparable to the quadrats used in the current study. Although when examining habitat use, previous studies have primarily focused on single point substratum assessments (Bonin, 2012; e.g., McCormick and Hoey, 2004; Pratchett et al., 2012; Wilson et al., 2008a) for exploring habitat selection and habitat selectivity measures (see Manly et al., 2002). However, these methods may miss broader habitat characteristics that influence fitness traits. For instance, *P. amboinensis* showed negative associations with the macroalga *Turbinaria*, significantly impacting survivorship, while *P. moluccensis* displayed a negative association with sand, which tended to increase their risk of mortality. Therefore, assessing the relative abundance of microhabitat characteristics and home ranges the fish interact with, rather than focusing solely on single substratum types, may lead to a more comprehensive understanding of the environmental factors influencing various fitness traits.

Habitat associations during the early life stage of coral reef fish may offer fitness benefits beyond their impact on early post-settlement survival. In the current study, species that associated with particular habitat characteristics received no survival benefits from such characteristics. However, they likely derive benefits in terms of faster growth from increased access to their preferred food sources. For instance, *P. moluccensis* is commonly found residing in corals high above the reef framework, positioning them close to plankton, which constitutes their primary diet (Pratchett et al., 2001). Similarly, unconsolidated substrata (e.g., sand and rubble) can foster higher densities of invertebrates (Wen et al., 2013b), which serve as a vital nutrient source for *P. amboinensis* (Debenay et al., 2011). Additionally, *P. chrysurus* is known to defend rubble-dominated territories, offering them access to the turf matrix that forms a significant part of their diet (Low, 1971). The increased availability of these food resources may enhance growth rates and potentially reduce predation risk, thereby contributing to overall fitness improvement.

Fish associations and their implications on survivorship

Grouping with conspecifics is an important trait to predict fitness outcomes in reef fishes (Booth, 1995; McCormick and Meekan, 2007). The current study highlights that species associations with characteristics of the fish community at settlement were indeed important, even though its importance was weaker than their associations with habitat characteristics. Our results show that *P. adelus*, *P. chrysurus* and *P. wardi* tended to settle in areas with higher abundances of congeneric species, while both *P. amboinensis* and *P. moluccensis* tended to settle in areas with higher abundances of conspecifics. These results align with previous works detailing grouping behaviours in both *P. amboinensis* and *P. moluccensis* (Öhman et al., 1998).

The formation of groups may be an ecological trade-off between survival and growth or may be due to preferential settlement. Grouping can have fitness impacts and is known to enhance survivorship in recently-settled reef fishes in some circumstances (Booth, 1995; McCormick and Meekan, 2007). Conversely, grouping can negatively impact the growth rates for recently-settled reef fish (Booth, 1995; Jones, 1990). For example, Booth (1995) showed that the time to reach maturity increased with group size, suggesting that living in smaller groups or living solitarily would allow an individual to more swiftly enter into the adult population. In the current study various associations with fish communities were found to impact the survivorship of each species. Congeneric adults significantly increased the risk of mortality in recently-settled *P. amboinensis*. Also, the abundance of adult territorial damselfishes (i.e., *Stegastes* and *Dischistodus*) similarly reduced the survivorship of *P. adelus*, another territorial or farming damselfish. Although newly-settled farming damselfish are unlikely to be directly competing for the same resources, adult damselfish may see them as future competitors. Alternatively, settling near other damselfish species has been shown to enhance early post-settlement survival (McCormick and Hoey, 2004), and may be an attempt to reduce conspecific group size, which can reduce growth rates (Booth, 1995). Interestingly, we found a positive relationship between congeneric juveniles and the survivorship of *P. moluccensis*. The additional prey items nearby may be reducing the predation pressure on *P. moluccensis* causing increased survivorship (Ferrari et al., 2020). Additionally, as densities increase, the optimal habitats will become saturated forcing newer recruits to suboptimal habitats. Our data suggests that early mortality rates are impacted by resident populations and probably in a density-dependent manner.

Although predators are known to be important regulators of coral reef fish communities (Chivers et al., 2019; Feeney et al., 2012; Webster, 2002), the abundance of local predators (*Pseudochromis*, *Thalassoma* and *Synodus*) was only associated with the survival rates of one of the five species, *P. chrysurus* (albeit non-significantly). This suggests that the mortality rates are not related to the predator densities recorded using visual surveys at the scale of the current study (Connell, 1996). This could be a result of local predators maintaining a larger home range than 1m perimeter surrounding the quadrat, resulting in underestimates of local predator and their activity from abundance. Additionally, a primary source of the mortality may be related to transient predators and/or cryptic predators (Hoey and McCormick, 2004; Mihalitsis et al., 2022) that may not be adequately quantified using visual surveys of specific sites. Also, due to the timing of the surveys (08:00-13:00) crepuscular or nocturnal fishes which may represent important predators may have been similarly underrepresented. While various components of the fish community impacted the survivorship of the studied species based on the species, it is clear that local communities do influence the survivorship at this life stage.

Morphological implications on survivorship

While habitat and fish communities were found to be important predictors of survival, morphological traits of individuals were found to be particularly informative. Although only two morphological variables (ocellus size and body depth) were used in the final survival analysis (of the total of 18 variables), these variables scored in the top 6 variables for relative importance for survival in three of the five species (Fig. S2), and for two of the five species they were the best predictors of survivorship. Interestingly, the morphological traits tended to be more important for species which are known territorial algae farmers (Hata and Ceccarelli, 2016). Our data suggests that in microhabitats with more aggressive residents, either size or dominance marking of larger ocellus may are providing survival benefits.

Although ocelli are hypothesised to confer some survival benefit (McPhail, 1977), few studies have explicitly demonstrated such benefits. We found the relative ocellus size to be the primary predictor of survival in *P. adelus*. The responsiveness of relative ocellus size to predator cues found in a recent study (McCormick et al., 2019a), also emphasizes their likely importance to survival. Interestingly, the ocellus was not found to be important predictors of survival in *P. amboinensis* or *P. chrysurus*. Indeed, while ocelli can provide certain advantages to some species in terms of predator deterrence and prey confusion, they may not be universally favored in all environments. Planktivores (e.g., *P. moluccensis*), for instance, are rarely found to possess ocelli (Hemingson et al., 2021), indicating that the benefits of ocelli in reducing predation risk may not apply universally across all ecological niches. Thus, ocelli may not serve as a broad and universally effective strategy for countering predation risks in all species and environments.

Body depth was also found to be a strong predictor in survival for *P. chrysurus*. Body depth is an important morphological trait which can enhance prey's escape responses (Domenici et al., 2008) and restrict predation by gape-limited predators (Mihalitsis and Bellwood, 2017). Together, our results support the long standing belief that bigger is better for coral reef fishes recruits (Vigliola and Meekan, 2002), as larger individuals of *P. chrysurus* had higher survival rates. In contrast to its importance, we only found size to predict survivorship of one species. This disparity may stem from the influence of local predators on *P. chrysurus* (although non-significant), which are smaller than transient predators and, consequently, more limited by gape size.

In summary, our study revealed contrasting habitat selection behaviors among different fish species during settlement, with some displaying specific preferences for certain microhabitat characteristics, while others exhibited a more generalized approach. Furthermore, we identified several environmental and morphological traits associated with the survival of recently settled fishes. Intriguingly, species that associated with particular microhabitats showed higher survival rates when they were not associated with specific habitat characteristics. While we initially anticipated that microhabitat characteristics promoting settlement would also enhance survival, the predictive power of such associations was generally weak within the scope of our investigation. Nonetheless, selecting specific habitat characteristics during settlement may yield longer-term fitness benefits, such as improved growth and faster maturation. This study underscores the diverse ways in which various habitat characteristics can influence populations.

Chapter 5: Habitat complexity and predator odours impact on the stress response and antipredation behaviour in coral reef fish

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5.1 Introduction

Globally, ecosystems are faced with a variety of climate-induced and local anthropogenic stressors that lead to degraded systems with reduced structural complexity and lower species diversity(Chapin et al., 2000; Pratchett et al., 2011). Coral reefs are particularly vulnerable to such degradation, largely due to the thermal sensitivities of the corals themselves (Marshall and Baird, 2000). As the frequency and severity of marine heatwaves is increasing worldwide, coral bleaching and subsequent mortality is predicted to intensify (Hughes et al., 2018b), likely leading to further reductions in habitat complexity (Pratchett et al., 2008). Reduction in the structural complexity of reef habitats has considerable implications for coral reef fishes, which are often closely tied to the physical characteristics of their habitat (Graham and Nash, 2013). For many reef fishes, structurally complex branching corals represent preferred settlement habitats due to the provision of refugia, and the loss of these corals leads to reduced abundances and diversity of reef fish assemblages as exposure to predators and predation risk increases (Jones et al., 2004). As many coral reef habitats are in a state of change, it is important to understand how structural complexity interacts with the perception of risk in reef fishes.

Habitat complexity can influence predator-prey interactions through the provision of shelter and by altering visibility, detection, and responses of both prey and predators, and thereby the probability of a predation event to occur and its success (Gregor and Anderson, 2016). Habitat complexity is therefore an important driver of the spatial variation in predation risk (Bleicher, 2017). The way prey perceive their environment is in part controlled by the fear of predation (Brown et al., 1999), and the spatial variation in predation risk throughout their environment i.e., ‘landscape of fear’ which describes the trade-off prey encounter across a risk gradient (Gaynor et al., 2019; Laundre et al., 2010). For example, following the reintroduction of wolves (*Canis lupus*) to the Yellowstone National Park (USA), the spatial distribution of elk (*Cervus elaphus*) increased in structurally more complex woodlands, highlighting that elk assessed open grasslands to be more dangerous when wolves were present (Creel et al., 2005).

This study emphasizes that structural complexity is an important component of both a prey's perceived and actual risk of predation within its environment.

While a lack of, or lagged response to a predator may be costly in terms of survival, continually responding to non-lethal threats is energetically costly as prey forego foraging and social opportunities (Gonzálvez and Rodríguez-Gironés, 2013; Ydenberg and Dill, 1986); therefore, the first step in avoiding predation is assessing the risk of predation. However, prey usually have incomplete information about their surrounding environment resulting in less than accurate assessments (Sih et al., 2000), forcing prey to over- or under-estimate risk (Van der Veen, 2002). The more information a prey has about predation threats, the better it will be able to optimize the balance between vigilance and other fitness-related behaviours. Consequently, prey often use multiple cues to identify potential predators, assess the level of risk they pose, and modify anti-predation behaviour appropriately (Amo et al., 2006; McCarthy and Fisher, 2000; McCormick and Lönnstedt, 2013).

Combining information from multiple cues can provide a more accurate assessment of risk, promoting optimal responses (Brown et al., 2004). In many fishes the main senses used to assess risk are vision, olfaction, and mechanoreception (e.g. hearing, vibrations) (Crane et al., 2022; Hartman and Abrahams, 2000; Kelley and Magurran, 2003). Using olfactory cues alone, prey can quickly detect predator odours but these cues may linger after a predator has departed the area, leading to overestimations of risk (Kats and Dill, 1998). In contrast, visual cues can provide immediate information on predator size, location and potentially motivation but prey may be more exposed to predation risk in obtaining visual cues (Smith and Belk, 2001; Van der Veen, 2002). When used in conjunction, odours and visual cues aid in positively identifying threats and determining the motivation of potential predators, and are therefore, important components within the decision matrix whereby prey determine the appropriate response (Amo et al., 2006; Mathis and Vincent, 2000).

If predator avoidance is unsuccessful and a potential prey is exposed to a predator, prey may reduce predation risk through behavioural responses such as maintaining a safe distance, decreasing activity, increased vigilance and/or initiating an escape response (Domenici and Hale, 2019; Tang et al., 2017). In fishes, a fast-start escape response is a common anti-predation behavior which corresponds with a short but high energy swimming burst typically in a C-shaped motion (Domenici and Blake, 1997; Domenici and Hale, 2019). Faster escape responses have been found to increase the likelihood of prey escaping a predator (Walker et al., 2005) and be good predictors of survivorship of fishes in the wild (McCormick et al., 2018). Fishes can optimize their escape response to a predatory threat depending upon the information available, including the presence and motivation of a predator (Domenici, 2010; Langerhans et al., 2004; Ramasamy et al., 2017, 2015). In particular, the presence of chemical alarm cues, such as odours from the damaged skin of conspecifics, prior to a predatory strike has been found to ‘forewarn’ prey, leading to more effective escape responses (McCormick and Allan, 2017; Ramasamy et al., 2015).

This optimized escape response through forewarning may have at its basis a cortisol stress response (Clinchy et al., 2013; Lima, 1998) as the synthesis of cortisol can prime decisions and movements (Øverli et al., 2002). As such, it has been hypothesized that increased cortisol synthesis in response to predators and/or cues may regulate the sensory processing used in detecting predators and ultimately shape prey’s responses (Bleicher, 2017; Harris and Carr, 2016; Lima, 1998). The mechanism that facilitates this improved antipredator response may be associated with the stress response (Barreto et al., 2014; Wendelaar Bonga, 1997). In vertebrates, the primary response to stress is a rapid elevation in glucocorticoids (such as cortisol or corticosterone), which quickly releases glucose into the blood priming the body for increased activity if required (secondary stress response) (Wendelaar Bonga, 1997). As such, olfactory cues of risk have been shown to alter the behaviour and physiology in fishes (Miyai et al., 2016; Sanches et al., 2015). For instance, risk cues have been shown to increase cortisol concentrations and induce defensive behaviours in zebrafish (*Danio rerio*) (Oliveira et al., 2014), likely in anticipation of an imminent threat. To some extent, the stress response may have a positive effect on prey by improving reaction times to a strike, and hence their probability of escape.

It is expected that the degradation of coral reefs will influence how predators interact with their prey and the relative balance of senses prey use to judge risk during predator-prey interactions (Chivers et al., 2016). As habitat complexity decreases, the visibility and olfactory cues of prey to predators (and vice versa) will increase (Catano et al., 2016; Large et al., 2011), potentially making degraded environments perceived as riskier for prey. While it is known that risk cues can cause an increase in cortisol levels (Oliveira et al., 2014; Sanches et al., 2015), habitat complexity levels have resulted in various impacts on cortisol levels; with reduced complexity decreasing (von Krogh et al., 2010), increasing (Giacomini et al., 2016; Woodley and Peterson, 2003), or having no impact (Pounder et al., 2016; Wilkes et al., 2012) on cortisol concentrations. A better understanding of the interaction between habitat complexity and risk assessment is required to interpret how the dynamics of predator-prey interactions may shift in degraded ecosystems (Crane et al., 2019).

