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The effects of suspended sediments on the physiology and performance of coral reef fishes

Thesis submitted by
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Statement of Acknowledgement

I acknowledge the Traditional Owners and Custodians of the land and sea country on which this research was conducted and recognise their continuing connection to land, waters and culture. I pay my respect to their Elders past, present and emerging.

Statement of contribution of others

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Declaration of ethics

The research presented and reported in this thesis was conducted in compliance with the National Health and Medical Research Council (NHMRC) Australian Code of Practice for the Care and Use of Animals for Scientific Purposes, 7th Edition, 2004 and the Qld Animal Care and Protection Act, 2001. The proposed research project received animal ethics approval from the JCU Animal Ethics Committee Approval Number A2218.

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General Abstract

Declining water quality, in particular increasing suspended sediment concentrations, is a major factor causing the degradation of marine ecosystems. Anthropogenic activities such as coastal development, agriculture, cattle grazing, mining, dredging, and shipping have led to an increase in suspended sediments in coastal waters over the past decades. On coral reefs, suspended sediments have been linked to declines in species diversity and abundance of both benthic and reef fish communities. Changes in physiological performance have been hypothesized to represent a mechanism driving the observed declines in fish populations, but evidence for this is currently lacking. Moreover, the cumulative effects of suspended sediments and co-occurring environmental factors, such as elevated ocean temperatures caused by anthropogenic climate change and different water flow regimes, on coral reef fishes are unknown. This information is essential to understand responses of reef fish populations to current and future environmental conditions. The overall aim of this thesis was to examine the effects of suspended sediments, both in isolation and in combination with elevated water temperature and elevated water flow, on the physiology and performance of coral reef fishes.

Suspended sediments commonly induce changes in fish gill morphology that have been hypothesized to compromise gill function. In **chapter two**, the effects of suspended sediments on the gill morphology and aerobic performance of three damselfish species (*Amphiprion melanopus*, *Amphiprion percula*, and *Acanthochromis polyacanthus*) were examined. Following suspended sediment exposure (0, 45, 90, 135 or 180 mgL^{-1}), all three species exhibited reductions in the length of gill lamellae (a proxy for gill surface area) and/or reductions in gas diffusion distances. Yet, only *A. melanopus* exhibited changes in oxygen uptake rates, i.e., a decrease in maximum oxygen uptake rates ($\dot{M}O_{2max}$) and an increase in resting oxygen uptake rates ($\dot{M}O_{2rest}$). This resulted in a decreased aerobic scope in *A. melanopus*, indicative of a reduced capacity for aerobic activities, such as growth and locomotion. Results from this chapter indicate that *A. melanopus* and other species that exhibit a reduction in aerobic performance induced by suspended sediments may decline as reefs become more turbid. In contrast, species that are able to maintain aerobic performance

despite changes in gill morphology, such as *A. polyacanthus* and *A. percula*, may be able to persist or even gain a competitive advantage on turbid reefs.

The ability to avoid and escape predators is key to the survival for juvenile coral reef fishes. If fish undergo changes in gill morphology that result in decreased aerobic scope, as observed in chapter two, suspended sediments may potentially compromise predator escape performance and influence anti-predator behaviours (i.e., behaviours that reduce the likelihood of encountering a predator, such as vigilance, changes in activity levels and sheltering). In **chapter three**, juvenile *A. melanopus* were exposed to suspended sediments (0 or 180 mgL^{-1}) for 7 days (prolonged exposure), and their predator escape performance and anti-predator behaviours were assessed in both clear water and turbid water (acute exposure). Prolonged exposure to suspended sediments led to an enhanced predator-escape performance, i.e., fish responded faster to a mechanical stimulus, had faster turning rates, and escaped at higher speeds and from further away than control fish. This was regardless of whether juvenile *A. melanopus* were being tested in clear or turbid water. In addition, acute exposure to suspended sediments led to an enhanced anti-predator behaviour, with fish moving less and staying closer to the wall of the experimental arena, regardless of prolonged exposure to suspended sediments. The observed increases in predator-escape performance and anti-predator behaviours were likely strategies to compensate for an increased perceived predation risk in turbid water due to a reduced ability to detect approaching predators. While the observed changes are expected to increase juvenile survival, they are likely associated with costly non-consumptive effects that may compromise growth rates of juveniles living on turbid reefs.

Ocean temperatures in the tropics have already increased by 0.25 – 0.75°C since pre-industrial times and are predicted to continue increasing in the future. Coral reef fishes on inshore reefs are thus increasingly exposed to the combination of suspended sediments and elevated temperatures. In **chapter four**, *A. polyacanthus*, was reared under current day (29°C) or projected future temperature (32°C) for 4.5 weeks. During the final week of exposure, suspended sediments (0, 90, or 135 mgL^{-1}) were added to aquaria in a full factorial design to simulate a turbidity event. Following treatment exposure, gill morphology, aerobic performance, and predator escape performance of fish were examined. While exposure to 90 mgL^{-1} suspended sediments had no effects on gill morphology, 135 mgL^{-1} suspended sediments led to a reduction in gas diffusion distance relative to control fish (i.e., fish exposed to 29°C and 0 mgL^{-1} suspended sediments). Elevated temperature led to reductions in both gas diffusion distance and filament thickness relative to control fish, which would generally be expected to enhance gas exchange efficiency at the gills. Suspended sediments and elevated temperature combined did not have any interactive effects on gill morphology. The changes in gill morphology were not reflected by the observed changes in oxygen uptake rates; elevated temperature in isolation led to reductions in $\dot{M}O_{2max}$ and $\dot{M}O_{2rest}$, while suspended sediments had no effects on oxygen uptake rates in isolation. In fish exposed to both stressors combined, $\dot{M}O_{2max}$ were not different from control fish, resulting in an enhanced aerobic scope compared to control fish regardless of suspended sediment concentration. Predator escape performance was solely influenced by suspended sediments, with fish exhibiting enhanced fast starts following suspended sediment exposure (i.e., increased average and maximum escape speeds and greater escape distances at 90mgL^{-1} and shorter response latency at 135mgL^{-1} suspended sediments, respectively) compared to control fish. This response was likely driven by an increased perceived predation risk in turbid waters due to a compromised ability to detect predators. These surprising findings show that suspended sediments did not compound, but rather masked some of the negative effects of elevated temperature on juvenile *A. polyacanthus*. While this interaction between the two stressors may help *A. polyacanthus* to acclimate to future environmental conditions on inshore coral reefs, future studies will need to examine potential trade-offs with other important performance traits or functions.

Inshore reefs are often characterized by high tidal and current flows. Elevated water flow and an associated increase in swimming activity leads to an increased oxygen demand, which may potentially reduce the capacity of coral reef fishes to tolerate gill changes induced by suspended sediment exposure. In **chapter five**, juvenile five-lined cardinalfish, *Cheilodipterus quinquelineatus,* were exposed to three suspended sediments (0, 90, and 180 mgL^{-1}) and two water flow conditions (20.0 ± 5.0 cm s^{-1} flow speed during 6 hours day^{-1} or continuous < 2 cm s^{-1} flow speed) in a full factorial design. Elevated water flow led to an increase in $\dot{M}O_{2max}$ and aerobic scope compared to control fish (i.e., fish exposed to < 2 cm s^{-1} flow speed and 0 mgL^{-1} suspended sediments), likely driven by increased swimming activity. However, $\dot{M}O_{2max}$ and aerobic scope in fish exposed to both stressors combined were not different from control fish. This absence of any increases in $\dot{M}O_{2max}$ in fish exposed to both stressors was not linked to changes in gill morphology, which were trait-specific and did not show a consistent pattern across treatments. Regardless of the underlying mechanism, however, an inability to enhance aerobic performance suggests that these fish likely also lack the capacity to enhance their swimming performance in response to elevated water flow conditions. As such, fish exposed to both stressors may have a reduced capacity to sustain elevated water flow velocities compared to fish exposed to elevated water flow in isolation. This may result in fish exposed to both stressors spending more time sheltering and less time foraging, which may negatively affect their growth and survival. A reduced capacity to enhance aerobic performance induced by suspended sediments may thus ultimately lead to a decline in abundance of *C. quinquelineatus* on turbid inshore reefs exposed to currents or high tidal flows or may limit the distribution of this species to more sheltered reefs.

This thesis has provided the most extensive evidence to date that suspended sediments affect the physiology and performance of coral reef fishes. While generally there was no close link between gill morphology and fish performance, one of four examined species in this thesis appeared to be sensitive to changes in gill morphology induced by suspended sediments, as evident by a reduction in aerobic performance. The effects of suspended sediments on predator escape performance was more consistent, with both examined species in this thesis enhancing their fast start performance in response to suspended sediment exposure. However, the impact of suspended sediments on fish performance was influenced by other environmental factors, with suspended sediments masking some of the negative effects of elevated temperature on aerobic performance, while elevated water flow conditions compounded the effects of suspended sediments on aerobic performance. Aerobic performance and predator escape performance are both linked to fitness and survival of juvenile coral reef fishes. As any factor influencing survival of coral reef fishes during this early life history stage can have important effects on adult populations, it is likely that the changes in fish performance reported in the present thesis represent an underlying mechanism contributing to changes in the distribution and abundance of species sensitive to suspended sediments on turbid inshore reefs.

Contents

List of Figures

List of Tables

Chapter 1: General Introduction

1.1 Introduction

Increasing suspended sediment concentrations affect aquatic ecosystems worldwide (e.g., Abott et al. 2018, Halpern et al. 2015, Hovenga et al. 2016). Anthropogenic activities in riparian and coastal zones, including the removal of vegetation, the expansion of agriculture and cattle grazing, the construction of infrastructure, mining, shipping, and dredging have led to increased sediment inputs and re-suspension in lakes, streams, and coastal waters (De Mello et al. 2017, Halpern et al. 2015, Jackson et al. 2017). While suspended sediment concentrations can be naturally high in some ecosystems, anthropogenic increases in suspended sediments have been linked to changes in composition and health of benthic and fish communities (Donohue and Molinos 2009, Parker et al. 2016, Xu and Li 2019). A growing number of studies have aimed to identify the underlying behavioural and physiological mechanisms that drive the observed changes at the population level. A mechanistic understanding of the effects of suspended sediments on organisms can inform management and conservation strategies and improve predictions of the impacts that future changes in suspended sediment concentrations may have on populations (Cooke and O’Connor 2009).

Increases in suspended sediments may affect coral reef ecosystems in particular, with over 30% of global corals reefs already exposed to terrestrial-run off (Halpern et al. 2015). For example, annual suspended sediment loads draining into the Great Barrier Reef, the largest coral reef system in the world, have increased by 5.5 times since European settlement (Kroon et al. 2012) and the frequency of flood plumes reaching mid-shelf reefs, which characteristically have very low background suspended sediment concentrations, has more than tripled over the same time period (Lough et al. 2015). Gradients of suspended sediment concentrations, such as found with decreasing distance from the shore and river mouths, are associated with decreases in coral and fish diversity and abundance, and a shift in community composition (corals, Fabricius et al. 2005, Seeman et al. 2014; fish, Bejarano and Appeldoorn 2013, Mallela et al. 2008, Moustaka et al. 2018). The observed changes in reef fish communities are partly driven by sediment-induced changes to benthic communities (Fabricius et al. 2005, Hamilton et al. 2017). Increasing levels of suspended sediments have been associated with declines in coral cover and diversity and shifts in community composition (reviewed in Fabricius 2005), thereby altering shelter and food availability for coral reef fishes. Suspended sediments can also have direct effects on fishes themselves, potentially compounding the impacts of habitat degradation. In the following, the current knowledge of the effects of suspended sediments on the behaviour, physiology, and performance of fishes – with particular focus on coral reef fishes – will be reviewed with the aim to identify potential mechanisms that may contribute to changes in fish communities and to highlight important avenues for future research.

1.2 Behaviour

Suspended sediment particles scatter and absorb light (Agrawal et al. 2008, Babin et al. 2003), thereby potentially reducing visibility and visual acuity for fish and other aquatic organisms. Accordingly, suspended sediments have been shown to interfere with various activities in fish where relying on detecting visual cues is critical, including foraging, predator-prey interactions, habitat selection, and within-species interactions.

1.2.1 *Foraging*

Numerous studies have documented mostly negative effects of suspended sediments on foraging in fish, although magnitude of the effect seem to be influenced by prey type, habitat, species, and life stage (e.g., Benfield and Minello 1996, Gardner 1981). In both planktivores and piscivores, reaction distances to food items and prey, respectively, are reduced in suspended sediments (Sketwa and Hartman 2001, Utne 2005, Vogel and Beauchamp 1999), thereby reducing feeding efficiency and the amount of food consumed in a given time (Gregory and Northcote 1993, Wenger et al. 2012). Some fish species increase attack rates in an attempt to compensate for reduced feeding efficiency (Meager and Batty 2007, Sweka and Hartman 2000). Increased attack rates, however, would be expected to increase energetic costs associated with feeding (Sweka and Hartman 2000). Indeed, increased foraging activity in juvenile brook trout (*Salvenius fontinalis*) was linked to reduced growth rate in turbid water, despite comparable food consumption to fish held in clear water (Sweka and Hartman 2000).

Suspended sediments appear to have a greater impact on fish that capture small or mobile prey versus fish that capture large or immobile prey (Johansen and Jones 2013). Accordingly, it has been shown that some fish become less selective or switch to different prey types as turbidity increases (Helenius et al. 2013, Johansen and Jones 2013, Reid et al.

1999). For example, nine-spined stickleback (*Gasterosteus aculeatus*) preferred to prey on small copepods in clear water, but preferred larger daphnids under increasing turbidity (Helenius et al. 2013). A diet-shift toward less preferred, and thus likely lower quality prey, may reduce overall energy intake and thus growth and condition of fish (Reid et al. 1999).

The impact of suspended sediments on foraging also appears to differ between species and habitats. For example, suspended sediments affected the foraging efficiency of coral reef fish species commonly found on reefs with low turbidity levels (i.e., mid-shelf and offshore reefs) more than species from turbid inshore reefs (Johansen and Jones 2013). Similarly, feeding efficiency of greenback flounder (*Rhombosolea tapirine*) in suspended sediments improved significantly after they had been reared in suspended sediments as opposed to being reared in clear water (Shaw et al. 2006). The observed differences at the species level and by habitat are likely associated with acclimation and/or adaptation in vision. Illustrating this, guppies (*Poecilia reticulata*) reared in suspended sediments upregulated the expression of opsin genes important in detecting motion, but downregulated the expression opsin genes associated with detecting colour (Ehlman et al. 2015).

Despite the mostly negative effects of suspended sediments on feeding, low to moderate turbidity appears to benefit feeding during early larval stages of some species (Hasenbein et al. 2016, Reitan et al. 1993, Utne et al. 2002). The early larval stages of several marine species have been observed to feed at higher rates in low and moderate turbidity levels than in clear water (Hasenbein et al. 2016; Reitan et al. 1993, Tamaru et al. 1994). Most larval fishes have poorly developed eyes and a short visual field, and it has been suggested that low levels of turbidity enhance the contrast between the background and food items, thus increasing their feeding efficiency (Utne et al. 2002). Supporting this, larval *Amphiprion percula* (clownfish) reared in low suspended sediment concentrations had increased body condition at settlement compared to fish reared in clear water or higher turbidity levels (Wenger et al. 2014). However, it is not yet clear as to which developmental stage or turbidity level this positive effect on feeding is lost.

1.2.2 *Anti-predator behaviour (activity, habitat use, shoaling)*

Suspended sediments influence anti-predator behaviours that are common in fish, including activity levels, habitat use and sheltering, and shoaling; although, findings have been inconsistent between species, and the majority of studies have focused on species living in lakes and streams. Suspended sediments often lead to a reduction in swimming activity (Ajemian et al. 2015, Borner et al. 2015), and fish presented with predator cues show a greater reduction in feeding in suspended sediments compared to clear water (Figueiredo et al. 2016, Leahy et al. 2011). For example, spiny chromis (*Acanthochromis polyacanthus*), which is the only coral reef fish species that has been examined so far, reduced feeding activities in response to predator cues by 40% in suspended sediments but only by 17% in clear water (Leahy et al. 2011). Decreases in activity lower the likelihood of encountering a predator (Lima 1998) and may compensate for a reduced capacity to assess predation risk and detect an approaching predator in turbid waters (Leahy et al. 2011, Mazur and Beauchamp 2003, see also discussion below). These results also indicate that fish perceived predation risk to be increased in turbid waters (Leahy et al. 2011), which can be associated with non-consumptive costs, including a reduction of energy intake due to less time spent feeding, increased metabolic costs as a result of stress, and compromised immune responses (reviewed in Preisser et al. 2005).

In contrast to the findings discussed above showing an increase in anti-predator behaviours, several studies have documented the opposite response in fish exposed to suspended sediments. Fish spent less time close to vegetation or sheltering when in suspended sediments compared to clear water (Lehtiniemi et al. 2005 Miner and Stein 1996, Snickars et al. 2004). Further, responses to predator cues were less pronounced when exposed to suspended sediments than in clear water (Abrahams and Kattenfeld 1997, Gregory et al. 1993). Compromised predator detection in turbid waters may reduce the benefits of anti-predator behaviours for some species, leading prey fish to prioritize feeding over anti-predator behaviours (Abrahams and Kattenfeld 1997). Alternatively, the ability of some predators to detect prey may be compromised to a greater extent than the reverse (i.e., prey detecting predators), which may reduce perceived predation risk in prey and decrease anti-predator behaviours (Lehtiniemi et al. 2005, Miner and Stein 1996). The reasons for the contrasting responses to suspended sediments between species are currently not known. Given the bias in the available literature towards species from freshwater habitats, in particular lakes and streams, research on species from coral reefs may help shed light on the factors influencing anti-predator behaviour during suspended sediment exposure.

Suspended sediments can interfere with shoaling behaviours of fish. In suspended sediments, fish spend less time shoaling and form smaller and less cohesive shoals (i.e., increase in average distances between shoal members; Borner et al. 2015, Sommer-Trembo et al. 2015) than in clear water. Accordingly, in suspended sediments, escaping shoals were also more fragmented compared to clear water (Kimbell and Morrell 2015). It is likely that suspended sediments compromise the ability of individuals to remain in visual contact with other shoal members, thereby affecting shoal formation and movement (Berner et al. 2015, Kimbell and Morrell 2015). Indeed, this is supported by findings showing that ayu (*Plecoglossus altivelis*) and Japanese anchovy (*Engraulis japonicus*), which both have well developed eyes, increase shoaling in suspended sediments (Ohata et al. 2014). Their well-developed eyes allow them to feed under low light conditions in deep waters and likely also allow them to maintain and even enhance shoaling behaviours in turbidity (Ohata et al. 2014). Shoaling is an important anti-predator behaviour in fish; a shoal can detect an approaching predator earlier than individual fish, and an escaping shoal can confuse predators, further reducing predation risk (reviewed in Pitcher and Parrish 1993). Ultimately, a compromised ability to shoal in turbid waters would thus likely increase predation risk of fish.

1.2.3 *Predator escape performance*

Suspended sediments interfere with predator detection; prey detect predators later and from shorter distances when they are in turbid versus clear water (Mazur and Beauchamp 2003, Miner and Stein 1996, Meager et al. 2006). For example, juvenile bluegill (*Lepomis macrochirus*) detected an approaching model predator from further than 200 cm in clear water, but their reaction distance was reduced to 23 cm in turbid water (10 NTU turbidity; estimated visibility 50 – 55 cm, Miner and Stein 1996). These shorter reaction distances were shown to decrease the likelihood that juvenile Atlantic cod (*Gadhus morhua*) escape a predator attack from 73% in clear water to 21% in turbid water (Meager et al. 2006). Suspended sediments have also been shown to influence the responsiveness (i.e., proportion of escapes vs no response) of prey fish to predator attacks (Kimbell and Morrell 2015). In turbid water, prey fish are more likely to freeze than flee when presented with a predator attack, thereby increasing their risk of being captured (Kimbell and Morrell 2015). Similarly, fathead minnows (*Pimephales promelas*) were less likely to recognize a predator in suspended sediments compared to clear water, which may further compromise responsiveness (Ferrari et al. 2010). Reduced responsiveness because fish do not recognize a predator or because they detect a threat too late, and poorly timed escape responses, would be expected to increase predation risk of juveniles (Lima 1998). However, no information is currently available on the effects of suspended sediments on predator detection in coral reef fishes, and no information is available on predator escape performance in general (e.g., escape speeds and acceleration). Coral reefs are of high structural complexity, and the capacity of juvenile reef fishes to escape ambush predators is directly linked to their survival (McCormick et al. 2018). Examining the effects of suspended sediments on predator escape performance can thus provide information on the mechanisms underpinning changes in predator-prey interactions on coral reefs and will be an important avenue for future research.

1.2.4 *Predation rates*

Given that the outcome of a predator-prey interactions are influenced by various factors, including anti-predator behaviours, predator escape performance, and the ability of predators to detect and capture prey (Preisser et al. 2005), it is not surprising that the effects of suspended sediments on predation rates are mixed (e.g., Gregory et al. 1996, van Landeghem et al. 2011). Several studies have reported increased survival of prey fish in suspended sediments, indicating that turbidity may act as a refuge for certain species (Gregory and Levings 2011, Reid et al. 1999, Snow et al. 2018). For example, survival of pike-perch larvae (*Sander lucioperca*) exposed to perch (*Perca fluviatilis*) was higher in turbid water compared to clear water (Pekcan-Hekim and Lappalainen 2006). Similarly, migrating juvenile salmonids (*Oncorhynchus* spp.) were less likely to be captured by predators in turbid sections of a river compared to sections with clear water (Gregory and Levings 2011). When exposed to cutthroat trout (*Oncorhynchus clarkii*) in a mesocosm experiment, however, juvenile salmonids did not have an advantage in turbid water, highlighting that the effects of suspended sediments on predation rates are likely to differ between species (Gregory 1996). To date, only one study has examined the effects of suspended sediments on predation rates in a coral reef fish, documenting that survival of juvenile black-axil chromis (*Chromis atripectoralis*) preyed upon by an ambush predator, dottyback (*Pseuchochromis fuscus*) was decreased under moderate (45 mgL^{-1}) suspended sediment concentrations, but remained unchanged at lower (30 mgL^{-1}) and higher (60 mgL^{-1}) suspended sediment concentrations (Wenger et al. 2013).

1.2.5 *Habitat selection at settlement*

Suspended sediments have been shown to interfere with habitat selection in coral reef fish larvae at the end of their pelagic larval phase when they are settling onto coral reefs

(O’Connor et al. 2016, Wenger et al. 2011). Larval Ambon (*Pomacentrus amboinensis*) and lemon (*Pomacentrus moluccensis*) damselfish larvae exhibited a strong preference for live coral when settling in clear water, but this preference was lost when settling in suspended sediments larvae settled randomly on live coral, partially dead coral, or dead coral (Wenger et al. 2011). This response was likely driven by impaired olfactory and visual systems (Wenger et al. 2011, O’Connor et al. 2016). Ambon damselfish juveniles were not able to distinguish between olfactory cues from live and dead coral when suspended sediments were present (Wenger et al. 2011). Similarly, juvenile green chromis (*Chromis viridis*) did not distinguish between live and dead coral in suspended sediments when only visual cues were available (O’Connor et al. 2016). In coral reef fishes, selecting sub-optimal habitats at settlement can result in reduced fitness and survival (Jones 1991, McCormick 2009), and an impaired ability to select habitats may thus have negative consequences for recruitment success to turbid reefs.

1.2.6 *Courtship and mating*

Suspended sediments have been shown to interfere with courtship behaviour and mate selection in fishes (Ehlman et al. 2018, Gray et al. 2011). While only a few studies have assessed the effects of suspended sediments, the effects of turbidity more broadly (i.e., caused by algae) have been tested in a number of species, including sticklebacks (*Gasterosteus aculeatus;* Engstrom-Ost and Candolin 2007), sand goby (*Pomatoschistus minutus;* Jarvenpaa and Lindstrom 2004), pipefish (*Syngnathus typhle*; Sundin et al. 2010), and various cichlid species (Cichlidae; Maan et al. 2010, Seehausen et al. 1997). Regardless as to whether turbidity is caused by suspended sediments or unicellular algae, most studies found that fish spent less time inspecting potential mates than in clear water (Ehlman et al. 2018, Engstrom-Ost and Candolin 2007, Sundin et al. 2010). Additionally, selectivity between mates was more evenly distributed in turbid water compared to clear water, which is likely to have negative consequences for the fitness of offspring (Jarvenpaa and Lindstrom 2004, Seehausen et al. 1997). Interference with mate choice can even lead to hybridization, as has been observed between closely related cichlid species in Lake Victoria (African Great Lakes; Seehausen et al. 1997, Seehausen et al. 2008). In these species, females exhibit a strong mate preference based on male colouration, which has been driven by the adaptation of the visual system to differences in light levels and has led to the adaptive radiation into several hundred cichlid species (Seehausen et al. 2004, 2006). By impairing mate choice, turbidity has resulted in hybridization between species and the loss of species diversity in Lake Victoria (Seehausen et al. 1997, Seehausen et al. 2008). Similarly, turbidity was positively correlated with the occurrence of hybridization between the invasive red shiner (*Cyprinella lutrensis*) and the native blacktail shiner (*C. venusta stimatura*) in the upper Coosa River (USA; Walters et al. 2008). Future studies will need to examine whether these events are isolated occurrences or representative for a more wide-spread impact of turbidity on ecosystems.

1.3 Physiology and performance

1.3.1 *Primary and secondary stress response*

Suspended sediments have been shown to elicit an acute stress response in several salmonids and other commercially important species, either due to the perceived increase in turbidity or due to sediment particles interacting with fish skin or gills (Anderson et al. 1996; Servizi and Martens 1992). Following exposure to suspended sediments, changes in hormones, i.e., elevated catecholamine and corticosteroid levels, have been observed, such changes are typical of a primary stress response (Awata et al. 2011; Redding et al. 1987; Sutherland et al. 2008). Increases in these hormones generally lead to a secondary stress response, involving changes in the cardiovascular and respiratory systems to increase oxygen uptake, transport, and delivery, and the mobilization of energy substrates (Wendelaar Bonga 1998). Accordingly, fish exposed to suspended sediments have been found to show an increased gill ventilation rate (Horkel and Pearson 1997), an increased cardiac output (Bunt et al. 2004), and increases in haematocrit and reduced leukocrit (i.e., volume percentage of red and white blood cells in the blood, Becke et al. 2018; Lake and Hinch 1999; Michel et al. 2013; Reid et al. 2006). While these physiological changes are acute responses to a stressor that are aimed to regain homeostasis, chronic stress can lead to reduced growth, development, reproduction, and disease resistance (Wendelaar Bonga 1998).

1.3.2 *Growth and development*

Suspended sediment exposure has been associated with reduced growth rates and body condition in several fish species, including one coral reef fish species (Ardjosoeodira and Ramnarine 2002; Michel et al. 2013; Shrimpton et al. 2007, Wenger et al. 2012). Juvenile spiny chromis (*Acanthochromis polyacanthus*) were exposed to suspended sediment concentrations of 90 or 180 mgL^{-1} for six weeks, growth rates were less than half of those of their clear water counterparts (Wenger et al. 2012). Mechanisms that may underpin reductions in growth upon exposure to suspended sediments include the re-allocation of energy away from growth as part of the stress response (Reid et al. 2006), reductions in food intake due to reduced feeding efficiency (Wenger et al. 2012), as well as less time spent feeding as part of enhanced anti-predator behaviours in turbid water (Leahy et al. 2011).

Suspended sediments have also been found to affect development of at least one coral reef fish species so far. Metamorphosis and settlement of larval anemone fish (*Amphiprion percula*) reared under low (i.e., 15 mgL^{-1}) suspended sediment concentrations were delayed relative to control fish, which increased the variation and the median pelagic larval duration of larvae by one day (Wenger et al. 2014). In coral reef and other fish species, predation is highest during early life history stages (Bertram and Leggett 1994; Hoey and McCormick 2004), and reduced growth or delayed development prolongs the time spent vulnerable to predation, potentially increasing mortality of larvae and juveniles, thereby affecting recruitment success (Leggett and Deblois 1994).

1.3.3 *Susceptibility to pathogens*

Several studies indicate that fish exposed to suspended sediments exhibit an increased susceptibility to pathogens, including a study on a coral reef fish. Mortality caused by the bacterial pathogen, *Vibrio anguillarum*, was enhanced in yearling steelhead (*Salmo gairdneri*) exposed to suspended sediments (Redding et al. 1987). An increased occurrence of bacterial gill disease (epitheliocystis) was observed in juvenile snapper (*Pagrus auratus*) following suspended sediment exposure (Lowe et al. 2015). Similarly, suspended sediment exposure was linked to an increased number of pathogenic bacterial species on the gills of newly settled anemonefish (*Amphiprion percula*, Hess et al. 2015) and adult rainbow trout (*Oncorhynchus mykiss*; Becke et al. 2018). Gill lesions and changes in gill morphology, such as an increased secretion of mucous, have been suggested to promote the colonization and growth of pathogens on the gills (Llewellyn et al. 2015; Lowe et al. 2015). Further, bacteria attach to sediment particles, thereby increasing exposure to pathogens of fish living in suspended sediments (Dropo et al. 2009). Other reasons that may contribute to a decreased pathogen resistance include the suppression of the immune system following a stress response, as indicated by a reduction in white blood cells in fish exposed to suspended sediments (Becke et al. 2018; Lake and Hinch 1999; Reid et al. 2006).