The aim of this study was to examine how levels of habitat complexity interact with olfactory predator cues in modifying the fast-start escape response of a common coral reef damselfish, *Pomacentrus chrysurus*, and whether the mechanism underlying the response was cortisol-related. Newly-settled damselfish were chosen as prey because the transition from pelagic larvae to settled juveniles represents a critical bottleneck where mortality is extreme (Almany and Webster, 2006; Hoey and McCormick, 2004). Newly settled *P. chrysurus* were reared in tanks containing one of three levels of topographic complexity for two weeks and then their fast-start responses were measured in the presence or absence of odours from a known predator. We predicted that predator odours would heighten the escape response of *P. chrysurus* and that lower levels of complexity would further exacerbate this response. Specifically, we predicted that prey fish would recognize predator odours as a threat and enhance their fast-start response. Prey may associate a lower complexity environment with higher risk and therefore, be more vigilant and respond sooner and more strongly to any perceived threat. Additionally, we hypothesized that if altered, escape responses induced by predator odours and/or habitat complexity may be mediated through elevated cortisol concentrations.

5.2 Methods

Study species & housing conditions

Pomacentrus chrysurus, the whitetail damselfish is a rubble-associated, omnivorous fish that is common across the Indo-Pacific. Recent research has shown that the whitetail damselfish's ability to learn and respond to predators can be impacted by coral degradation (Chivers et al., 2021b). Fish were collected around Lizard Island (14°40'S, 145°28'E), northern Great Barrier Reef (GBR) in November of 2016. Naïve settlement-stage whitetail damselfish that had not being exposed to reef-based predators were collected with light traps moored at least 50 m off the fringing reef. Light traps were deployed at dusk and collected at dawn the following morning, whereby the catch was immediately sorted and all whitetail damselfish captured were haphazardly placed into 32 L plastic tanks (432 x 324 x 305mm) containing one of three levels of complexity for 2 weeks prior to the start of the experiment. Three replicate tanks of each complexity were established with ~45 fish per tank. The three levels of complexity were manipulated by altering the number of resin coral models (~8 × 3 × 5 cm) within the tanks: no corals (low complexity), 3 model corals (medium complexity), and 6 corals (high complexity). The structural complexity or rearing environments can play an important role in shaping the behavioral phenotypes in captive fishes (Crane et al., 2015; Näslund, 2021). The standard length (SL) of the whitetail damselfish (16.2 ± 0.7 mm) was measured following the 2-week housing, from kinematic videos. Fish were fed *ad libitum* with newly hatched *Artemia* twice daily, but not fed 12 h prior to commencement of the experimental trials to standardise for satiation in the trials.

The piscivorous rock cod, *Cephalopholis boenak*, was selected as a model predator as it is known to prey on juvenile damselfishes (Stewart and Jones, 2001). Two *C. boenak*, were caught using hand nets and an anaesthetic clove oil solution and transferred immediately to individual 68 L aquaria with 5-7 PVC tubes as shelter where they were housed until they were used in the trials. Each aquaria was supplied with fresh flow-through seawater and supplemental aeration. *C. boenak* were fed juvenile cardinalfish daily, which is phylogenetically distant from the target damselfish to avoid providing information from diet (McCormick et al., 2019b). Newly recruited cardinalfish were caught in light traps and housed in 32L plastic tanks with fresh flow-through seawater and fed as per whitetail damselfishes. Every day, one cardinalfish per *C. boenak* were euthanised through cold shock using an ice slurry, and a net was used to ensure fish did not encounter ice. This method of euthanasia has been shown to be fast (~7 s) and cause little distress in small fishes (J. M. Wilson et al., 2009). The herbivorous surgeonfish, *Acanthurus nigrofuscus,* were used as a behavioural control for olfactory cues of any non-threating heterospecific fish species. Two *A. nigrofuscus* were caught using a barrier net and hand nets, and transferred immediately to individual 68L aquaria supplied with fresh flow-through seawater, supplemental aeration and 5-7 PVC tubes as shelter. *A. nigrofuscus* were regularly provided with fresh pieces of coral rubble covered in algal turfs on which to graze. All fish were transported to the Lizard Island Research Stations aquarium facility in 68 L tanks within an hour of capture and no mortality occurred during transportation or housing periods. Following experimental trials, surgeonfish and rock cods were released near the original capture site, and the remaining damselfishes and cardinalfishes were distributed over multiple reefs.

Animal ethics

Research was carried out under approval of the James Cook University animal ethics committee (permit: A2005, A2080) and according to the University’s animal ethics guidelines.

Experimental overview

In summary, settlement-stage *P. chrysurus* were reared within tanks that had one of three levels of habitat complexity (low, medium, or high) for 15 days. All fish were conditioned to recognized predator odours as a threat (see pre-conditioning below for details). Following rearing, individual *P. chrysurus* were placed into fast-start escape trial tank with complexity that matched the rearing environments and exposed (5 min) to one of three olfactory cues (predator odour, herbivore odour, or a saltwater control). Then a weighted stimulus dropped overhead to elicit their fast-start escape response, which was recorded at high speed for kinematic analysis. A subset of fish were collected following the odour acclimation, but prior to the stimulus dropping, to quantify cortisol concentrations. See Figure 5.1 for overview of timeline and experimental design. Odours from the piscivore, *C. boenak,* were used to represent a threat while odours from the herbivore, *A. nigrofuscus,* were used as a behavioural control to account for the effect of exposing *P. chrysurus* to olfactory cues of any non-threating heterospecific fish species. The saltwater treatment was used to control for the injection of cues.

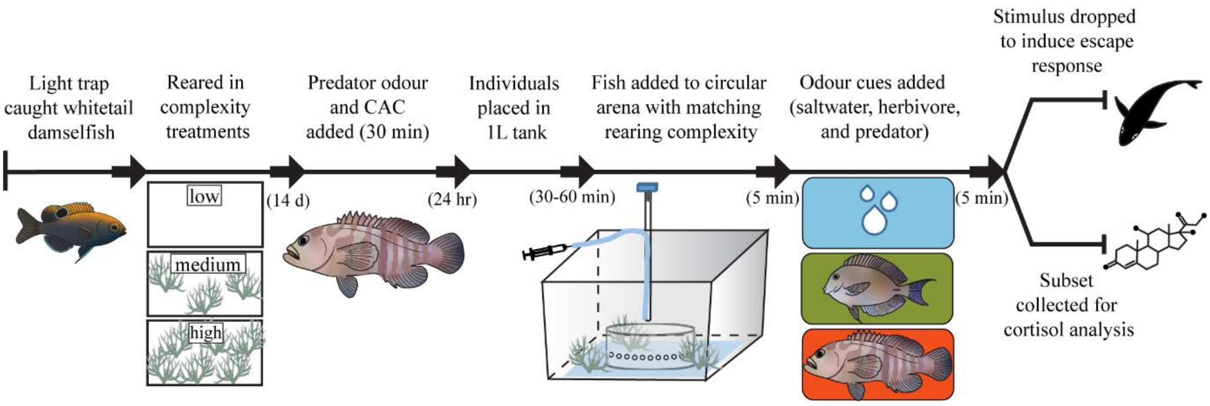

Figure 5.1 *Schematic diagram of the timeline for treatment conditioning and experimental design. On the 15th day in complexity treatments all juvenile P. chrysurus were trained to associate predator odours (C. boenak) as a threat with chemical alarm cues (CAC). After acclimation in circular arena, either saltwater, herbivore (A. nigrofuscus) or predator odour was introduced for 5 minutes. Most fish were startled by a stimulus to induce fast-start escape response, which were recorded from below, while a subset of saltwater and predator samples were collected for cortisol analysis. See Figure D1 in Appendix D for more details on experimental tank.*

Pre-conditioning

Fifteen days after being placed into one of three topographic complexity treatments (see above), fish were pre-conditioned to associate the smell of *C. boenak* as a threat using associative learning. This was done to ensure that the experimental whitetail damselfish associated *C. boenak* as a threat because these fish were naïve to reef-based predators. Pre-conditioning occurred only once and was conducted the day prior to experimental trails. This involved pairing alarm cues released by damaged juvenile whitetail damselfish with the odour of the predator. Chemical alarm cues (CAC) were obtained through 5 superficial cuts to both sides of 4 euthanised (as described above) whitetail damselfish and each rinsed with 15 ml of seawater (Chivers et al., 2021a). The odour from the predator was prepared by turning off the flowing seawater and leaving the tank containing the predators with aeration for 3 h. Prey were exposed to 1 L of water from the predator tank (to their 32 L tank) plus 60 ml of CAC for 30 min (Chivers et al., 2019). These were injected simultaneously into the tanks housing the *P. chrysurus* to be used in all experimental trials for the day, which was left without water flow (just aeration) for 30 min. This coupling of the predator odour cues with a CAC leads to the assignment of risk to the cues through a process known as associative learning. Previous research has shown that newly settled damselfish can learn to associate the olfactory cue of a predator with risk after only one exposure when paired with a CAC (Ferrari et al., 2010).

Experimental protocol

Escape responses were examined in a transparent circular acrylic arena (diameter 200mm; height 100 mm), within a large opaque-sided plastic tank (585 length x 420 width x 330 mm height; 60 L) with a transparent Perspex bottom to allow responses to be filmed as a silhouette from below. The circular arena was used to ensure that the focal individual remained roughly in the center of the tank where the stimulus was dropped. The large tank was illuminated by a LED light strip wrapped around the outside of the tank with light penetrating with even illumination through the opaque sides. The water level was maintained at 80 mm (~ 20 L) to reduce movements in the vertical plane, and the water in the tank was emptied and refilled with flow-through seawater after every trial to avoid a build-up of odours and to maintain temperature. The same plastic corals used in the rearing tanks were randomly placed within the experimental tank (excluding arena), representing various complexity levels each individual were reared in. See Figure S1 for more details on experimental tank.

At the beginning of each trial, fish were carefully transferred to a 1 L acclimation tank (one fish/tank) held within a water bath (to maintain the same temperature) and left for 30 – 60 min. Fish were then carefully transferred individually to the arena and allowed to acclimate to the tank and complexity level for 5 min. The complexity level in each trial matched the individuals' rearing complexity level and complexity was the coral models were placed around but not inside the fast-start arena. Odours were then introduced into the arena through a clear plastic tube attached to the stimulus weight tube that hung over the center of the arena and fish were left for an additional 5 min. It has been previously shown that 5 minutes is sufficient to induce an acute stress response, measured as cortisol, in marine fishes (Grutter and Pankhurst, 2000). Holes in the arena allowed flow of water during acclimation. Predator and herbivore odour treatments involved the slow injection of 15 ml of seawater from either the *C. boenak* or *A. nigrofuscus* holding tanks (prepared as described above) and flushed with seawater (15 ml) to ensure all the odours entered the arena. The saltwater control consisted of slowly injecting 30 ml of seawater. All odours introduced within the escape trials excluded CAC. All olfactory injection tubes were flushed with 60 ml of seawater between trials. After the second 5 min acclimation within the arena, fish were startled with the release of the stimulus weight, which was suspended within a 48.5 mm diameter white PVC pipe placed directly over the center of the arena. The stimulus was released into the water using an electromagnet and remained invisible to the juvenile fish until the falling stimulus touched the water surface. The stimulus was only released once the fish swam into the central region of the tank which allowed all individuals to move an equal distance in any direction and standardized for fish position relative to the stimulus. A monofilament line prevented the stimulus from hitting the bottom of the arena, thus ensuring the escape response was triggered by the stimulus breaching the water surface. To ensure a standardized protocol, prey escape variables were only measured when prey performed a C-start (commencement of fast-start that results in the individual forming a C-shape). High-speed (480 fps) videos of fast starts were filmed using a Casio ZR1000 camera. See Figure S1for visualization and description of kinematic trial setup. All trials were conducted between 10:00 and 17:00 h. Salinity (35 ppt) and temperature (29˚C) were kept constant throughout the study period and trials. The sample sizes for low complexity trials were n = 20, 20, and 19 for control, herbivore and predator, respectively. For medium complexity trials n = 20, 20, and 21 and lastly for high complexity trials n = 20, 20, and 23 (control, herbivore, predator), respectively.

To determine if the predator odor cue caused a physiological stress response, a subsample of fish were collected for whole-body cortisol analysis via enzyme-linked immunosorbent assay (ELISA). These fish were subjected to the exact same protocol up until when they were startled with the stimulus (see above). Just prior to the startle but following the 5 min acclimation to odour treatments, fish were quickly removed from the arena, rapidly killed with cold shock (as described above) and stored in liquid nitrogen for subsequent cortisol analysis. The herbivore treatment was used as a behavioural control and therefore no herbivore samples were undertaken for cortisol analysis. Sample sizes for cortisol from low complexity trials were n = 10 and 12 (control and predator respectively) and for medium complexity trials n = 9 and 11 and high complexity trials n = 9 and 11.

Kinematic analysis

Kinematic variables associated with the fast-start escape responses were analysed using the image-analysis software Image-J, with the manual tracking plug-in (imagej.nih.gov/ij/). The centre of mass of each fish was tracked through stage 1 and 2, (i.e., the first two axial bends, defined based on Domenici and Blake (Domenici and Blake, 1997)), which is the period considered crucial for avoiding predator attacks. The following kinematic variables were measured:

1. Response latency (s) was measured as the time interval between the stimulus onset and the first detectable movement leading to the escape of the animal.
2. Response speed (m s^{-1}) was measured as the distance covered within a fixed time (41 ms). This fixed duration was based on the average duration of stage 1 and 2 from juveniles of a similar species (Hess et al., 2019).
3. Maximum response speed (m s^{-1}) was measured as the maximum speed achieved during any frame during stage 1 and stage 2 (Domenici and Blake, 1997).
4. Maximum acceleration (m s^{-2}) was measured as the maximum increase in speed between frames during stage 1 and stage 2.
5. Response distance (m) is a measure of the total distance covered by the fish during stage 1 and 2.

Cortisol extraction and ELISA validations

Whole-body cortisol was extracted using a method described by Allan et al., (2020) and was measured with a commercially available cortisol ELISA kit (Cayman Chemical Item Number 500360), in March of 2019. Briefly, individual fish were freeze-dried (Christ Alpha 1-2 LDplus, 0.2 mbar, >16 h) and weighed (Mettler Toledo UMX2 Ultra-Microbalance, 0.1 µg readability) prior to being homogenized in 2 ml Eppendorf vials, using a glass bead and 0.5 ml phosphate-buffered saline (PBS, pH 7.4) in a shaking mill (MP Biomedical FastPrep24) for 3 min. The homogenate was then transferred to a 10 ml glass vial and rinsed with an additional 0.4 ml of PBS and then ethyl acetate was added at a 1:9 ratio. The samples were vortexed for 1 min before being centrifuged (Eppendorf 5810 R) at 3,500 rpm for 5 min at 4 °C. The supernatant was collected, and the extraction steps were performed four times, pooling each extraction step. The ethyl acetate was dried off in a centrifugal vacuum concentrator (Thermo Savant SpeedVac SC110A, 43°C) and the samples were reconstituted within 48 hours using 1 ml assay buffer. The samples were analysed in triplicates with a spectrophotometer (SpectraMax Plus 384 Microplate Reader, Molecular Devices) and the average absorbance was calculated from readings between 405 and 420 nm. See Allan et al., (2020) for more details on cortisol extraction.

Prior to measuring cortisol concentrations, assay validations steps (parallelism, accuracy and precision) were performed for the cortisol ELISA kit, following recommendations by Metcalfe et al., (2018). Parallelism was confirmed by an ANCOVA's homogeneity of slopes assumption by comparing dose–response curves of diluted samples for each fish against a standard curve (ANCOVA, P>0.05, n=3; Fig S2). In brief, the reconstituted samples (n=3) were diluted (1:1, 1:2, 1:4, 1:8, 1:16, 1:32, 1:64, and 1:128) and compared against the cortisol standard curve (Cayman Chemical ELISA kit, 6.6–4000 pg ml^{-1} range). The optimal dilution for the samples fell between the dilutions 1:8 and 1:16; therefore, a sample dilution of 1:12 was chosen to achieve a 50% relative maximum binding and only sample dilutions falling within 20–80% B/B_0 relative maximum binding were accepted. The accuracy or recovery rate of the extraction method was assessed by spiking samples (n=4) with 800 pg cortisol ml^{-1}. For each of the four samples, two fish were homogenized, pooled and split into halves, with one half receiving the spike and the other the assay buffer. Both halves were then processed in the same way as all other samples. The spike's recovery (percentage) was expressed as spiked sample result−unspiked sample result×100/known spike (800 pg ml^{-1}), and the mean recovery (78.5%, n=4) was used as a correction factor for calculating the samples' cortisol concentration. Intra-assay precision of triplicate samples was determined using the coefficient of variation (CV) and found to be 3.8±3.6 (mean±s.d., n=62). See Fig S2 for dose–response curves, comparison between standard curve and recovery rate from method of extraction.

Statistical analyses

Escape response

Of our 183 kinematic trials, 143 (78.1% across all treatments) performed C-start escape responses. To investigate if the frequency of responsiveness (Domenici and Hale, 2019) was impacted by treatments, a Pearson's Chi-square test was used and found no association between performing escape responses and treatments (χ^2_8=12.13, p=0.146; See Table S1 for sample sizes). A similar level of responsiveness of *P. chrysurus* to burst stimulus has been shown in other studies (Velasquez Jimenez et al., 2020). The latency to respond to the stimulus was positively related to distance to the stimulus (ANCOVA: $F_{1,133}$= 77.09, p<0.001) and the nature of the relationship was consistent among treatments (homogeneous slopes: $F_{4,125}$ =0.64, p=0.637). Therefore, the residuals of this relationship were used in subsequent analyses to remove the influence of the distance to the stimulus on latency alone. No other variables were impacted by distance to the stimulus. A two-way multivariate analysis of covariance (MANCOVA) was performed to test whether there were differences in the escape response variables among complexity treatments (low, medium, and high), potential forewarning odours (predator, herbivore, and saltwater control), and their interaction. Complexity and odour treatments were fixed factors, the five escape variables were used as dependent variables, and the standard length of the fish was used as a covariate. Box's M test was satisfied (P=0.095). All dependent variables from the MANCOVA were further examined using a two-way analysis of covariance (ANCOVA) with Bonferroni Bonferroni-corrected comparison post-hoc tests to determine the nature of the significant difference found within the ANCOVA's. Post-hoc comparisons from significant ANCOVA's are provided in supplemental materials (see Table S2). Partial eta-squared (η^2) are given as an estimate of effect size. The assumption of normality was visually examined (Q-Q plots) and homogeneity of variance tested (Levene's test). Response and kinematic analyses were performed in SPSS (IBM, version 27).

Physiological stress response

A two-way ANOVA was used to examine the effect of complexity (low, medium, high) and odours (control, predator) on whole-body cortisol concentrations. Tukey's HSD post-hoc tests were conducted to determine the nature of any significant differences from the ANOVA.

Post-hoc comparisons are provided in supplemental materials (see Table S3). Partial eta-squared are given as an estimate of effect size. The assumption of normality was visually examined (Q-Q plots) and homogeneity of variance tested (Levene's test). Cortisol analysis was performed in R, version 3.5.1 (R Core Team, 2023).

5.3 Results

Escape response

A two-way MANCOVA revealed a significant difference in the escape performance based on complexity (Pillai's trace 0.14, $F_{10,260} = 1.89$, $p = 0.047$) and odour (Pillai's trace 0.20, $F_{10,260} = 2.85$, $p = 0.002$), but no interaction effect was observed (Pillai's trace 0.19, $F_{20,528} = 1.28$, $p = 0.188$). The nature of these differences was further explored by two-way ANCOVAs. These indicated that nearly all fast-start variables were impacted by the level of complexity and the forewarning odour, however, no interaction effects were observed (Table 5.1). High complexity resulted in faster response speed (Figure 5.2; $F_{2,133} = 3.83$, $p = 0.024$), higher maximum speed ($F_{2,133} = 5.62$, $p = 0.005$) and an increase in response distance ($F_{2,133} = 3.83$, $p = 0.024$), when compared to low levels of complexity. Compared to the saltwater controls, exposure to predator odour significantly increased the response speed ($F_{2,133} = 4.45$, $p = 0.013$), and escape distance ($F_{2,133} = 4.45$, $p = 0.013$), while exposure to herbivore odour reduced maximum acceleration ($F_{2,133} = 4.77$, $p = 0.010$). Neither complexity levels nor forewarning odours were found to have an impact on response latency ($F_{2,133} = 0.74$, $p = 0.481$; $F_{2,133} = 1.22$, $p = 0.299$, respectively).

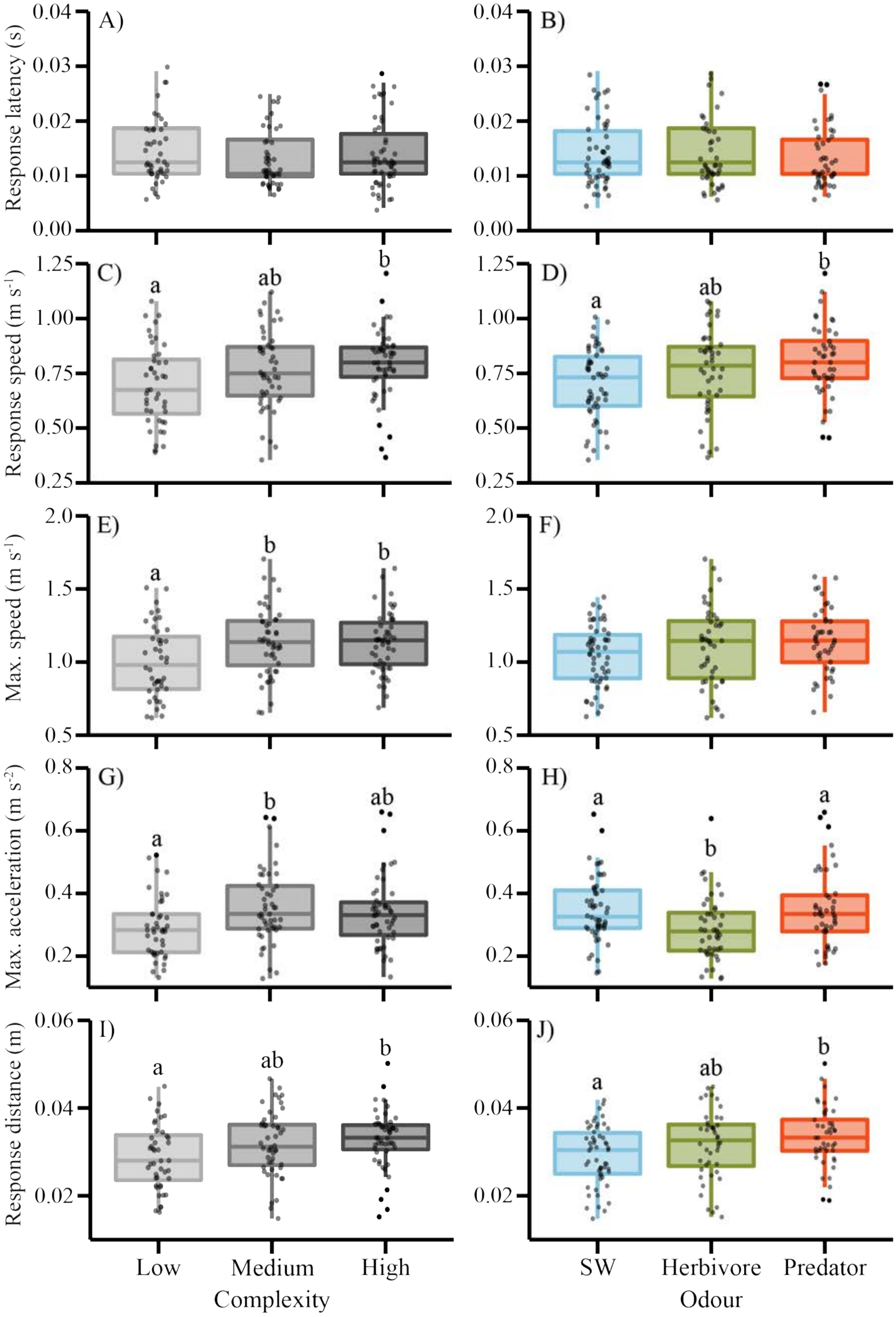

Figure 5.2 *Effect of complexity and forewarning odours on escape response and kinematic of Pomacentrus chrysurus. Boxplots show the median and 25% quantiles, black dots are outliers,*

grey dots are raw data points for response latency (A,B), response speed (C,D), maximum speed (E,F), maximum acceleration (H,I), and response distance (J,K). Letters above bars represent LSD groupings of means. Sample sizes for complexity trials were n= 44, 48, and 51 (low, medium, high), and for predator odour trials n= 54, 44, and 45 (control, herbivore, predator), respectively.

Table 5.1 *Results of two-way ANCOVA's on the fast-start response variables for Pomacentrus chrysurus. Summary of the fast-start escape response variables: response latency (s), speed (m s^{-1}), maximum speed (m s^{-1}), maximum acceleration (m s^{-2}), and response distance (m) in juvenile P. chrysurus reared with varying levels of complexity (low, medium and high) and presented with potential forewarning odours (predator, herbivore, and salt water). Standard length was used as a covariate. Degree of freedom are presented with each model and bold values are significant at alpha = 0.05. Partial eta-squared (η^2) are given as an estimate of effect size.*

	Complexity $_{(2,133)}$			Odour $_{(2,133)}$			Complexity*Odour $_{(4,133)}$		
Variable	F	p	η^2	F	p	η^2	F	p	η^2
Latency	0.74	0.481	0.011	1.22	0.299	0.018	1.37	0.249	0.039
Speed	3.83	**0.024**	0.054	4.45	**0.013**	0.063	1.19	0.318	0.035
Max Speed	5.62	**0.005**	0.078	2.15	0.121	0.031	1.55	0.191	0.045
Max Accel	5.19	**0.007**	0.072	4.77	**0.010**	0.067	0.85	0.495	0.025
Distance	3.83	**0.024**	0.054	4.45	**0.013**	0.063	1.19	0.318	0.035

Physiological Stress response

A two-way ANOVA revealed a significant interaction between complexity and odour on the concentration of cortisol (Table 5.2: $F_{2,56} = 6.66$, $p = 0.003$). Juvenile *P. chrysurus* reared in the low complexity treatment and exposed to predator odours prior to the burst stimulus had significantly higher cortisol levels than fish from low complexity saltwater controls and from the medium complexity predator odour treatment (Figure 5.3). In the low complexity treatments, the mean (±SE) cortisol concentrations of fish treated with sea water and predator odour water were 39.56± 6.80 pg mg^{-1} and 69.54± 6.76 pg mg^{-1}, respectively. The presence of predator odour altered cortisol concentrations only at low structural complexity, resulting in a context-dependent physiological response.