1.3.4 *Gill morphology*

Gills are a multi-functional organ, playing an important role for gas exchange, osmo/ionregulation, acid-base balance, nitrogen excretion, and the immune system (Evans et al. 2005). Gills have a large surface area and a relatively thin gill epithelium (as thin as 0.5 μm), which allows for efficient gas exchange (Nilsson et al. 2007, 2012), but can also enhance exposure to water-borne particles. Suspended sediments can affect gill morphology in several ways. Sediment particles bind to the mucous layer on the gill epithelium, which may be compounded by an increased mucous secretion in response to acute (< 72 hours) exposure to suspended sediments (Li et al. 2012; Sutherland and Meyer 2007). This may interfere with gas exchange at the gills and, at high suspended sediment concentrations, can lead to the clogging of gill lamellae and suffocation (Baba et al. 2006; Baiyinbaoligao et al. 2013; Swinkels et al. 2004). Sediment particles can also irritate or damage the gill epithelium following prolonged (>3 days) exposure, leading to gill lesions, the shortening of gill lamellae, and reduced lamellar density (Cumming and Herbert 2016; Humborstad et al. 2006), and thereby resulting in a reduction in gill surface area available for gas exchange. Prolonged exposure to suspended sediments can further induce changes in the number and composition of cells on the lamellar and filament epithelium (epithelium in between lamellae), including the proliferation of pavement cells (hyperplasia; Lowe et al. 2015; Wong et al. 2013), an increase in cell size (hypertrophy; Humborstad et al. 2006; Sutherland and Meyer 2007), and an increased number of mucous cells (Hess et al. 2015; Humborstad et al. 2006) and mitochondria-rich cells (also termed chloride cells; Au et al. 2004; Wong et al. 2013). These changes increase the gas diffusion distance (i.e., distance between the water and blood sinus) and reduce the space between the lamellae for water flow, thereby reducing the efficiency of gas exchange (Lappivaara et al. 1995). In contrast to these changes, however, a reduction in gas diffusion distance was also observed in response to suspended sediments, which would be expected to enhance gas exchange efficiency (Au et al. 2004).

The vast majority of studies to date examining the effects of suspended sediments on gill morphology have focused on commercially important temperate species, such as rainbow trout (*Oncorhynchus mykiss*) (e.g., Michel et al. 2013, Swinkels et al. 2004). The only two coral reef fish species that have been studied so far include anemonefish (*Amphiprion percula*) larvae (Hess et al. 2015) and orange spotted grouper (*Epinephelus coioides*) (Au et al. 2004, Wong et al. 2013). Compared to temperate species that often can tolerate high suspended sediment concentrations (i.e., exceeding 500mgL^{-1}) without showing any changes in gill morphology (e.g., Lake and Hinch 1999, Michel et al. 2013), the two examined coral reef fishes appeared to be more sensitive, with both species exhibiting gill morphological changes at concentrations of 50 mgL^{-1} suspended sediments or less (Au et al. 2004, Hess et al. 2015, Wong et al. 2013). The observed changes in response to suspended sediments compared to control fish included an increase in the density of mitochondria-rich cells and mucous cells, mucous secretion, and gas diffusion distance, respectively, in both species (Au et al. 2004, Hess et al. 2015, Wong et al. 2013). Interestingly, while Wong et al. 2013 found an increased gas diffusion distance in *E. coioides* in response to suspended sediments, Au et al. 2004 reported the opposite response in the same species. The underlying reasons for these contradicting results are not yet understood, and require further investigation. Changes in gas diffusion distance and the density of different cell types, such as mitochondria-rich cells, are generally assumed to be associated with changes in ion/osmoregulation and gas exchange (Evans et al. 2005), and may thus potentially have cascading effects on whole-animal performance (see sections below).

1.3.5 *Ion/osmoregulation*

Several studies have examined the effects of suspended sediments on ion/osmoregulation of fish using changes in the number of mitochondria-rich cells on gill lamellae and changes in Na^+K^+ATPase activity to indicate osmoregulatory stress (Au et al. 2004; Li and Shen 2012; Shrimpton et al. 2007; Wong et al. 2013). In marine fishes, Na^+K^+ATPase creates an ionic and electrical gradient across the membrane of mitochondria-rich cells, allowing the excretion of excessive salts (Avella and Bornancin 1989; McCormick 1993). Suspended sediments have been found to lead to an increased number of mitochondria-rich cells and/or a decrease in Na^+K^+ATPase activity (Au et al. 2004; Li and Shen 2012; Shrimpton et al. 2007; Wong et al. 2013). These changes may be due to direct damage to mitochondria-rich cells induced by sediment particles, or reduced efficiency of ion/osmoregulation as a result of other changes in gill morphology, such as an increased mucous secretion (Au et al. 2004). However, currently no information is available on the direct effects of suspended sediments on ion/osmoregulation, and future studies should therefore examine potential impacts on blood chemistry, such as plasma ion concentrations and plasma osmolality.

1.3.6 *Gas exchange and aerobic performance*

Although it is well established that gill morphology is linked to gill function (e.g., Evans et al. 2005, Sollid et al. 2003, 2005), little is known as to whether suspended sediments, via changes in gill morphology, may affect gas exchange and therefore aerobic performance of fishes. The exceptions to this are studies that have associated high suspended sediment concentrations to fish suffocation (e.g., Baba et al. 2006, Skwinkels et al. 2004, Whitefield and Paterson 1995). A reduction in gas exchange efficiency due to changes in gill morphology (i.e., the binding of particles to the gill epithelium, increases in gas diffusion distance, and reductions in gill surface area) would be expected to decrease maximum oxygen uptake rates ($\dot{M}O_{2max}$) achieved during peak performance of fish or during recovery from exercise. The only study examining the effects of suspended sediments on $\dot{M}O_{2max}$ to date has been on juvenile snapper (*Pagrus auratus*), and results indicated no changes when compared to control fish, despite changes in gill morphology (i.e., reduced lamellar density and increased gas diffusion distance, Cumming and Herbert 2016).

While $\dot{M}O_{2max}$ sets the upper limit for aerobic performance, oxygen uptake rates at rest ($\dot{M}O_{2rest}$) represent the amount of oxygen (as a proxy for energy) required to sustain maintenance or baseline metabolic processes, such as protein turn-over (Fry 1971, reviewed in Norin and Clark 2016). Suspended sediment exposure may lead to an increase in $\dot{M}O_{2rest}$ as a consequence of increased energetic costs associated with reduced gas exchange efficiency, changes in gill morphology (e.g., tissue repair and increased mucous secretion), and ion/osmoregulation, but studies examining the effects of suspended sediments on $\dot{M}O_{2rest}$ have had mixed results. Adult rainbow trout (*Oncorhynchus mykiss*) exposed to suspended sediments for a short period (30 h) exhibited an increase in $\dot{M}O_{2rest}$ (Reid et al. 2006), while brown trout (*Salmo trutta*; Greer et al. 2015), three blackline shriner (*Notropis* spp. Gray et al., 2016), and snapper (*Pagrus auratus*, Cumming and Herbert 2016) exposed to suspended sediments for prolonged periods (30 days to several months) did not show any changes in $\dot{M}O_{2rest}$ relative to control fish. Aerobic scope is calculated as the difference between $\dot{M}O_{2max}$ and $\dot{M}O_{2rest}$ and represents an organism's capacity for aerobic activities, including growth, development, and swimming (Fry 1971, Norin and Clark 2016). Aerobic scope is linked to fitness and survival of fish (Norin and Clark 2016), and more studies are required to examine the potential impact of suspended sediments on this important trait.

1.3.7 *Hypoxia tolerance*

Elevated suspended sediment concentrations on coastal reefs often co-occur with nutrient enrichment, which can lead to a decline in dissolved oxygen concentration (i.e., hypoxia) due to bacterial respiration (Breitburg et al. 2018, Doney et al. 2010). As water oxygen levels decline, the concentration gradient of oxygen across the gill epithelium decreases, thereby decreasing the efficiency of oxygen diffusion (Schurmann and Steffensen 1997). Fish can compensate a reduction in oxygen diffusion efficiency to a certain extent by increasing gill ventilation and perfusion (Nilsson 2007). The critical oxygen tension (P_{crit}) is the lowest dissolved oxygen concentration at which fish can still maintain their $\dot{M}O_{2rest}$ and is used as a measure to describe hypoxia tolerance; below P_{crit}, fish have to engage in anaerobic metabolism for ATP production to support the energy demand of tissues, which is less energetically efficient than aerobic respiration and leads to the build-up of toxic by-products including lactate (Nilsson 2007, Schurmann and Steffensen 1997). It would be expected that gill morphological changes that reduce gas diffusion efficiency, such as an increase in gas diffusion distance, would negatively affect the hypoxia tolerance of fish. Yet, although an increase in gas diffusion distance was observed in Australasian snapper (*Pagrus auratus*) in response to suspended sediments, this was not associated with any changes in hypoxia tolerance. However, suspended sediment exposure led to a decrease in hypoxia tolerance (i.e., increase in P_{crit}) in brown trout (*Salmo trutta*; Reid et al. 2003) and two blackline shiner species (*Notropis* spp.; Gray et al. 2016). While no changes in gill morphology were observed in brown trout, and gill morphology was not analysed in blackline shiner species, both studies attributed the observed negative effects on hypoxia tolerance to sediment particles attaching to the gill surface, thereby interfering with gas exchange (Gray et al. 2016, Reid et al. 2003). Reductions in hypoxia tolerance may affect the capacity of individuals to exploit hypoxia-prone habitats, which may influence species abundance and distribution (Doney et al. 2010). Examining the relationship between suspended sediment exposure and hypoxia tolerance is thus an important avenue for future research.

The available literature to date suggests that suspended sediments influence behaviour, physiology, and performance of fishes. Numerous studies have established the negative effects of suspended sediments on the perception of visual cues and the consequences this has for fish behaviour, including feeding, predator-prey interactions, and intraspecific interactions. In contrast, the effects of suspended sediments on physiological performance have received less attention. Moreover, most of the physiological studies to date have focused on identifying the changes in gill morphology occurring in response to suspended sediments. Although changes in gill morphology may have severe consequences for fish performance, aerobic performance in particular, our knowledge about the potential effects of suspended sediments on fish performance is limited and represents an important avenue for future research. Reviewing the literature has also brought to light that the majority of studies have been conducted on large-bodied, cold-water fish species, and especially those important for fishing and aquaculture industries, while coral reef fishes are underrepresented. Coral reefs naturally have low background levels of suspended sediments (Larcombe et al. 2001, Wenger et al. 2016), and coral reef fishes may thus be especially vulnerable to increasing levels of suspended sediments.

Lastly, while past studies have provided important baseline information about the impacts of suspended sediments on fish, fish in the wild are increasingly exposed to multiple environmental stressors simultaneously (Halpern et al. 2015). To gain a more realistic understanding as to how fish respond to current and future environmental conditions, it is thus critical to consider the cumulative effects of suspended sediments and other environmental stressors on coral reef fishes. Stressors that influence the aerobic performance of fishes are of particular interest, such as elevated water temperatures and elevated water flow conditions, as they may potentially have interactive effects with suspended sediments or may increase the vulnerability of fish to suspended sediments. Elevated water temperatures have been shown to compromise aerobic scope of coral reef fishes (e.g., Gardiner et al. 2010, Rummer et al. 2014), which may be further compounded by the negative effects of suspended sediments on gill morphology. In contrast, exposure to elevated water flow conditions caused by currents or tides leads to an increased oxygen demand and enhanced aerobic performance of fish as a consequence of increased swimming activity (Binning et al. 2015). An increased oxygen demand and aerobic performance may leave fish particularly vulnerable to the potential impacts of suspended sediments on gill morphology and aerobic scope. Yet, currently no information exists about the cumulative effects of suspended sediments and other environmental factors on the physiology and performance of fishes.

1.4 Research aims and thesis structure

The overall aim of this thesis was to examine the effects of suspended sediments, in isolation and in combination with elevated water temperature and elevated water flow conditions, on the gill morphology, physiology, and performance of coral reef fishes.

Changes in performance are linked to changes in fitness and survival and establishing the effects of suspended sediments on physiological performance is thus fundamental to understand the drivers underpinning the observed responses of coral reef fish populations and coral reef communities to changes in suspended sediment concentrations. The specific aims of this thesis were to:

1. Examine the effects of suspended sediments on the gill morphology and aerobic performance of coral reef fishes;

2. Test the effects of suspended sediments on anti-predator behaviour and predator escape performance in coral reef fishes;

3. Assess the cumulative effects of suspended sediments and elevated temperature on gill morphology, aerobic performance, and predator escape performance in coral reef fishes; and

4. Determine the cumulative effects of suspended sediments and elevated water flow on gill morphology and aerobic performance in coral reef fishes. Each of these aims is addressed in a separate chapter.

In Chapter 2, I exposed the juveniles of three damselfish species, i.e., two species common on turbid inshore reefs, *Amphiprion melanopus* and *Acanthochromis polyacanthus*, and one species typically found on reefs with low background turbidity, *Amphiprion percula*, to a range of suspended sediment concentrations representative of conditions found on turbid inshore reefs of the Great Barrier Reef. I hypothesized that suspended sediments would lead to changes in gill morphology, and that these changes would result in a reduction in gas exchange efficiency and compromised aerobic performance.

In Chapter 3, I focused on the effects of acute (2 min) and prolonged exposure (7 days) to suspended sediments on a common anti-predator behaviour (i.e., changes in activity patterns) and predator escape performance of juvenile *A. melanopus*. I hypothesized that acute exposure to suspended sediments would interfere with the detection of a mechanical stimulus and would lead to an enhanced anti-predator behaviour; whereas prolonged exposure to suspended sediments would compromise predator escape performance due to a reduction in aerobic scope.

In Chapter 4, I investigated the combined effects of suspended sediments and elevated temperature on the gill morphology, aerobic performance, and predator escape performance of juvenile *A. polyacanthus*. I hypothesized that exposure to suspended sediments and elevated temperature in isolation would have negative effects on gill morphology and compromise aerobic scope and predator escape performance, while exposure to both stressors combined would compound these impacts on gill morphology and fish performance.

In Chapter 5, I assess the cumulative effects of suspended sediments and elevated water flow conditions on the gill morphology and aerobic performance of juvenile five-lined cardinalfish, *Cheilodipterus quinquelineatus*, a species common on turbid inshore reefs. I hypothesized that suspended sediments in isolation would negatively affect gill morphology and compromise aerobic scope. In contrast, I hypothesized that elevated water flow in isolation would lead to changes in gill morphology beneficial to gas exchange and would enhance aerobic scope as a consequence of increased swimming activity and increased oxygen demand. I further hypothesize that exposure to both stressors combined would interfere with the capacity of fish to adjust gill morphology and aerobic scope to elevated water flow conditions.

Chapter 2: Species-specific impacts of suspended sediments on gill structure and function in coral reef fishes

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2.1 Abstract

Reduced water quality, in particular increasing suspended sediments, has been linked to declines in fish abundance on coral reefs. Changes in gill structure induced by suspended sediments have been hypothesized to impair gill function and may provide a mechanistic basis for the observed declines; yet, evidence for this is lacking. Juveniles of three reef fish species (*Amphiprion melanopus, Amphiprion percula*, and *Acanthochromis polyacanthus*) were exposed to suspended sediments (0 - 180mgL^{-1}) for 7 days and examined changes in gill structure and metabolic performance (i.e. oxygen consumption). Exposure to suspended sediments led to shorter gill lamellae in *A. melanopus* and *A. polyacanthus* and reduced oxygen diffusion distances in all three species. While *A. melanopus* exhibited impaired oxygen uptake upon suspended sediment exposure, i.e., decreased maximum and increased resting oxygen consumption rates resulting in decreased aerobic scope, the oxygen consumption rates of the other two species remained unaffected. These findings imply that species sensitive to changes in gill structure such as *A. melanopus* may decline in abundance as reefs become more turbid, while species that are able to maintain metabolic performance despite suspended sediment exposure, such as *A. polyacanthus* or *A. percula*, may be able to persist or gain a competitive advantage.

2.2 Introduction

Declining water quality is one of the leading causes of aquatic ecosystem degradation globally (Halpern et al. 2015, Syvitski et al. 2005). In particular, the input and resuspension of sediments are having a dramatic effect on both sessile and mobile organisms (Wenger et al. 2017). Coastal development, agriculture, overgrazing, mining, removal of riparian vegetation, dredging, and shipping have increased suspended sediments in coastal waters over the past decades (Bartley et al. 2014, Foley et al. 2005, Syvitski et al. 2005). Continued population growth in coastal areas, especially in the tropics (Lutz and Samir 2010), is likely to lead to further increases in suspended sediments. Increases in suspended sediments have already led to biodiversity loss and fundamental changes in benthic and fish assemblages on coral reefs (Cheal et al. 2013, Fabricius 2005, Wenger et al. 2015); however, the mechanistic basis for these declines is not fully understood.

Declining water quality has been associated with reductions in abundance and/or diversity of reef fishes on coastal reefs (e.g., Great Barrier Reef; Cheal et al. 2013, Fabricius et al. 2005, Caribbean; Mallela et al. 2007, Pacific Islands; Beger et al. 2008, Letourneur et al. 1998). It is not clear, however, if these changes in fish assemblages are the result of the direct effects of suspended sediments on fish behavior or physiology, and/or the indirect effects of suspended sediments that manifest through the degradation of benthic habitats. The negative effects of sediments on the abundance and composition of corals is well established (Erftemeijer et al. 2012, Fabricius 2005), and numerous studies have documented declines in fish assemblages following coral loss (Pratchett et al. 2011, Wilson et al. 2008). However, the indirect effects of sediment-induced coral loss may be compounded by any direct impacts of suspended sediments on fishes (Hamilton et al. 2017, Wenger et al. 2017). For example, suspended sediments have been shown to interfere with visual acuity and olfaction in some coral reef fishes, thereby affecting larval settlement (O’Connor et al. 2016, Wenger et al. 2011) and prey capture (Wenger et al. 2012, Johansen and Jones 2013). Suspended sediments, however, may also have important impacts on physiological processes, such as respiration, that could lead to negative effects at the level of performance and fitness.

Most fish species rely on their gills to extract oxygen from water (Wilson and Laurent 2002), and suspended sediments and other pollutants have been hypothesized to interfere with this process (Heath 1995). Several studies have shown that suspended sediments and other pollutants can irritate and damage gill tissues, leading to changes in gill structure (Au et al. 2004, Cumming and Herbert 2016, Lowe et al. 2015). Specifically, exposure to suspended sediments has been shown to reduce the length of gill lamellae, thereby reducing gill surface area, and to damage the gill epithelium (Lake and Hinch 1999, Sutherland and Meyer 2007), which is the primary site for oxygen uptake in most fishes (Evans et al. 2005). To repair tissue damage and to reduce the impact of pollutants, gills often secrete mucous and grow additional cell layers on the lamellae (referred to as hyperplasia), which increases the thickness of the gill epithelium and oxygen diffusion distances (Herbert and Merkens 1961, Servizi and Martens 1987). Reductions in gill surface area and increases in oxygen diffusion distances are assumed to decrease the efficiency of gas exchange and reduce the capacity of the gills for oxygen uptake (Hughes and Morgan 1973, Lappivaara et al. 1995). Indeed, some species are known to undergo extensive modifications in gill surface area and oxygen diffusion distances to regulate ion- and oxygen transport across the gills in response to changes in temperature, hypoxia, and air exposure (LeBlanc et al. 2010, Ong et al. 2007, Sollid et al. 2003). However, little is known as to whether changes in gill structure induced by pollutants such as suspended sediments directly affect gill function and the metabolic performance of fish (but see Cumming and Herbert 2016).

The capacity to perform vital aerobic activities is tied to the capacity of the gills for oxygen uptake (Killen et al. 2016, Metcalfe et al. 2016). For example, the maximum rate of oxygen uptake of individuals (i.e., $\dot{M}O_{2\mathrm{max}}$) has been correlated with various activities linked to fitness and survival in fish, including locomotion and competitive ability (Metcalfe et al. 2016, Norin and Clark 2016). Gill damage induced by suspended sediments may reduce the capacity for oxygen uptake and limit oxygen delivery to tissues, especially during activities requiring high levels of oxygen (Roberts 2012), thus compromising fish performance and ultimately reducing fitness and survival of fish living on turbid reefs. The potential link between gill structure, metabolic performance, and individual fitness may be an important - albeit understudied - driver of the observed changes in fish assemblages with declining water quality (Cumming and Herbert 2016, Hess et al. 2015, Wong et al. 2013). Determining the extent to which structural gill changes induced by suspended sediments affect the metabolic performance of individual species is critical to understand current and future changes in coastal fish assemblages as water quality decreases.

The aim of this study was to investigate whether changes in gill structure resulting from exposure to suspended sediments will compromise gill function and metabolic performance of juvenile coral reef fishes, thereby providing a mechanistic link between declining water quality and fish health. To do so, I examined the effects of a range of ecologically relevant suspended sediment concentrations on the gill morphology and oxygen consumption rates of three common damselfish species using histology and intermittent-flow respirometry, respectively. The suspended sediment concentrations used reflect those currently experienced on inshore reefs of the Great Barrier Reef during re-suspension events such as currents, waves, and flood plumes (Larcombe et al. 2001, Wolanski et al. 2008). These suspended sediment concentrations are likely to become more frequent in the future as population growth, coastal development, and associated terrestrial run-off in tropical coastal areas rapidly increase (Halpern et al. 2015, Lutz and Samir 2010).

2.3 Materials and methods

2.3.1 *Larval and juvenile rearing conditions*

Ten breeding pairs each of *Amphiprion melanopus* (cinnamon clownfish), *Acanthochromis polyacanthus* (spiny chromis), and *Amphiprion percula* (false orange clownfish) were maintained in 60L outdoor aquaria supplied with flow-through, UV-filtered seawater (28.5 ± 0.5°C) (for each species, juveniles from at least six breeding pairs were used for experiments). Breeding pairs were provided with half a terracotta pot for egg deposition and fed twice daily with INVE Aquaculture NRD 1.2 mm food pellets (ProAqua Pty Ltd, Queensland, Australia) to satiation. Terracotta pots in the aquaria of *A. melanopus* and *A. percula* breeding pairs were examined every morning for newly deposited egg clutches. On the day when hatching was predicted, i.e. 6 - 8 days after egg deposition for *A. melanopus* (Gordon et al. 2002) and 9 – 11 days for *A. percula*, respectively, terracotta pots were transferred to 40L static indoor aquaria, and egg clutches were kept well aerated. Aquaria were painted black to reduce glare and maintained in a water bath at 28.5 ± 0.3°C under a 12:12 light – dark cycle. Larvae generally hatched a couple of hours after lights went out, and were reared following a standard protocol. For the first 4 days post-hatch, aquaria were flushed with filtered seawater for 1 hour every morning before the lights turned on, after which *Branchionus sp*. rotifers at a density of 15 rotifers ml^{-1} and *Nannochloropsis sp*. algal paste (ProAqua Pty Ltd, QLD, Australia) was added to the water until the bottom of the tank was no longer visible. On day 5 post-hatch, aquaria were supplied with flow-through, UV-filtered seawater and *Artemia sp*. nauplii were added to tanks at a density of 1 nauplii ml^{-1} twice daily. Rotifers were supplied at a reduced density of 5 rotifers ml^{-1} until day 8 post-hatch. A filter with 50 µm mesh prevented the loss of rotifers and *Artemia*. On day 14 post-hatch, larvae were fed flakes NRD 0.5 – 0.8 mm (Pro Aqua Pty Ltd) twice daily, and supplied with *Artemia spp*. nauplii at a density of 1 nauplii ml^{-1} once daily until day 18 post-hatch.

In contrast to *A. melanopus* and *A. percula, A. polyacanthus* do not have a pelagic larval stage, and start feeding on *Artemia sp*. nauplii immediately after hatching. *A. polyacanthus* were thus transferred to 40L indoor aquaria with flow-through, UV filtered seawater (28.5 ± 0.3°C) on the day of hatching, and were fed *Artemia sp*. nauplii at a density of 1 nauplii ml^{-1} twice daily until 14 days post-hatch. At this point they were fed NRD 0.5 - 0.8 flakes twice daily and were supplied with *Artemia* spp. nauplii once daily until day 18 post-hatch.

2.3.2 *Experimental set-up and protocol*

At 30 days after hatching, juvenile *A. melanopus* and *A. polyacanthus* were randomly assigned to one of five suspended sediment concentrations (i.e., 0, 45, 90, 135 or 180 mgL^{-1}) corresponding to turbidity levels of 0.5 ± 0.5, 7.0 ± 2.7, 14.2 ± 3.0, 21.3 ± 3.4, and 30.1 ± 3.7 NTU, respectively. Juvenile *A. percula* were randomly allocated to one of three suspended sediment concentrations (i.e., 0, 135 or 180 mgL^{-1}) due to the limited number of larvae available. Water temperature was maintained at 28.5 ± 0.5°C for all fish. For each of the three species, four to six replicate aquaria were established for each of the suspended sediment concentrations, and five randomly selected individuals were placed in each aquarium. Fish were maintained in the experimental aquaria for seven days and fed with flakes NRD 0.5 – 0.8 mm (Pro Aqua Pty Ltd) twice daily. Sediment was maintained in suspension using a series of 200 L sumps fitted with two submersible pumps and a grid of airlines, with a single sump for each of the sediment concentrations (Fig. 2.1). Each sump supplied water to six 3L aquaria (however, only 4 aquaria were used per species). Water was delivered to the bottom of each experimental aquarium at a rate of 210 ± 30 mL min^{-1} and drained through an overflow outlet back into sumps (Fig. 2.1). Water temperature was maintained at 28.5 ± 0.5°C using a 1000 W heater (Eheim Gmbh & Co. KG, Deizisau, Germany) in each sump. Sumps were flushed with UV-filtered seawater for 1 hour (>100 % water exchange) daily, after which sediment was added to sumps. Temperature was monitored daily (Thermo Fisher Scientific, QLD, Australia), and turbidity was monitored twice weekly (WP88 turbidity meter, Thermo Fisher Scientific, QLD, Australia) throughout the experiments.

Australian bentonite, a clay with a small particle size range (< 63 µm) and representative of sediments found in suspension on the Great Barrier Reef (Bainbridge et al. 2012), was used as the sediment. The selected suspended sediment concentrations and turbidity levels, and length of exposure to suspended sediments (i.e., 7 days), represent conditions that are currently observed on inshore reefs of the GBR during regular re-suspension events (e.g., waves and currents) and periodic events such as flood plumes (Bainbridge et al. 2012, Wolanski et al. 2008, Wenger et al. 2016).

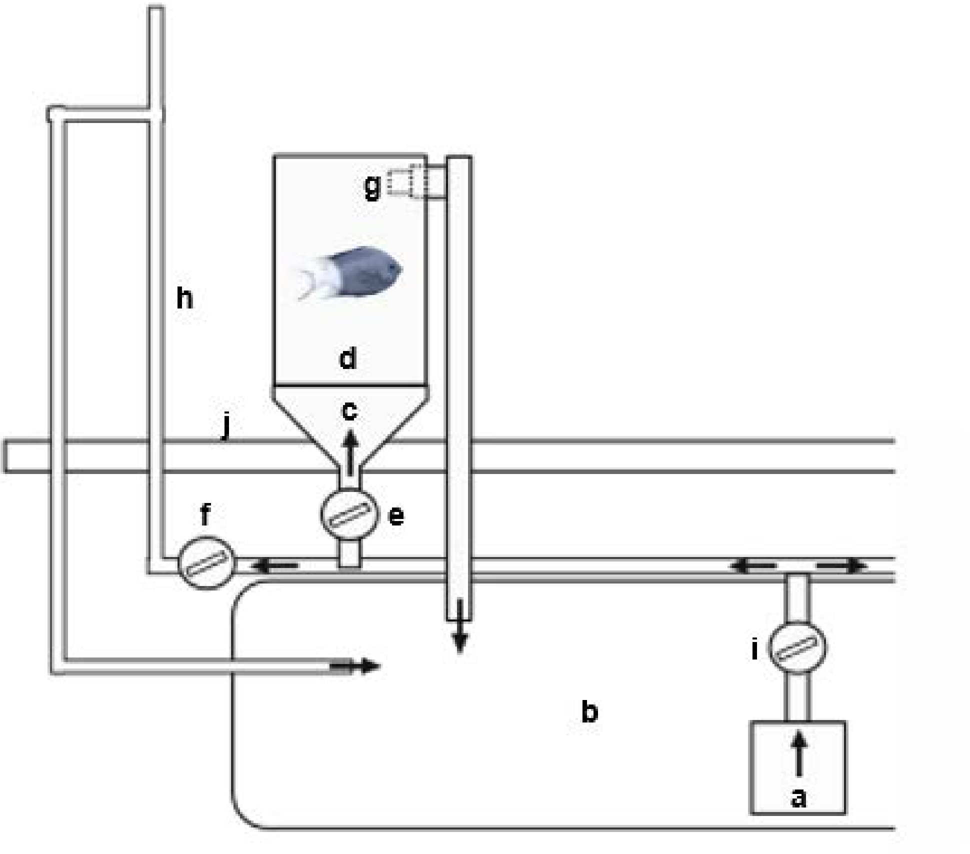

Figure 2.1. Schematic of the experimental set-up used to suspend sediment. A submersible pump **a)** delivered water from the sump **b)** to experimental tanks, which consisted of a plastic funnel **c)** attached to a piece of PVC pipe (14 cm outer diameter, 30 cm height) **d).** Water flow inside tanks was regulated by individual valves **e),** and was further regulated via a bypass **f).** Water drained from tanks back to sumps via an overflow outlet **g).** A stand-pipe **h)** and a one-way valve **i)** prevented the draining of tanks when the main pump was switched off. Tanks were stabilized because they rested in holes drilled into a table **j).** Sediments were re-suspended inside sumps via re-suspension pumps and a set of airstones (not pictured). Water temperature was controlled using a heater submerged in the sump.