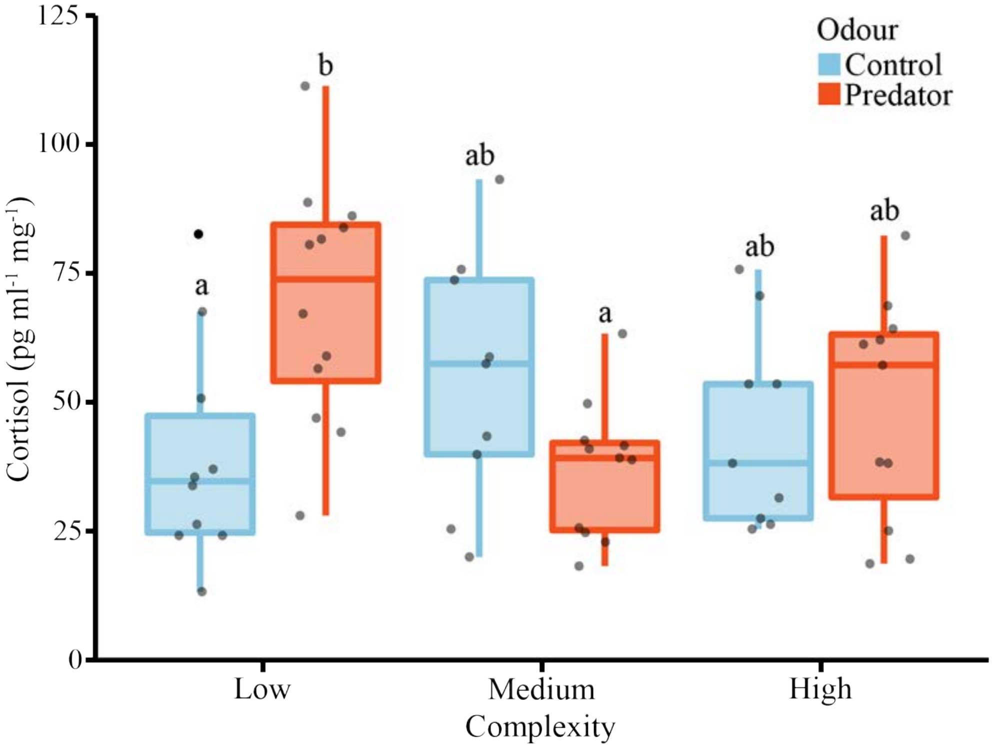

Figure 5.3 *Effect of complexity and forewarning odours on cortisol concentrations of juvenile Pomacentrus chrysurus. Boxplots show the median and 25% quantiles, black dots are outliers, grey dots are raw data points and letters above bars represent Tukey's HSD groupings of means. Sample sizes for control odours were n = 10, 9, and 9 (low, medium, high) and for predator odours n = 12, 11, and 11 (low, medium, high), respectively.*

Table 5.2 *Results of two-way ANOVA on cortisol concentrations of juvenile Pomacentrus chrysurus. Summary of the effect of complexity and forewarning odours on cortisol concentrations from whole-body homogenate of juvenile P. chrysurus. Degree of freedom are presented with each model and bold values are significant at alpha = 0.05. Partial eta-squared (η^2) are given as an effect size.*

Source	F	p	η^2
Complexity $_{(2,56)}$	1.14	0.327	0.039
Odour $_{(1,56)}$	1.11	0.297	0.019
Complexity*Odour $_{(2,56)}$	6.66	**0.003**	0.192

5.4 Discussion

Predation risk can play an important part in predator-prey dynamics (Brown et al., 1999; Creel et al., 2005; Leris et al., 2022) and can also have cascading effects on community composition and ecosystem functions (Catano et al., 2016; Gregor and Anderson, 2016; Rilov et al., 2007). Our results show that a common coral reef damselfish (*P. chrysurus*) perceives risk based on available information and environmental context. Both the level of habitat complexity and olfactory predator cues can behaviorally modify escape responses in juvenile fishes, although we found the effects of complexity and olfactory cues on the behavioral response to be independent and not additive/interactive as hypothesized. Our results suggest that trends in the fast-start response may in part be associated with context-dependent cortisol concentrations and their influence on response motivation. The current study shows that the whitetail damselfish can modify anti-predation behaviours when presented with risk cues odours from a predator and similarly that increasing levels of complexity were perceived as increased risk.

Our results also highlight the important role that environmental context plays in antipredation responses. In contrast to expectations, our results suggest that whitetail damselfish perceived increased complexity as increased risk, as their escape responses were heightened with increasing complexity. For example, between the low and high complexity treatments, the mean response speed of prey increased by 13.7% and the maximum speed increased by 13.3%. While we hypothesized that a lack of complexity would leave prey exposed and more vigilant, our data indicates fish reared in higher complexity environments had enhanced kinematic performance, potentially due to reduced visual information of the surrounding environment causing prey to over-estimate the risk. Although shelter is known to provide many benefits to prey (Crane et al., 2019; Woodley and Peterson, 2003), complex habitats may challenge their ability to accurately assess risk and occasionally impede escape pathways (Metcalfe, 1984). The lack of visual information from increased turbidity has been shown to reduce exploration, activity levels and is associated with higher perception of predation risk in a freshwater minnow (*Pelasgus stymphalicus*) (Leris et al., 2022). Similarly, Hess et al. (2019), found that juvenile damselfish *Amphiprion melanopus* exposed to turbid environments displayed heightened escape response when compared to clear water controls. By reducing visual information, habitat complexity can increase the uncertainty of predation risk which can impact fitness. For instance, Rilov and colleagues (2007) found that artificially reducing the field of view for the bicolor damselfishes (*Stegastes partitus*) decreased mating attempts and distance ventured from their nest. The low complexity environment in the current experiment offers more visual information which is preferably used by prey to gauge the intentions and motivation of any approaching threat (Van der Veen, 2002), and therefore, may allow them to respond in an optimal manner.

Olfactory cues used in risk assessment provided forewarning and improved escape performance. In our study, juvenile whitetail damselfish perceived the predator odour of the rockcod to be a threat and responded by escaping faster. For example, when exposed to predator odours prior to the simulated predator strike, the mean response speed of prey increased by 14.9% compared to control treatment while exposure to odours of a non-threating herbivore had no significant impact on response speeds. These results support earlier findings on the whitetail damselfish (McCormick and Allan, 2016), which showed juveniles appropriately assessed olfactory, visual, and combined cues of another rockcod (*C. microprion*) as a predation threat and enhanced both mean and maximum response speed in response, while exposure to a non-predatory butterflyfish (*Chaetodon trifasciatus*) had a substantially lower impact. Similarly, Ramasamy et al., (2015) found that exposing damselfish (*Acanthochromis polyacanthus*) to olfactory, visual, and combined cues of a common predator (*Pseudochromis fucus*) improved escape responses. Together these results show that as levels of apparent predation risk increase, prey prime their escape performance to optimize energy use, which highlights the context-dependent nature of fishes' escape response (Domenici, 2010).

Although no interaction occurred between complexity and odours in the kinematic analysis, it is of interest to explore the relative impacts each treatment had on the escape response of *P. chrysurus*. When comparing the escape response between saltwater control and predator odours, the forewarning of a threat led to a more risk adverse response, escaping faster and further. However, when comparing the complexity treatments, we found that the escape responses of higher complexity treatment matched the magnitude that was induced by the predator odour treatment. Similarly, the intensity of escapes in low complexity treatments aligned with saltwater controls (See Fig 2). Using the partial eta-squared as an estimate of effect size we can see that complexity and odours had similar impacts on the escape variables (e.g. distance; complexity $\eta^2 = 0.054$ and odour $\eta^2 = 0.063$; See Table 1), indicating that prey perceived the rearing environment and lack of visual information to be roughly as threatening as olfactory cues of a known threat. Our data suggests that prey over-estimate the risk when visual information is restricted and that the potentially alleviating influence of shelter does not outweigh the uncertainty of predation risk in the whitetail damselfish.

A short-term rapid elevation in cortisol may be adaptive by increasing survival-related behaviors (Harris and Carr, 2016), since energy stores are utilized and redirected to improve cardiovascular, muscular, and cognitive abilities (Wendelaar Bonga, 1997). For example, exogenously increasing corticosterone levels in tree lizards (*Urosaurus ornatus*) and subsequently exposing them to a predator enhanced their anti-predation behaviors (Thaker et al., 2009). Tree lizards with higher corticosterone levels had a faster latency to respond to the predator and hid longer than control lizards. In fishes, exogenous cortisol implants in the frillfin goby (*Bathygobius soporator*) were found to increase survival-related behaviours, such as reduced activity and increased sheltering (Barreto et al., 2014). Similarly, micropredation events by gnathiids have been shown to increase cortisol concentrations in the Ambon damselfish (*P. amboinensis*) and consequently, reduce activity levels (Allan et al., 2020). However, this study found fast-start escape responses to be negatively impacted by increased cortisol. An explanation to the disparity in our results compared to those of Allan et al. (2020) may be that the cortisol levels induced by the gnathiids were much higher than in our study, suggesting that their stress response might represent a chronic effect which can become maladaptive (Wendelaar Bonga, 1997). The interaction effect between olfactory cues and habitat complexity on the cortisol response observed in the current study only partially aligns with improved survival behaviours. As our cortisol data indicates that fish from both predator odours and lower complexity treatments would be stressed, suggesting their response would be similar and advantageous to survival. Although most kinematic fast-start escape variables were enhanced with the addition of predator odour, in the lower complexity treatments escape responses appear unaffected as they aligned similarly with the saltwater controls. Our physiological results indicate that the structural environment can alleviate the stress of predation risk. However, to better understand the role cortisol plays in modifying fast-start escape responses we suggest future studies explicitly test the effects of exogenously induced cortisol on the kinematic responses of fishes.

In addition to affecting behavioral responses, the threat of predation can also induce a stress response, however, environmental characteristics can interact with prey perception to mediate such responses. Woodley and Peterson (2003) found that in the absence of shelter, the visual presence of a predator elevated cortisol levels by four-fold, and altered the metabolism and growth of the longnose killifish (*Fundulus majalis*). However, these effects were not observed when adequate shelter was provided (Woodley and Peterson, 2003). Their study highlights both the impact predators can have on important hormonal, physiological, and whole-animal performance traits as well as the ability of shelter to mediate these impacts during predator-prey interactions. In the current experiment we found that predator odours and structural complexity interacted to influence cortisol concentrations in a context-dependent manner. The whitetail damselfish experienced higher cortisol levels when exposed to predator odours but only when structural complexity was low. In accordance with Woodley and Peterson (2003), our data shows that the whitetail damselfish recognizes the threat of a predator odour, but only perceives it as a physiological stressor when shelter is lacking.

While our physiological data shows that the structural environment is important to mediate a stress response, a considerable amount of variability is present in our cortisol data, which may arise for a variety of reasons. Although 5 min has been shown to induce an acute stress response in the blackeye thicklip wrasse (*Hemigymnus melapterus*) from a physical stressor (Grutter and Pankhurst, 2000), the limited exposure time (5 min) of odours may have limited the rise in cortisol levels, considering we used a psychological or anticipatory stressor (Clinchy et al., 2013). However, if too much time is provided between the odour and burst stimulus, prey may realize there is no imminent threat. Additionally, although care was taken to transfer fish to arena tanks, this handling may have induced some stress and increased variability among all treatments. Likewise, allowing for additional acclimation time may have reduced this variability. Even with this variability, our finding of significant context-dependent physiological response between habitat complexity and threat of predation suggests the 5 min time period used was a sufficient timeframe for the current study, although longer would likely reduce this variability.

While many coral reef areas are transitioning from complex coral ecosystems to more homogenous algal dominated seascapes, the impact of this degradation on predator-prey relationships is poorly understood. It has been suggested that as habitat complexity is lost, consumptive effects would increase (Graham and Nash, 2013), however, with reduced structural complexity comes the provision of more sensory information about potential threats.

The current study suggests that higher levels of complexity can be perceived to be higher risk and that the increased visual information in low complexity environments may help prey to appropriately assess risk and respond more effectively. With the capacity to better assess risk and modify escape responses, prey may be able to partly alleviate the risk of increased predator-prey interactions in more degraded environments. Additional work should attempt to understand how complexity may alter the rates of predator strikes and their success to provide a more complete picture of the ecology of predator-prey interactions in changing environments.

In conclusion, we found that the whitetail damselfish modified its fast-start escape response when forewarned with olfactory cues of a predator and that the degree of habitat complexity could additionally shape these responses. Higher habitat complexity supported improved escape performance, likely a result of overestimating risk as visual information became limited. Our whole-body cortisol levels suggested that whitetail damselfish became stressed when forewarned with predator odours, but only when complexity levels were low. The present study did not allow us to elucidate cortisol’s role in directly modifying anti-predator responses, representing an area of interest to explore in future studies.

Chapter 6: General Discussion

Determining how dependent species are on particular habitat features, and how changes in those habitat features can influence individual fitness levels is central to predicting the likely future composition and structure of communities. This thesis addressed an underrepresented area of research, focusing on the sublethal effects of habitat degradation on coral-associated fishes (Chapter 2, 3 and 5). While such sublethal impacts of changes in coral cover have been observed and studied in corallivorous butterflyfishes (e.g., Pratchett et al., 2004), highlighting that the loss of coral cover can reduce their fitness and abundances, these impacts are also relative to their specialization for using corals as a food source (Berumen et al., 2005; Pratchett et al., 2006). However, little research has been conducted to link such impacts on fishes that heavily rely on live coral as a shelter. This thesis investigated whether variations in habitat characteristics like live coral cover or structural complexity were linked to stress-hormonal changes (Chapter 2 and 3) as a mechanistic pathway in which fishes may suffer sublethal consequences, such as increased cortisol, and subsequentially altered behaviour, body condition and survival. Additionally, by examining how the structural rugosity of environments influences the survival of juvenile coral reef fishes (Chapter 4 and 5), this thesis confirms the importance of such a feature to support the replenishment of new individuals to a population (Fontoura et al., 2020; Graham and Nash, 2013).

In Chapter 2, I studied whether natural variations in habitat characteristics related to cortisol levels and whether cortisol levels were related to various fitness metrics, ultimately testing the cortisol fitness hypothesis (Bonier et al., 2009a). Chapter 2 is the first known study to examine the cortisol fitness hypothesis in coral reef fishes. This chapter found that variations in habitat characteristics were related to cortisol concentration which was in turn related to individuals' condition in some cases, while in other instances, variations in habitat characteristics were directly related to individuals' conditions but not mediated through cortisol. The observation that increased cortisol levels had a negative relationship with fitness measures in two of three species provides some support for the cortisol fitness hypothesis and that inclusion of relevant resources improved these relationships (Breuner and Berk, 2019). However, habitat characteristics which were unrelated to cortisol but influenced fitness implied other, non-stress related mechanisms were also contributing to these findings. Broadly these results highlight that the relationships and effects varied among species which may relate to their various associations with habitat characteristics and that habitats can influence reef fishes fitness via the stress response.