2.3.3 *Gill histology*

Following respirometry trials to measure oxygen consumption rates (see below), twelve individuals (out of 20 exposed individuals) for each species and sediment treatment were randomly selected for histological analyses. These fish were euthanized in an ice-water slurry, fixed in Bouin's solution for 24 - 48 hours, and then transferred to 70% ethanol. Fish were then serially dehydrated (Shandon Southern Duplex Processor BS5), embedded in paraffin wax blocks (Shandon Histocentre 3, Thermo Electron Corporation), and sectioned (5 μm thick) longitudinally with a microtome. Sections (5 μm thick) were stained with Mayer's Haematoxylin and Eosin to allow the primary gill filaments, secondary lamellae, gill epithelium, and supporting pillar cell system to be distinguished visually. Lamellae were photographed (Olympus DP12 Microscope Digital Camera System) at 400x magnification,

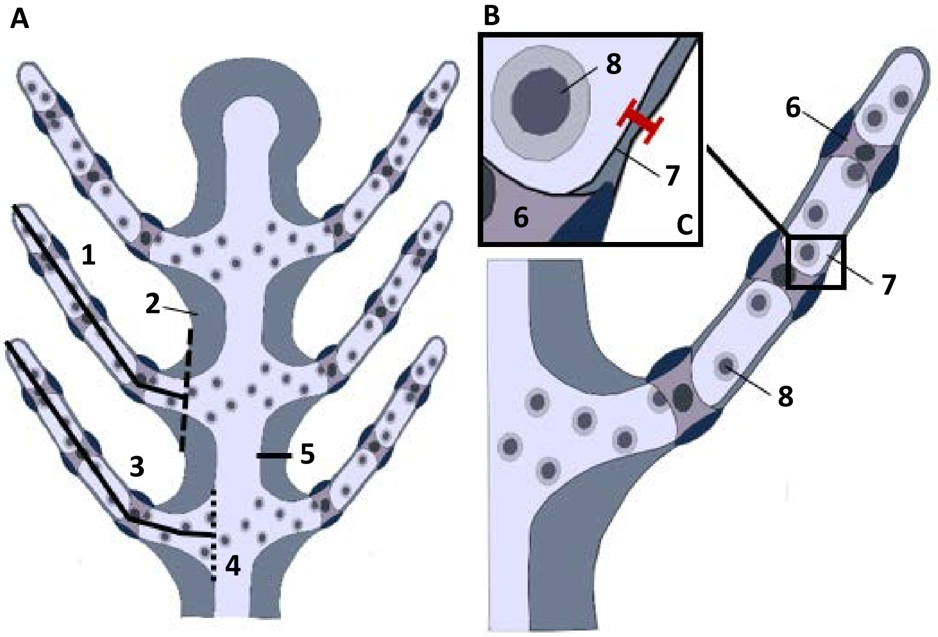

Figure 2.2 Schematic of a cross-section of fish gills. **A** Gill filament and protruding secondary lamellae; **1)** functional lamellar length (solid line) denotes the part of the lamellum above the edge of the **2)** filament epithelium (broken line); **3)** total lamellar length (solid line) is defined as the length of the lamellum between the lamellar tip and the edge of the **4)** blood sinus (dotted line); **5)** filament thickness. **B** Gill lamellum, **6)** pillar cell supporting the **7)** gill/lamellar epithelium, **8)** blood cell. **C** Close-up of the lamellar epithelium. The red line indicates the oxygen diffusion distance, and **6)** pillar cell, **7)** lamellar epithelium, and **8)** blood cell.

and 15 randomly selected lamellae per fish were analyzed in ImageJ (Version 1.48, National Institute of Health, USA).

Morphological features of the lamellae (i.e., total and functional lamellar length, filament thickness, and thickness of the lamellar epithelium/oxygen diffusion distance) were measured following (Hess et al. 2015; Fig. 2.2 A-C). In brief, total length of lamellae was measured from the tip of the lamellae to the base (including the filament epithelium) and functional length from the tip to the edge of the filament epithelium (Fig. 2.2 A). The thickness of the filament epithelium was measured in between two lamellae (Fig. 2.2 A). The total area of the lamellae and the area of the pillar cell system of the functional lamellar length was measured, and the difference between the two was divided by twice the functional length to determine oxygen diffusion distance (Fig. 2.2 C). Note that the oxygen diffusion distance is different from the thickness of the lamellar epithelium, as it includes non-tissue space caused by epithelial lifting (i.e., detachment of the epithelium from the pillar cell system, Mallatt 1985). All filaments were analyzed blindly with respect to treatments.

2.3.4 *Oxygen consumption rates*

Oxygen consumption rates ($\dot{M}O_2$) of individuals were determined using intermittent-flow respirometry (Steffensen 1989). Prior to respirometry, each fish was fasted for 24 hours to ensure a post-absorptive state (Niimi and Beamish 1974). Then, each individual fish was manually chased in a circular container (60 cm diameter, 10 cm water depth) until exhaustion (Roche et al. 2013). Individuals were deemed exhausted when they no longer displayed burst swimming, which always occurred within 2 - 3 min. This method assumes that maximum oxygen uptake rates are achieved while fish recover from exhaustive anaerobic exercise (Reidy et al. 1995). Upon exhaustion, fish were placed immediately into glass respirometry chambers (40 mm length, 18 mm inner diameter; 15.7 ml total volume including tubing) that were sealed with Perspex caps and immersed in a temperature-controlled (28.5 ± 0.3°C) water bath. Respirometry chambers were covered by black sleeves to reduce visual disturbance of fish. Water oxygen levels (in mgO_2) in each chamber were measured every 2 seconds (0.5 Hz) over the 3-hour experimental period with a FireSting Oxygen Optical Oxygen Meter connected to fibre-optic sensors focused on 2 mm^2 sensor spots of REDFLASH green dye affixed on the inside of the recirculation loops of each chamber. Initially when oxygen levels decreased to 90% air saturation, flush pumps (Loligo Systems, Denmark) supplied each chamber with aerated, UV-filtered seawater from the water bath.

Thereafter, flush pumps would switch on every 10 min for 2 min, as controlled by a relay timer. Each chamber was also connected to its own recirculation pump and gas impermeable tubing to ensure homogenous oxygen tension throughout the chamber. The water flow rates (20 ml min^{-1}) for both recirculating and flush pumps were controlled using a variable-voltage power source (Loligo Systems, Denmark). Background microbial respiration of each chamber was measured before and after each trial.

Fish were left in respirometry chambers to recover for 3 hours while oxygen levels were continuously recorded. While adult fish are usually monitored over 24 hours (Clark et al. 2013), smaller fish recover much faster from exhaustive exercise and are commonly measured for 2 - 3 hours only to minimize stress and risk of starvation (e.g. Killen et al. 2014, McLeod et al. 2013). After the trial, fish were euthanized in an ice-water slurry, blotted dry, and fish standard length (to the nearest mm) and mass (to the nearest 0.001 g) were recorded. Fish length and mass (mean ± s.e.), respectively, were as follows: *A. melanopus*, 13.5 ± 0.3 mm and 98 ± 6 mg; *A. polyacanthus*, 17.6 ± 0.3 mm and 174 ± 8 mg; *A. percula*, 19.1 ± 0.3 mm and 153 ± 6 mg. Sampling size per treatment was as follows: For *A. melanopus*, 0 mgL^{-1}: 21 individuals, 45 mgL^{-1}: 11, 90 mgL^{-1}: 12, 135 mgL^{-1}: 13, 180 mgL^{-1}: 15. For *A. polyacanthus,* 0 mgL^{-1}: 30 individuals, 45 mgL^{-1}: 22, 90 mgL^{-1}: 20, 135 mgL^{-1}: 12, 180 mgL^{-1}: 12, and for *A. percula,* 0 mgL^{-1}: 12 individuals, 135 mgL^{-1}: 10, 180 mgL^{-1}: 11.

Oxygen consumption rates (mg O_2 h^{-1}) during closed respirometry phases (i.e., non-flushing) were calculated using linear least square regression in Labchart v. 6.1.3 (ADinstruments, Dunedin, New Zealand). The R^2 values of slopes were calculated in Excel and were always greater than 95%. Slopes with R^2 values of less than 95% were discarded. Background microbial respiration was subtracted from total respiration in chambers to derive oxygen consumption rates of fish (Clark et al. 2013). The highest value of oxygen consumption rates (30s intervals) after exercise was taken as maximum oxygen consumption rate and usually occurred during the first measurement cycle. Resting oxygen consumption rate was estimated as the mean of the lowest 10% of all values, excluding outliers below or above 2 standard deviations (Clark et al. 2013). Aerobic scope was calculated as the difference between maximum and resting oxygen consumption rate.

2.3.5 *Statistical analysis*

All analyses were performed in R (v. 3.3.2, R Core Team 2013). Linear and generalized linear mixed models (lme4 package, Bates et al. 2015) were used to analyze the effects of suspended sediment concentrations on gill morphology. Total and functional lamellar length, and oxygen diffusion distance were used as dependent variables, sediment treatment as a fixed effect and standard length of fish as covariate, allowing for interactions between treatments and standard length. Standard length was mean-centered to help with the interpretation of model intercepts. Fish identity was included as random factor to account for repeated measurements per fish. Assumptions of normality and homogeneity of residuals were visually assessed with Q-Q plots and frequency distributions. Parameters were estimated using Restricted Maximum Likelihood, and *P*-values were generated using Kenward-Rogers approximation (Bolker 2008). If residuals of models did not meet assumptions, generalized linear mixed models were used with a gamma distribution and log-link function. Residuals were assessed for homogeneity, and checked for overdispersion. Parameters were estimated using Laplace approximation, and *P*-values were generated using Wald Z test (Bolker 2008). Interactions between the fixed factor and the covariate were dropped when not significant. The effects of suspended sediments on oxygen consumption rates were analyzed using linear or generalized linear models as described above. Maximum and resting oxygen consumption rates, and aerobic scope (in mg O_2 h^{-1}) were dependent variables, sediment treatment was a fixed effect, and body mass a covariate. Clutch identity was included as a random effect with random intercepts.

To display results visually, intercepts and standard errors produced by the model (i.e. after correcting for fish size or mass) were plotted for each treatment. Parameters on log-scales were transformed to facilitate visual interpretation.

2.4 Results

2.4.1 *Gill morphology*

Exposure to suspended sediments significantly altered the gill structure of all three species investigated, with the extent of the changes varying among species and suspended sediment concentrations. Total lamellar length and functional length of the lamellae of both *A. melanopus* and *A. polyacanthus* were shorter following exposure to sediments, while no changes were observed for *A. percula*. *For A. melanopus,* the functional lamellae length was on average between 20.5% and 29.6% shorter exposed to any of the four suspended sediment concentrations when compared to gills from control fish (45 mgL^{-1}: t = -2.02, p = 0.0436, 90 mgL^{-1}: t =-2.25, p = 0.0245, 135 mgL^{-1}: t = 3.62, p = 0.0002, 180 mgL^{-1}: t = -3.04, p = 0.002, Fig. 2.3 A, Table 2.1). For *A. polyacanthus*, functional lamellar length was on average between 21.7% and 30.6% shorter upon exposure to three of four suspended sediment concentrations when compared to control fish (45 mgL^{-1}: t = -3.26, p = 0.0011, 135 mgL^{-1}: t = -3.07, p = 0.0021, 180 mgL^{-1}: t = -2.20, p = 0.027, Fig. 2.3 A, Table 2.2).

All three species exhibited significant reductions in oxygen diffusion distances upon suspended sediment exposure (Fig. 2.3 B, Tables 2.1-2.3). In *A. melanopus*, oxygen diffusion distance was reduced by 24.1% and 28.8%, respectively, in fish exposed to the two highest suspended sediment concentrations (135 mgL^{-1}: t = -1.96, p = 0.049, 180 mgL^{-1}: t = -2.28, p = 0.02, Fig. 2.3 B, Table 2.1). Similarly, oxygen diffusion distance was between 24.9% and 28.8% shorter in *A. polyacanthus* exposed to three of four suspended sediment concentrations when compared to control fish (45 mgL^{-1}: t = -2.68, p = 0.007, 135 mgL^{-1}: t = -2.69, p = 0.007, 180 mgL^{-1}: t = -2.20, p = 0.02, Fig. 2.3 B, Table 2.2). In *A. percula,* reductions in oxygen diffusion distance by as much as 18.3% were evident in fish from both examined suspended sediment treatments (135 mgL^{-1}: t = -2.12, p = 0.04, 180 mgL^{-1}: t = -3.25, p = 0.003, Fig. 2.3 B, Table 2.3).

For the three species investigated, changes to filament thickness were highly variable, increasing in some (e.g., *A. melanopus* exposed to 45 mgL^{-1}), decreasing in others (e.g., *A. polyacanthus* exposed to 45 mgL^{-1}), and showing no change in the majority of instances (Tables 2.1-2.3).

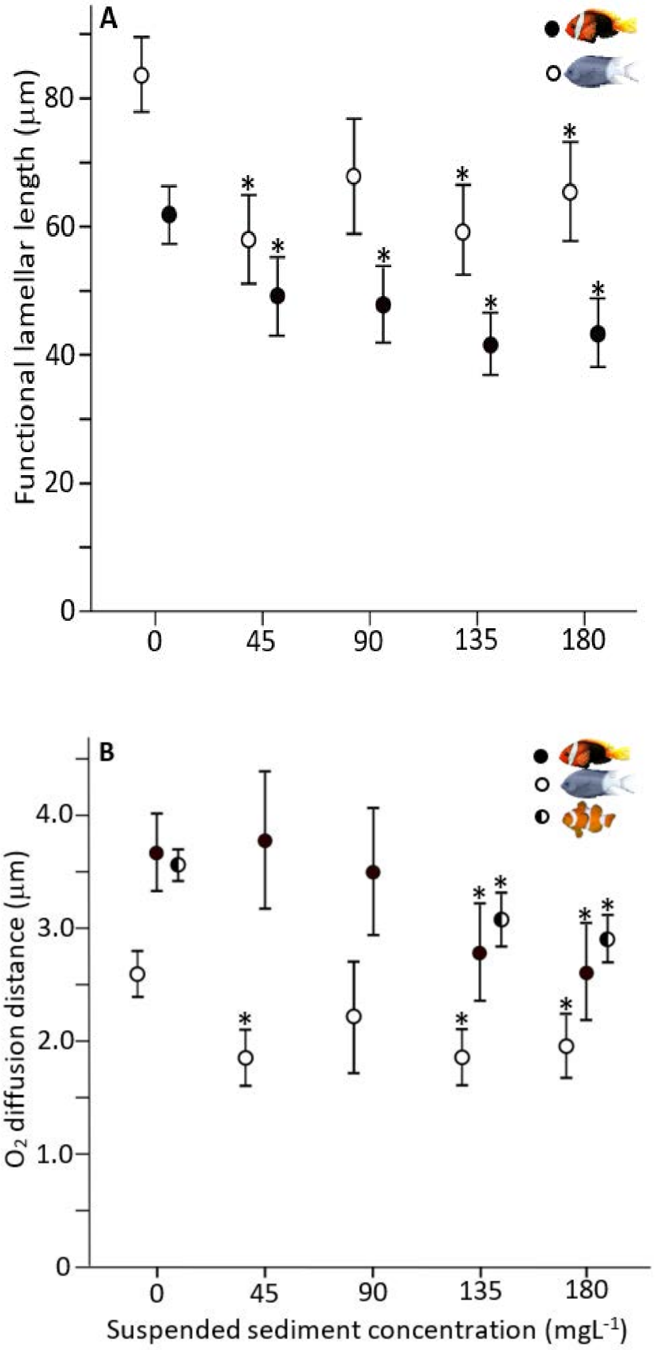

Figure 2.3 Effects of suspended sediments on the gill morphology of three coral reef fishes. **A)** functional lamellar length and **B)** oxygen diffusion distance in *A. melanopus* (black circles), *A. polyacanthus* (white circles), and *A. percula* (black and white circles). Circles and bars represent intercepts and standard errors, respectively, as estimated by general- and linear mixed models after accounting for fish length. Asterisks indicate significant differences when compared to the control group at α < 0.05.

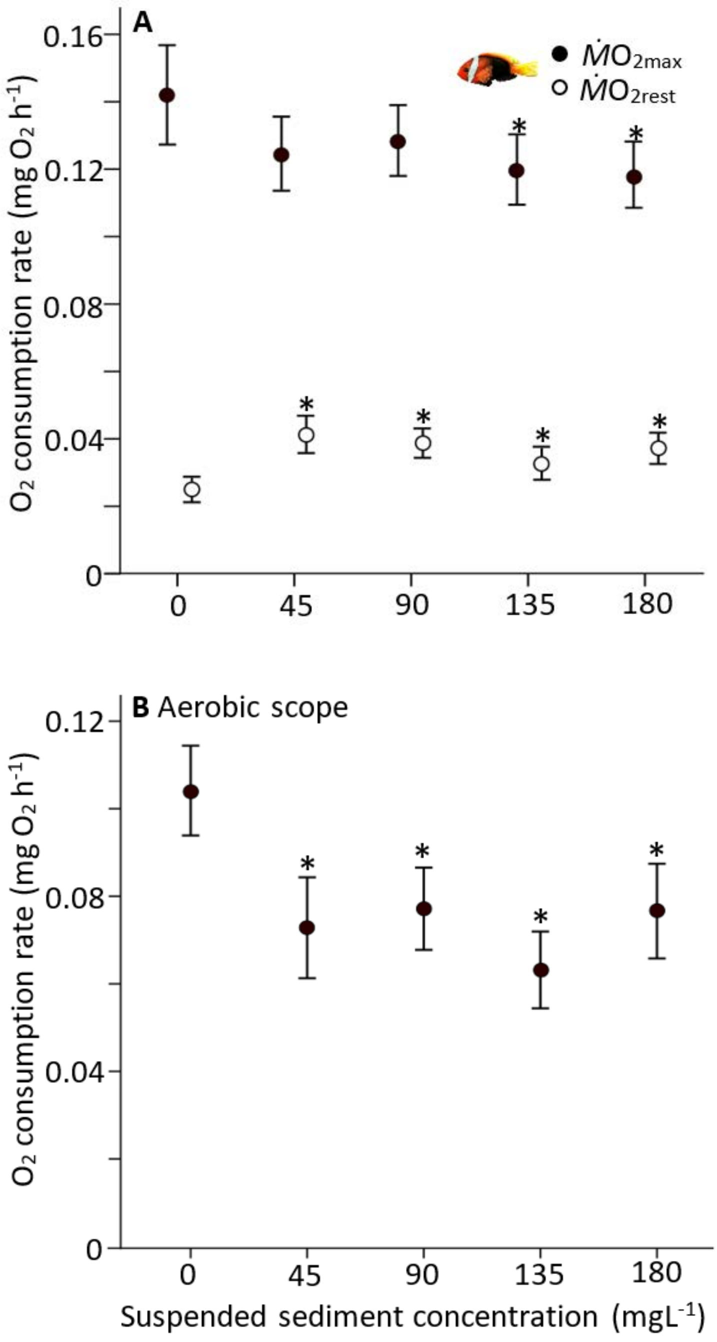

Figure 2.4 Effects of suspended sediments on oxygen consumption of *A. melanopus*. **A** Maximum oxygen consumption rates ($\dot{M}O_{2max}$, black circles) and resting oxygen consumption rates ($\dot{M}O_{2rest}$, white circles), and **B** aerobic scope. Circles and bars represent intercepts and standard errors, respectively, as estimated by general- and linear mixed models after accounting for fish mass. Asterisks indicate significant differences when compared to the control group at $\alpha < 0.05$.

Table 2.1 Generalized and linear mixed models to estimate the effects of suspended sediments on the gill structure of *A. melanopus*. Estimates and standard errors (SE) represent the effects of treatments on a fish of mean length relative to the control group (i.e., "intercept (0 mgL^{-1})") after accounting for fish length (fish length was mean-centred to facilitate interpretation of intercepts). Note that parameters of models using a log-link are on a logarithmic scale. Bold p-values indicate significant factors (α < 0.05).

Response variables	**Factors**	**Estimates**	**SE**	***t***	***p***
Total lamellar length	Intercept (0 mgL^{-1})	4.3108	0.0666	64.64	<0.0001
(log link)	45 mgL^{-1}	-0.1327	0.1098	-1.21	0.2269
	90 mgL^{-1}	-0.1801	0.1096	-1.64	0.1004
	135 mgL^{-1}	-0.3358	0.1044	-3.21	**0.0013**
	180 mgL^{-1}	-0.3427	0.1121	-3.06	**0.0022**
	standard length	0.0316	0.0174	1.82	0.0688
Functional lamellar length	Intercept (0 mgL^{-1})	4.1248	0.0691	59.64	<0.0001
(log link)	**45 mgL^{-1}**	-0.2299	0.1139	-2.02	**0.0436**
	90 mgL^{-1}	-0.2558	0.1137	-2.25	**0.0245**
	135 mgL^{-1}	-0.3926	0.1084	-3.62	**0.0002**
	180 mgL^{-1}	-0.3510	0.1154	-3.04	**0.0023**
	standard length	0.0454	0.0180	2.52	**0.0116**
Filament thickness	Intercept (0 mgL^{-1})	12.2890	0.8327	14.563	<0.0001
	45 mgL^{-1}	3.6163	1.3683	2.439	**0.0193**
	90 mgL^{-1}	1.8416	1.3644	1.202	0.2383
	135 mgL^{-1}	-0.6944	1.3086	-0.515	0.6089
	180 mgL^{-1}	-2.7750	1.3554	-1.960	0.0561
	standard length	-0.3166	0.2161	-1.374	0.1818
Gas diffusion distance	Intercept (0 mgL^{-1})	1.3009	0.0893	14.555	<0.0001
(log link)	45 mgL^{-1}	0.0291	0.1468	0.199	0.8426
	90 mgL^{-1}	-0.0482	0.1466	-0.329	0.7422
	135 mgL^{-1}	-0.2761	0.1407	-1.963	**0.0497**
	180 mgL^{-1}	-0.3400	0.1487	-2.287	**0.0222**
	standard length	-0.0030	0.0231	-0.130	0.8968

Table 2.2 Generalized and linear mixed models to estimate the effects of suspended sediments on the gill structure of *A. polyacanthus*. Estimates and standard errors (SE) represent the effects of treatments on a fish of mean length relative to the control group (i.e. “intercept (0 mgL^{-1})”) after accounting for fish length (fish length was mean-centred to facilitate interpretation of intercepts). Note that parameters of models using a log-link are on a logarithmic scale. Bold p-values indicate significant factors ($\alpha < 0.05$).

Response variable	**Factors**	**Estimates**	**SE**	***t***	***p***
Total lamellar length	Intercept (0 mgL^{-1})	4.5644	0.0662	68.94	<0.0001
(log link)	**45 mgL^{-1}**	-0.3570	0.1109	-3.22	**0.0012**
	90 mgL^{-1}	-0.2089	0.1225	-1.71	0.0880
	135 mgL^{-1}	-0.3287	0.1099	-2.99	**0.0027**
	180 mgL^{-1}	-0.2554	0.1099	-2.32	**0.0201**
	standard length	0.0443	0.0124	3.56	**0.0003**
Functional lamellar length	Intercept (0 mgL^{-1})	4.4277	0.0672	65.88	<0.0001
(log link)	**45 mgL^{-1}**	-0.3667	0.1125	-3.26	**0.0011**
	90 mgL^{-1}	-0.2102	0.1243	-1.69	0.0907
	135 mgL^{-1}	-0.3416	0.1113	-3.07	**0.0021**
	180 mgL^{-1}	-0.2456	0.1115	-2.20	**0.0276**
	standard length	0.0505	0.0126	4.01	**<0.0001**
Filament thickness	Intercept (0 mgL^{-1})	2.4479	0.0741	33.02	<0.0001
(log link)	**45 mgL^{-1}**	-0.2689	0.1240	-2.17	**0.0302**
	90 mgL^{-1}	-0.1982	0.1374	-1.44	0.1493
	135 mgL^{-1}	-0.1951	0.1232	-1.58	0.1133
	180 mgL^{-1}	-0.2586	0.1231	-2.10	**0.0357**
	standard length	-0.0072	0.0139	-0.52	0.6043
Gas diffusion distance	Intercept (0 mgL^{-1})	0.9574	0.0756	12.66	<0.0001
(log link)	**45 mgL^{-1}**	-0.3409	0.1269	-2.68	**0.0072**
	90 mgL^{-1}	-0.1633	0.2011	-0.81	0.4168
	135 mgL^{-1}	-0.3391	0.1260	-2.69	**0.0071**
	180 mgL^{-1}	-0.2863	0.1299	-2.20	**0.0275**
	standard length	0.0051	0.0224	0.22	0.8192
	45*standard length	0.1285	0.0549	2.33	**0.0193**
	90*standard length	0.0333	0.0576	0.57	0.5631
	135*standard length	-0.0219	0.0339	-0.64	0.5179
	180*standard length	0.0156	0.0410	0.38	0.7024

Table 2.3 Generalized and linear mixed models to estimate the effects of suspended sediments on the gill structure of *A. percula*. Estimates and standard errors (SE) represent the effects of treatments on a fish of mean length relative to the control group (i.e. "intercept (0 mgL^{-1})") after accounting for fish length (fish length was mean-centred to facilitate interpretation of intercepts). Note that parameters of models using a log-link are on a logarithmic scale. Bold p-values indicate significant factors ($\alpha < 0.05$).

Response variable	**Factors**	**Estimate**	**SE**	***t***	***p***
Total lamellar length	Intercept (0 mgL^{-1})	60.0224	2.0530	29.236	<0.0001
	135 mgL^{-1}	-0.2384	3.3375	-0.071	0.9437
	180 mgL^{-1}	0.3032	2.9377	0.103	0.9187
	standard length	2.3903	0.8530	2.919	**0.0079**
Functional lamellar length	Intercept (0 mgL^{-1})	50.7906	2.1674	23.434	<0.0001
	135 mgL^{-1}	-0.8723	3.5247	-0.247	0.8068
	180 mgL^{-1}	-0.9577	3.1023	-0.309	0.7605
	standard length	2.3740	0.9010	2.635	**0.0151**
Filament thickness	Intercept (0 mgL^{-1})	9.3039	0.4388	21.203	<0.0001
	135 mgL^{-1}	0.2608	0.7142	0.365	0.7184
	180 mgL^{-1}	1.1850	0.6276	1.888	0.0723
	standard length	0.1254	0.1823	0.688	0.4987
Gas diffusion distance	Intercept (0 mgL^{-1})	3.5577	0.1402	25.372	<0.0001
	135 mgL^{-1}	-0.4843	0.2281	-2.123	**0.04522**
	180 mgL^{-1}	-0.6520	0.2006	-3.250	**0.00368**
	standard length	0.1276	0.0582	2.189	**0.03951**

2.4.2 *Oxygen uptake rates*

Despite the observed changes in gill morphology in all three species of fish exposed to suspended sediments, oxygen consumption rates were only affected in one of the three species (*A. melanopus*). Specifically, maximum oxygen consumption rates were reduced by as much as 17.5% following exposure to the two highest suspended sediment concentrations relative to control fish (135 mgL^{-1}: $t = -2.39$, $p = 0.016$, 180 mgL^{-1}: $t = -2.01$, $p = 0.044$), but no changes were evident in fish exposed to 45 or 90 mgL^{-1} (Fig. 2.4 A, Table 2.4). Resting oxygen consumption rates in *A. melanopus* were elevated on average by 36.3% to 64.3% for three of the four suspended sediment concentrations when compared to control fish (45 mgL^{-1}: $t = 2.92$, $p = 0.004$, 90 mgL^{-1}: $t = 3.00$, $p = 0.003$, 180 mgL^{-1}: $t = 2.61$, $p = 0.011$, Fig. 2.4 A, Table 2.4). Reflecting changes in maximum and resting oxygen consumption rates, aerobic scope was reduced by as much as 39.3% in fish upon exposure to all suspended sediment concentrations when compared to control fish (45 mgL^{-1}: $t = -2.42$, $p = 0.015$, 90 mgL^{-1}: $t = -2.60$, $p = 0.009$, 135 mgL^{-1}: $t = -3.85$, $p = 0.001$, 180 mgL^{-1}: $t = -2.31$, $p = 0.020$, Fig. 2.4 B, Table 2.4). No effects on maximum or resting oxygen consumption rates or aerobic scope were detected for *A. polyacanthus* or *A. percula* exposed to suspended sediments (Tables 2.4 and 2.5).

To further explore the connection between oxygen consumption rates and changes in gill morphology, I analyzed the relationship between aerobic scope and the squared mean functional lamellar length of *A. melanopus* exposed to 0 and 180 mgL^{-1} suspended sediments, respectively, using simple linear regression. Square transformed values were used because aerobic scope has been shown to correlate linearly with gill surface area [62]. While there was a significant correlation between aerobic scope and squared mean functional lamellar length in *A. melanopus* juveniles exposed to control conditions ($F(1, 13) = 6.82$, $R^2 = 0.29$, $p = 0.02$), these two variables were not significantly correlated in juveniles exposed to 180 mgL^{-1} suspended sediments ($F(1,8) = 0.03$, $R^2 = -0.12$, $p = 0.85$).

Table 2.4 Generalized and linear mixed models to estimate the effects of suspended sediments on oxygen consumption rates of *A. melanopus*. Estimates and standard errors represent the effects of treatments on a fish of mean weight relative to the control group (i.e. "intercept (0 mgL^{-1})") after accounting for fish mass (fish mass was mean-centred to facilitate interpretation of intercepts). Note that parameters of models using a log-link are on a logarithmic scale. Bold p-values indicate significant factors ($\alpha < 0.05$).

Response variables	**Factors**	**Estimates**	**SE**	***t***	***p***
$\dot{M}O_{2max}$	Intercept (0 mgL^{-1})	-2.0580	0.0679	-30.279	<0.0001
(log link)	45 mgL^{-1}	-0.1019	0.0808	-1.262	0.207
	90 mgL^{-1}	-0.1229	0.0746	-1.647	0.099
	135 mgL^{-1}	-0.1922	0.0802	-2.397	**0.016**
	180 mgL^{-1}	-0.1621	0.0805	-2.014	**0.044**
	fish mass	8.1109	1.1197	7.243	**<0.0001**
$\dot{M}O_{2rest}$	Intercept (0 mgL^{-1})	0.0249	0.0035	7.003	<0.0001
	45 mgL^{-1}	0.0163	0.0053	2.928	**0.004**
	90 mgL^{-1}	0.0128	0.0042	3.004	**0.003**
	135 mgL^{-1}	0.0078	0.0046	1.639	0.106
	180 mgL^{-1}	0.0122	0.0045	2.613	**0.011**
	fish mass	0.4106	0.0850	4.579	**<0.0001**
	45*fish mass	0.0936	0.1697	0.539	0.592
	90*fish mass	0.0794	0.1214	0.636	0.527
	135*fish mass	-0.4176	0.1093	-3.693	**0.0005**
	180*fish mass	-0.0912	0.1137	-0.779	0.4394
Aerobic scope	Intercept (0 mgL^{-1})	-2.2623	0.0940	-24.06	<0.0001
(log link)	**45 mgL^{-1}**	-0.3582	0.1476	-2.42	**0.0152**
	90 mgL^{-1}	-0.3006	0.1155	-2.60	**0.0092**
	135 mgL^{-1}	-0.5008	0.1300	-3.85	**0.0001**
	180 mgL^{-1}	-0.3070	0.1324	-2.31	**0.0204**
	fish mass	6.8899	2.6766	2.57	**0.0100**
	45*fish mass	-3.7202	4.7614	-0.78	0.4346
	90*fish mass	4.2197	3.5235	1.19	0.2310
	135*fish mass	6.7437	3.1108	2.16	**0.0301**
	180*fish mass	-2.8901	3.1587	-0.91	0.3602

Table 2.5 Generalized and linear mixed models to estimate the effects of suspended sediments on oxygen consumption rates of *A. polyacanthus*. Estimates and standard errors (SE) represent the effects of treatments on a fish of mean weight relative to the control group (i.e. "intercept (0 mgL^{-1})") after accounting for fish mass (mass was mean-centred to facilitate interpretation of intercepts). Note that parameters of models using a log-link are on a logarithmic scale. Bold p-values indicate significant factors (α < 0.05).

Response variables	**Factors**	**Estimates**	**SE**	***t***	***p***
$\dot{M}O_{2max}$	Intercept (0 mgL^{-1})	-1.5114	0.0667	-22.649	<0.0001
(log link)	45 mgL^{-1}	-0.0722	0.0631	-1.144	0.253
	90 mgL^{-1}	0.0176	0.0650	0.270	0.787
	135 mgL^{-1}	0.0114	0.0995	0.115	0.908
	180 mgL^{-1}	-0.0336	0.0997	-0.337	0.736
	fish mass	4.2656	0.5624	7.584	**<0.0001**
$\dot{M}O_{2rest}$	Intercept (0 mgL^{-1})	-2.9279	0.0898	-32.58	<0.0001
(log link)	45 mgL^{-1}	0.1421	0.1118	1.27	0.204
	90 mgL^{-1}	0.1597	0.1140	1.40	0.161
	135 mgL^{-1}	0.2171	0.1424	1.52	0.127
	180 mgL^{-1}	0.1136	0.1469	0.77	0.439
	fish mass	4.0678	0.6858	5.93	**<0.0001**
Aerobic scope (log link)	Intercept (0 mgL^{-1} SS)	-1.8842	0.0909	-20.709	<0.0001
	45 mgL^{-1} SS	-0.1338	0.1156	-1.158	0.2470
	90 mgL^{-1} SS	-0.0687	0.1181	-0.582	0.5603
	135 mgL^{-1} SS	0.1887	0.1448	1.303	0.1924
	180 mgL^{-1} SS	0.1309	0.1459	0.897	0.3695
	fish mass	2.2117	0.7459	2.965	**0.0030**

Table 2.6 Generalized and linear mixed models to estimate the effects of suspended sediments on oxygen consumption rates of *A. percula*. Estimates and standard errors (SE) represent the effects of treatments on a fish of mean weight relative to the control group (i.e. “intercept (0 mgL^{-1}) after accounting for fish mass (mass was mean-centred to facilitate interpretation of intercepts). Note that parameters of models using a log-link are on a logarithmic scale. Bold p-values indicate significant factors (α < 0.05).

Response variables	**Factors**	**Estimates**	**SE**	***t***	***p***
$\dot{M}O_{2max}$	Intercept (0 mgL^{-1})	-1.7198	0.0913	-18.826	<0.0001
(log link)	135 mgL^{-1}	-0.0433	0.1468	-0.295	0.768
	180 mgL^{-1}	0.0128	0.1414	0.091	0.927
	fish mass	6.3604	1.0489	6.064	**<0.0001**
$\dot{M}O_{2rest}$	Intercept (0 mgL^{-1})	0.0569	0.0070	8.097	<0.0001
	135 mgL^{-1}	-0.0056	0.0108	-0.519	0.366
	180 mgL^{-1}	-0.0097	0.0105	-0.926	0.307
	fish mass	0.2586	0.0907	2.850	**0.0047**
Aerobic scope	Intercept (0 mgL^{-1})	-2.0894	0.0950	-21.987	<0.0001
(log link)	135 mgL^{-1}	-0.0275	0.1482	-0.185	0.853
	180 mgL^{-1}	0.0835	0.1417	0.582	0.560
	fish mass	7.0351	1.4263	4.932	**<0.0001**

2.5 Discussion

Changes in the gill structure of fishes exposed to elevated suspended sediment concentrations have been hypothesized to reduce metabolic performance and thereby lead to declines in the diversity and abundance of fish assemblages on turbid reefs (Cumming and Herbert 2016, Hess et al. 2015, Wong et al. 2013). Our results confirm that short-term exposure to suspended sediments led to changes in the gill structure of all three examined species; however, these morphological changes only translated to impaired metabolic performance (i.e., oxygen consumption rates) in one species (*A. melanopus*). These findings imply that species that are sensitive to changes in gill structure such as *A. melanopus* may decline in abundance as reefs become more turbid, while species that are able to maintain metabolic performance despite suspended sediment exposure, such as *A. polyacanthus* or *A. percula*, may be able to persist or gain a competitive advantage. Our results highlight that suspended sediment can have direct physiological consequences for some coral reef fish and provide further insight into how reduced water quality can shape coral reef communities.

The interspecific variation in the effect of suspended sediment exposure on oxygen uptake rates observed in this study may be related to differences in environmental tolerances among species. Following suspended sediment exposure, *A. melanopus* exhibited increases in resting oxygen consumption rates and reductions in both maximum oxygen consumption rates and aerobic scope. In contrast, the congener *A. percula* and confamilial *A. polyacanthus* both maintained oxygen uptake rates despite similar changes in gill structure. The widespread distribution pattern of *A. polyacanthus* spanning both turbid inshore and clear offshore reefs may explain the high tolerance of this species to suspended sediments (Brolund et al. 2004). The differential responses of the closely related *A. percula* and *A. melanopus,* however, is counter to expectations based on their current distributions. While the current distribution of *A. melanopus* includes turbid reefs (Fautin et al. 1992), this species was the most heavily affected by suspended sediments in the present study. In contrast, the species least affected by suspended sediments, *A. percula*, is typically rare in turbid environments (Brolund et al. 2004), and the effects of suspended sediments on gills may hence not influence the distribution of this species. Understanding which species are sensitive versus those that are tolerant to changes in suspended sediment concentrations will be critical for the effective conservation of inshore coral reefs.

Our results document that juvenile *A. melanopus* exposed to elevated suspended sediment concentrations had a reduced capacity for oxygen uptake, as indicated by reductions in maximum oxygen consumption rates (i.e. $\dot{M}O_{2max}$). This may have been the result of gill damage, leading to the observed reductions in lamellar length and thus a reduction in gill surface area available for gas exchange. Aerobic scope was positively correlated with (the squared) functional lamellar length in control fish, supporting the idea that the reduction in $\dot{M}O_{2max}$ may have been caused by gill damage. Interestingly, there was no significant relationship between aerobic scope and functional lamellar length in fish upon suspended sediment exposure. The reasons for this are currently not known, however, it is important to note that *A. melanopus* also showed reductions in oxygen diffusion distances in response to suspended sediments; this would have been likely to enhance oxygen uptake efficiency, i.e. it may have compensated partly for reductions in gill surface area (Lappivaara et al. 1995) and may have weakened the relationship between aerobic scope and functional lamellar length (see also discussion below). An alternative hypothesis for the drivers of the observed reduction in $\dot{M}O_{2max}$ could be that suspended sediment exposure may have elicited a stress response, which may have influenced $\dot{M}O_{2max}$. However, there is limited information available on the effects of stress on $\dot{M}O_{2max}$. Considering the multiple stressors that fish experience on degraded reefs, this would be an interesting avenue for further research. Regardless of the underlying mechanisms, a reduced $\dot{M}O_{2max}$ suggests that oxygen delivery to tissues may be insufficient during activities with elevated oxygen demand, such as during swimming (Metcalfe et al. 2016, Pörtner and Farrell 2008).

Individuals exposed to suspended sediments exhibited increased oxygen consumption at rest (i.e., $\dot{M}O_{2rest}$), which is commonly observed in fish exposed to poor water quality (e.g. Baum et al. 2016, Davison et al. 1992, Reid et al. 2003). This finding indicates that inhabiting areas with elevated suspended sediments incurs an energetic cost. The increase in $\dot{M}O_{2rest}$ may have been the result of a stress response, behavioral and physiological acclimation to suspended sediments, tissue repair at the gills, an enhanced immune response, and/or inefficient oxygen uptake (Beyers et al. 1999, Bonga 1997). Further, the reduction in aerobic scope of *A. melanopus* exposed to suspended sediments suggests a compromised capacity to perform vital aerobic activities, such as growth, development, and locomotion (Claireaux and Lefrançois 2007). A reduction in aerobic scope may be especially problematic in juvenile fish, which exhibit exceptionally high growth rates (Suthers 1998) but low survival rates (McCormick and Hoey 2004). Factors that reduce growth or survival during early life history stages can have strong effects on recruitment patterns (Jones 1990, Schmitt and Holbrook 1999). While *A. melanopus* is common on turbid reefs today (Fautin et al. 1992) our findings suggest that this species could decline in abundance as suspended sediments continue to increase into the future, or may shift to alternative/less suitable habitats, potentially increasing interspecific competition. However, as a habitat specialist, *A. melanopus* relies on the availability of symbiontic anemone species (Fautin et al. 1992), and may thus be especially vulnerable to declines in habitat quality associated with increases in suspended sediments.

In contrast to *A. melanopus*, exposing juvenile *A. percula* to suspended sediments had no detectable effect on the lamellar length and hence, surface area for gas exchange, or oxygen consumption rates. This is counter to expectations, given the current distribution of *A. percula.* The reason why *A. percula*, unlike *A. melanopus* and *A. polyacanthus*, did not exhibit any reductions in lamellar length upon suspended sediment exposure remains unclear. However, *A. percula* juveniles were on average larger in size than *A. melanopus* and *A. polyacanthus*, and may thus potentially have been more tolerant (Wenger et al. 2017). Regardless of the underlying mechanism, these findings highlights the inter-specific variation in response to suspended sediments. Considering the limited impact of suspended sediments on the physiology of *A. percula* in the present study, its absence on turbid reefs is most likely driven by other factors, such as the habitat preferences of its primary host, the sea anemone, *Heteractis magnifica*, which requires clear water (Brolund et al. 2004). However, the present study may have underestimated the effects of suspended sediments on oxygen consumption rates of fish. It is well known that suspended sediments adhere to the mucous layer on the gill epithelium (Heath 1995); it was not possible in the present study to examine whether this may have reduced oxygen uptake, since oxygen consumption rates were determined in the absence of suspended sediments. It will be important for future studies to examine whether the presence of suspended sediments may affect oxygen uptake, and whether this may compound the negative effects of gill changes induced by suspended sediments.

Following exposure to elevated suspended sediments, *A. polyacanthus* exhibited similar changes in gill structure (i.e., reduced functional lamellar length) to *A. melanopus*; however, these changes did not translate to any negative effects on metabolic performance, and the reasons behind this are currently unclear. As mentioned above, it is possible that the observed reductions in oxygen diffusion distances (i.e., a thinner gill epithelium) may have compensated for declines in oxygen uptake due to reductions in surface area. Further, oxygen uptake capacity is also influenced by factors other than gill structure, including haemoglobin oxygen carrying capacity and affinity, heart rate, stroke volume, and cardiac output (Servizi and Martens 1987); some or all of these traits may have also been modified to enhance oxygen transport. It is also possible that the capacity for oxygen uptake at the gills in this species may be much higher than either the capacity to deliver oxygen to tissues or the maximum oxygen demand of tissues (Goolish 1995, Nilsson 2007). Not all species perfuse all of the available lamellae, and thus do not use their gill surface area to its full potential (Bowden et al. 2014, Randall and Daxboeck 1984). This may allow them to tolerate some gill damage without adversely affecting oxygen uptake (Cumming and Herbert 2016). While the underlying mechanisms are not fully resolved, it is likely that the ability to tolerate or compensate for changes in gill structure may allow *A. polyacanthus* to persist on reefs that become increasingly more turbid, which could provide an important competitive advantage to this species.

The underlying mechanisms that led to a reduction in oxygen diffusion distances upon suspended sediment exposure in all three species are not clear. The onset and intensity of gill changes in response to suspended sediments varies considerably between species, life-stages, angularity of sediment particles, sediment concentration, and exposure duration (Newcombe and MacDonald 1991, Wenger et al. 2017) The reported structural changes induced by suspended sediments, however, are nonspecific (Heath 1995, Mallatt 1985), and the majority of studies examining the effects of prolonged exposure to suspended sediments (> 3 days) have documented the growth of protective cell layers, i.e. an increase - rather than a decrease - in oxygen diffusion distances (e.g. Cumming and Herbert 2016, Sutherland and Meyer 2007). For example, an increase in oxygen diffusion distances was observed in a previous study on settlement-stage *A. percula* exposed to the same sediment type, exposure duration, and concentration (yet at an earlier developmental stage) as used in the present study (Hess et al. 2015). The only study that has reported a reduction in oxygen diffusion distances in response to prolonged suspended sediment exposure (6 weeks) examined this in green grouper (*Epinephelus coioides*), a coral reef fish living on turbid inshore reefs (Au et al. 2004). The observed reductions in oxygen diffusion distances in green grouper and the three coral reef fishes examined in the present study may have been caused by tissue abrasion (Au et al. 2004). However, when exposed to hypoxia (Heath 1995, Sollid et al. 2003), elevated temperatures (Sollid and Nillson 2006), air (LeBlanc et al. 2010, Ong et al. 2007) and even some pollutants (Berry et al., *in prep.*), some fish species are known to actively alter oxygen diffusion distances to regulate oxygen uptake. As suggested by (Au et al. 2004), the observed reductions in oxygen diffusion distances may have thus been the result of a re-arrangement of cell layers to enhance oxygen uptake, rather than gill damage (see also Nilsson 2007, Nilsson et al. 2012). Regardless of the underlying mechanisms, reductions in oxygen diffusion distances may enhance the susceptibility of fish to parasites and pathogens and may interfere with ion- and osmoregulation (Nilsson et al. 2012).

Up to half of the world's coral reefs are threatened by poor water quality (Burke et al. 2011), with continued and rapid expansions of coastal human populations likely to exacerbate the inputs and resuspension of terrestrial sediments (Halpern et al. 2015, Lutz and Samir 2010). Identifying the mechanisms that drive changes in coral reef fish assemblages in response to human impacts is of key interest for the conservation of coral reefs (McKenzie et al. 2016, Illing and Rummer 2017). Our findings suggest that *A. melanopus*, a species frequently found on turbid reefs today (Brolund et al. 2004), may decline in abundance or disappear from turbid reefs as suspended sediments increase, due to the impacts of suspended sediments on the oxygen uptake rates of juveniles. Other species, such as *A. polyacanthus* or *A. percula*, may be insensitive to short-term exposure to suspended sediments and may remain unaffected or even gain a competitive advantage under certain conditions. However, while juvenile *A. polyacanthus* and *A. percula* did not suffer any reductions in metabolic performance upon suspended sediment exposure, the observed changes in gill structure may potentially compromise their capacity to cope with other environmental factors that affect oxygen demand or oxygen availability, such as increasing water temperatures, ocean acidification or falling oxygen levels caused by climate change (Altieri and Gedan 2015). The combined effects of suspended sediments, elevated temperature and hypoxia on gill structure and function of reef fish will thus be important to consider in future studies. The present study shows that investigating the effects of suspended sediments on physiological processes plays an important role in the identification of winners and losers under future suspended sediment conditions and can provide crucial information for the conservation of coral reefs.

Chapter 3: Enhanced fast-start performance and anti-predator behaviour in a coral reef fish in response to suspended sediment exposure

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3.1 Abstract

Declining water quality, in particular elevated suspended sediments, pose a significant threat to coastal coral reefs. Juvenile anemonefish (*Amphiprion melanopus*) were exposed to two suspended sediment concentrations (0 or 180 mgL^{-1}) for 7 days and examined their predator escape performance and anti-predator behaviour in both clear water and suspended sediments (0 and 180 mgL^{-1}, i.e., acute exposure). After 7-day exposure to suspended sediments, fish responded faster to a mechanical stimulus and exhibited enhanced fast starts compared to individuals reared in clear water, regardless of acute exposure. Fish were also less active and avoided open areas when exposed to elevated suspended sediments in the test arena when compared to clear water, irrespective of prior 7-day exposure. While these changes are likely strategies to compensate for an increased perceived predation risk in suspended sediments, they may also be associated with non-consumptive costs for juveniles living on turbid reefs.

3.2 Introduction

Suspended sediment concentrations in tropical coastal waters have increased substantially over the past few decades as a result of human activities including agriculture, dredging, and shipping (Syvitski et al. 2005) and are altering the composition of biological communities (Fabricius et al. 2005). Elevated levels of suspended sediments can influence coral reef fish assemblages indirectly through changes in the composition and structural complexity of benthic habitats (Brown et al. 2017; Hamilton et al. 2017). Recent studies, however, suggest that suspended sediments may also affect the physiology and behaviour of reef fishes, which may compound the negative effects of habitat degradation (reviewed in Wenger et al. 2017).

Elevated suspended sediments reduce visual acuity and can thereby influence important ecological processes such as predator-prey interactions (Wenger et al. 2017). Reduced visual acuity can delay the detection of approaching predators, potentially altering predation rates (Meager et al. 2006). While the early detection of predators is crucial, the ability to escape a predator attack is equally important for prey survival (McCormick et al. 2018; Walker et al. 2005). Most prey fish escape predatory attacks via fast starts, i.e., short, high-energy swimming bursts elicited by a sudden stimulus (Domenici and Blake 1997). Prolonged exposure to elevated suspended sediments, however, has been shown to reduce the aerobic scope (i.e., the capacity for aerobic activities) of some reef fishes (Chapter 2); this may compromise their capacity to engage in or recover from energetically costly activities such as fast starts (Killen et al. 2015). Yet, the effects of suspended sediments on the fast start performance of reef fishes are unknown.

A reduced ability to detect predators may decrease prey survival (Meager et al. 2006), and, as a consequence, may increase the perceived risk of predation in suspended sediments. Fish commonly respond to increases in predation risk with strategic changes in anti-predator behaviour or fast start performance (Domenici 2010; Lima 1998; Ramasamy et al. 2015). Such changes can be linked to considerable non-consumptive costs (Hawlena and Schmitz 2010; Preisser et al. 2005). For example, enhanced vigilance is associated with increased metabolic costs and can trade-off with foraging (Lima 1998; Killen et al. 2015; Preisser et al. 2005). Non-consumptive costs can result in reduced growth and condition (Hawlena and Schmitz 2010; Lima 1998) and can have equal or greater effects on prey population dynamics than predation itself (Preisser et al. 2005). Non-consumptive costs are particularly important to consider for juvenile reef fishes, as survivorship is typically low during this critical life-history stage and has been related to the growth and condition of individuals (Hoey and McCormick 2004). Despite its potential importance in shaping juvenile - and hence adult - populations, our understanding of the effects of suspended sediments on perceived predation risk and associated changes in performance or anti-predator behaviour of juvenile reef fishes is limited.

The aim of this study was to test the effects of suspended sediments on the fast start performance and anti-predator behaviour of a common coral reef fish. Specifically, juvenile cinnamon clownfish (*Amphiprion melanopus*) were exposed to suspended sediments for 7 days (0 or 180 mgL^{-1}) and examined their fast start performance and anti-predator behaviour in both 0 and 180 mgL^{-1} (i.e., after 5 min habituation time, referred to as “acute exposure” hereafter) suspended sediment concentrations. Exposing fish to suspended sediments both acutely and for 7 days allowed us to distinguish between potential effects on fast starts and anti-predator behaviour as a result of turbidity (e.g., sensory impairment) and potential effects driven by physiological changes such as reduced aerobic scope following prolonged exposure to suspended sediments.

3.3 Materials and methods

3.3.1 *Suspended sediment exposure*

Amphiprion melanopus larvae were sourced from five captive breeding pairs maintained at 28.5 ± 0.5°C and reared following standard protocols (see Chapter 2). At 35-days post-hatch, juveniles were exposed to 0 or 180 mgL^{-1} of Australian bentonite (0.7 ± 0.5 or 30.0 ± 4 NTU, respectively) for 7 days. Bentonite clay is representative of the size range of suspended sediments common to the Great Barrier Reef (GBR). The sediment concentration and exposure duration were selected to represent conditions frequently observed on inshore reefs of the GBR (Larcombe et al. 2001; Wenger et al. 2016). Sediment was suspended in external 200 L sumps fitted with submersible pumps. Each sump delivered water at a rate of 210 ± 30 mL min^{-1} to the bottom of 18 replicate conical experimental aquaria (3L). The water drained through an overflow outlet at the top of the experimental aquaria back into sumps. Water temperature was maintained at 28.5 ± 0.5°C via 1000 W heaters (Eheim Gmbh & Co. KG, Deizisau, Germany) in each sump. Sumps were flushed daily for 1 hour (> 100 % water exchange) to remove sediment and waste, after which new sediment (Australian Bentonite) was added to the sumps. Temperature was monitored daily and turbidity was monitored every four days (WP88 turbidity meter, Thermo Fisher Scientific, QLD, Australia) throughout the experiments.

3.3.2 *Larval rearing*

Breeding pairs of *Amphiprion melanopus* laid eggs on terracotta pots, which were transferred to individual, black painted 40L hatching aquaria on the day hatching was predicted (i.e. 6 – 8 days after egg deposition, Gordon et al. 2002). Aquaria were kept in a 1000L water bath to maintain temperature at 28.5 ± 0.5 °C and were flushed every morning (prior to lights turning on) with filtered seawater for an hour to remove waste. To reduce glare, algal paste (*Nannochloropsis* spp.) (ProAqua Pty Ltd, QLD, Australia) was added to the water until the bottom of the aquaria was no longer visible. *Branchionus* spp. rotifers were added at a density of 15 rotifers ml^{-1} until 5 days post-hatch and were then reduced to a density of 5 rotifers ml^{-1} until day 8 post-hatch. Starting at day 5 post-hatch, *Artemia* spp.

nauplii were added to aquaria at a density of 1 nauplii ml^{-1} twice daily until day 14 post-hatch, at which point larvae were fed flakes NRD 0.5 – 0.8 mm (Pro Aqua Pty Ltd) twice daily. Most fish settled at approximately 8 days post-hatch. At 35 days post-hatch juveniles were randomly assigned to experimental aquaria at a density of 5 individuals per aquaria. Trials were run sequentially until a total number of 60 individuals per treatment was reached.

3.3.2 *Experimental set-up*

After 7-day exposure, juvenile *A. melanopus* (18.7 ± 2.1 mm standard length; mean ± SE) were introduced into a circular (21 cm diameter) test arena contained within an aquarium (Fig. 3.1). The base of the arena was transparent, allowing the position of the juveniles to be recorded as a silhouette via a mirror positioned at an angle of 45° below the arena (Fig. 3.1). Juveniles were left undisturbed for five minutes, after which their behaviour was recorded for two minutes (Casio ex-fh20 camera). Following this, individuals were startled by a mechanical stimulus (a small weight), which was positioned above the arena and released via an electromagnet. The release commenced when fish were at least two body lengths away from the stimulus and the wall of the arena (following Allan et al. 2014). Responses of fish were recorded at 480 frames $second^{-1}$. Each individual was tested twice, i.e. both in 0 and 180 mgL^{-1} suspended sediments in the arena (Fig. 3.2), and the presentation order of these trials was randomized. In between the two trials, fish were housed in individual aquaria for 1.5 hours to recover from escape responses.

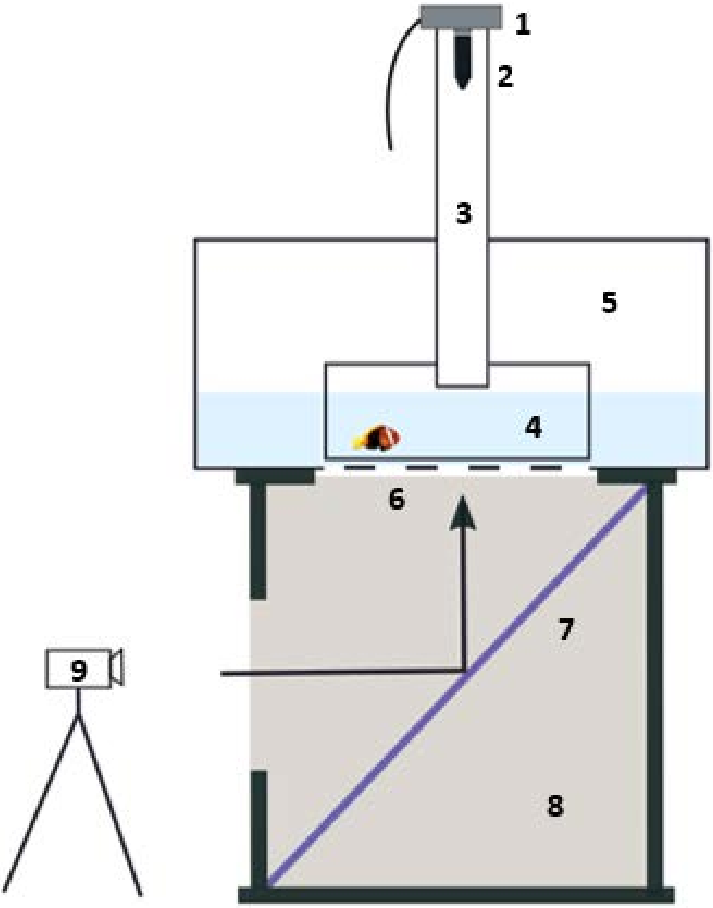

Figure 3.1 Experimental set up to determine fast start performance. **1)** Electromagnet, accessible via a remote switch; **2)** black weight with tapered end used as a startling stimulus; **3)** the stimulus fell through a tube ending 5mm above the water surface to prevent fish from detecting the stimulus before entering the water surface. The stimulus was released once the juveniles swam at least two body lengths away from the wall and one body length away from the tube to avoid edge effects; **4)** test arena (210 mm diameter) sitting in **5)** an aquarium with opaque walls and top, illuminated by external LED lights (not shown), and filled to 8 cm with water to minimize vertical displacement; **6)** the aquarium had a transparent bottom (indicated by the broken line), so the fish could be observed via a **7)** mirror, sitting at an angle of 45° inside a **8)** box; **9)** high speed camera pointed at the mirror.

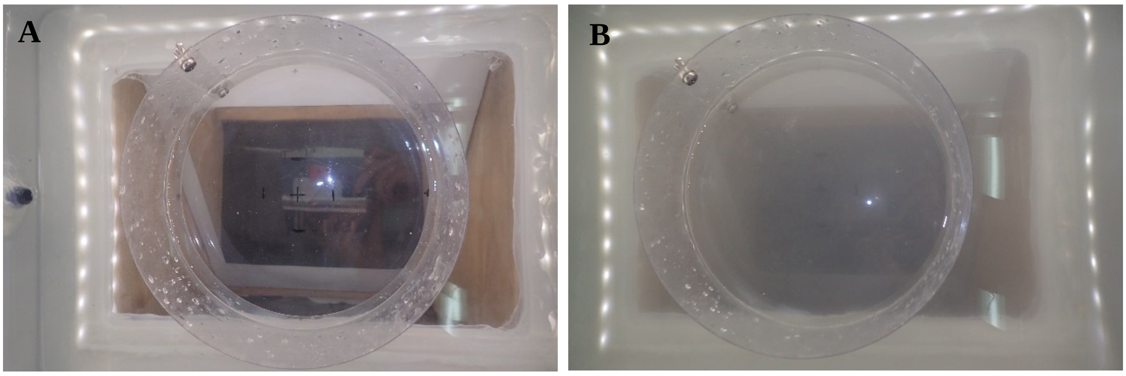

Figure 3.2 Photographs of the experimental fast start arena. **A)** Arena filled with clear water from the control and **B)** filled with water from the suspended sediment treatment.