Another primary aim of this thesis was to shed light on the role of the living tissues of corals on coral-associated damselfish species. Prior work by Garcia-Herrera and colleagues (2017) suggested that highly specialised coral-dwelling species (*Dascyllus marginatus*) sheltering in dead coral displayed a higher standard metabolic rate (19%), and it was suggested this may be a sublethal impact induced by stress response. Therefore, this primary stress response (Barton, 2002) was experimentally explored as a potential mechanistic pathway in which reef degradation may regulate metabolism, physiological condition, behaviour, and ultimately populations (Chapter 3). However, I found no substantial impact of living in degraded environments (i.e., dead coral rubble and algae) on the metabolism of three coral associated damselfishes (Chapter 3). Notably, this study was conducted in the absence of predators and therefore the necessity and true importance of refuge may have been underrepresented. At the same time, this chapter (Chapter 3) did reveal that the behavioural habitat-use and morphological traits of these fishes were impacted and, in a gradient-like response, with the specialised species being more impacted than the species with lower associations to coral. Additional efforts should be made to clarify the conflicting results between this thesis and prior research (Garcia-Herrera et al., 2017) and further elucidate the hormonal findings. In accordance with our findings, hormonal data from Chapter 2 found that basal cortisol concentrations were poorly predicted by the percentage of coral cover in individuals home ranges. Overall, some aspects including sheltering behaviour and growth-related traits were directly influenced by the presence of live coral tissues which have been previously shown to be altered in degraded environments (Boström-Einarsson et al., 2018; Feary et al., 2009). However little evidence was discovered for live coral tissue influencing the fishes at the endocrine and metabolic level.

This thesis builds on and expands the current literature which highlights the importance of physical structure created by scleractinian corals. It is acknowledged that the structural rugosity of a reef allows a higher abundance and diversity of fishes to coexist (Almany, 2004; Ceccarelli et al., 2016; Jones and Syms, 1998). A primary role of the structural rugosity of reefs is to mediate direct mortality through predator-prey interactions, which largely impacts the early juvenile life-stage (Fontoura et al., 2020). Examining this process *in situ*, on newly settled juveniles showed that settlement sites with higher structural rugosity was a strong predictor of survivorship in multiple species (Chapter 4). I extended this work to show that this improved survivorship may be a result of altered anti-predation behaviours, as newly settled juveniles from environments with higher levels of rugosity had improved escape responses (e.g., max speed and distance travelled) from a potential predator (Chapter 5). This indicates physically structure may not only impede predators strikes, but also provides environmental context in which prey used to gauge the level of threat a nearby predators and influences the preys overall stress response. By impacting this early life-stage, rugosity can influence immigration of new individuals into populations and therefore has the potential to regulate communities. Additionally, this thesis expands the current knowledge of the importance of rugosity by exploring its influence on fitness levels and the endocrine system (e.g., cortisol production), a promising area for future research. Interestingly, the results of this thesis show that the responses to rugosity varied among species with juvenile *P. amboinensis* from more rugous habitats having substantially higher lipid content, while juvenile *P. moluccensis* from lower rugosity habitats had higher stress levels (Chapter 2). Overall, the results of this thesis emphasize that the physical environment of the habitats can influence the behaviour, condition, hormones and ultimately survivorship of coral reef fishes.

When coral loss occurs, many species tend to decline, however species which associate with live coral are often disproportionately affected and a broad aim of this thesis was to investigate if this disproportional response was induced by coral-associated fishes recognizing habitat degradation as stressor. As the pressures of climate change intensify, environmental challenges, including degradation events, are expected to increase (Emslie et al., 2024). With these challenges, stress levels in organisms are anticipated to rise, while overall fitness declines (Bonier et al., 2009a). This thesis hypothesizes that reef degradation will lead to elevated cortisol levels and reduced fitness in coral-associated fishes. This could lead to selective mortality towards less fit coral-associated individuals (Hoey and McCormick, 2004) and therefore providing a mechanistic cause for the disproportional changes in abundance for these species following coral loss. Although this thesis showed that being in preferred habitats can reduce cortisol levels (Chapter 2), the impact of degraded habitats was not apparently strong (Chapter 3). This highlights that habitat quality is important but its impact on stress is likely incapable of eliciting profound impacts on fitness and indirectly altering survivorship. Instead, this thesis generally identified other factors which may lead to increased direct mortality specially for coral associated fishes. This thesis along with other works has shown that for coral associated fishes, residing in degraded environments can leave them comparatively smaller (Chapter 3; Feary et al., 2009) and body size is known to be an important indicator of survivorship (Chapter 4; Goatley and Bellwood, 2016). Additionally, coral associated fish were found to spend more time further away from their shelter (Chapter 3; Boström-Einarsson et al., 2018) ultimately leaving these fishes more vulnerable to predators (McCormick, 2009). Together, this suggests changes in habitat may be manifested in growth and behavioural changes, which may be the main influential factor increasing mortality rates and resulting in the observed changes in abundance for coral associated fishes following coral loss.

As coral reefs are in a state of change, its critically important that we fully understand the relationships between habitat features and individual condition, and hence the impact of habitat degradation on the persistence of fish populations. The importance of scleractinian corals is often down played when looking at the coarseness of community and ecosystem level analysis (Muruga et al., 2024; Yan and Bellwood, 2023). However, many works that focus on the biology of organisms and populations do find clear links between corals and particular species (Coker et al., 2015; Doll et al., 2021; Noonan et al., 2012; Pratchett et al., 2012; Streit et al., 2021). This thesis is in agreement with the latter works, that a finer scale approach provides more accurate assessment of whether fishes are reliant on live coral or not, suggesting these relationships are scale dependent. Therefore, to progress our understanding of reef fish and their reliance on benthic habitats we need to move beyond community wide metrics and quantitatively reassess fishes use of habitat at an ecologically relevant scale. For example metrics commonly used to classify community composition (abundance, richness and diversity), have been identified as performing poorly at detecting changes in community composition following coral loss (Bellwood et al., 2006). Unsurprisingly, a recent meta-analysis also shows that the abundance and richness of fishes is weakly related to the cover of live corals and coral cover at both the family and trophic group level (Muruga et al., 2024). This disconnect between community composition traits and actual changes in community composition after environmental disturbances arises from; 1) some species benefiting from the new habitat made available by the disturbance (Bellwood et al., 2006; Wilson et al., 2006) and 2) rarely will a species go entirely extinct following disturbances (Munday, 2004). For this reason, assessing habitat selection and use should be conducted at the individual level and not community wide assessments.

The disconnect between reported relationships in coral cover and fish abundance and richness is likely related to spatial scale. Almost everything in ecology depends on the scale at which it is examined (Madin et al., 2023). Often fish and benthic communities are quantified at different scales. For example, many studies and monitoring programs quantify fish assemblages within a set area or volume (e.g., belt transects 5x50m; Hoey et al., 2022; Morais and Bellwood, 2019), benthic communities are typically quantified along a 1-dimensional line (e.g., point intercept transect; refs). While these 1-dimensional assessments of benthic community are efficient and broadly representative of the broader habitat, it will homogenize the distinct habitats at the scale in which many fish actually use its microhabitat with the total environment. Additionally, many fishes which associate with benthic habitats tend to have relatively small home ranges (~2m^2; Streit et al., 2021). Therefore, I suggest this type of data is not collected at the appropriate scale to make fair assessments of habitat associations or use. Instead, this thesis provides a novel way to comparatively assess habitat associations and level of specialisation by quantifying the percent cover of microhabitat features within an estimated home range (Chapter 2, 4). However, other metrics to measure habitat associations (i.e., resource selectivity; See Manly et al., 1994) have also been long used with success to detect when species do and do not associate with live coral and other microhabitat features (Coker et al., 2015; Doll et al., 2021; Hoey et al., 2007).

There appears to be some discrepancy between cortisol concentrations from field caught fishes to aquaria-reared conspecifics in this thesis, as samples collected from aquaria-reared studies had cortisol levels 10-17 times higher than field studies. However, these results may not be directly comparable. One main aim of this thesis was to assess fishes stress levels in relation to the quality of their habitat. While some of the exploration of this data was interesting and revealed links between baseline stress and habitat features (Chapter 2), other chapters struggled to find clear basal stress responses. When comparing these data chapters against each other (Chapter 2 vs Chapter 3 and 5), Chapter 3 and 5 showed some signs of elevated baseline stress levels. For example regardless of species or treatments, cortisol levels in Chapter 2, averaged 3.21 (SE ±0.19) pg/mg of fish carcass, while in Chapter 3 it averaged 29.68 (SE ±2.74) pg/mg in whole-body homogenates and similarly in Chapter 5 was 49.44 (SE ±2.90) pg/mg whole-body homogenates.

There are many factors that may contribute to such differences: 1) different tissue sampling methods, 2) size and/or life stage, and 3) tank and confinement factors. Firstly, in Chapter 2 fish carcasses were used as otoliths were removed for potential sampling meaning a vertical dissection line was applied directly behind the operculum while in Chapters 3 and 5 the entire body was used as a sample. Although the production of cortisol molecules does not occur in the head region for fishes (i.e., kidneys) removing the head resulted in a loss of blood (i.e., medium of cortisol transport), and may have led to lower absolute cortisol levels in Chapter 2. Secondly, the fish from Chapter 2 were subadults (3.12 cm standard length) while fish from Chapter 3 and 5 were recently settled (~3 weeks; 1.47 and 1.62 cm SL respectively) and it is expected that blood volume and therefore circulating levels of cortisol would increase with body size, however it is unknown whether this relationship is linear in fishes. Lastly, tank size and/or confinement stress is well known in fishes (Parker et al., 2012; Pavlidis et al., 2013) and may have caused higher cortisol levels in aquaria-reared fishes (Chapter 3 and 5). Some evidence to support this is found in Chapter 3 as *D. aruanus* was reared as trios in larger tanks (5.3 L) than *P. moluccensis* and *P. amboinensis* (2.6 L) and *D. aruanus* was found to have much lower cortisol levels (See Chapter 3). Although it is not possible to disentangle the potential social impact of having two conspecifics in these tanks. In total, this means the absolute levels of cortisol concentrations found in Chapter 2 may not be directly comparable with other chapters (3 and 5), but it does provide important considerations for future works. It seems imperative that this be further explored, as it could have huge ramifications for all past and future lab-based studies assessing growth rates, behaviours and various other metrics. This would simply require assessing whole-body homogenate samples such as recruits which natural settled and comparing them to aquaria-reared samples. Additionally, the stock density and overall housing volume and size of fish could be experimentally manipulated to see at what density or space is required to maintain basal cortisol levels and avoid confinement stress.

Finally, while this thesis explored habitat characteristics on the stress response of fishes, through a longer time frame, it may be that fishes stress response is more regulatory during the changing in habitat or initial stressor (i.e., during a bleaching event). Throughout this thesis I explored whether residing in degraded habitats influenced stress levels but did not explore whether living through a degradation event actually induced a stress response. Fortunately for my study site (Lizard Island Research Station), no mass degradation events took place during my thesis timeline, but unfortunately a recent mass bleaching event just occurred during the summer 2024 and ongoing research projects are exploring the impact of a large-scale degradation event on the stress response in coral reef fishes.

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Appendix A

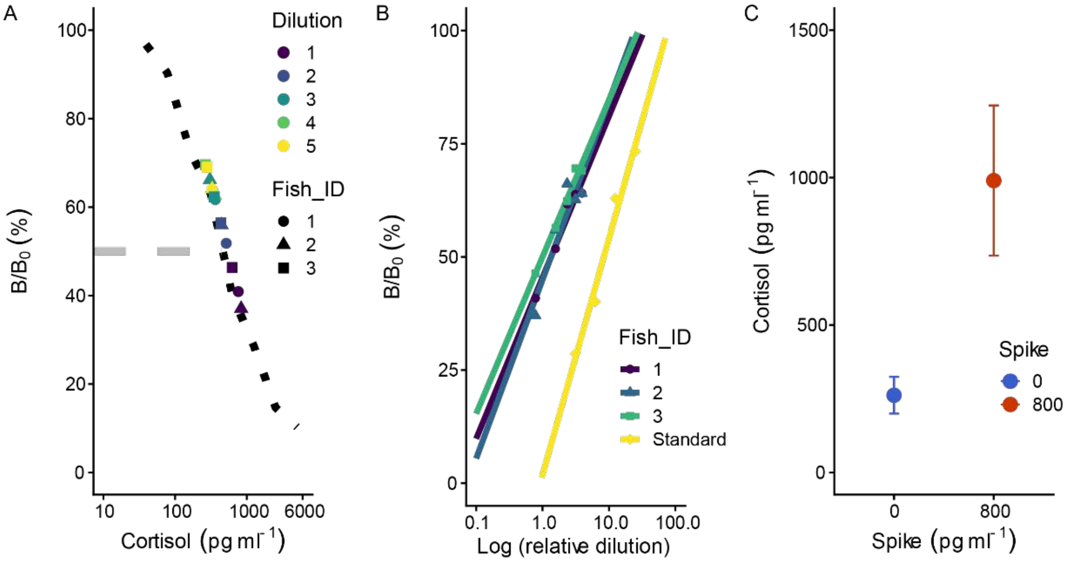


Figure A1. *Validation steps for ELISA cortisol analysis of whole-body homogenates. The optimal sample dilution (50 % B/B$_0$) was determined, and compared with the standard curve (A, dotted line). Based on the tested dilutions, an optimal dilution factor of 1.5 was used for analysing the remaining samples. Second, parallelism was confirmed by comparing the slopes of the standard curve and the diluted samples (B). Third, the accuracy or extraction efficiency of cortisol from the fish samples (n = 4) was tested (C). The extraction efficiency of (91.0 %) was determined.*

Table A1. *Summary of benthic, community and morphological variables measured for the three Pomacentrid species and control quadrats. Samples sizes (n = 40, 37, 37, 140) from left to right.*

Variable	***P. moluccensis***		***P. amboinensis***		***P. chrysurus***		**Control**	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Pavement (% cover)	27.43	17.24	20.86	14.49	32.81	12.75	32.43	15.27
Rubble (%)	21.85	25.19	23.16	24.15	31.38	20.52	18.96	17.74
Sand (%)	7.68	12.06	29.35	19.69	15.38	10.27	11.79	14.10
Macro algae (%)	3.15	4.49	1.76	2.59	3.73	3.99	3.53	3.70
Soft coral (%)	12.10	12.74	13.19	19.13	8.22	8.71	15.73	16.40
Coarse branching coral (%)	0.83	2.01	0.86	1.83	0.49	1.39	2.01	6.35
Fine branching coral (%)	13.10	11.88	5.16	6.38	2.78	3.49	6.46	6.60
Mounding coral (%)	13.18	19.21	5.43	10.52	4.81	5.65	7.37	9.06
Rugosity	1.91	0.37	1.66	0.25	1.58	0.30	1.80	0.42
Depth (m)	2.43	0.70	2.79	0.68	1.75	0.30	2.06	0.62