3.3.3 *Analysis of videos*

Videos were analyzed in ImageJ (Version 1.48, National Institute of Health, USA) using a manual tracking plug-in. To determine fast start performance, the following variables were measured: 1) response latency, 2) turning rate, 3) maximum acceleration, 4) maximum escape speed, 5) average escape speed, 6) distance travelled. Variables 3 – 6 were measured within the first 41 ms of the response, i.e. during the first two caudal fin flips (Domenici and Blake 1997). Response latency was defined as the time interval between the weight entering the water and the first movement of the individual. Turning rate was defined as the ratio of stage 1 angle and stage 1 duration (Domenici et al. 2008). Stage 1 angle was defined as the angle between the position of the fish (i.e. a line was drawn connecting the center of mass and the tip of the head) prior to the first detectable movement and at the end of stage 1. The end of stage 1 was reached when the turning rate of the fish reached zero for the first time (i.e., before a change in the direction of head rotation occured). The remaining variables were determined based on stages 1 and 2 of the escape response (i.e., the first two axial bends, Domenici and Blake 1997), which lasted 41 ms in this species. The position of the fish was determined for every frame (corresponding to a time period of 2 ms between two frames) throughout the escape response. The distance moved and speed was calculated for each pair of consecutive frames. Acceleration was calculated as the change in speed over time. Based on these measurements, the escape distance (i.e., sum of distances moved within 41 ms), average and maximum escape speed, and maximum acceleration were determined.

To determine anti-predator behaviour, the position of individuals within the arena was determined every three seconds from the videos of the initial 2-min observation period. These data were used to derive the total distance moved by individuals (as a proxy for activity) and the percent of time they spent within 2.5 body lengths of the wall of the arena (i.e., thigmotaxis/avoidance of open areas; Schnörr et al. 2012). Thigmotaxis, or “wall hugging” is a proxy for the shyness of an individual in a novel environment (Schnörr et al. 2012).

3.3.4 *Statistical analyses*

Generalized linear mixed models were used to analyze the effects of acute and 7-day exposure to suspended sediments and their interaction on fast starts, activity, and thigmotaxis of juveniles in R (v.3.3.2) using the package lme4 (Bates et al. 2015). Fish standard length and distance between fish and the stimulus were mean-centered and included as covariates, and presentation order of trials and fish identity were included as random factors (ESM). Significance of fixed effects was evaluated via model comparison.

3.3.5 *Distance to the stimulus*

When suspended sediments were present in the test arena, fish were on average further away from the stimulus (5.3 ± 1.4 cm) when it entered the water than when the water was clear (4.2 ± 1.1 cm) (n = 65, $\chi^2(1) = 14.72$, $p = 0.001$), irrespective of 7-day exposure to suspended sediments ($\chi^2(1) = 0.02$, $p = 0.89$). To test whether distance to the stimulus had an effect on fast start variables, distance to the stimulus was initially included as covariate in models. However, distance to the stimulus did not have a significant effect on any of the variables, i.e. response latency, n = 65, $\chi^2(1) = 2.98$, $p = 0.08$; average escape speed, $\chi^2(1) = 1.18$, $p = 0.28$; maximum escape speed, $\chi^2(1) = 0.01$, $p = 0.91$; and distance travelled $\chi^2(1) = 1.36$, $p = 0.24$, and did not change the outcomes of any model. Therefore, distance to stimulus was not included in the final models.

3.4 Results and discussion

After 7-day exposure to elevated suspended sediments (180 mgL^{-1}), juvenile *A. melanopus* were faster to respond to the stimulus (11.3 ± 1.2 ms vs 14.2 ± 1.0 ms, mean ± SE; n = 65, $\chi^2(1) = 5.36$, $p = 0.02$, Fig. 3.3 A), exhibited higher turning rates (10.2 ± 0.3 deg ms^{-1} vs 9.2 ± 0.3 deg ms^{-1}, $\chi^2(1) = 4.26$, $p = 0.03$, Fig. 3.3 B), higher average escape speeds (72.1 ± 3.5 cm sec^{-1} vs 62.7 ± 3.4 cm sec^{-1}; $\chi^2(1) = 6.38$, $p = 0.01$, Fig. 3.3 C), and moved further away from the stimulus within the first 41 ms of the simulated attack (34.4 ± 1.6 mm vs 29.5 ± 1.5 mm, $\chi^2(1) = 6.51$, $p = 0.01$, Fig. 3.3 D) than fish exposed to clear water (0 mgL^{-1} suspended sediments) for 7 days. Maximum escape speeds and maximum acceleration were not influenced by 7-day exposure to suspended sediments (Figs. 3.3 E and F). Maximum acceleration, however, was influenced by acute exposure to suspended sediments, with fish achieving higher maximum acceleration (40.8 ± 2.3 cm sec^{-2} vs 36.8 ± 2.0 cm sec^{-2}, $\chi^2(1) = 5.2$, $p = 0.02$, Fig. 3.3 F) when the water in the test arena was turbid as opposed to when it was clear. Acute exposure to suspended sediments did not influence any other fast start trait. The shorter response latencies suggest that fish were more vigilant after 7-day exposure to suspended sediments, and coupled with the higher escape speeds, turning rates, and acceleration, these changes would be expected to increase chances of a fish to survive a predator attack (Domenici and Blake 1997; Walker et al. 2005).

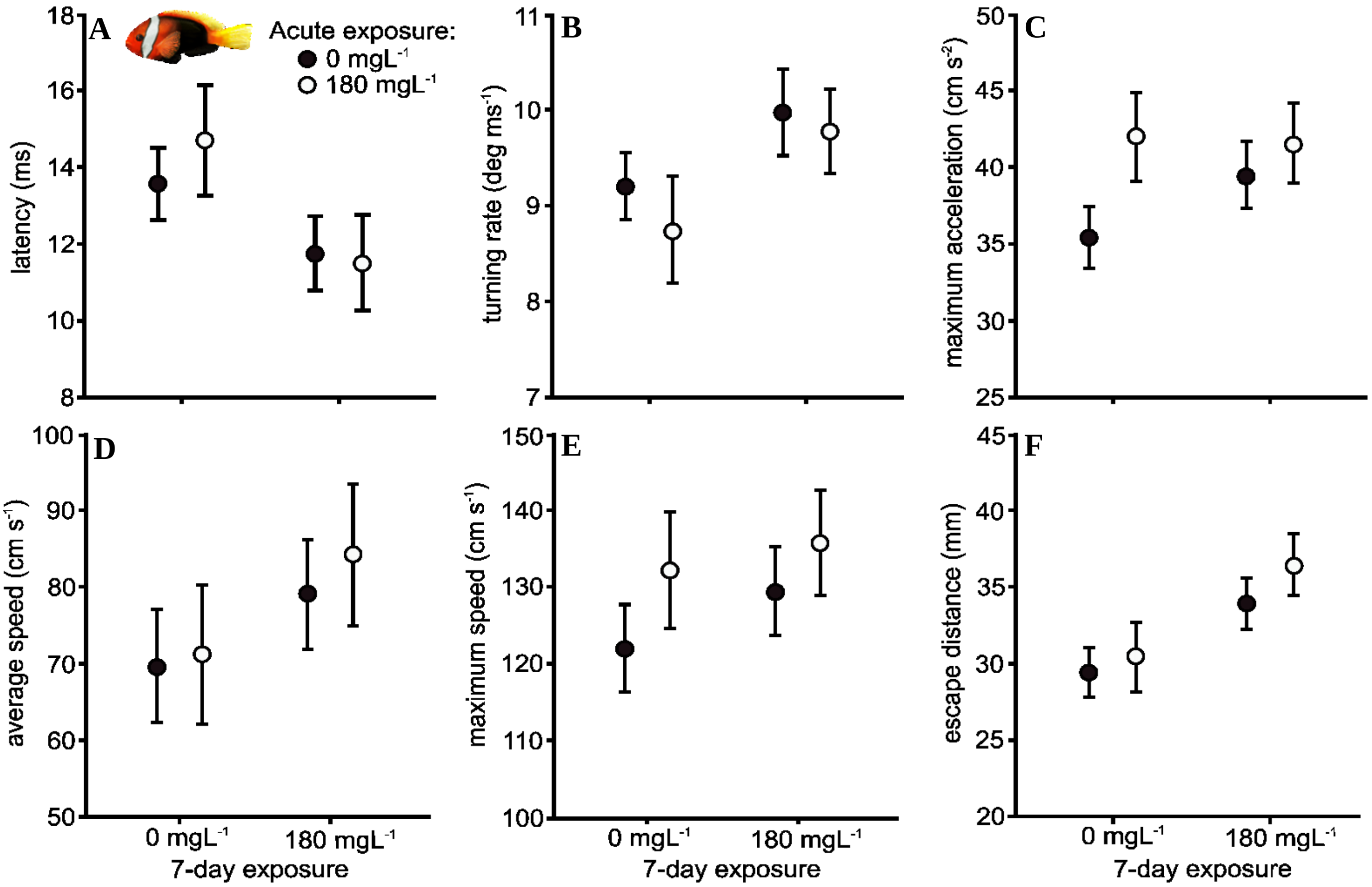

Figure 3.3 Variables associated with fast starts of juvenile *Amphiprion melanopus* exposed to 0 or 180 mgL^{-1} suspended sediments for 7 days and tested in both clear (closed circles) and turbid water (open circles), i.e. “acute exposure” (effect plots and associated adjusted means ± SE). **A)** Latency to respond to a stimulus, **B)** turning rate, **C)** average escape speed, **D)** distance escaped within 41 ms of the reponse, **E)** maximum escape speed, and **F)** maximum acceleration.

Acute exposure to suspended sediments led to changes in anti-predator behavior of juvenile *A. melanopus*. Fish moved significantly less (38.6 ± 4.8 cm vs 51.8 ± 4.8 cm; n = 78, $\chi^2(1) = 13.47$, $p = 0.0002$, Fig. 3.4 A) and spent less time in the open area of the arena (i.e., enhanced thigmotaxis) (11.6 ± 2.4 % vs 28.2 ± 3.5 %; $\chi^2(1) = 11.16$, $p = 0.0008$, Fig. 3.4 B) when exposed to suspended sediment in the arena compared to clear water. Fish exhibited these changes regardless of whether they were previously exposed to suspended sediments or clear water for 7 days. Exposure for 7-days would be expected to be sufficient for fish to habituate to suspended sediments, suggesting that the observed changes in behaviour (and fast start performance) are not short-term reactions to a novel threat (i.e., turbidity), but rather adjustments to changes in the environment. Juvenile *A. melanopus* are commonly found on turbid inshore reefs (Fautin 1992), where turbidity levels can be variable. Short periods (minutes to hours) of elevated suspended sediments are caused by resuspension from waves and currents (Larcombe et al. 2001), while periodic events such as flood plumes can increase suspended sediment concentrations for days or weeks at a time (Wenger et al. 2016). Reductions in activity and enhanced thigmotaxis are strategies to decrease the likelihood of predator interactions (Lima 1998), suggesting that juvenile *A. melanopus* may adopt more cautious behavior when suspended sediment levels are high.

Contrary to expectations, neither 7-day nor acute exposure to suspended sediments had negative effects on the fast start performance of fish. Exposure to suspended sediments for 7 days was found to compromise the aerobic performance of juvenile *A. melanopus* (Chapter 2), yet the locomotor performance of fish in the present study was enhanced rather than constrained. Similarly, acute exposure to suspended sediments did not influence the ability of fish to detect a stimulus that was a short distance away (~5cm), simulating an ambush attack. This distance may have not been sufficient for suspended sediments to significantly reduce visual cues. Alternatively, fish may have used sound and/or water displacement, in addition to visual cues, to detect the stimulus. It is well established, however, that suspended sediments delay the visual detection of predators (Wenger et al. 2017) and attenuate sound transmission over longer distances (Brown et al. 1998). The observed enhanced fast starts and anti-predator behaviour following suspended sediment exposure are typical responses to increases in perceived predation risk (Domenici 2010; Lima 1998) and were likely an attempt to compensate for this reduced ability to detect visual and/or auditory cues associated with predators. Supporting this, a closely related species, *Acanthochromis polyacanthus,* has been found to enhance their anti-predator response to olfactory cues in suspended sediments to compensate for reduced visual cues (Leahy et al. 2011).

Whether the enhanced fast start performance and antipredator behaviour would translate into changes in predation rates in suspended sediments compared to clear water is not known, since suspended sediments may also affect the detection of prey by predators (e.g. Higham et al. 2015). A study on newly settled *Chromis atripectoralis* preyed upon by an ambush predator, *Pseudochromis fuscus,* documented that suspended sediments indeed influenced predation rates, with increased predation at medium (30 mgL^{-1}) suspended sediment levels, compared to low predation rates at both lower or higher levels of suspended sediment (Wenger et al. 2013).

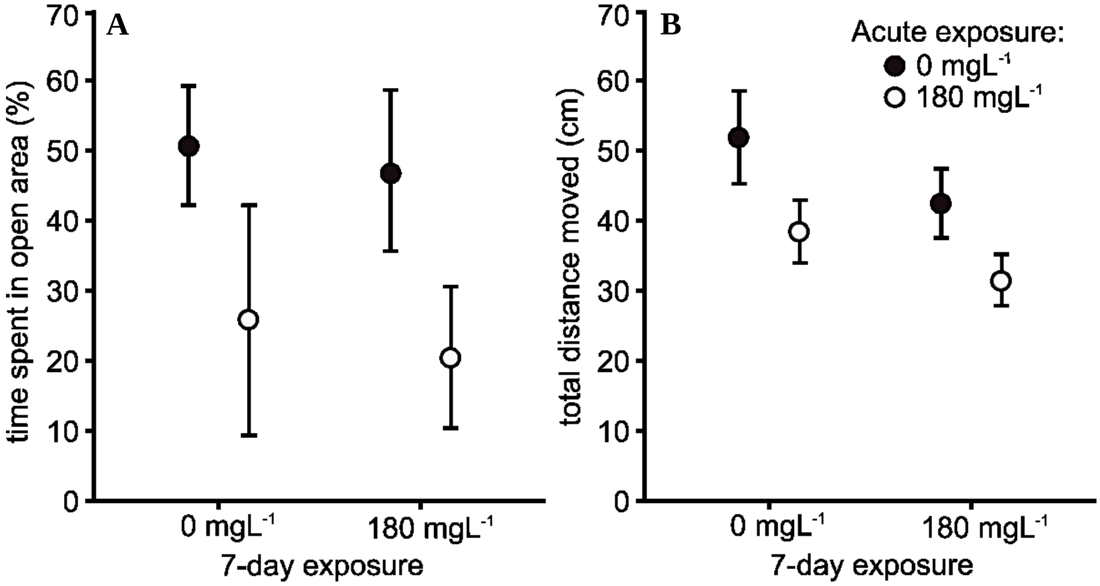

Figure 3.4 Anti-predator behaviour of juvenile *A. melanopus* exposed to 0 or 180 mgL^{-1} suspended sediments for 7 days and tested in both clear (closed circles) and turbid water (open circles), i.e. “acute exposure” (effect plots and associated adjusted means ± SE). **A)** Percent of time spent > 2.5 body lengths away from the wall of the arena, and **B)** total distance moved during the 2 min trial.

Enhanced fast starts and anti-predator behaviour, as observed in response to suspended sediments, may lead to non-consumptive costs (Preisser et al. 2005). While *A. melanopus* lives symbiotically with sea anemones (Fautin and Allen 1992), the area they use for foraging (i.e., their home range) extends considerably beyond the area covered by their host (Ross 1978). Juveniles also frequently migrate between sea-anemones (Ross 1978). More cautious behaviour, such as observed in elevated suspended sediments, may not only reduce movement within their home range, potentially limiting access to food, but may also reduce the ability of juveniles to find a suitable anemone. Indeed, another damselfish species, *Pomacentrus moluccensis*, has been found to reduce movement between settlement habitats in elevated suspended sediments (Wenger and McCormick 2013). Furthermore, an increase in perceived predation risk itself is associated with numerous negative effects, such as impaired growth efficiency and immunocompetence (Hawlena and Schmitz 2010). While no studies, to date, have examined the metabolic costs of enhanced locomotor performance during fast starts, shorter response latencies have been linked to non-consumptive costs. Shorter response latencies are a result of enhanced vigilance, which is associated with increased metabolic costs (Killen et al. 2015) and is well known to trade-off with feeding in fish (Godin and Smith 1988; Preisser et al. 2005). Thus, while the enhanced fast start performance and anti-predator behaviours can be perceived as beneficial when suspended sediment concentrations are high, such changes are likely associated with considerable costs for *A. melanopus* juveniles living on turbid reefs.

Chapter 4: Cumulative effects of suspended sediments and elevated temperature on fish gills, oxygen uptake and predator escape performance

4.1 Abstract

Increasing sediment inputs and ocean warming pose major threats to the health of coastal coral reefs. While each factor in isolation can affect the physiology and performance of coral reef fishes, little is known of their cumulative effects. In this chapter, a common coral reef fish, *Acanthochromis polyacanthus*, was reared under current day (29°C) or projected future temperature (32°C) for 4.5 weeks. During the final week of exposure, suspended sediments (0, 90, or 135 mgL^{-1}) were added to aquaria in a full factorial design to simulate a turbidity event (such as a flood plume). Following treatment exposure, gill morphology, aerobic performance, and predator escape performance of fish were examined. While exposure to 90 mgL^{-1} suspended sediments had no effects on gill morphology, 135 mgL^{-1} suspended sediments led to a reduction in gas diffusion distance (i.e., between -5.4% and -21.7%; 95% credibility interval ($CI_{95\%}$)), relative to control fish (i.e., fish exposed to 29°C and 0 mgL^{-1} suspended sediments). Elevated temperature led to reductions in both gas diffusion distance ([-8.8%, -24.2%] $CI_{95\%}$) and filament thickness ([-5.4%, -21.7%] $CI_{95\%}$) relative to control fish, which are changes generally associated with improved gas exchange efficiency. Suspended sediments and elevated temperature combined did not have any interactive effects on gill morphology. The changes in gill morphology were not reflected by the observed changes in oxygen uptake rates; elevated temperature in isolation led to reductions in maximum ([-23.6%, +1.1%] $CI_{95\%}$) and resting oxygen uptake rates [-5.4%, -40.5%] $CI_{95\%}$), while suspended sediments had no effects on oxygen uptake rates in isolation. In fish exposed to both stressors combined, maximum oxygen uptake rates were not different from control fish, resulting in an enhanced aerobic scope compared to control fish regardless of suspended sediment concentration (i.e., [-1.7%, +50.4%] $CI_{95\%}$ at 90 mgL^{-1} and [-1.4%, +52.4%] $CI_{95\%}$ at 135 mgL^{-1}). Predator escape performance was solely influenced by suspended sediments, with fish exhibiting enhanced fast starts following suspended sediment exposure (i.e., increased average ([+1.1%, +16.0%] $CI_{95\%}$) and maximum escape speeds ([-1.4%, +17.0%] $CI_{95\%}$), and greater escape distances ([+0.7%, +15.3%] $CI_{95\%}$) at 90mgL^{-1} and shorter response latency at 135mgL^{-1} ([-11.6%, -50.8%] $CI_{95\%}$), respectively) compared to control fish. This response was likely driven by an increased perceived predation risk in turbid waters due to a compromised ability to detect predators. These surprising findings show that suspended sediments did not compound, but rather masked some of the negative

effects of elevated temperature on juvenile *A. polyacanthus*. While this interaction between the two stressors may help *A. polyacanthus* to acclimate to future environmental conditions on inshore coral reefs, future studies will need to examine potential trade-offs with other important performance traits or functions.

4.2 Introduction

Coastal ecosystems are increasingly exposed to the cumulative impacts of local anthropogenic activities and global climate change (Halpern et al. 2015, Hughes et al. 2017). Over the past century, many nearshore coral reefs have experienced decreasing water quality, in particular increasing concentrations of suspended sediments, due to coastal development, land conversion, mining, shipping, and dredging (Foley et al. 2005, Syvitksi et al. 2005). In addition, sea surface temperatures in the tropics have already increased by 0.25 – 0.75°C since pre-industrial times and are projected to continue increasing due to anthropogenic greenhouse-gas emissions (Hughes et al. 2017, Stocker et al. 2013). Increasing suspended sediment concentrations and water temperatures are leading to changes in the composition of fish assemblages on coastal reefs (Bejarano and Appeldoorn 2013, Cheal et al. 2013, Moustaka et al. 2018), either indirectly through changes in benthic composition and/or directly through impacts on the fishes themselves (Fabricius et al. 2005, Hamilton et al. 2017). Past studies have established that suspended sediments and elevated temperatures in isolation can significantly affect the physiology and performance of coral reef fishes (e.g., Munday et al. 2012, Allan et al. 2015, Wenger et al. 2017). However, to better understand and predict responses of coral reef fish populations to current and future environmental conditions, it is critical to consider the cumulative effects of both suspended sediments and elevated temperature.

Elevated temperature and suspended sediments both influence the gill morphology of reef fishes. Gill morphology is linked to the efficiency of gas exchange and other functions, including acid-base and osmo-regulation (Evans et al. 2005). Indeed, numerous fish species are known to remodel their gills to regulate oxygen uptake (Nilsson 2007, 2012). For example, fishes often decrease the thickness of the gill epithelium (i.e., gas diffusion distance) and increase gill surface area in response to increases in temperature, thereby enhancing gas exchange efficiency (e.g., Sollid et al. 2005, Phuong et al. 2017, Gibbons et al. 2018). Similarly, a decrease in gas diffusion distance has been observed in response to suspended sediments (Chapter 2, Wong et al. 2013). Suspended sediments, however, can also damage the gill epithelium, leading to the shortening of gill lamellae (Lake and Hinch 1999, Sutherland et al. 2007), the growth of protective cell layers with a subsequent increase in gas diffusion distance (Cumming and Herbert 2016, Lowe et al. 2015), and an increase in mucous secretion (Humborstad et al. 2006, Li et al. 2012). All of these changes can reduce gas exchange efficiency and interfere with oxygen uptake across the gills (Chapter 2, Evans et al. 2005: Lappivaara 1995).

Given the effects of suspended sediments and elevated temperature on gill morphology, it is not surprising that they both influence the aerobic performance of fishes. Exposure to elevated levels of suspended sediments decreased maximum oxygen uptake rates ($\dot{M}O_{2max}$) achieved by juvenile anemone fish, *Amphiprion melanopus*, while increasing oxygen uptake rate at rest ($\dot{M}O_{2rest}$, Chapter 2), which is a proxy for metabolic costs required to sustain basic metabolic functions (Claireaux and Lefrancois 2007, Pörtner and Farrell 2008). These changes resulted in a reduction in aerobic scope (i.e., difference between $\dot{M}O_{2max}$ and $\dot{M}O_{2rest}$) and thus a reduced capacity for aerobic activity (Chapter 2). In contrast, two confamilial species, *Amphiprion percula* and *Amphiprion melanopus,* were able to maintain aerobic performance despite changes in gill morphology following suspended sediment exposure (Chapter 2). Compared to suspended sediments, the effects of elevated temperatures on aerobic performance is more consistent among species, with $\dot{M}O_{2rest}$ generally increasing with acute increases in temperature (Claireaux and Lefrancois 2007, Pörtner and Farrell 2008), while $\dot{M}O_{2max}$ often decreases, thereby compromising aerobic scope (Gardiner et al. 2010, Rummer et al. 2014). A reduction in aerobic scope can affect aerobic activities such as growth and development, with negative consequences for the survival and fitness of fishes (Norin and Clark 2016).

Elevated temperatures and suspended sediments both also influence the predator escape performance of fishes. Coral reef fishes reared at elevated temperatures mostly exhibit a reduction in predator escape performance, with fish responding slower to a mechanical stimulus, escaping at lower speeds and covering shorter escape distances than fish reared at control temperature, although responses may vary between species (Allan et al. 2015, Motson et al. 2017, Warren et al. 2017). In contrast, *Amphiprion melanopus* exposed to suspended sediments showed the opposite response, i.e., individuals responded faster to a mechanical stimulus, achieved higher escape speeds and acceleration, and escaped further away following suspended sediment exposure (Chapter 3). Predator escape performance plays an important role in predator-prey interactions and any changes in predator escape performance are likely to directly influence survival of juvenile fishes on coral reefs (McCormick et al. 2018).

The aim of the present study was to investigate the cumulative effects of elevated suspended sediments and elevated water on the gill morphology, aerobic performance, and predator escape performance of juvenile spiny chromis, *Acanthochromis polyacanthus* (Pomacentridae). This species was selected due to their wide distribution range, which includes both turbid inshore reefs and clear water offshore reefs (Thresher 1985). Juveniles were the focus, as mortality at this life-stage is high (e.g., Hoey and McCormick 2004) and even small changes in survival have consequences for adult populations (Jones 1990, Schmitt and Holbrook 1999). I hypothesize that:

i) suspended sediments in isolation will lead to a suite of gill morphological changes that are associated with contrasting effects on oxygen uptake efficiency (i.e., such as reduced functional lamellar length and decreases in gas diffusion distance), a compromised aerobic performance, and enhanced predator escape performance;

ii) elevated temperature in isolation will lead to gill morphological changes that enhance oxygen uptake efficiency, a compromised aerobic performance, and a compromised predator escape performance; and

iii) suspended sediments and elevated temperature combined will have additive or synergistic effects on gill morphology, aerobic performance, and predator escape performance.

4.3 Materials and methods

4.3.1 *Experimental protocol*

Juvenile *Acanthochromis polyacanthus* (Bleeker, 1855) were sourced from five captive breeding pairs originating from the northern Great Barrier Reef (GBR). Breeding pairs were maintained at 29.0 ± 0.5°C in 60 L aquaria, provided with half a terracotta pot for egg deposition, and fed commercial food pellets NRD 2 mm (ProAqua, Brisbane, Queensland, Australia) *ad libitum* twice daily. Pots were checked daily for egg clutches, and hatching usually occurred eight to ten days after egg deposition. At the day of hatching, juveniles (*A. polyacanthus* does not have a dispersive larval phase) were collected and randomly allocated to one of two temperature treatments, i.e. 29.0 ± 0.5°C (control) or 32.0 ± 0.5°C (elevated temperature) and placed in replicate 20L aquaria with a maximum of 30 individuals in each aquarium. To acclimate individuals to the elevated temperatures (i.e., 32°C) treatment, the temperature of 20 L aquaria (n = 5 per temperature) was raised by 1°C per day. Fish were fed *Artemia* sp. nauplii until 10 days after hatching, and then commercial flakes (NRD 0.5 – 0.8 mm; ProAqua, Brisbane, Queensland, Australia) twice daily throughout the remainder of the experiment.

At four weeks post-hatch, juveniles were randomly allocated to one of three suspended sediment treatments, i.e. 0, 90 or 135 mgL^{-1}, corresponding to turbidity levels of 0.5 ± 0.3, 14.5 ± 1.5 and 22.4 ± 1.7 NTU for seven days. The suspended sediment concentrations were selected to simulate conditions that are observed on inshore reefs of the GBR during frequent re-suspension events such as waves and currents, and periodic events such as flood plumes (Bainbridge et al. 2012; Wolanksi et al. 2008; Wenger et al. 2016). Juveniles were held in 3 L aquaria (n = 6 aquaria were used per treatment) at a density of 5 individuals per aquarium maintained at their respective acclimation temperature. Due to logistic constraints and the timing of clutches, individual clutches were split across two to three of the six treatments at a time, and the experiment was conducted sequentially over the course of three months.

Sediments were re-suspended in external sumps and delivered to aquaria via submersible pumps (as described in Chapter 2). Briefly, water entered through the conical-shaped base of the aquaria and drained back into sumps via an outlet at the top of aquaria, which aided in keeping sediment in suspension. Sumps and aquaria were flushed daily with fresh seawater for one hour (> 100% water exchange) to remove waste and sediment, after which sediment was again added to sumps (i.e., turbidity levels were below respective treatment levels for a maximum of 1.5 hours a day). Turbidity levels were monitored every second day using a hand-held turbidity meter (ThermoFisher Scientific, Scoresby,Victoria, Australia). Sterile Australian bentonite was used as sediment, as it is a clay with a small particle size range (< 63 μm) representative of sediments found in suspension in inshore waters of the GBR (Larcombe et al. 2001).

4.3.2 *Gill morphology*

Following exposure to suspended sediments and temperature treatments, 10 individuals per treatment were randomly selected for histological analyses of the gills. Juveniles were euthanized in an ice-water slurry, and their entire body (gills were too small to be excised) was fixed in Bouin's solution for 24 hours and then transferred to 70% ethanol. Fish were then serially dehydrated using ethanol, embedded longitudinally in paraffin wax, and sectioned (5 μm) with a microtome (ThermoFisher, Australia). Sections were mounted on glass slides and stained with Mayer's Haematoxylin and Eosin (H&E) (Merck, Frenchs Forest, New South Wales, Australia). Due to varying quality of processed samples, some treatments resulted in 7 – 9 samples. Sections were photographed using a light microscope equipped with a camera (Olympus, Sydney, New South Wales, Australia) at 400x magnification.

Photographs were analyzed using ImageJ (Version 1.48, National Institute of Health, USA). Ten lamellae per fish were randomly selected, excluding lamellae that were not fully developed (i.e., lamellae at the tip of a branch) or showed sectioning errors. For each lamellae the following morphological features were quantified: 1) total lamellar length (including the base of the lamellae covered by the filament epithelium), 2) thickness of the filament epithelium, 3) functional lamellar length, calculated by subtracting the thickness of the filament epithelium from the total lamellar length, and 4) gas diffusion distance (following Hess et al. 2015). To determine gas diffusion distance, the area of the pillar cell system was subtracted from the total area of the lamellae (of the functional lamellar length), which was divided by twice the functional length. All measurements were taken blindly with respect to treatments.

4.3.3 *Oxygen uptake rates*

After exposure to suspended sediment and temperature treatments, oxygen uptake rates ($\dot{M}O_2$) of 9 – 12 individuals per treatment were determined using intermittent-flow respirometry (Steffensen 1989). Individuals were fasted for 24 hours prior to trials to ensure a post-absorptive state (Niimi and Beamish 1974). To determine maximum oxygen uptake rates ($\dot{M}O_{2max}$), individuals were chased by hand for two minutes in a large circular container (60 cm diameter, 10 cm water depth, maintained at the treatment temperature of individuals), which was sufficient to fully exhaust fish. After the chase, fish were immediately transferred to individual glass respirometry chambers (40 mm length x 18 mm diameter, 13.3 ml volume including tubing), and covered with black sheeting to minimize visual disturbances. Oxygen levels in chambers were recorded every 2 seconds using a cell of oxygen sensitive dye and a fiber-optic oxygen meter (Pyro-Science, Aachen, Germany). This method assumes that fish will achieve maximum oxygen uptake rates ($\dot{M}O_{2max}$) as they recover from exhaustive anaerobic exercise (Roche et al. 2013). Chambers were connected to a recirculation pump via tubing to ensure homogenous oxygen concentration inside chambers (Rodgers et al. 2016) and were flushed every 7 min for 5 min with fully aerated, UV- sterilized and particle size (1µm) filtered water from the surrounding water bath maintained at the respective treatment temperature of individuals. Oxygen levels were monitored continuously for 9 h during which time oxygen levels within chambers remained above 90% air saturation at all times. Background respiration in empty chambers was measured for 15 min before and after each trial (Rodgers et al. 2016). After each trial the respirometry chambers, tubing, pumps, and the water bath were cleaned with freshwater and a 10% bleach solution to minimize microbial growth. At the end of each trial, fish were euthanized using an ice-water bath, and standard length (to the nearest mm) and blotted wet mass (to the nearest 0.0001g) were measured.

Oxygen consumption rates during closed phases of the respirometry trials were determined using linear least squares regression of 1-minute intervals in Labchart v. 6.1.3 (ADinstruments, Auckland, New Zealand). MS Excel was used to derive R^2 of slopes, which was >95%. Proportional background $\dot{M}O_2$ was subtracted from each measurement to obtain $\dot{M}O_2$ of fish (Rodgers et al. 2016). The highest $\dot{M}O_2$ measured was taken as $\dot{M}O_{2max}$, and usually occurred during the first 7-min cycle of each trial. Resting oxygen consumption rates were calculated following the "Mean of the Lowest Normal Distribution" (MLND) method described in Chabot et al. (2016). The coefficient of variation of the mean of the lowest normal distribution was determined for each fish, and was generally low (< 5). This method assumes that $\dot{M}O_2$ of fish at rest are normally distributed around the true $\dot{M}O_{2rest}$ (i.e., the mean of the lowest normal distribution), while $\dot{M}O_2$ of fish associated with bouts of spontaneous activity cluster together in separate normal distributions characterized by higher means (Chabot et al. 2016). The first three hours of each trial were discarded; three hours were sufficient for fish to fully recover from the exercise and get accustomed to respirometry chambers (i.e., $\dot{M}O_2$ had stabilized and did not further decline). Aerobic scope was calculated by subtracting $\dot{M}O_{2rest}$ from $\dot{M}O_{2max}$.

4.3.4 *Fast start performance*

Fast start performance of at least 19 juveniles per treatment (mean = 23 fish per treatment) was assessed immediately after the 7-day exposure to suspended sediment and temperature treatments. Fish were introduced into an experimental arena that consisted of a thin (1.5 mm) transparent plastic circular arena (diameter 20 cm, filled to 80 mm with water to minimize vertical displacement) within a 60 L white-sided container (350 x 400 x 250 mm) maintained at the respective acclimation temperature of fish (following Allan et al. 2014). Individuals were left to habituate to the new surroundings for ten minutes, which was sufficient time to resume routine behavior, as evidenced by exploratory behavior spanning the length and intersect of the experimental arena. Fast start responses were elicited using a mechanical stimulus (a black weight with a tapered end) placed 500 mm above the aquarium (following Allan et al. 2014). The stimulus was released remotely via an electromagnet and travelled through a PVC pipe that ended 10 mm above the water surface to prevent fish detecting the stimulus before it entered the water. The stimulus was released only once the fish was at least 2 body lengths away from the wall of the arena to avoid potential edge effects. The base of the arena was transparent so juveniles could be seen as a silhouette via a mirror positioned at an angle 45° below the aquarium and recorded using a camera (Casio EX-ZR1000) at 480 frames second^{-1}.

The videos were analyzed using ImageJ (Version 1.48, National Institute of Health, USA). Fast start variables were only measured when individuals performed a C-start (Domenici and Blake 1997). The following variables were measured by tracking the center of mass of each fish; 1) response latency (in ms), defined as the time interval between the stimulus entering the water surface and the first movement of the fish, 2) mean escape speed (cm s^{-1}), 3) maximum escape speed (cm s^{-1}), and 4) escape distance (cm). The latter three were measured within the first 24 ms of the response, i.e. the first two caudal fin flips, which are considered crucial for escaping ambush attacks (Domenici and Blake 1997). Videos were analyzed blindly with respect to treatments.

4.3.5 *Statistical analysis*

All analyses were carried out in R (v. 3.4.4). The effects of temperature, suspended sediments, and their interaction on measured traits were examined with Bayesian linear hierarchical models. To account for differences in fish size, standard length was included as a covariate in models for gill morphology and fast start models, and fish mass was included as a covariate in oxygen consumption models. While distance between fish and the stimulus at the onset of fast starts (54.3 ± 0.8 mm) was not significantly different between treatments ($F_{5,139} = 0.4$, $p = 0.7$), it was positively correlated with response latency and improved model fit (based on leave-one-out information criteria) and was thus included in the response latency model as a covariate. All covariates were mean-centered. Identity of parents was included in all models, and fish identity was included as an additional random effect in gill morphology models to account for multiple measurements per fish. Vague, non-informative normal priors were specified for the intercept, the main effects, covariates and random effects, and half-cauchy priors were specified for the variance. Posterior draws were built using three Markov chains with 20 000 iterations per chain, a thinning interval of 5, and a warm-up of 50%, resulting in a total of 6000 samples. All Rhat values were < 1.05 and along with the trace plots suggested that chains had converged on stable, well-mixed posteriors. Autocorrelation function (ACF) plots were used to check for autocorrelation, which was < 0.25 for all models. Priors/posterior plots were visually assessed to check whether posterior distributions were constrained by priors. Deviance residuals were checked for normality and heterogeneity of variance. Posterior means and 95% credibility intervals of effect sizes relative to the control group (i.e., fish raised at 29°C and exposed to 0 mgL^{-1} suspended sediments) were computed using higher posterior density (HPD) intervals. An effect/difference between groups was considered significant if it was greater than 0 at a probability of 95% or higher, which was determined using specific contrasts on the posteriors.

4.4 Results

4.4.1 *Gill morphology*

Exposure to elevated temperature or suspended sediments independently led to changes in the gill morphology of juvenile *A. polyacanthus*; however, the combined effect of both stressors was trait-specific. Gas diffusion distance across the lamellae was reduced in fish exposed to elevated temperature (32°C) in isolation (>99% probability of a reduction, and a 95% probability that this reduction was between -8.8% and -24.2%, hereafter written as >99% probability, [-8.8%, -24.2%] $CI_{95\%}$), and fish exposed to 135 mgL^{-1} suspended sediments (>99% probability, [-5.4%, -21.7%] $CI_{95\%}$) when compared to fish from the control (29°C and 0 mgL^{-1}) group (Fig. 4.1A and B, 4.2A). Fish exposed to 90 mgL^{-1} suspended sediments showed no change in gas diffusion distance, irrespective of temperature. Fish exposed to both elevated temperature and 135 mgL^{-1} suspended sediments combined did not show any further reduction in gas diffusion distance when compared to fish exposed to either of these stressors in isolation (Fig. 4.2A, 97.4% probability). In contrast to gas diffusion distance, filament thickness of fish was influenced solely by temperature, with fish exposed to elevated temperatures exhibiting a thinner filament epithelium (>99% probability, [-17.4%, -40.3%] $CI_{95\%}$) compared to fish from the control group (Fig. 4.2B).

Neither elevated temperatures nor suspended sediments had independent effects on total or functional lamellar length. Elevated temperature and 90 mgL^{-1} suspended sediment, however, had significant interactive effects on both traits, resulting in increased total (>99% probability, [+2.9%, +15.8%] $CI_{95\%}$) and functional (>99% probability, [+2.2% to +15.6%] $CI_{95\%}$) lamellar length when compared to fish from the control group (Fig. 4.2C).

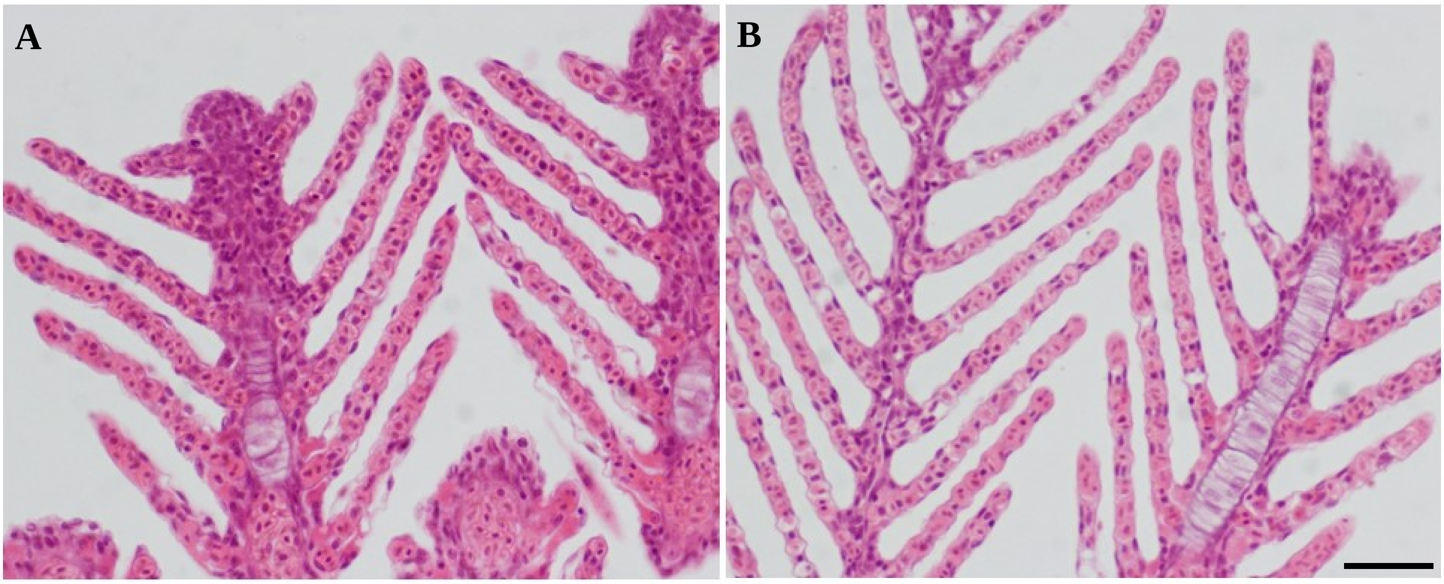

Figure 4.1 Micrographs of gill lamellae of *A. polyacanthus* following exposure to **A)** 0 mgL^{-1} suspended sediments at 29 ± 0.5°C and **B)** 135 mgL^{-1} suspended sediments at 32 ± 0.5°C. Haematoxylin and Eosin stain, scale bar; 20 μm.

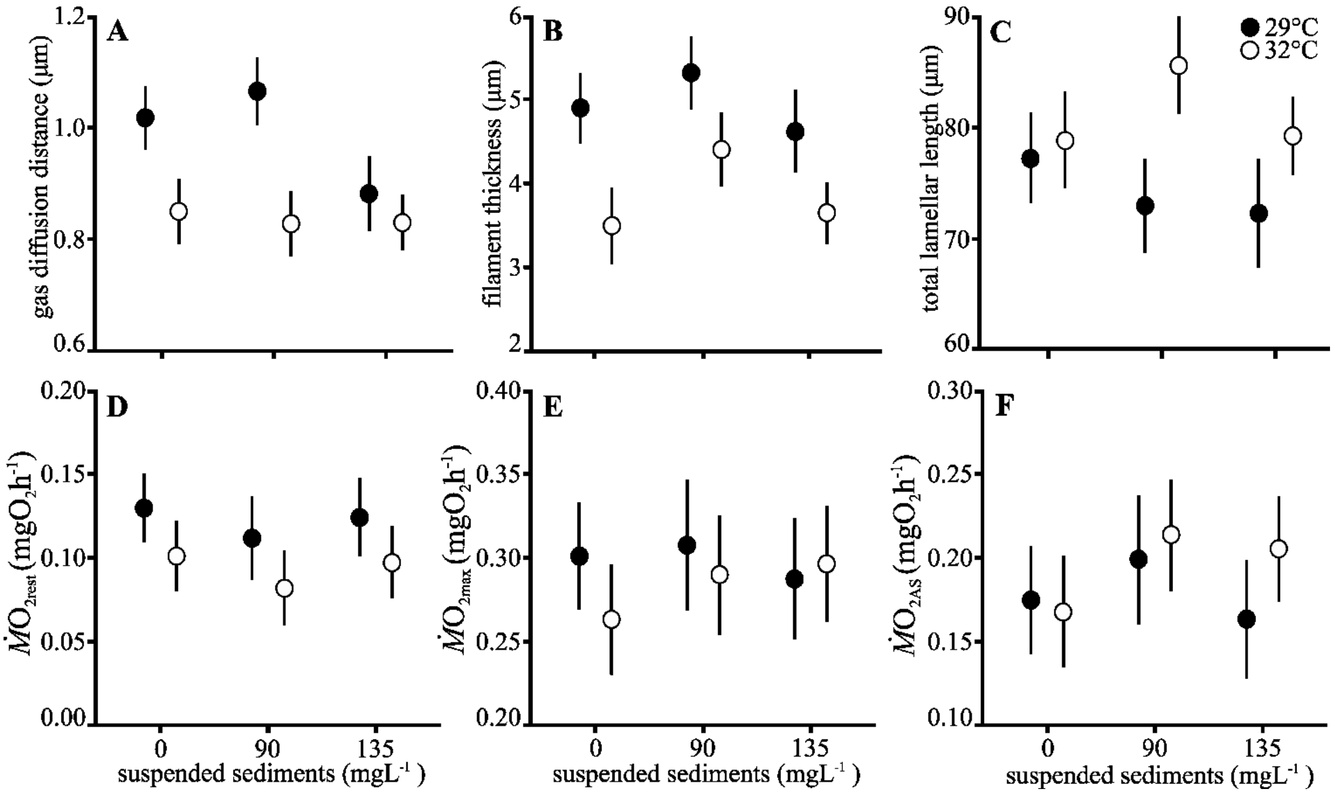

Figure 4.2 Effects of suspended sediments and elevated temperatures on gill morphology (**A-C**) and oxygen uptake rates (**D-F**) on juvenile *Acanthochromis polyacanthus*. Black and white circles represent fish reared under control (29 ± 0.5°C) or elevated temperature (32 ± 0.5°C), respectively. Circles represent posterior means, while bars represent 95% credibility intervals for a fish of mean length (A-C) and mean mass (D-F).

4.4.2 *Oxygen uptake rates*

Elevated temperature, but not suspended sediments, had independent effects on oxygen uptake rates ($\dot{M}O_2$) of juvenile *A. polyacanthus*; however, there were several trait-specific interactive effects between the two stressors. Fish exposed to elevated temperatures exhibited a lower $\dot{M}O_{2\text{rest}}$ (98.0% probability, [-5.4%, -40.5%] $CI_{95\%}$) and lower $\dot{M}O_{2\text{max}}$ (96.7% probability, [-23.6%, +1.1%] $CI_{95\%}$) when compared to fish from the control group, while suspended sediment exposure in isolation had no effect (Fig. 4.2D, 4.2E). When fish were exposed to elevated temperature and suspended sediments combined, their $\dot{M}O_{2\text{max}}$ was not different to that of the fish from the control group.

The aerobic scope of fish exposed to the elevated temperature in isolation was not different from that of fish from the control group, with the reduction in $\dot{M}O_{2\text{rest}}$ in this group having compensated for the observed reduction in $\dot{M}O_{2\text{max}}$ (Fig. 4.2F). Suspended sediments in isolation had no effects on aerobic scope of fish. Fish exposed to the elevated temperature and 90 mgL^{-1} suspended sediments combined had a higher aerobic scope (96.9% probability, [-1.7%, +50.4%] $CI_{95\%}$) when compared to the control group, although this did not result in an interaction between the two stressors (Fig. 4.2F). Aerobic scope of fish exposed to the elevated temperature and 135 mgL^{-1} suspended sediments was not significantly different from fish from the control group, but it was higher (98.0% probability, [-1.4%, +52.4%] $CI_{95\%}$) than the aerobic scope of fish exposed to elevated temperature in isolation (Fig. 4.2F).

4.4.3 *Fast start performance*

Suspended sediment exposure influenced fast start performance of juvenile *A. polyacanthus,* while elevated temperature had no effect, and there were no interactions between the two stressors. Fish exposed to 90 mgL^{-1} suspended sediments had higher maximum escape speeds (95.9% probability, [-1.4%, +17.0%] $CI_{95\%}$), higher average escape speeds (>99% probability, [+1.1%, +16.0%] $CI_{95\%}$), and moved further away from the stimulus (>99% probability, [+0.7%, +15.3%] $CI_{95\%}$), compared to fish from the control group, irrespective of temperature exposure (Fig. 4.3A-C). Exposure to 135 mgL^{-1} suspended sediments had no effect on escape speeds or escape distance, but fish had a shorter response latency (>99% probability, [-11.6%, -50.8%] $CI_{95\%}$) when compared to fish from the control group, irrespective of temperature exposure (Fig. 3.4D).

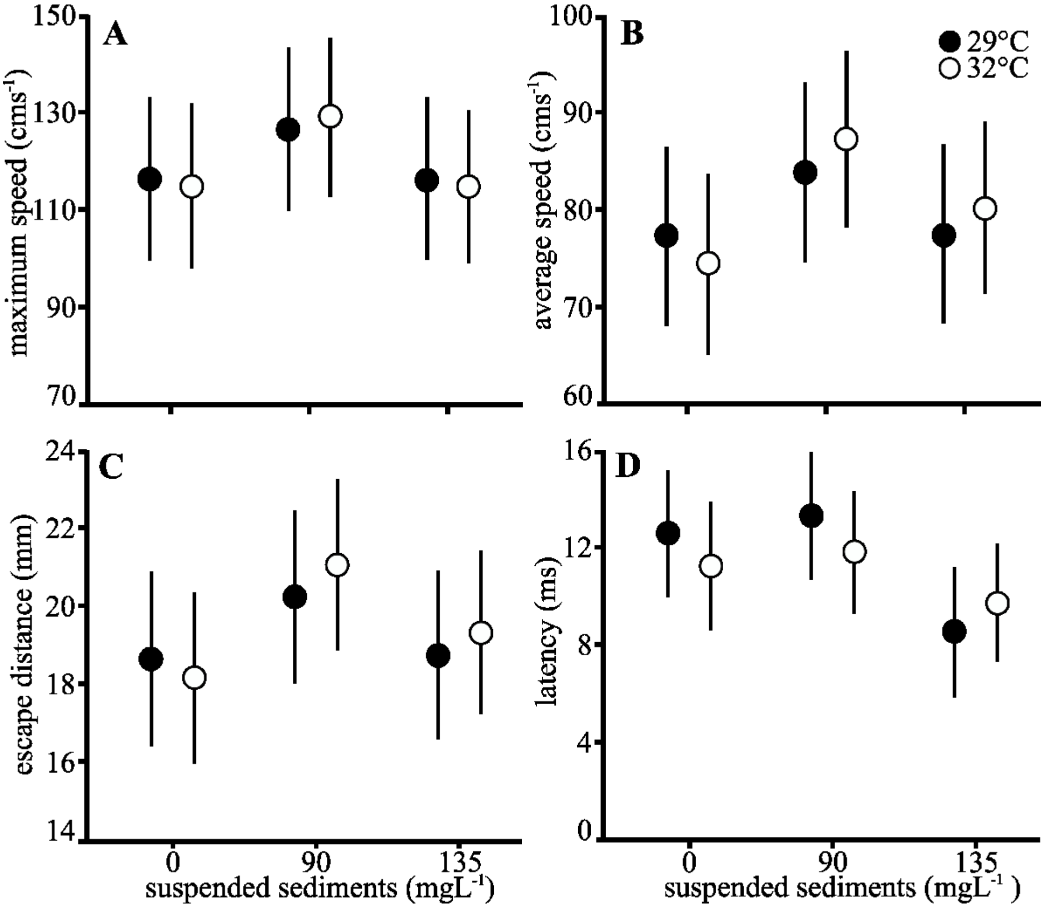

Figure 4.3 Effects of suspended sediments and elevated temperatures on fast start performance of juvenile *Acanthochromis polyacanthus*, i.e., **A)** maximum escape speed, **B)** average escape speed, **C)** distance escaped within the first 24 ms of the escape response, and **D)** latency to respond to the stimulus. Black and white circles represent fish reared under control (29 ± 0.5 °C) or elevated temperature (32 ± 0.5°C), respectively. Circles represent posterior means and bars 95% credibility intervals.

4.5 Discussion

In the present study, suspended sediments and elevated temperature acted mostly independently or antagonistically on gill morphology, aerobic performance, and predator escape performance of juvenile *A. polyacanthus*. Suspended sediments and/or elevated temperature independently led to morphological changes of the gills (i.e., reductions in filament thickness and gas diffusion distance) that are likely beneficial for gas exchange efficiency (Evans et al. 2005, Lappivaara 1995). In contrast, suspended sediments and elevated temperature acted antagonistically on aerobic performance, with suspended sediments masking some of the effects of elevated temperature, resulting in an enhanced aerobic scope in fish exposed to both stressors combined compared to control fish. Lastly, predator escape performance was solely influenced by suspended sediments, with fish exposed to suspended sediments exhibiting an enhanced predator escape performance, indicating an increased perceived predation risk in turbid water (Domenici et al. 2010, Ramasamy et al. 2015). Interestingly, the observed changes in gill morphology, aerobic performance, and predator escape performance did not appear to be linked; rather, the cumulative effects of suspended sediments and temperature were trait-specific, highlighting the importance of examining responses of fish at multiple levels of organization.

Suspended sediments and elevated temperature, whether in isolation or in combination, led to similar changes in gill morphology. Exposure to the highest suspended sediment concentration (135 mgL^{-1}) induced a reduction in gas diffusion distance, while elevated temperature led to a reduction in gas diffusion distance as well as a reduction in filament thickness compared to control fish. Exposure to suspended sediments and elevated temperature combined did not amplify these changes, yet resulted in an increase in lamellar length at moderate suspended sediment concentration (90mgL^{-1}), thereby increasing the surface area available for gas exchange. These changes would be expected to enhance gas exchange efficiency and are similar to findings of previous studies documenting gill remodeling in response to elevated temperature and suspended sediments in isolation (e.g., Chapter 2, Phuong et al. 2017, Sollid et al. 2006). Suspended sediments in the present study, however, did not lead to gill changes that may decrease gas exchange efficiency, contrasting the majority of earlier studies (e.g., Cumming and Herbert 2016; Lowe et al. 2015), which may be due to differences in tolerance to suspended sediments between species or life stages (Wenger et al. 2017). While the observed gill remodeling in the present study may be adaptive under conditions of elevated temperatures and suspended sediments, they are likely to come at the cost of a lower efficiency of ion- and osmoregulation, and an increased vulnerability to pathogens (Nilsson et al. 2012).

Despite suspended sediments in isolation not having an effect on aerobic performance, suspended sediments masked the effects of elevated temperature on $\dot{M}O_{2max}$ of juvenile *A. polyacanthus*. Fish exposed to elevated temperature in isolation exhibited a decrease in $\dot{M}O_{2max}$ compared to control fish, which is a response that is often observed in coral reef fishes that live at the upper end of their thermal range (e.g., Gardiner et al. 2011, Rummer et al. 2014). Juvenile fish exposed to both stressors combined, however, exhibited no differences in $\dot{M}O_{2max}$ relative to control fish, which - in combination with the observed reduction in $\dot{M}O_{2rest}$ – resulted in an enhanced aerobic scope. While an enhanced aerobic scope would be expected to increase a fish’s capacity for aerobic activities such as locomotion (Norin and Clark 2016), the underlying mechanisms that would lead to such a masking effect are unclear. It can be speculated that an increase in perceived predation risk in suspended sediments (see below) may have driven a re-allocation of resources under elevated temperature, leading to an increased oxygen demand. The associated costs of such a re-allocation of resources are unknown, but may potentially involve the suppression of the immune system, which is a common response to chronic stress (reviewed in Tort 2011). This topic warrants further investigation.

Elevated temperature reduced $\dot{M}O_{2rest}$ in juvenile *A. polyacanthus*, regardless of suspended sediment exposure. This result contradicts the majority of previous studies, which report an exponential increase in $\dot{M}O_{2rest}$ with acute increases in temperature across various taxa and temperature ranges (Schulte 2015, Rummer and Munday 2017). However, recent studies have found that acclimation to elevated temperatures can change the relationship between $\dot{M}O_{2rest}$ and temperature considerably. For example, $\dot{M}O_{2rest}$ of *Chromis viridis* (blue-green damselfish) acclimated to elevated temperatures for 6 weeks increased from 23°C to 25°C and then plateaued until 33°C (Habary et al. 2017). Similarly, *Fundulus heteroclitus* (mummichog), following temperature acclimation for 1 week, displayed a continuous increase in $\dot{M}O_{2rest}$ from 5°C to 30°C with a trend towards a decline in $\dot{M}O_{2rest}$ thereafter to 35°C (Healy and Schulte 2012). Further, *A. polyacanthus* has shown partial and complete recovery of $\dot{M}O_{2rest}$ to control values following developmental and transgenerational exposure to elevated temperatures, respectively (Donelson et al. 2011 and 2012). A lower $\dot{M}O_{2rest}$ typically indicates lower metabolic costs associated with maintenance or baseline metabolic processes, and is thus generally considered beneficial (Fry 1971, Norin and Clark 2016). However, a reduction in $\dot{M}O_{2rest}$ in juvenile fish may potentially also indicate a depression in growth or development, which has been observed in response to other unfavorable environmental conditions, such as low food availability (e.g., O'Connor et al. 2005). Future studies should aim to unravel the underlying mechanisms that may have driven a decrease in $\dot{M}O_{2rest}$ under elevated temperature in *A. polyacanthus*.

Interestingly, there seemed to be no clear connection between changes in gill morphology and oxygen uptake of juvenile *A. polyacanthus*. In particular, the reduction in gas diffusion distance in fish exposed to 135mgL^{-1} suspended sediments would have been expected to lead to an enhanced oxygen uptake capacity, yet $\dot{M}O_{2max}$ in this treatment group was not different from control fish. There are several possible explanations for this. First, it cannot be excluded that $\dot{M}O_{2max}$ was underestimated in the present study, as oxygen uptake was examined under the assumption that fish would reach maximum oxygen uptake rates during recovery from exhaustive exercise (see e.g., Roche et al. 2013). Future studies should therefore measure oxygen uptake rates directly during maximum sustained swimming speeds using a swim respirommeter (Roche et al. 2013). Second, suspended sediments may have caused an initial decrease in $\dot{M}O_{2max}$ by binding to mucous on gill surfaces (e.g., Li et al. 2012, Sutherland and Meyer 2007), but this decrease may have then been compensated for by gill remodeling. Alternatively, the oxygen uptake capacity of the gills in some species is much higher than the capacity of the cardiovascular system to transport oxygen and/or the maximum oxygen demand of tissues (Hillman et al. 2013, Nilsson et al. 2007). Oxygen uptake may thus be relatively tolerant to changes in gill morphology, which is supported by a growing number of studies failing to find a strong link between changes in gill morphology and oxygen uptake rates (Chapter 2, Cumming and Herbert 2016). It has been suggested, however, that CO_2 removal may be limited by diffusion across the gill epithelium (Hillman et al. 2013). While no changes in $\dot{M}O_{2max}$ were observed in the present study, it may thus be possible that the reduction in gas diffusion distance may have led to changes in CO_2 excretion, which is an interesting avenue for future research.

Despite the antagonistic effects of elevated temperature and suspended sediments on aerobic scope, predator escape performance was solely influenced by suspended sediments. Fish exposed to 90 mgL^{-1} suspended sediments escaped at higher average and maximum speeds and further away from a mechanical stimulus compared to control fish, while fish exposed to 135 mgL^{-1} suspended sediments responded faster to the stimulus. Although the underlying reasons for these differences between suspended sediment concentrations remain unclear, the results overall support the findings of a previous study in another damselfish species (Chapter 3). Suspended sediments interfere with the detection of predators approaching from a distance (Meager et al. 2006), and the observed enhanced predator escape performance was thus likely driven by an increased perceived predation risk in turbid waters (Leahy et al. 2011). An improved predator escape performance increases survivorship of juvenile coral reef fishes (McCormick et al. 2018), but is likely also associated with non-consumptive costs such as increased energy expenditure and prolonged recovery time (Killen et al. 2015, Preisser et al. 2005). In contrast to suspended sediments, elevated temperature did not affect fast start performance of fish. This finding is not surprising, as previous studies on coral reef fishes have reported mixed effects of elevated temperature on predator escape performance; while a 2-3°C increase in temperature compromised the escape performance of several species of wrasses (Labridae; Motson et al. 2017) and a damselfish (*Pomacentrus wardi*; Allan et al. 2015), the escape performance of two other damselfishes, *P. moluccensis* and *P. amboinensis*, remained unaffected upon similar increases in temperature (Warren et al. 2017).