Table A2. *Summary of output for linear models of time to capture until euthanasia was tested against the cortisol concentrations. From Figure 1, main text.*

Species	**Coeff**	**SE**	**t**	**df**	**P**	**Adj R^2**
All	0.011	0.006	1.87	1, 112	0.064	0.022
Amboinensis	0.015	0.011	1.43	1, 35	0.161	0.028
Chrysurus	0.013	0.011	1.16	1, 35	0.254	0.010
Moluccensis	0.001	0.008	0.10	1, 38	0.923	-0.026

Table A3. *Linear coefficients from the structural equations model exploring habitats characteristics influence on cortisol concentrations, total lipids, and Fulton's K. from Figure 3A-C, main text. Bold values denote significant at alpha = 0.05.*

Species	**Response**	**Predictor**	**Estimate**	**SE**	**P-value**
P. moluccensis	Lipids	Cortisol	**-0.4467**	**0.1415**	**0.0016**
	Fulton's K	Lipids	**0.6480**	**0.1444**	**<0.0001**
	Fulton's K	Cortisol	**0.4039**	**0.1433**	**0.0048**
P. amboinensis	Lipids	Cortisol	-0.0909	0.1426	0.5239
	Fulton's K	Lipids	0.3133	0.1897	0.0985
P. chrysurus	Lipids	Cortisol	**-0.3691**	**0.1528**	**0.0157**
	Fulton's K	Lipids	-0.1114	0.1625	0.4931

Table A4. *Linear coefficients from the structural equations model exploring habitats characteristics influence on cortisol concentrations, total lipids, and Fulton's K. from Figure 3D-F, main text. Bold values denote significant at alpha = 0.05 and ~ denotes covariances.*

Species	**Response**	**Predictor**	**Estimate**	**SE**	**P-value**
P. moluccensis	Cortisol	Rugosity	**-0.4102**	**0.1479**	**0.0056**
	Cortisol	Mounding	-0.1409	0.1389	0.3105
	Cortisol	Fine branching	-0.0025	0.1570	0.9872
	Lipids	Cortisol	**-0.4220**	**0.1338**	**0.0016**
	Lipids	Mounding	**0.2994**	**0.1338**	**0.0252**
	Fulton's K	Lipids	**0.6480**	**0.1444**	**<0.0001**
	Fulton's K	Cortisol	**0.4039**	**0.1433**	**0.0048**
	~~Rugosity	~~Fine branching	**0.4109**		**0.0084**
P. amboinensis	Cortisol	Rugosity	-0.0469	0.1466	0.7646
	Cortisol	Sand	**-0.5236**	**0.1675**	**0.0018**
	Cortisol	Depth	**0.4046**	**0.1675**	**0.0157**
	Lipids	Cortisol	-0.0614	0.1368	0.6537
	Lipids	Rugosity	**0.3856**	**0.1591**	**0.0157**
	Fulton's K	Lipids	0.2918	0.2019	0.1485
	Fulton's K	Depth	**-0.3292**	**0.1469**	**0.0250**
	~~Sand	~~Depth	**0.4887**		**0.0021**
P. chrysurus	Cortisol	Rubble	**-0.4997**	**0.1395**	**0.0003**
	Cortisol	Rugosity	-0.2268	0.1410	0.1077
	Cortisol	Sand	-0.1124	0.1425	0.4302
	Lipids	Cortisol	**-0.3691**	**0.1528**	**0.0157**
	Fulton's K	Lipids	-0.1419	0.1541	0.3571
	Fulton's K	Rubble	**0.3664**	**0.1541**	**0.0174**

Appendix B

Cortisol validation

Parallelism was confirmed by an ANCOVA's homogeneity of slopes assumption by comparing dose–response curves of diluted samples for each fish against a standard curve (ANCOVA, P>0.05, n=3; Supp Fig 1B). In brief, the reconstituted samples (n=3) were diluted (1:2, 1:3, and 1:4,) and compared against the cortisol standard curve (Cayman Chemical ELISA kit, 39.1–5000 pg ml^{-1} range). The optimal dilution for the samples fell around 1:2 (Supp Fig1A); therefore, a sample dilution of 1:2 was chosen to achieve a 50% relative maximum binding and only sample dilutions falling within 20–80% B/B0 relative maximum binding were accepted. The accuracy or recovery rate of the extraction method was assessed by spiking samples (n=3) with 500 pg cortisol. For each of the three samples, two fish were homogenized, pooled and split into halves, with one half receiving the spike and the other the assay buffer. Both halves were then processed in the same way as all other samples. The spike's recovery (percentage) was expressed as spiked sample result−unspiked sample result×100/known spike (500 pg ml^{-1}), and the mean recovery (95.3% ±sd 22.6%).

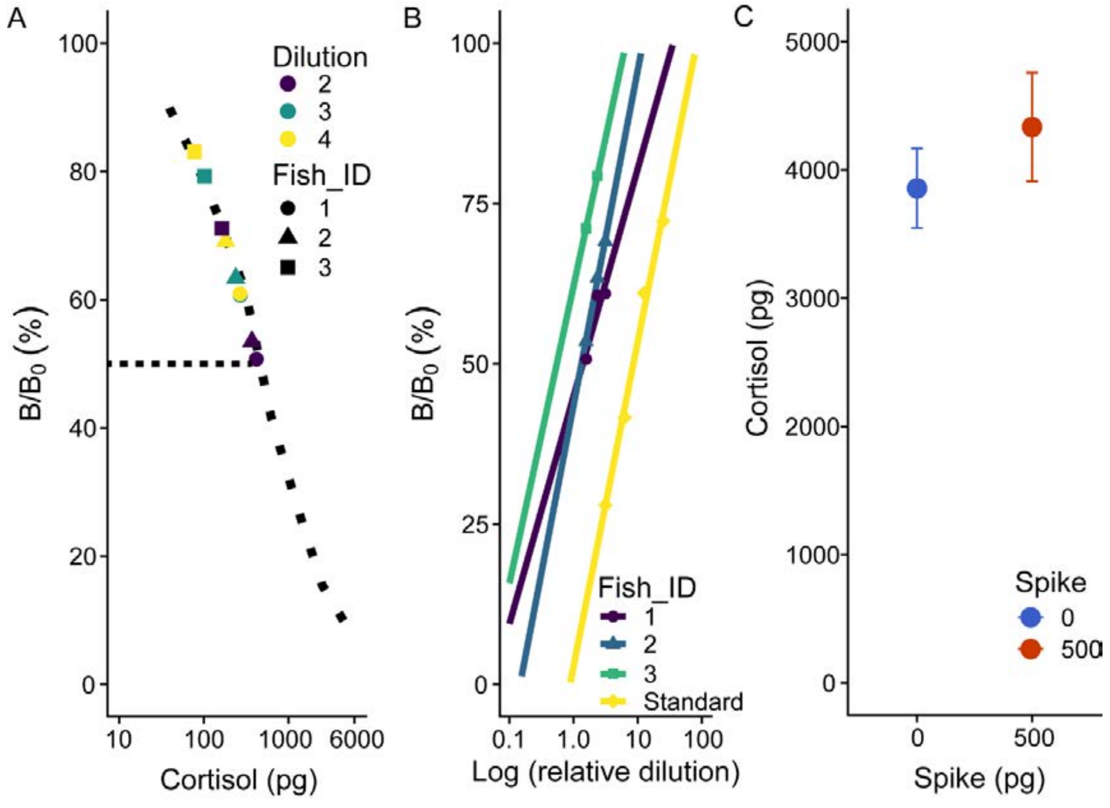


Figure B1. *Validation steps for ELISA cortisol analysis of whole-body homogenates. The optimal sample dilution (50 % B/B$_0$) was determined, and compared with the standard curve (A, dotted line). Based on the tested dilutions, an optimal dilution factor of 2 was used for analysing the remaining samples. Second, parallelism was confirmed by comparing the slopes of the standard curve and the diluted samples (B). Third, the accuracy or extraction efficiency of cortisol from the fish samples (n = 3) was tested (C). The extraction efficiency of (95.3 %) was determined.*

Table B1. *Results of linear mixed effects model on cortisol concentration. Summary of GLMM's on cortisol concentration in juvenile damselfish reared in various habitat treatments (live coral, mixed and degraded coral). Estimate of effect size of fixed factor (habitat treatment) is presented as marginal-*R^2 *(*R^2_m*) and random factors as (Tank+Plate) as conditional-*R^2 *(*R^2_c*). Bold values are significant at alpha = 0.05.*

Species	**Source**	χ^2	**p**	R^2_m	R^2_c
P. amboinensis	Cortisol $_{(2)}$	1.67	0.435	0.09	0.10
P. moluccensis	Cortisol $_{(2)}$	8.75	**0.013**	0.14	0.72
D. aruanus	Cortisol $_{(2)}$	0.46	0.794	0.01	0.34

Table B2. *Bonferroni corrected post-hoc comparisons for cortisol measures in Figure 3.1.*

Species	**Trait**	**Contrast**	**Contrast**	**Estimate**	**Std. Error**	**z value**	**p**
P. amboinensis	Cortisol	Healthy	Mixed	16.50	23.2	0.71	0.759
		Healthy	Degraded	-14.60	22.4	-0.65	0.794
		Mixed	Degraded	-31.10	24.1	-1.29	0.419
P. moluccensis	Cortisol	Healthy	Mixed	-9.54	7.58	-1.38	0.363
		Healthy	Degraded	-20.16	6.91	-2.94	**0.018**
		Mixed	Degraded	-10.62	8.16	-1.21	0.460
D. aruanus	Cortisol	Healthy	Mixed	2.07	3.79	0.55	0.849
		Healthy	Degraded	1.66	2.96	0.56	0.841
		Mixed	Degraded	-0.41	3.97	-0.10	0.994

Table B3. *Results of linear mixed effects model on the metabolic response variables. Summary of the metabolic variables: standard metabolic rate (SMR), maximum metabolic rate (MMR), and aerobic scope (AS) in juvenile damselfishes reared in various habitat treatments (live coral, mixed and degraded coral). Random effect of Tank ID is included in all models. Estimate of effect size of fixed factor (habitat treatment) is presented as marginal-*R^2 *(*R^2_m*) and random factors as (Tank ID) as conditional-*R^2 *(*R^2_c*). Bold values are significant at alpha = 0.05.*

Species	**Source**	χ^2	**p**	R^2_m	R^2_c
P. amboinensis	SMR $_{(2)}$	3.43	0.180	0.07	0.07
	MMR $_{(2)}$	3.89	0.143	0.08	0.38
	AS $_{(2)}$	6.96	**0.031**	0.14	0.47
P. moluccensis	SMR $_{(2)}$	1.25	0.535	0.02	0.02
	MMR $_{(2)}$	6.80	**0.033**	0.12	0.12
	AS $_{(2)}$	3.96	0.138	0.07	0.07
D. aruanus	SMR $_{(2)}$	2.03	0.363	0.05	0.22
	MMR $_{(2)}$	7.92	**0.019**	0.10	0.10
	AS $_{(2)}$	4.72	0.094	0.06	0.06

Table B4. *Bonferroni corrected post-hoc comparisons for metabolic variables in Figure 3.2.*

Species	Trait	Contrast	Contrast	Estimate	Std. Error	z value	Pr (>|z|)
P. amboinensis	SMR	Healthy	Mixed	-56	86	-0.654	0.791
		Healthy	Degraded	-152	84	-1.809	0.178
		Mixed	Degraded	-96	81	-1.180	0.471
	MMR	Healthy	Mixed	281	143	1.973	0.130
		Healthy	Degraded	148	140	1.056	0.546
		Mixed	Degraded	-134	136	-0.987	0.588
	AS	Healthy	Mixed	331	135	2.445	**0.047**
		Healthy	Degraded	285	132	2.148	0.091
		Mixed	Degraded	-46	129	-0.357	0.932
P. moluccensis	SMR	Healthy	Mixed	-52	113	-0.463	0.889
		Healthy	Degraded	76	109	0.696	0.767
		Mixed	Degraded	128	116	1.107	0.515
	MMR	Healthy	Mixed	-257	174	-1.472	0.313
		Healthy	Degraded	210	169	1.243	0.434
		Mixed	Degraded	460	179	2.607	**0.032**
	AS	Healthy	Mixed	-204	166	-1.229	0.442
		Healthy	Degraded	134	161	0.831	0.686
		Mixed	Degraded	338	171	1.982	0.127
D. aruanus	SMR	Healthy	Mixed	66	75	0.883	0.653
		Healthy	Degraded	106	75	1.407	0.343
		Mixed	Degraded	40	75	0.530	0.857
	MMR	Healthy	Mixed	70	133	0.530	0.857
		Healthy	Degraded	357	134	2.665	**0.026**
		Mixed	Degraded	287	134	2.140	0.089
	AS	Healthy	Mixed	4	125	0.033	0.999
		Healthy	Degraded	240	126	1.904	0.145
		Mixed	Degraded	236	126	1.872	0.156

Table B5. *Results of linear mixed effects model on the morphometric variables. Summary of the morphological variables: mass, standard length, body depth and Fulton's K in juvenile damselfish reared in habitat treatments (live coral, mixed and degraded coral). Random effect of Tank ID is included in all models. Estimate of effect size of fixed factor (habitat treatment) is presented as marginal-*R^2 *(*R^2_m*) and random factors as (Tank) as conditional-*R^2 *(*R^2_c*). Models noted with (*) have a covariate of the initial length or body depth, from day 0 photographs. Bold values are significant at alpha = 0.05.*