Rapid population growth in coastal areas and climate change are projected to lead to further increases in suspended sediment concentrations and water temperatures on inshore coral reefs globally (Halpern et al. 2015, Stocker et al. 2013). The findings of the present study show that suspended sediments did not compound the effects of elevated temperature on the gill morphology, aerobic performance, and predator escape performance of a common coral reef fish. Rather, these two stressors acted mostly independently and even antagonistically, appearing to reduce the overall impact on individuals. Future studies should focus on investigating the cumulative impacts of suspended sediments and elevated temperature on species at higher trophic levels, in particular predators, and should consider potential interactions with other environmental factors that are expected to change under predicted future climate change scenarios, such as elevated pCO_2 and hypoxia.

Chapter 5: Effects of suspended sediments and elevated water flow on gill structure and function of a coral reef fish

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5.1 Abstract

Increasing sediment inputs and ocean warming pose major threats to the health of coastal coral reefs. While each factor in isolation can affect the physiology and performance of coral reef fishes, little is known of their cumulative effects. In this chapter, a common coral reef fish, *Acanthochromis polyacanthus,* was reared under current day (29°C) or projected future temperature (32°C) for 4.5 weeks. During the final week of exposure, suspended sediments (0, 90, or 135 mgL^{-1}) were added to aquaria to simulate a turbidity event (such as a flood plume) under both current day and projected future temperatures, in a full factorial design. Following exposure, gill morphology, aerobic performance, and predator escape performance of fish were examined. While exposure to 90 mgL^{-1} suspended sediments had no effects on gill morphology, 135 mgL^{-1} suspended sediments led to a reduction in gas diffusion distance (i.e., between -5.4% and -21.7%; 95% credibility interval ($CI_{95\%}$)), relative to control fish (i.e., fish exposed to 29°C and 0 mgL^{-1} suspended sediments). Elevated temperature led to reductions in both gas diffusion distance ([-8.8%, -24.2%] $CI_{95\%}$) and filament thickness ([-5.4%, -21.7%] $CI_{95\%}$) relative to control fish, which are changes generally associated with improved gas exchange efficiency. Suspended sediments and elevated temperature combined did not have any interactive effects on gill morphology. The changes in gill morphology were not reflected by the observed changes in oxygen uptake rates; elevated temperature in isolation led to reductions in maximum ([-23.6%, +1.1%] $CI_{95\%}$) and resting oxygen uptake rates [-5.4%, -40.5%] $CI_{95\%}$), while suspended sediments had no effects on oxygen uptake rates in isolation. In fish exposed to both stressors combined, maximum oxygen uptake rates were not different from control fish, resulting in an enhanced aerobic scope compared to control fish regardless of suspended sediment concentration (i.e., [-1.7%, +50.4%] $CI_{95\%}$ at 90 mgL^{-1} and [-1.4%, +52.4%] $CI_{95\%}$ at 135 mgL^{-1}). Predator escape performance was solely influenced by suspended sediments, with fish exhibiting enhanced fast starts following suspended sediment exposure (i.e., increased average ([+1.1%, +16.0%] $CI_{95\%}$) and maximum escape speeds ([-1.4%, +17.0%] $CI_{95\%}$), and greater escape distances ([+0.7%, +15.3%] $CI_{95\%}$) at 90mgL^{-1} and shorter response latency at 135mgL^{-}

^{1} ([-11.6%, -50.8%] $CI_{95\%}$), respectively) compared to control fish. This response was likely driven by an increased perceived predation risk in turbid waters due to a compromised ability to detect predators. These surprising findings show that suspended sediments did not compound, but rather masked some of the negative effects of elevated temperature on juvenile *A. polyacanthus*. While this interaction between the two stressors may help *A. polyacanthus* acclimate to future environmental conditions on inshore coral reefs, future studies will need to examine potential trade-offs with other important performance traits or functions.

5.2 Introduction

Declining water quality, in particular increasing suspended sediment concentrations, threatens the health of many inshore coral reefs worldwide (Burke et al. 2011). Suspended sediments on inshore waters have been increasing due to coastal development, agriculture, mining, dredging, shipping and other anthropogenic activities (Foley et al. 2005, Syvitski et al. 2005). Suspended sediments affect the diversity and biomass of fish assemblages, both indirectly through changes in benthic communities (Bejarano and Appeldorn 2013, Cheal et al. 2013, Moustaka et al. 2018) and through direct effects on the fish themselves, including changes in feeding efficiency (De Robertis et al. 2003, Johansen and Jones 2013) and predator prey interactions (Meager et al. 2006, Wenger et al. 2013, Chapter 3). Suspended sediments have also been shown to reduce the oxygen uptake capacity in some fish species via changes in gill morphology, thereby reducing the capacity of fish for aerobic activities (Chapter 2, Humborstad et al. 2006, Lowe et al. 2015). A reduced capacity for aerobic activities may have particularly severe consequences for fish with high activity levels and oxygen demands, such as species swimming long distances (e.g., tuna, Korsmeyer et al. 1996) and fish living under elevated water flow conditions (Binning et al. 2015). As elevated water flow promotes the re-suspension of sediments (Larcombe et al. 2001, Wilson et al. 2008), fish on inshore coral reefs are commonly exposed to elevated water flow conditions and suspended sediments combined (Wenger et al. 2016). Yet, studies examining the potentially interactive effects between suspended sediments and elevated water flow on the physiological performance of coral reef fishes are currently missing.

Suspended sediments have had mixed effects on gill morphology and oxygen uptake rates in previous studies (e.g., Lake and Hinch 1999, Wong et al. 2013, Chapter 1).

Suspended sediments can reduce gas exchange efficiency by binding to the mucous layer on the gill surface (Li et al. 2012, Sutherland and Meyer 2007), reducing lamellar length (Lake and Hinch 1999), and inducing the growth of protective cell layers on the gill epithelium with a subsequent increase in gas diffusion distance (i.e., the distance between the water and blood sinus) (Hess et al. 2015, Wong et al. 2013). Some fish species, however, have also exhibited gill changes that may enhance gas exchange efficiency following suspended sediment exposure, such as a reduction in gas diffusion distance (Au et al. 2004, Chapter 2). Accordingly, the effects of suspended sediments on oxygen uptake processes varies among species. Some species are able to maintain maximum oxygen uptake rates during peak performance (or recovery thereof, $\dot{M}O_{2\mathrm{max}}$) despite changes in gill morphology induced by suspended sediments (Cumming and Herbert 2016, Chapter 2). This indicates that oxygen uptake capacity in these species is likely limited by factors other than gill morphology, such as the oxygen carrying capacity of the blood or the aerobic capacity of muscle tissue (discussed in Chapter 2). In contrast, a damselfish species, *Amphiprion melanopus*, exposed to suspended sediments exhibited a reduction in $\dot{M}O_{2\mathrm{max}}$, which resulted in a reduction in aerobic scope (i.e., the difference between $\dot{M}O_{2\mathrm{max}}$ and oxygen uptake rate at rest) and thus a reduce capacity for aerobic activities (Chapter 2).

Inshore reefs are often characterized by moderate to high tidal and current flows (Larcombe et al. 2001). Several fish species have been shown to remodel their gill morphology in response to elevated water flow and associated increases in swimming activity, including decreases in gas diffusion distances and increases in gill surface area (Fu et al. 2014, Perry et al. 2012). By enhancing gas exchange efficiency at the gills, changes in gill morphology – in combination with other changes in the oxygen cascade, such as increases in cardiac output via increased stroke volume and/or heart rate and increases in haematocrit - allow for an increased oxygen supply to muscle tissues (Brauner et al. 2011, Fu et al. 2014). Swimming activity further induces long-term changes, such as an increased number of mitochondria, that enhance the capacity of muscle tissues for oxygen utilization, thereby leading to an increase in $\dot{M}O_{2\mathrm{max}}$ and aerobic scope (Brauner et al. 2011, Fu et al. 2014). An enhanced aerobic capacity of muscle tissue allows fish to sustain higher swimming speeds for longer periods and thus to inhabit areas characterized by high water flow velocities (Binning et al. 2014, Herskin and Steffensen 2005). A high oxygen demand, however, may also potentially increase the vulnerability of fish to changes in gill morphology that reduce or limit oxygen uptake rates induced by suspended sediment exposure. Further, increased swimming activity would be expected to increase the rate of suspended sediment particles passing over the gill surface area due to increased gill ventilation. This may potentially compound any negative effects of suspended sediments on the gill morphology.

The aim of this chapter was to investigate the effects of suspended sediments and elevated water flow on the gill morphology and aerobic performance of juvenile five-line cardinalfish *Cheilodipterus quinquelineatus*. This species is commonly found on turbid inshore coral reefs of the Great Barrier Reef (Randall et al. 1997), including reef flats and seaward reefs (Paxton et al. 1989), which are also typically exposed to tidal currents and/or waves. The present study is the first to examine the effects of suspended sediments on a coral reef fish in context of different water flow conditions, which will improve our understanding of the effects of local environmental conditions on the responses of coral reef fish communities to declining water quality. I hypothesize that:

i) *suspended sediments in isolation will lead to a suite of gill morphological changes that are associated with contrasting effects on oxygen uptake efficiency (i.e., such as reduced functional lamellar length and decreases in gas diffusion distance) and a compromised aerobic performance;*
ii) *elevated water flow in isolation will lead to gill morphological changes that enhance oxygen uptake efficiency and enhanced aerobic performance; and*
iii) *suspended sediments and elevated water flow combined will have additive or synergistic effects on gill morphology and aerobic performance.*

5.3 Materials and methods

5.3.1 *Experimental protocol*

Settlement-stage *C. quinquelineatus* were collected with light traps (BellaMare, San Diego, CA) on inshore reefs surrounding Orpheus Island (18°36'S, 146°29'E) on the Great Barrier Reef (GBR), Australia. Fish were maintained at 30.0° ± 0.5°C in 20 L aquaria provided with pieces of PVC pipes for shelter and were fed *Artemia* sp. nauplii (ProAqua, Brisbane, Queensland, Australia) twice daily. After two days, fish were randomly allocated to one of three suspended sediment treatments, i.e. 0, 90 or 180 mgL^{-1} (0.5 ± 0.3, 15.2 ± 0.7, and 30.0 ± 1.7 NTU) and one of two water flow treatments, i.e. low (< 2 cms^{-1}) or increased water flow velocity (20.0 ± 5 cms^{-1} for six hours day^{-1}), in a full factorial design for three weeks. To habituate fish to the increased water flow conditions, water flow velocity was step-wise increased over the course of four days at the beginning of treatment exposure. Australian bentonite was used as sediment, as it is a clay with a small particle size range (< 63 µm) and is representative of terrestrial-derived sediments found in suspension on inshore reefs on the GBR (Larcombe et al. 2001, Wolanski et al. 2008). The selected suspended sediment concentrations simulated conditions observed on inshore reefs on the GBR during periodic events such as flood plumes (Wolanksi et al. 2008; Bainbridge et al. 2012; Wenger et al. 2016), and the increased water flow velocity and duration is within average values recorded at inshore reefs exposed to currents (Hamner and Hauri 1981, Johansen 2013).

To maintain suspended sediment levels, bentonite was re-suspended in external sumps using submersible pumps. Water was delivered to the conical base of 3L aquaria (three tanks per treatment, with five fish per tank) and drained through an overflow outlet at the top of the aquaria back into the sumps (as described in Hess et al. 2017). The conical base of aquaria prevented the accumulation of sediment and also facilitated the regulation of water flow velocity. Sumps and aquaria were flushed daily with fresh seawater for an hour (>100% water exchange) to remove waste and sediments. Sediment was then added again to the sumps and turbidity levels returned to respective treatment levels in aquaria within 20 min. Turbidity levels were checked daily with a turbidity-meter (WP88, ThermoFisher Scientific, Scoresby, Victoria, Australia). In the elevated water flow treatment, water flow was increased in the morning to 20.0 ± 5 cm s^{-1} and was returned to control levels (< 2 cm s^{-1}) six hours later. Water flow within each individual aquarium was highest at the inlet (i.e., at the base of aquarium; 22.5 ± 2.5 cm s^{-1}) and lowest near the outlet (i.e., near the top of the aquarium;

17.5 ± 2.5 cm s^{-1}). Reported water flow velocity was determined at the outlet. No information is currently available regarding the maximum sustained swimming speed of post-settlement *C. quinquelineatus* juveniles; however, mean maximum sustained swimming speeds of 38.2 cm s^{-1} and 26.3 cms^{-1} have been reported for settlement-stage larvae and post-settlement juveniles, respectively, for three pomacentrid species of similar size ranges to the individuals used in the present study (Stobutzki and Bellwood 1994). Juvenile *C. quinquelineatus* were able to swim in aquaria for six hours without any visible signs of struggle or exhaustion. No shelter was provided in aquaria to prevent individuals seeking refuge from water flow exposure.

5.3.2 *Oxygen uptake rates*

Following the 3-week treatment exposure, oxygen uptake rates ($\dot{M}O_2$) of fish (n = 9 – 12 per treatment) were measured using intermittent-flow respirometry (Steffensen 1989). Fish were fasted for 24 hours prior to trials (Niimi and Beamish 1974) during which time fish of all treatments were kept at control water flow to reduce risk of energy depletion and starvation. A chase protocol was used to determine maximum $\dot{M}O_2$ (i.e., $\dot{M}O_{2max}$), which assumes that fish achieve $\dot{M}O_{2max}$ while recovering from exhaustive exercise (Steffensen 2005). Fish were chased by hand for 2 min in a circular container (60 cm diameter, 10 cm water depth). This was sufficient to exhaust fish (i.e., they ceased to display burst swimming). After the chase, fish were immediately transferred to glass respirometry chambers (40 mm length x 18 mm diameter, 13.3 ml volume including recirculation loop) that were fitted with black sleeves to prevent visual disturbance of fish. Each respirometry chamber was connected to a recirculation loop and pump to ensure homogenous oxygen levels throughout chambers (Rodgers et al. 2016). Oxygen levels were recorded at 2 Hz using an oxygen-sensitive sensor spot inside the recirculation loop and fibre-optic oxygen meter (Pyro-Science, Aachen, Germany), and laptop with associated software (Labchart v. 6.1.3, ADinstruments, Auckland, New Zealand). Chambers were flushed every 5 min with fully aerated, UV-filtered water from the surrounding water bath (oxygen levels remained >90% oxygen saturation at all times) for 2 min. Fish were left undisturbed for 9 hours as oxygen levels were continuously monitored. Oxygen uptake rates of all fish stabilized within 2 hours after the chase, indicating that fish had fully recovered within that time. Oxygen uptake rates of early life history stages of fishes, as used in the present study are usually recorded for < 12 hours to reduce risk of starvation due to their high growth rates (e.g., Killen et al. 2014). Prior to and after each trial (i.e., after completing the hypoxia challenge, as described below), background (microbial) $\dot{M}O_2$ in blank chambers was recorded for 15 min (Rodgers et al. 2016).

Oxygen uptake during closed respirometry phases were derived using linear least square regression of oxygen levels over time. Only slopes with $R^2 > 0.95$ were retained. Background respiration was subtracted from total respiration in chambers to calculate oxygen uptake rates of fish (Rodgers et al. 2016). The highest $\dot{M}O_2$ value of each individual was taken as $\dot{M}O_{2max}$ and always occurred during the first closed phase after the chase. Resting $\dot{M}O_2$ of fish ($\dot{M}O_{2rest}$) was derived as the average of the 20% lowest $\dot{M}O_2$ values of the trial, after excluding the first 2 hours of the trial while fish were recovering from the chase (the mean of the lowest normal distribution method was not used because the coefficient of variation was too high (>5), as advised in Chabot et al. 2016). Aerobic scope was calculated as the difference between $\dot{M}O_{2max}$ and $\dot{M}O_{2rest}$.

5.3.3 *Gill morphology*

Changes in gill morphology of 8-10 randomly selected fish per treatment was determined using histology and light microscopy. Whole fish were dehydrated, cleared, embedded in paraffin wax, and then sectioned at 5 µm thickness; three sections per individual were stained with Mayer’s hematoxylin and eosin (H & E). Gills were photographed (Olympus DP12 Microscope Digital Camera System) at 400 x magnification.

Measurements of gill morphology were performed using ImageJ (v. 1.51n, National Institutes of Health, Rockville, MD, USA) on n = 10 lamellae (i.e., secondary filaments) per fish. The following measurements were made; 1) total length of lamellae, 2) functional length of lamellae, defined as the lamellar length not covered by the filament epithelium, 3) thickness of the filament epithelium, and 4) gas diffusion distance. To determine gas diffusion distance, the area of the lamellar pillar cell system above the filament epithelium (i.e., functional length) was measured and subtracted from the total area of the lamellum (resulting in the area of the epithelium), which was then divided by twice the functional lamellar length (see Hess et al. 2015 for further details). Epithelial lifting was not measured, as it was present in < 5% of all lamellae. Lamellae were measured blindly with respect to treatments.

5.3.4 *Statistical analyses*

Analyses were carried out in R (v. 3.4.3, The R Foundation for Statistical Computing 2017). Linear mixed models and linear models were used to examine the effects of suspended sediments, water flow, and their interaction on gill morphology and oxygen uptake rates ($\dot{M}O_{2max}$, $\dot{M}O_{2rest}$ and aerobic scope). The mean-centered standard length of fish was included as a covariate in models examining gill morphology, while the mean-centered mass of fish was included as a covariate in models examining oxygen uptake rates. Fish identity was included as a random factor in models examining gill morphology to account for multiple measurements per individual. Significance of fixed effects and interactions were determined using Wald tests and post-hoc Tukey tests. Model residuals were visually assessed for normality and homogeneity with quantile-quantile plots. Figures depict estimated means and standard errors of a fish of mean length or mass, respectively.

5.3 Results

5.4.1 *Fish mass and standard length*

Mass and length of juvenile *C. quinquelineatus* differed significantly between treatments (mass, $F_{5,57} = 5.5$, $p < 0.001$; length, $F_{5,57} = 6.6$, $p < 0.001$, Fig. 5.1). This was driven by an increased mass and length of fish reared under elevated water flow and 0 mgL^{-1} suspended sediments compared to fish from the other treatments, while fish reared under elevated water flow and 180 mgL^{-1} suspended sediments exhibited a reduced mass and length, respectively (Fig. 5.1A and B).

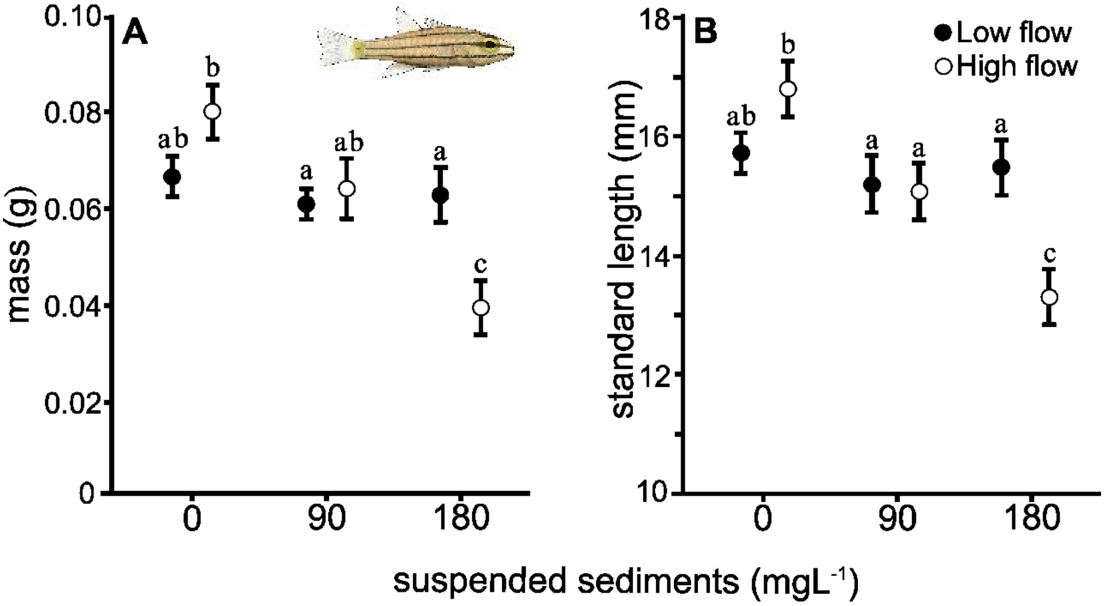

Figure 5.1 Mass **A)** and standard length **B)** of juvenile *Cheilodipterus quinquelineatus* following exposure to suspended sediments under low water flow (< 2 cm s^{-1}; filled circle) and high water flow (20.0 ± 5.0 cms^{-1} for 6 hours day^{-1}; open circle). Circles and bars represent means and standard errors, respectively. Groups with different letters were significantly different at $\alpha < 0.05$.

5.4.2 *Oxygen uptake rates*

Elevated water flow led to an enhanced aerobic performance in juvenile *C. quinquelineatus* compared to fish living under control conditions (i.e., reared under 0 mgL^{-1} suspended sediments and low flow conditions), while fish reared under elevated water flow and suspended sediments combined did not show any changes in aerobic performance. More specifically, $\dot{M}O_{2max}$ was significantly increased in fish reared under elevated water flow conditions and 0 mgL^{-1} suspended sediments relative to control fish (95% confidence interval of difference between groups, $CI_{95\%}$ = [0.004 mgO$_2$h^{-1}, 0.058 mgO$_2$h^{-1}], $t = 2.35$, $p = 0.02$, Table 5.1, Fig. 5.2). In contrast, $\dot{M}O_{2max}$ of fish reared under elevated water flow and suspended sediments, regardless of concentration, did not differ compared to control fish (Table 5.1, Fig. 5.2). Neither elevated water flow nor suspended sediments had any effects on $\dot{M}O_{2rest}$ of fish (Table 5.1). Reflecting the changes observed in $\dot{M}O_{2max}$, aerobic scope in fish reared under elevated water flow conditions and 0 mgL^{-1} suspended sediments was enhanced compared to control fish ($CI_{95\%}$ = [0.003 mgO$_2$h^{-1}, 0.058 mgO$_2$h^{-1}], $t = 2.23$, $p = 0.02$, Table 5.1, Fig. 5.2), while none of the other treatment groups exhibited any changes in aerobic scope compared to control fish (Table 5.1).

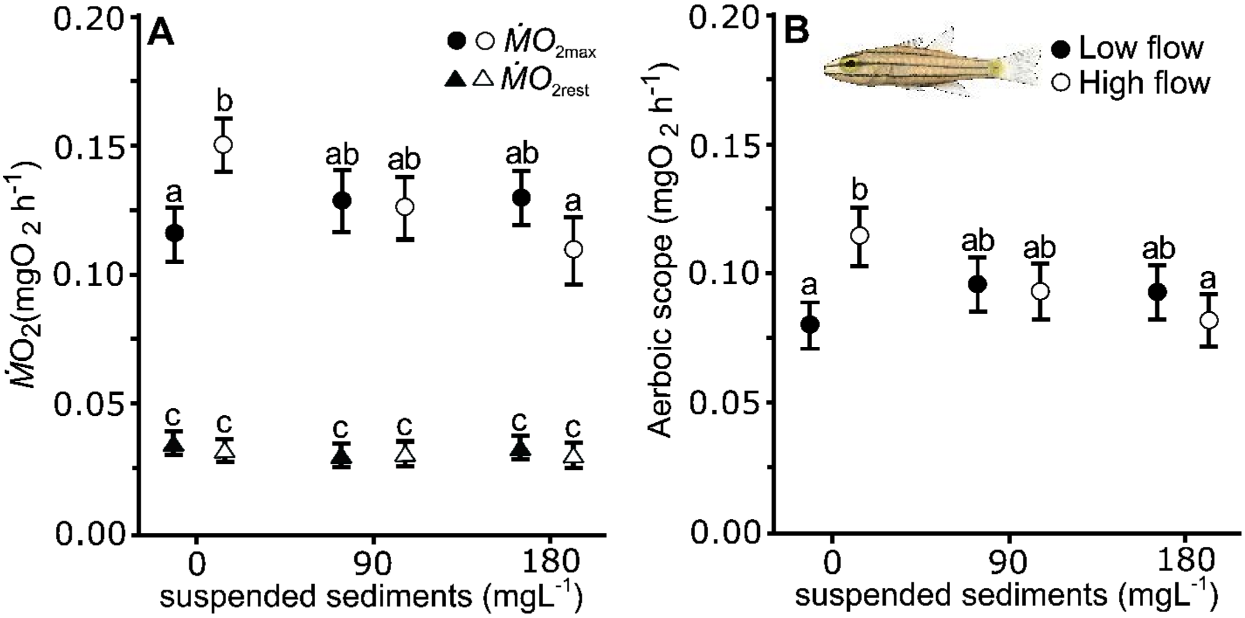

Figure 5.2 Aerobic performance of *C. quinquelineatus* juveniles reared under suspended sediments at low (< 2 cm s^{-1}; filled symbols) and elevated (20.0 ± 5.0 cms^{-1} for 6 hours day^{-1}; open symbols) water flow, respectively. Estimated mean **A)** maximum oxygen uptake rates ($\dot{M}O_{2max}$; circles), resting oxygen uptake rates ($\dot{M}O_{2rest}$; triangles) and **B)** aerobic scope (circles) of a fish of mean mass. Bars represent standard errors. Groups with different letters were significantly different at $\alpha < 0.05$.

Table 5.1 Estimates, standard errors (SE) and 95% confidence intervals (lower (CI_L) and higher (CI_H) limits, respectively) of the effects of suspended sediments and elevated water flow on the oxygen uptake rates of juvenile *C. quinquelineatus* from linear models. Bold *p*-values indicate significant factors ($\alpha < 0.05$). Intercepts represent estimates of a fish of the control group (i.e., fish exposed to 0 mgL^{-1} suspended sediments under low flow) of average mass, while estimates of the remaining factors represent differences relative to the intercept.

Response variables	**Factors**	**Estimates**	**SE**	**CI_L**	**CI_H**	***t***	***p***
$\dot{M}O_{2max}$ (mgO_2h^{-1})	Intercept (0 mgL^{-1} and low flow)	0.119	0.010	0.100	0.137	11.73	<0.001
	90 mgL^{-1}	0.010	0.014	-0.019	0.038	0.68	0.49
	180 mgL^{-1}	0.012	0.014	-0.014	0.039	0.84	0.40
	High flow	**0.037**	**0.014**	**0.004**	**0.058**	**2.59**	**0.01**
	90 mgL^{-1}:Elevated water flow	-0.034	0.020	-0.075	0.006	-1.68	0.09
	180 mgL^{-1}:Elevated water flow	**-0.052**	**0.020**	**-0.094**	**-0.010**	**-2.52**	**0.01**
	Mass	**1.142**	**0.239**	**0.661**	**1.623**	**4.77**	**<0.001**
$\dot{M}O_{2rest}$ (mgO_2h^{-1})	Intercept (0 mgL^{-1} and low flow)	0.034	0.002	0.029	0.041	13.29	<0.001
	90 mgL^{-1}	-0.003	0.004	-0.014	0.005	-0.83	0.40
	180 mgL^{-1}	0.002	0.004	-0.007	0.011	0.61	0.54
	High flow	-0.001	0.004	-0.008	0.010	-0.21	0.82
	90 mgL^{-1}:Elevated water flow	0.000	0.006	-0.015	0.012	0.05	0.96
	180 mgL^{-1}:Elevated water flow	-0.006	0.007	-0.021	0.006	-0.85	0.39
	Mass	**0.437**	**0.080**	**0.289**	**0.617**	**5.44**	**<0.001**
Aerobic scope (mgO_2h^{-1})	Intercept (0 mgL^{-1} and low flow)	0.083	0.009	0.064	0.102	8.46	<0.001
	90 mgL^{-1}	0.014	0.015	-0.014	0.044	0.98	0.33
	180 mgL^{-1}	0.010	0.014	-0.016	0.038	0.76	0.44
	High flow	**0.035**	**0.014**	**0.003**	**0.058**	**2.54**	**0.01**
	90 mgL^{-1}:Elevated water flow	-0.038	0.021	-0.074	0.009	-1.77	0.08
	180 mgL^{-1}:Elevated water flow	**-0.048**	**0.021**	**-0.087**	**-0.002**	**-2.20**	**0.03**
	Mass	**0.763**	**0.250**	**0.198**	**1.179**	**3.05**	**0.003**

5.4.3 *Gill morphology*

The observed changes in oxygen uptake in juvenile *C. quinquelineatus* were not reflected by changes in gill morphology. While suspended sediments had some independent trait-specific effects on gill morphology, and suspended sediments and elevated water flow had significant interactive effects on lamellar length, there was no consistent pattern across treatments (Table 5.2, Fig. 5.3A and B, Fig. 5.4A). More specifically, suspended sediments had a significant effect on filament thickness. Filament thickness of fish from the control group was not different than in fish from any other treatment group. Filament thickness of fish reared under 180 mgL^{-1} suspended sediments (regardless of water flow conditions), however, was significantly increased when compared to fish reared under elevated water flow conditions in the absence of suspended sediments ($CI_{95\%}$ = [0.17 μm, 1.74 μm], $t = 2.27$, $p =$ 0.02, Table 5.2, Fig. 5.4A). Functional lamellar length of fish reared under 180 mgL^{-1} suspended sediments under high water flow conditions was significantly decreased compared to fish from the control group ($CI_{95\%}$ = [0.052 μm, 5.97 μm], $t = 2.03$, $p = 0.04$, Table 5.2, Fig. 5.4B). Total lamellar length of fish reared under 180 mgL^{-1} suspended sediments and high water flow conditions was not significantly different than in control fish (Table 5.2, Fig. 5.4C). However, total lamellar length of fish reared under 180 mgL^{-1} suspended sediments and low water flow was significantly increased when compared to all other treatment groups (e.g., relative to fish reared in the absence of suspended sediments under high water flow conditions: $CI_{95\%}$ = [-5.62 μm, -0.10 μm], $t = -2.07$, $p = 0.04$), with the exception of the control group (Table 5.2, Fig. 5.4C). Neither suspended sediments nor elevated water flow had any effects on gas diffusion distance (Table 5.2).