Species	**Source**	χ^2	**p**	R^2_m	R^2_c
P. amboinensis	Mass $_{(2)}$	0.04	0.979	0.00	0.00
	Length* $_{(2)}$	0.26	0.880	0.22	0.22
	Body depth* $_{(2)}$	0.44	0.802	0.21	0.21
	Futlon's K $_{(2)}$	1.89	0.388	0.02	0.10
P. moluccensis	Mass $_{(2)}$	1.70	0.427	0.02	0.02
	Length* $_{(2)}$	4.67	0.097	0.30	0.47
	Body depth* $_{(2)}$	2.97	0.225	0.37	0.66
	Futlon's K $_{(2)}$	2.57	0.276	0.03	0.67
D. aruanus	Mass $_{(2)}$	9.92	**0.007**	0.07	0.07
	Length $_{(2)}$	5.04	0.081	0.04	0.04
	Body depth $_{(2)}$	5.54	0.063	0.04	0.05
	Futlon's K $_{(2)}$	1.07	0.585	0.02	0.18

Table B6. *Bonferroni corrected post-hoc comparisons for morphometric variables in Figure 3.4.*

Species	**Trait**	**Contrast**	**Contrast**	**Estimate**	**Std. Error**	**z value**	**Pr (>|z|)**
P. amboinensis	Mass	Healthy	Mixed	-0.14	6.41	-0.02	1.000
		Healthy	Degraded	-1.19	6.47	-0.18	0.982
		Mixed	Degraded	-1.06	6.33	-0.17	0.984
	Length	Healthy	Mixed	-0.01	0.03	-0.40	0.915
		Healthy	Degraded	0.00	0.03	0.04	0.999
		Mixed	Degraded	0.01	0.03	0.46	0.889
	Depth	Healthy	Mixed	0.01	0.02	0.30	0.952
		Healthy	Degraded	0.01	0.02	0.66	0.786
		Mixed	Degraded	0.01	0.02	0.38	0.991
	Fulton K	Healthy	Mixed	-0.56	0.64	-0.88	0.655
		Healthy	Degraded	-0.87	0.64	-1.36	0.366
		Mixed	Degraded	-0.31	0.62	-0.50	0.872
P. moluccensis	Mass	Healthy	Mixed	2.80	5.37	0.52	0.861
		Healthy	Degraded	6.79	5.22	1.30	0.399
		Mixed	Degraded	3.99	5.46	0.73	0.746
	Length	Healthy	Mixed	0.03	0.03	1.19	0.464
		Healthy	Degraded	0.06	0.03	2.15	0.086
		Mixed	Degraded	0.02	0.03	0.87	0.663
	Depth	Healthy	Mixed	0.02	0.02	1.52	0.288
		Healthy	Degraded	0.02	0.02	1.43	0.329
		Mixed	Degraded	-0.01	0.02	-0.13	0.991
	Fulton K	Healthy	Mixed	0.62	0.71	0.87	0.659
		Healthy	Degraded	-0.54	0.69	-0.78	0.716
		Mixed	Degraded	-1.16	0.73	-1.60	0.250
D. aruanus	Mass	Healthy	Mixed	6.18	3.62	1.71	0.206
		Healthy	Degraded	11.45	3.64	3.15	**0.006**
		Mixed	Degraded	5.27	3.64	1.45	0.320
	Length	Healthy	Mixed	0.02	0.02	1.08	0.530
		Healthy	Degraded	0.05	0.02	2.24	0.068
		Mixed	Degraded	0.02	0.02	1.17	0.471
	Depth	Healthy	Mixed	0.02	0.01	1.70	0.211
		Healthy	Degraded	0.03	0.01	2.26	0.066
		Mixed	Degraded	0.01	0.01	0.57	0.835
	Fulton K	Healthy	Mixed	0.81	1.07	0.76	0.731
		Healthy	Degraded	1.07	1.08	0.99	0.585
		Mixed	Degraded	0.25	1.08	0.24	0.970

Appendix C

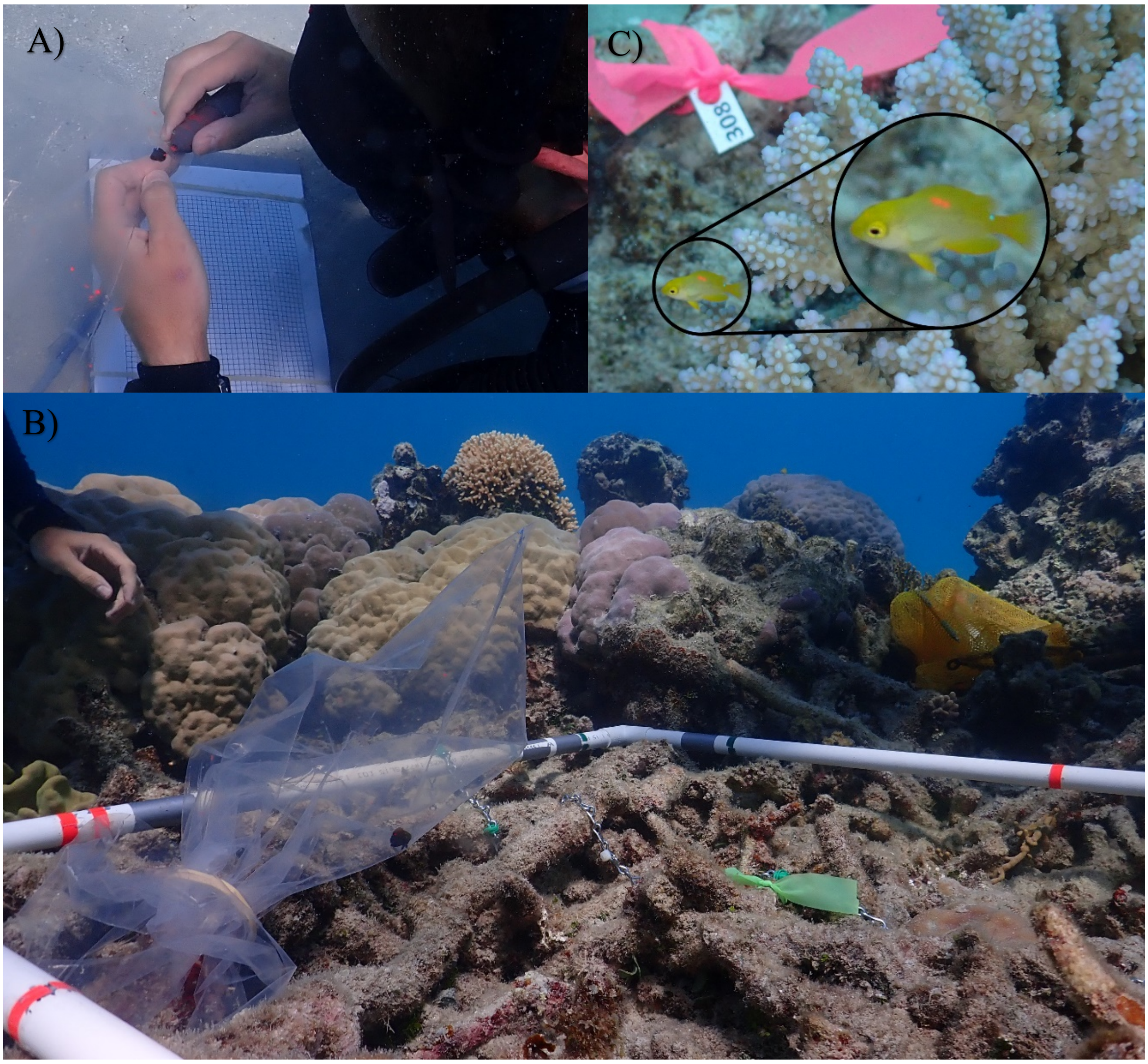


Figure C1. *Photographs of field protocol, highlighting A) the tagging process and lateral photo grid, B) acclimation phase of recruit and field sampling (i.e. rugosity measure) and C) individual tagged with an orange, fluorescent elastomer tag and site marked with flagging tape and identification number. Photo credit: Mila Grinblat A, B; Maddi Craig C.*

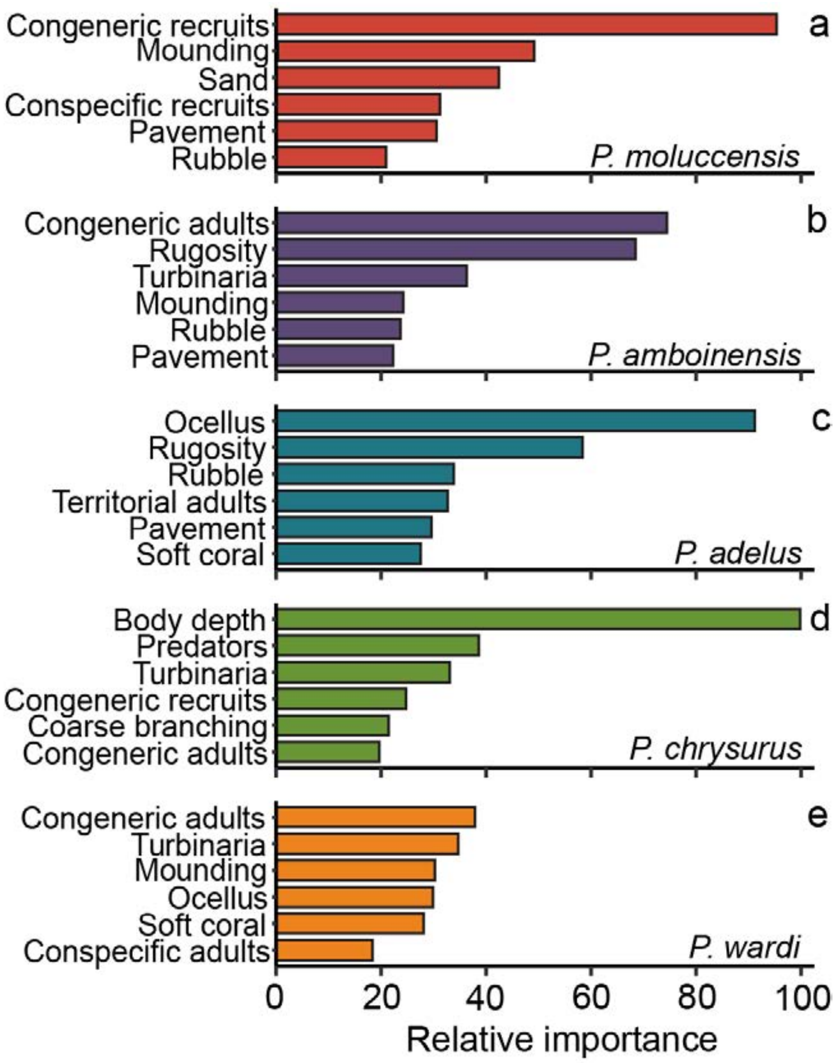


Figure C2. *Relative importance of the top six variables in predicting survivorship of newly-settled individuals of five pomacentrid species across 14 days. Importance is calculated from the sum of weights (≤ 0.95) from each species' Cox proportional-hazard regression analysis.*

Table C1. *Summary of benthic, community and morphological variables measured for five Pomacentrid species and control quadrats. Samples sizes (n = 67, 63, 61, 72, 57, 70) from left to right.*

Variable	***P. moluccensis***		***P. amboinensis***		***P. adelus***		***P. chrysurus***		***P. wardi***		**Control**	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Pavement (% cover)	31.07	13.46	24.24	17.68	35.52	15.79	33.50	12.84	37.98	14.54	35.16	13.67
Rubble (%)	10.72	9.57	11.30	9.39	22.97	19.53	32.76	14.46	25.96	16.42	16.43	14.97
Sand (%)	7.91	9.35	35.16	20.03	15.59	15.22	20.36	11.70	10.25	11.11	10.78	12.52
Turbinaria (%)	0.82	2.16	0.37	1.04	1.41	1.83	1.71	2.31	2.21	2.74	2.11	2.59
Soft coral (%)	15.61	18.53	13.17	14.09	9.36	10.77	4.32	7.14	8.16	11.78	14.86	15.57
Coarse branching coral (%)	0.36	1.44	0.13	0.42	0.67	1.34	0.63	1.33	0.95	2.28	1.47	4.70
Fine branching coral (%)	15.69	7.13	5.13	8.35	4.46	4.23	2.11	3.41	4.23	4.93	5.81	6.12
Mounding coral (%)	14.16	13.46	8.90	10.57	6.72	7.80	2.82	4.00	6.65	9.15	9.41	8.90
Rugosity	2.14	0.60	1.63	0.33	1.62	0.32	1.41	0.18	1.65	0.29	1.74	0.35
Depth (m)	2.16	0.71	2.99	0.56	2.22	0.62	1.90	0.41	2.04	0.56	1.89	0.59
Conspecific adults (counts)	2.57	1.94	2.02	1.69	1.67	0.99	2.53	1.67	0.68	0.70		
Conspecific juveniles (counts)	1.82	1.56	1.81	1.91	0.43	0.66	1.00	1.25	0.93	0.88		
Congeneric adults (counts)	2.36	1.43	2.97	2.44	2.59	2.03	2.11	1.95	3.60	1.80		
Congeneric juveniles (counts)	1.54	1.86	1.19	1.31	1.85	1.71	1.39	1.54	1.60	1.50		
Territorial adults (counts)	0.60	0.98	0.10	0.43	0.38	0.66	0.07	0.25	0.44	0.82		
Predators (counts)	0.67	0.97	0.65	0.72	0.59	0.84	1.22	1.45	1.02	1.16		
Standard length (mm)	1.28	0.07	1.38	0.09	1.35	0.07	1.47	0.09	1.55	0.09		
Body depth (mm)	0.48	0.04	0.57	0.07	0.55	0.05	0.61	0.07	0.62	0.06		
Ocellus size area (mm)			0.021	0.004	0.027	0.005	0.025	0.006	0.044	0.010		

Table C2. *Summary of pairwise comparisons of associated habitat characteristics between Pomacentrid species. Adjusted P-value uses a Bonferroni correction for multiple comparisons. Bold values are significant at alpha = 0.05.*