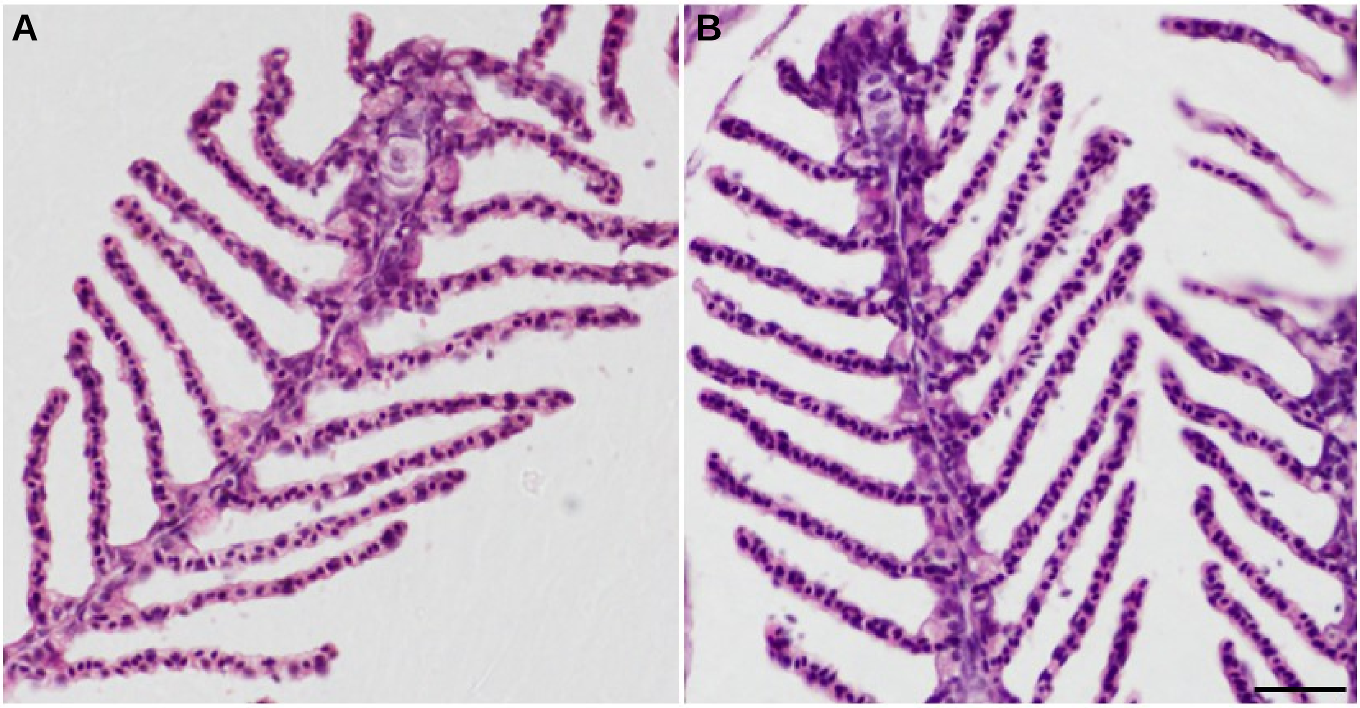

Figure 5.3 Micrographs depicting secondary lamellae of gills of *C. quinquelineatus* juveniles following exposure to **A)** 0 mgL^{-1} suspended sediments at low water flow (< 2 cm s^{-1}) and **B)** 180 mgL^{-1} suspended sediments at elevated water flow (20.0 ± 5.0 cms^{-1} for 6 hours day^{-1}). Tissues were stained with Haematoxylin and Eosin, scaling bar; 15 µm.

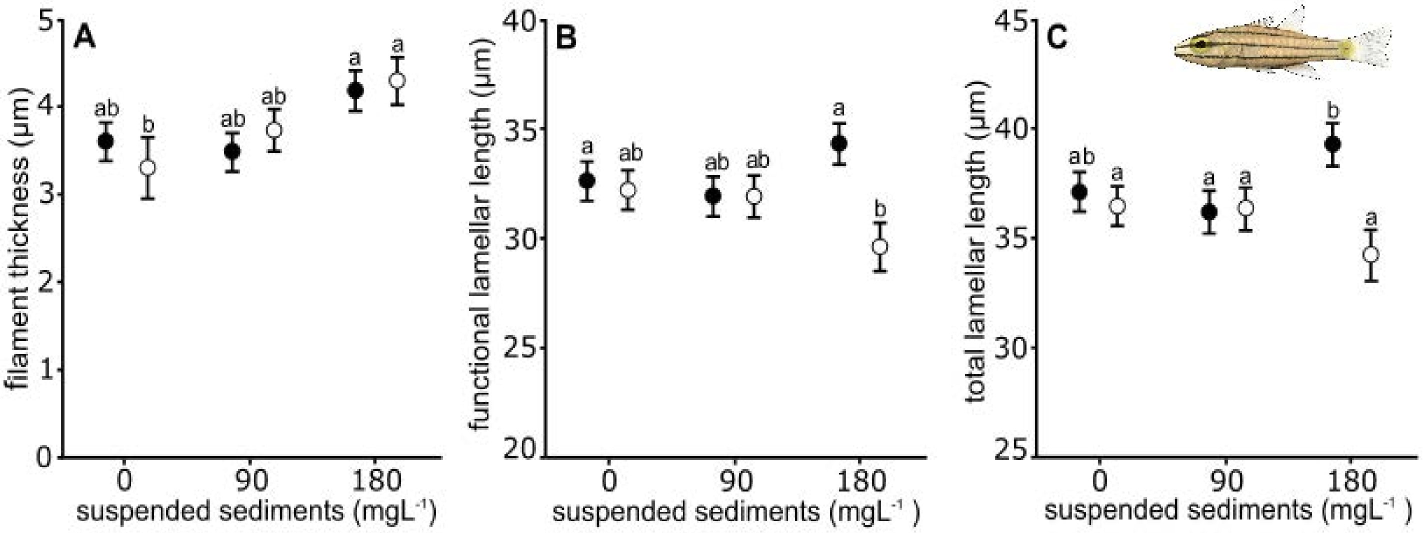

Figure 5.4 Effects of suspended sediments at low (< 2cm s^{-1}; filled circle) and elevated (20.0 ± 5.0 cms^{-1} for 6 hours day^{-1}; open circle) water flow, respectively, on the gill morphology of juvenile *C.quinquelineatus,* i.e., **A)** thickness of the filament, **B)** functional length of gill lamellae and **C)** total length of gill lamellae. Circles and bars represent estimated means and standard errors after accounting for differences in standard length and random effects. Groups with contrasting letters were significantly different at $\alpha < 0.05$.

Table 5.2 Estimates, standard errors (SE) and 95% confidence intervals (i.e., lower limit (CI$_{L}$) and higher limit (CI$_{H}$), respectively) of the effects of suspended sediments and elevated water flow on the gill morphology of juvenile *C. quinquelineatus* from linear mixed effects models. Bold *p*-values indicate significant factors ($\alpha < 0.05$). Intercepts represent the estimates for a fish of average length from the control group (i.e., 0 mgL^{-1} suspended sediments under low water flow conditions), after accounting for random effects (with exception of filament thickness, where the intercept represents fish exposed to 0 mgL^{-1} suspended sediments under high water flow conditions). The remaining factors represent estimates of differences relative to the intercept.

Response variables	**Factors**	**Estimates**	**SE**	**CI$_{L}$**	**CI$_{H}$**	***t***	***p***
Functional lamellar length (µm)	Intercept (0 mgL^{-1} and low flow)	32.66	0.90	30.86	34.46	36.16	<0.001
	90 mgL^{-1}	-0.75	1.26	-3.28	1.77	-0.59	0.55
	180 mgL^{-1}	1.66	1.32	-0.97	4.30	1.25	0.21
	Elevated water flow	-0.39	1.27	-2.92	2.14	-0.31	0.75
	90 mgL^{-1}:Elevated water flow	0.37	1.87	-3.35	-4.10	0.20	0.84
	180 mgL^{-1}:Elevated water flow	**-4.28**	**1.93**	**-8.14**	**-0.43**	**-2.21**	**0.03**
	standard length	0.83	0.41	0.00	1.66	1.99	0.05
Total lamellar length (µm)	Intercept (0 mgL^{-1} and low flow)	36.97	0.91	35.13	38.79	40.27	<0.001
	90 mgL^{-1}	-0.78	1.29	-3.35	1.78	-0.60	0.54
	180 mgL^{-1}	2.29	1.34	-0.38	4.97	1.70	0.09
	Elevated water flow	-0.57	1.29	-3.14	2.00	-0.44	0.65
	90 mgL^{-1}:Elevated water flow	0.73	1.90	-3.05	4.52	0.38	0.70
	180 mgL^{-1}:Elevated water flow	**-4.27**	**1.96**	**-8.19**	**-0.36**	**-2.17**	**0.03**
	standard length	0.68	1.90	-0.15	1.53	0.38	0.70
Filament thickness (µm)	Intercept (0 mgL^{-1} and elevated flow)	3.33	0.25	2.87	3.80	13.34	<0.001
	90 mgL^{-1}	0.39	0.37	-0.29	1.09	1.07	0.28
	180 mgL^{-1}	**0.95**	**0.41**	**0.17**	**1.74**	**2.27**	**0.02**
	Low flow	0.35	0.33	-0.27	0.99	1.05	0.29
	90 mgL^{-1}:Low water flow	-0.60	0.49	-1.53	0.32	-1.20	0.23
	180 mgL^{-1}:Low water flow	-0.46	0.51	-1.42	0.49	-0.90	0.37
	standard length	-0.60	0.49	-0.38	0.03	-1.20	0.23
Gas diffusion distance (µm)	Intercept (0 mgL^{-1} and low flow)	0.48	0.02	0.43	0.53	18.38	<0.001
	90 mgL^{-1}	0.04	0.03	-0.25	0.11	2.29	0.24
	180 mgL^{-1}	0.01	0.03	-0.05	0.08	0.32	0.74
	Elevated water flow	-0.01	0.03	-0.08	0.05	-0.29	0.76
	90 mgL^{-1}:Elevated water flow	-0.01	0.05	-0.11	0.09	-0.20	0.83
	180 mgL^{-1}:Elevated water flow	0.06	0.05	-0.04	0.16	1.10	0.27
	standard length	-0.01	0.01	-0.03	0.01	-0.84	0.40

5.4 Discussion

The capacity to acclimate physiological performance is of fundamental importance for fish to persist and succeed under changing environmental conditions. The present Chapter provides evidence that suspended sediments may affect the capacity of juvenile *C. quinquelineatus* to acclimate their aerobic performance to elevated water flow conditions. Fish reared under elevated water flow conditions in the absence of suspended sediments increased both $\dot{M}O_{2max}$ and aerobic scope compared to those reared under low water flow and 0 mgL^{-1} suspended sediments. This increase in $\dot{M}O_{2max}$ and aerobic scope was likely driven by an increased swimming activity (e.g., Binning et al. 2015, Gallaugher et al. 2001, Zhang et al. 2011). This result supports previous studies documenting the effects of exercise on aerobic scope, including in fish subjected to regular swimming activity under laboratory conditions (Gallaugher et al. 2001, Zhang et al. 2011) and in fish experiencing differences in water flow conditions in the wild (Binning et al. 2015). Swimming activity generally leads to an increase in the density of mitochondria in muscle tissue, growth of aerobic muscle fibre, and has been shown to lead to increased overall growth (Shrivastava et al. 2018, Moya et al. 2019). In support of this, fish reared under elevated water flow conditions in the present Chapter had a greater body length and mass compared to control fish. However, body length and mass were only measured after treatment exposure, and it can thus not be excluded that these differences were already present before treatment exposure. Increased growth of muscle fibre and mitochondria densities increase the amount of oxygen that can be utilized by muscle tissue at any given time, thereby enhancing the aerobic capacity of muscle tissue and, as a consequence, $\dot{M}O_{2max}$ of fish (Stoiber et al. 2002, Zhang et al. 2011). Ultimately, these changes enhance the capacity for aerobic swimming, which means that fish will be able sustain higher water flow velocities for longer before the onset of energetically costly anaerobic metabolism and the production of toxic by-products (Blasco et al. 2015, Gallaugher et al. 2001). An enhanced aerobic capacity allows fish to spend less time sheltering and recovering from swimming and more time feeding and performing other fitness-related activities. Juvenile *C. quinquelineatus* often inhabit reef flats and seaward reefs, and would thus typically be exposed to water flow velocities driven by tidal currents and waves that exceed flow velocities used in the current study (e.g., an average flow velocity of 40 cm s^{-1} was reported on the reef flat at a reef of the Lizard Island Group in the northern Great Barrier Reef; Fulton and Bellwood 2005). An enhanced aerobic capacity would therefore be likely to increase their ability to survive and thrive in such high flow environments.

While fish reared under elevated water flow conditions enhanced their aerobic performance, no such increases in $\dot{M}O_{2max}$ or aerobic scope were observed in fish reared under elevated water flow and suspended sediments combined. Surprisingly, however, the observed increases in aerobic scope and the absence thereof, respectively, were not linked to changes in gill morphology; changes in gill morphology were mostly trait-specific and did not show a consistent pattern between treatments (i.e., increased filament thickness in fish exposed to 180 mgL^{-1} suspended sediments, increased total lamellar length in fish exposed to 180 mgL^{-1} under low water flow, and reduced functional lamellar length under elevated water flow, respectively). Although the underlying mechanism is not clear, a reduced capacity of fish to enhance their aerobic performance in suspended sediments will likely impair the ability of fish to cope with high water flow velocities, as aerobic scope is closely linked to swimming performance (Binning et al. 2015). The majority of inshore coral reefs are regularly, or at least periodically, exposed to elevated water flow conditions due to currents, tidal flows and storms (Larcombe et al. 2001, Wolanski et al. 2008), and a reduced capacity of juvenile *C. quinquelineatus* to cope with these conditions may thus have far-reaching consequences for the abundance and distribution of this species on turbid reefs.

In previous studies on temperate fishes, an increased oxygen demand – either as a result of increased swimming activity or changes in environmental factors - often induced changes in gill morphology beneficial to gas exchange (e.g., Brauner et al. 2011, Fu et al. 2013). Although juvenile *C. quinquelineatus* exposed to elevated water flow conditions in the present Chapter showed an increased oxygen demand, this was not linked to any changes in gill morphology. These results are in contrast to findings in reef fish species examined in earlier Chapters that displayed gill morphological changes that may have enhanced gas exchange efficiency in response to suspended sediments or elevated temperature, such as decreases in filament thickness and gas diffusion distances (Chapter 2 and Chapter 4, see also Au et al. 2004). Further, the results of the present study do not support the idea that increased oxygen demand and the resulting increase in gill ventilation would compound the effects of suspended sediment particles on gill morphology by increasing the contact between sediment particles and the gill epithelium. However, the absence of clear changes in gill morphology in juvenile *C. quinquelineatus* in response to elevated water flow supports the results of a previous study, which concluded that this species has little capacity for gill remodelling (Bowden et al. 2014). Changes in gill morphology, however, are only one of many aspects that influence oxygen transport to tissues, and it is possible that fish in the present Chapter may have undergone other changes in the cardio-respiratory system to support an increase in oxygen demand, such as increases in haemoglobin oxygen carrying capacity or affinity, heart rate, stroke volume, and cardiac output (Farrell et al. 2003, Thorarensen et al. 1993, Servizi and Martens 1987). Future studies should therefore examine the effects of elevated water flow on these other components of the oxygen cascade.

Juvenile *C. quinquelineatus* were unable to increase $\dot{M}O_{2\text{max}}$ and aerobic scope in response to elevated water flow conditions when they were reared in suspended sediments, which may have several potential explanations. Although there was overall no close link between gill morphology and oxygen uptake rates in the present Chapter, it is possible that other gill changes occurred that were not measured that may have interfered with gas exchange. For example, suspended sediments have been observed to increase mucous secretion at the gills (e.g. Hess et al. 2015, Humborstad et al. 2006, Li et al. 2012). Mucous can trap sediment particles and block the spaces between lamellae, thereby reducing gas exchange efficiency (Humborstad et al. 2006). Alternatively, suspended sediments may have interfered with the growth of muscle tissue through other ways. In particular, although fish swam continuously against the water flow during a pilot study, and no shelter were available in aquaria that may have allowed fish to avoid swimming, it cannot completely be excluded that fish in suspended sediments found ways to reduce their exposure to water flow (e.g., by strategically positioning themselves along the wall) and may have thus had a lower swimming activity than fish reared in the absence of suspended sediments. Future studies should therefore expose fish to an exercise regime in individual flume chambers to control for potential differences in activity levels.

This Chapter provides first evidence that the impacts of suspended sediments on *C. quinquelineatus*, and potentially other reef fishes, may depend strongly on local water flow conditions prevailing on individual coral reefs. The results of this Chapter suggest that fish living in elevated water flow environments may be more vulnerable to increases in suspended sediments, as suspended sediments can interfere with their ability to acclimate to, and thus likely cope with, high water flow velocities. As such, increases in suspended sediments levels may particularly affect juvenile *C. quinquelineatus* on inshore reefs frequently exposed to currents, high tidal flow, or storms, potentially leading to a decline in species abundance on these reefs, or a re-distribution of fish to more sheltered reefs.

Chapter 6: General Discussion

Declining water quality (including increasing suspended sediment concentrations), together with climate change and overfishing are regarded as the three main threats facing coral reefs today (Halpern et al. 2015). There is now strong evidence that coastal development, including changes to natural vegetation cover and dredging, causes long-term changes in suspended sediment regimes in coastal waters (De Mello et al. 2017, Halpern et al. 2015, Jackson et al. 2017). Increasing levels of suspended sediments can have transformative effects on coral reef fish species assemblages via declines in species abundance and diversity and changes in community composition (Donohue and Molinos 2009, Parker et al. 2016, Xu and Li 2019). The body of research presented in this thesis provides the most extensive evidence to date that suspended sediments induce changes in the physiological performance of juvenile coral reef fishes, which may potentially be contributing to observed changes at the population level.With previous studies mainly focusing on the effects of suspended sediments on reef fish behaviour (Wenger et al. 2017), this thesis addresses an important knowledge gap in the field of conservation physiology.

Conservation physiology aims to establish cause-and-effect relationships between changes in animal physiological performance and changes in population dynamics, with the purpose to inform policy and management (Cooke and O'Connor 2009, Illing and Rummer 2017). The present thesis focused on the relationship between changes at the level of the gills and changes in two metrics of fish performance, namely aerobic performance and predator escape performance. Changes in gill morphology, induced by suspended sediments, have widely been assumed to affect aerobic performance and other aspects of fish performance, including predator escape performance (e.g., Lowe et al. 2015, Sutherland and Meyer 2007). Changes in aerobic scope can have cascading effects on various aerobic activities linked to fitness (Clark et al. 2013, Norin and Clark 2016), while predator escape performance is a crucial aspect of predator-prey interactions that is linked to survivorship of fish (Domenici et al. 2010, McCormick et al. 2018). Due to their links to fitness and survival, changes in these two performance metrics can serve as important indicators of whole-animal performance and the impacts of increasing suspended sediments on a species.

One of the most significant findings of the present research is that suspended sediments compromised the aerobic performance in one out of four coral reef fish species, *Amphiprion melanopus* (Chapter 2). Such species-specific differences may potentially lead to winners and losers on coral reefs and could translate into changes in the composition of fish assemblages. A reduction in aerobic scope introduces a fundamental constraint on fish performance and may thus profoundly change the way a fish functions in its environment (Clark et al. 2013, Farrell et al. 2008). For instance, a reduced aerobic scope may potentially compound the known effects of suspended sediment on fish behaviour. Previous studies have documented a reduction in feeding efficiency in turbid waters, which means that fish likely need to dedicate a greater amount of time to feeding in order to meet their energy demand (Swetka and Harman 2001, Utne 2005, Wenger et al. 2013). A reduction in aerobic scope due to suspended sediments would be expected to restrict a fish's capacity to increase feeding efforts, which may negatively affect their growth and development, and may thus restrict their ability to adjust to and cope with suspended sediments. Similarly, a reduction in aerobic scope may interfere with various other aspects of a fish's performance, such as swimming performance, competitive ability and the capacity to recover from exercise (Clark et al. 2013, Norin and Clark 2016). Although our understanding of how changes in aerobic scope link to individual activities relevant for fish survival and fitness is still limited (Clark et al. 2013, Norin and Clark 2016), the relationship between suspended sediments and aerobic scope established in the present research provides a basis for future studies to investigate in further detail the consequences this may have for different performance traits.

The present research also provides first evidence that suspended sediments can alter the predator escape performance of coral reef fishes (Chapters 3 and 4). Predator escape performance plays an integral role for the survival of coral reef fishes during the juvenile stage (McCormick et al. 2018). Mortality of juvenile coral reef fishes is high (McCormick and Hoey 2004, Almany and Webster 2006), and any factor influencing survival at this stage can have considerable effects on adult populations (Jones 1990). Previous studies have found that turbidity interferes with the detection of predators and the assessment of predation risk (Mazur and Beauchamp 2003, Miner and Stein 1996, Meager et al. 2006). This thesis expands on these studies by showing that fish attempt to compensate for their reduced ability to detect predators by enhancing their predator escape performance and by adopting more cautious anti-predator behaviours (i.e., reducing their activity and willingness to venture into open, unprotected areas). A large body of literature documents the significant trade-offs between anti-predator behaviours and fitness-related traits such as growth (e.g., reviewed in Lima 1998, Preisser et al. 2005). The observed increase in escape performance and anti-predator behaviour thus likely represents a shift in how juvenile reef fishes on turbid reefs allocate resources (i.e., time and energy). An increased investment into escape performance and anti-predator behaviours will therefore have to be considered as a cost associated with suspended sediment exposure in future studies. Such additional costs arising during this critical life history stage may negatively affect juvenile survival, thereby potentially influencing adult populations on turbid reefs. However, future studies should also examine the potential effects of suspended sediments on predators, as changes in predator performance that influence the capacity to capture prey would have important consequences for juvenile survival.

The present research also provides first evidence that the effects of suspended sediments on coral reef fishes depend on environmental conditions (Chapters 4 and 5). Climate change poses the greatest threat to the persistence of coral reefs (Hughes et al. 2017), and investigating whether suspended sediments may compound the effects of rising water temperatures on coral reef fishes is crucial to understanding their capacity to acclimate and adapt to future conditions. Surprisingly, suspended sediments did not compound, but rather masked some of the negative effects of elevated water temperature on aerobic performance. In contrast, however, the present research also showed that suspended sediments can adversely affect the capacity of fish to acclimate to changes in water flow conditions. The process of enhancing aerobic scope – and associated swimming performance – is an important function that allows fish to inhabit reefs characterized by increased water flow velocities (Binning et al. 2015). A reduced capacity to enhance aerobic scope is likely to leave fish with a lower capacity to sustain high water flow velocities (Binning et al. 2015, Gallaugher et al. 2001, Zhang et al. 2011), which means that fish would need to spend more time sheltering and may not be able to spend enough time foraging to meet their energy demands (Johansen et al. 2007, Johansen et al. 2008). As a consequence, a reduced capacity to acclimate aerobic scope to increased water flow conditions due to effects of suspended sediments may potentially lead to reductions in some coral reef fish populations from some reefs, thereby influencing overall species distribution. Water flow is a factor that is rarely considered in experimental studies investigating the effects of anthropogenic stressors on fish species. The present research highlights that the impacts of suspended sediments, and potentially other anthropogenic stressors, will likely be underestimated if they are not examined in context of local water flow conditions.

The present research represents the most extensive examination to date of the relationship between changes in gill morphology induced by suspended sediments and aerobic performance. Gill morphology has an important influence on gill functions (Evans et al. 2005, Nilsson et al. 2012); accordingly, changes in gill morphology induced by suspended sediments and other stressors have been widely regarded as indicator of changes in oxygen uptake capacity (e.g., Lake and Hinch 1999, Li et al. 2012, Lowe et al. 2015). Indeed, in one of four examined species in the present research, gill changes following suspended sediment exposure were associated with a reduced oxygen uptake capacity and aerobic scope (Chapter 2 and discussed above). Surprisingly, however, there was no clear link between changes in gill morphology and oxygen uptake rates in the other species; most importantly, some species were able to tolerate significant reductions in functional lamellar length, which is a proxy for gill surface area available for gas exchange. These results indicate that the capacity of the gills for oxygen uptake in these tolerant species likely far exceeds the capacity of the cardiovascular system to transport oxygen to tissues and/or the capacity of tissues to consume oxygen, meaning that gill morphology may not be the limiting factor for oxygen uptake (see also Cumming and Herbert 2016). A high tolerance to gill changes may allow these species to retain their aerobic capacity and to thereby acclimate to increasing suspended sediment concentrations. In contrast, species with a low tolerance to gill changes and associated reductions in aerobic scope may be particularly vulnerable to increases in suspended sediments. The absence of a clear link between changes in gill morphology and oxygen uptake rates also shows that changes in gill morphology are not necessarily a reliable proxy for changes in aerobic performance. Yet, changes in gill morphology are still be a useful metric when assessing the impacts of suspended sediments on fish; although changes in gill morphology may not directly translate into changes in oxygen uptake in all species, they may still indicate changes to the overall capacity of the gills for oxygen uptake (even though other factors may be limiting realized oxygen uptake capacity), which may be important to consider in context of changing environmental conditions that can lead to an increase in oxygen demand or affect oxygen uptake efficiency, such as hypoxic events. Further, gills fulfil many functions other than oxygen uptake (e.g., nitrogen excretion, ion/osmoregulation, acid-base balance Evans et al. 2005), and it is possible that those functions may be affected under suspended sediment conditions, even if no changes in oxygen uptake are observed, which will be an interesting avenue for future research.

An important aim of this thesis, and conservation physiology in general, is to produce research that informs management and policy. The present research has provided extensive evidence as to the impacts of suspended sediments on the physiological performance of coral reef fishes during a vulnerable life history stage. Juvenile coral reef fishes exhibit an extraordinarily high growth rate and feeding activity, and it is estimated that juveniles exhaust their aerobic scope most of the time (Cunha et al. 2007). This is in contrast to older fish, which may reach their full aerobic potential only occasionally, such as during digestion or burst swimming (Norin and Clark 2016). This means that even short periods (i.e, days) of reductions in aerobic scope due to suspended sediment exposure are likely to adversely affect juvenile reef fishes of some species. Further, the need to enhance both predator escape performance and anti-predator behaviours in turbid waters is likely to impose considerable costs (e.g., reduced time spent feeding, Preisser et al. 2005) on reef fish juveniles that may trade-off with growth, and thus survival (McCormick and Hoey 2004). Managing stressors that change the survival of juvenile reef fishes is of particular importance, as changes in juvenile survival can have a strong influence on adult populations (Jones 1990, Schmitt and Holbrook 1999). As such, reducing activities that increase suspended sediment concentrations, including capital and maintenance dredging, near habitats that act as fish nurseries and during fish spawning periods can likely mitigate the impacts of these activities on fish populations.

Focusing on the juvenile phase of the examined reef fish species led to some limitations to the present research that are important to mention. The size of the fish (< 1g) added technical challenges to many methods or even rendered them impossible. In particular, oxygen uptake rates in all chapters were determined using clear water (i.e., 0 mgL^{-1} suspended sediments) during respirometry trials, as sediment particles interfered with recording of oxygen levels in respirometry chambers. The findings of this thesis research are thus conservative, as the presence of sediment particles in the water may have potentially interfered with gas exchange at the gills by adhering to gill surfaces. Another limitation of the present research is that maximum oxygen uptake rates were determined using one protocol only, i.e., an exhaustive chase protocol, which assumes that maximum oxygen uptake rates are achieved during recovery from exhaustive exercise (Clark et al. 2013, Roche et al. 2013; Rummer et al., 2016). Ideally, oxygen uptake rates should have been examined using both a chase protocol and a swim tunnel with a step-wise increase of swimming speeds to obtain more robust results (Clark et al. 2013, Norin and Clark 2016; Rummer et al., 2016). However, equipment used in the present research was designed and constructed specifically for this project. It was particularly challenging to find a pump that would produce water flow gentle enough to not disturb juvenile fish, yet robust enough to run for months with unwavering accuracy under the extreme conditions of a tropical marine laboratory. Although it was attempted, building a swim tunnel for juvenile fish was outside the scope of the present project.

The importance of healthy coral reefs for the global community cannot be overstated. Over 250 million people – over 3% of the population – depend on coral reefs directly or indirectly for their livelihoods (Burke et al. 2011). Beyond economic benefits, however, coral reefs hold immeasurable ecological, social, spiritual and heritage value. Although representing less than 1% of the ocean floor, coral reefs harbour over 25% of marine biodiversity (Reaka-Kudla 1997). Over 80% of Australians, and over 37% of international tourists, state that the Great Barrier Reef is an important part of their identity (Marshall et al. 2016). Understanding the factors and underlying mechanisms that lead to changes in species assemblages on coral reefs is thus of interest not only to policy and management, but to the wider community, as it can inspire stewardship and empower people. The present research has provided evidence for the impact of suspended sediments on the physiological performance of coral reef fishes, thereby adding another facet to the various impacts of declining water quality on coral reef ecosystem being documented by a rapidly growing number of studies (e.g., Brodie et al. 2019, Wenger et al. 2017). Together, these studies paint a picture of increasing clarity of the pervasive consequences of human activities in coastal areas, and the decline in water quality they cause, for the species communities making up coral reef ecosystems.

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