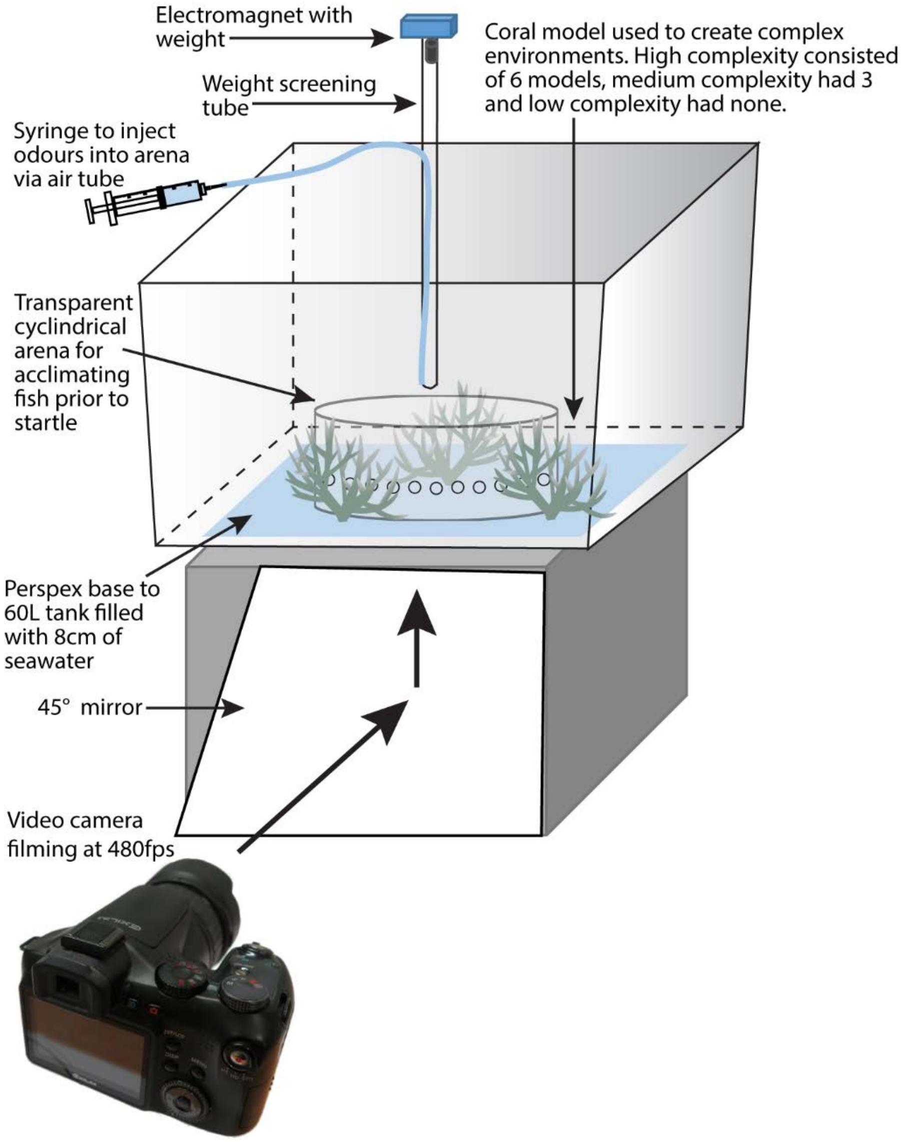
Comparison	**df**	**SS**	**F**	**R^2**	**Adj. P**
Adelus vs Chrysurus	1	0.709	9.786	0.070	**0.015**
Adelus vs Wardi	1	0.149	1.648	0.014	1
Adelus vs Moluccensis	1	1.612	17.470	0.122	**0.015**
Adelus vs Amboinensis	1	1.816	18.365	0.131	**0.015**
Adelus vs Control	1	0.323	3.334	0.025	0.225
Chrysurus vs Wardi	1	0.692	10.423	0.076	**0.015**
Chrysurus vs Moluccensis	1	4.388	62.640	0.314	**0.015**
Chrysurus vs Amboinensis	1	2.902	38.454	0.224	**0.015**
Chrysurus vs Control	1	2.068	27.701	0.165	**0.015**
Wardi vs Moluccensis	1	1.891	21.825	0.152	**0.015**
Wardi vs Amboinensis	1	2.943	31.547	0.211	**0.015**
Wardi vs Control	1	0.481	5.264	0.040	**0.015**
Moluccensis vs Amboinensis	1	3.005	31.589	0.198	**0.015**
Moluccensis vs Control	1	0.850	9.115	0.063	**0.015**
Amboinensis vs Control	1	2.330	23.419	0.152	**0.015**

Table C3. *Summary of pairwise comparisons of associated fish community characteristics between Pomacentrid species. Adjusted P-value uses a Bonferroni correction for multiple comparisons. Bold values are significant at alpha = 0.05.*

Comparison	**df**	**SS**	**F**	**R^2**	**Adj. P**
Adelus vs Chrysurus	1	0.888	5.878	0.043	**0.01**
Adelus vs Wardi	1	1.306	10.251	0.081	**0.01**
Adelus vs Moluccensis	1	1.103	7.514	0.056	**0.01**
Adelus vs Amboinensis	1	0.861	5.575	0.044	**0.01**
Chrysurus vs Wardi	1	2.674	19.260	0.132	**0.01**
Chrysurus vs Moluccensis	1	0.634	4.068	0.029	**0.03**
Chrysurus vs Amboinensis	1	0.420	2.573	0.019	0.63
Wardi vs Moluccensis	1	1.857	13.874	0.102	**0.01**
Wardi vs Amboinensis	1	1.422	10.065	0.079	**0.01**
Moluccensis vs Amboinensis	1	0.222	1.396	0.011	1

Appendix D

Figure D1. *Overview of experimental tank.*



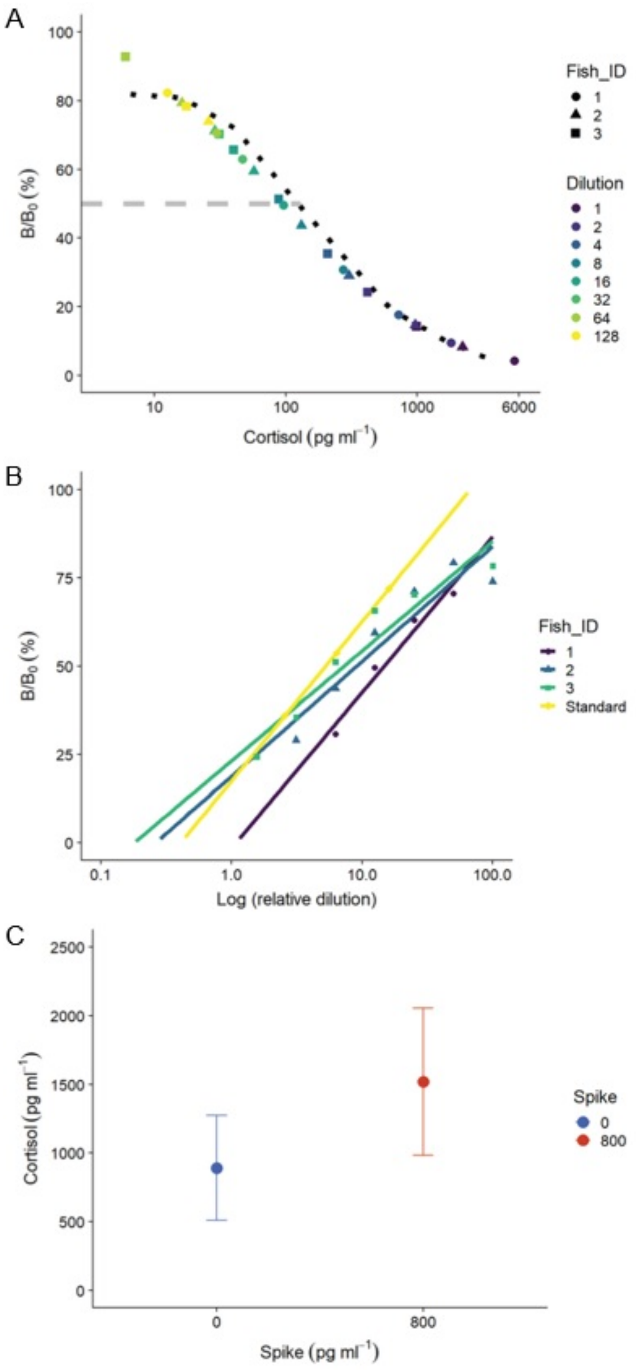


Figure D2. *Validation steps for ELISA cortisol analysis of whole-body homogenates of the white tail damselfish (Pomacentrus chrysurus). The optimal sample dilution (50 % B/B$_0$) was determined, and compared with the standard curve (A, dotted line). Based on the tested dilutions, an optimal dilution factor of 12 was used for analysing the remaining samples (A, dashed line). Second, parallelism was confirmed by comparing the slopes of the standard curve and the diluted samples (B). Third, the accuracy or extraction efficiency of cortisol from the fish samples was tested (C, means±SD, n = 4). The extraction efficiency (78.5 %) was used as a correction factor for the samples.*

Table D1. *Sample sizes for responsiveness of juvenile Pomacentrus chrysurus performing a C-start escape response compared by treatments, level of complexity and odour.*

Complexity	Odour	Responsiveness	Non-responsiveness
Low	Control	17	3
Low	Herbivore	13	7
Low	Predator	14	5
Medium	Control	17	3
Medium	Herbivore	18	3
Medium	Predator	14	7
High	Control	20	0
High	Herbivore	14	6
High	Predator	17	6

Table D2. *Bonferroni corrected post-hoc comparisons for kinematic variables in Figure 5.2. Bold values are significant at alpha = 0.05.*

Variable	Treatment	Term (1)	Term (2)	Mean Diff (1-2)	Std. Error	Adj. P	LCI 95%	UCI 95%
Speed	Complexity	Low	Medium	-.076	.035	.090	-.160	.008
			High	-.088	.034	**.033**	-.172	-.005
		Medium	Low	.076	.035	.090	-.008	.160
			High	-.013	.034	1.000	-.094	.069
		High	Low	.088	.034	**.033**	.005	.172
			Medium	.013	.034	1.000	-.069	.094
	Odour	Predator	Herbivore	.040	.035	.788	-.046	.126
			SW	.099	.034	**.012**	.017	.180
		Herbivore	Predator	-.040	.035	.788	-.126	.046
			SW	.059	.034	.244	-.022	.140
		SW	Predator	-.099	.034	**.012**	-.180	-.017
			Herbivore	-.059	.034	.244	-.140	.022
Max. Speed	Complexity	Low	Medium	-.143	.046	**.007**	-.254	-.031
			High	-.123	.046	**.024**	-.233	-.012
		Medium	Low	.143	.046	**.007**	.031	.254
			High	.020	.045	1.000	-.088	.129
		High	Low	.123	.046	**.024**	.012	.233
			Medium	-.020	.045	1.000	-.129	.088
Max. Acceleration	Complexity	Low	Medium	-.072	.022	**.005**	-.126	-.018
			High	-.042	.022	.182	-.096	.012
		Medium	Low	.072	.022	**.005**	.018	.126
			High	.030	.022	.513	-.023	.083
		High	Low	.042	.022	.182	-.012	.096
			Medium	-.030	.022	.513	-.083	.023
	Odour	Predator	Herbivore	.061	.023	**.026**	.005	.116
			SW	.001	.022	1.000	-.051	.054
		Herbivore	Predator	-.061	.023	**.026**	-.116	-.005
			SW	-.059	.022	**.021**	-.112	-.007
		SW	Predator	-.001	.022	1.000	-.054	.051
			Herbivore	.059	.022	**.021**	.007	.112
Distance	Complexity	Low	Medium	-.003	.001	.090	-.007	.000
			High	-.004	.001	**.033**	-.007	.000
		Medium	Low	.003	.001	.090	.000	.007
			High	-.001	.001	1.000	-.004	.003
		High	Low	.004	.001	**.033**	.000	.007
			Medium	.001	.001	1.000	-.003	.004
	Odour	Predator	Herbivore	.002	.001	.789	-.002	.005
			SW	.004	.001	**.012**	.001	.007
		Herbivore	Predator	-.002	.001	.789	-.005	.002
			SW	.002	.001	.245	-.001	.006
		SW	Predator	-.004	.001	**.012**	-.007	-.001
			Herbivore	-.002	.001	.245	-.006	.001

Table D3. *Tukey's HSD post-hoc comparisons for cortisol concentration presented in Figure 5.3. Bold values are significant at alpha = 0.05.*

Term (1)	Term (2)	Mean Diff (1-2)	Std. Error	P	LCI 95%	UCI 95%
Low / Control	High / Control	-5.174	9.590	.994	-33.472	23.124
	High / Predator	-9.170	9.120	.914	-36.080	17.740
	Low / Predator	-29.979	8.937	**.017**	-56.349	-3.608
	Medium / Control	-14.657	9.590	.648	-42.955	13.641
	Medium / Predator	2.463	9.120	1.000	-24.447	29.373
Low / Predator	High / Control	24.805	9.204	.092	-2.353	51.963
	High / Predator	20.809	8.713	.178	-4.900	46.517
	Low / Control	29.979	8.937	**.017**	3.608	56.349
	Medium / Control	15.322	9.204	.560	-11.836	42.480
	Medium / Predator	32.442	8.713	**.006**	6.734	58.151
Medium / Control	High / Control	9.483	9.840	.927	-19.550	38.516
	High / Predator	5.487	9.382	.992	-22.195	33.169
	Low / Control	14.657	9.590	.648	-13.641	42.955
	Low / Predator	-15.322	9.204	.560	-42.480	11.836
	Medium / Predator	17.120	9.382	.459	-10.562	44.803
Medium / Predator	High / Control	-7.638	9.382	.964	-35.320	20.045
	High / Predator	-11.634	8.900	.780	-37.895	14.628
	Low / Control	-2.463	9.120	1.000	-29.373	24.447
	Low / Predator	-32.442	8.713	**.006**	-58.151	-6.734
	Medium / Control	-17.120	9.382	.459	-44.803	10.562
High / Control	High / Predator	-3.996	9.382	.998	-31.678	23.686
	Low / Control	5.174	9.590	.994	-23.124	33.472
	Low / Predator	-24.805	9.204	.092	-51.963	2.353
	Medium / Control	-9.483	9.840	.927	-38.516	19.550
	Medium / Predator	7.638	9.382	.964	-20.045	35.320
High / Predator	High / Control	3.996	9.382	.998	-23.686	31.678
	Low / Control	9.170	9.120	.914	-17.740	36.080
	Low / Predator	-20.809	8.713	.178	-46.517	4.900
	Medium / Control	-5.487	9.382	.992	-33.169	22.195
	Medium / Predator	11.634	8.900	.780	-14.628	37.895