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

Authors: Sybille Hess\textsuperscript{1,2}, Leteisha Prescott\textsuperscript{1,2}, Andrew S. Hoey\textsuperscript{1}, Shannon McMahon\textsuperscript{1,2}, Amelia S. Wenger\textsuperscript{3} & Jodie L. Rummer\textsuperscript{1}

Affiliations:

\textsuperscript{1}ARC Centre of Excellence for Coral Reef Studies, Townsville, 4811 Queensland, Australia
\textsuperscript{2}College of Science and Engineering, James Cook University, Townsville, 4811 Queensland, Australia
\textsuperscript{3}School of Earth and Environmental Sciences, University of Queensland, St. Lucia, 4072 Queensland, Australia

Corresponding author and institution:

Sybille Hess, email: sybille.hess@my.jcu.edu.au, ARC Centre of Excellence for Coral Reef Study and College of Science and Engineering, James Cook University, Townsville, 4811 Queensland, Australia

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. We exposed juveniles of three reef fish species (\textit{Amphiprion melanopus}, \textit{Amphiprion percula}, and \textit{Acanthochromis polyacanthus}) to suspended sediments (0 - 180mgL\textsuperscript{-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 \textit{A. melanopus} and \textit{A. polyacanthus} and reduced oxygen diffusion distances in all three species. While \textit{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 \textit{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 \textit{A. polyacanthus} or \textit{A. percula}, may be able to persist or gain a competitive advantage.
1. Introduction

Declining water quality is one of the leading causes of aquatic ecosystem degradation globally [1, 2]. In particular, the input and resuspension of sediments are having a dramatic effect on both sessile and mobile organisms [3]. Coastal development, agriculture, overgrazing, mining, removal of riparian vegetation, dredging, and shipping have increased suspended sediments in coastal waters over the past decades [1, 4, 5]. Continued population growth in coastal areas, especially in the tropics [6], 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 [7-11]; 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 [9, 10], Caribbean [11], Pacific Islands [12, 13]). 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 [7, 14], and numerous studies have documented declines in fish assemblages following coral loss [15, 16]. However, the indirect effects of sediment-induced coral loss may be compounded by any direct impacts of suspended sediments on fishes [3, 17]. For example, suspended sediments have been shown to interfere with visual acuity and olfaction in some coral reef fishes, thereby affecting larval settlement [18, 19] and prey capture [20-22]. 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 [23], and suspended sediments and other pollutants have been hypothesized to interfere with this process [24]. Several studies have shown that suspended sediments and other pollutants can irritate and damage gill tissues, leading to changes in gill structure [25-28]. 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 [29, 30], which is the primary site for oxygen uptake in most fishes [31]. 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 [32, 33]. 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 [34, 35]. 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 [36-41]. 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 [28]).

The capacity to perform vital aerobic activities is tied to the capacity of the gills for oxygen
uptake [42, 43]. For example, the maximum rate of oxygen uptake of individuals (i.e., $\bar{M}O_2\max$) has
been correlated with various activities linked to fitness and survival in fish, including locomotion and
competitive ability [43, 44]. 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 [45], 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 [26, 28, 46]. 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, we 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 [47, 48]. 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 [2, 6].
2. Methods

Experimental protocol

Larvae of *Amphiprion melanopus* (cinnamon clownfish), *Amphiprion percula* (false orange clownfish), and *Acanthochromis polyacanthus* (spiny chromis) were sourced from captive breeding pairs between January and May 2016 and maintained until experimentation (see Supplemental Materials for detailed description). 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. Sediments were re-suspended in external sumps and delivered to aquaria via submersible pumps (figure S1, see also supplementary materials for further information). Australian bentonite, a clay with a small particle size range (< 63 μm) and representative of sediments found in suspension on the Great Barrier Reef [49], was used as the sediment. The selected suspended sediment concentrations, 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 [47, 48, 50].

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.
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 [26] (figure S3a-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 (figure S3a). The thickness of the filament epithelium was measured in between two lamellae (figure S3a). 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 (figure S3c) [26]. 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, [51]). All filaments were analyzed blindly with respect to treatments.

Oxygen consumption rates

Oxygen consumption rates ($\dot{M}O_2$) of individuals were determined using intermittent-flow respirometry [52]. Prior to respirometry, each fish was fasted for 24 hours to ensure a post-absorptive state [53]. Then, each individual fish was manually chased in a circular container (60 cm diameter, 10 cm water depth) until exhaustion [54]. 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 [55]. Upon exhaustion, fish were placed immediately into their respective respirometry chambers (15.7 ml volume including tubing) and left to recover for 3 hours (see Supplementary Materials for detailed description) while oxygen levels were continuously recorded. While adult fish are usually monitored over 24 hours [56], 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. [57, 58]). Flush pumps supplied each chamber with (clear) aerated, UV-filtered seawater from the surrounding water bath every 10 min for 2 min, thus preventing oxygen levels from falling below 90% air saturation. Recirculation pumps ensured homogenous oxygen tensions throughout chambers [56]. Background microbial respiration in empty chambers was measured before and after each trial [56]. 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 ± SE), 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.

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). Background microbial respiration was subtracted from total respiration in chambers to derive oxygen consumption rates of fish [56]. 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 [56]. Aerobic scope was calculated as the difference between maximum and resting oxygen consumption rate (see [57]).

**Statistical analyses**

All analyses were performed in R (v. 3.3.2, R Core Team 2013). Linear and generalized linear mixed models (lme4 package, [59]) 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 as a 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 [60]. 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 [60]. 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.

3. Results

Gill structure

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 (see Supplementary Materials) 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\(^{-1}\): \(t = -3.04, p = 0.002\), figure 1a, table S1).

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\(^{-1}\): \(t = -2.20, p = 0.027\), figure 1a, table S2).

All three species exhibited significant reductions in oxygen diffusion distances upon suspended sediment exposure (figure 1b, tables S1-S3). 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\(^{-1}\): \(t = -2.28, p = 0.02\), figure 1b, table S1). 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\(^{-1}\): \(t = -3.26, p = 0.0011\), 135 mgL\(^{-1}\): \(t = -3.07, p = 0.0021\), 180 mgL\(^{-1}\): \(t = -2.20, p = 0.027\), figure 1a, table S2). 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\(^{-1}\): \(t = -3.25, p = 0.003\), figure 1b, table S3).

Oxygen consumption 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 (figure 2a, table S4). 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: \( t = 3.00, p = 0.003 \), 135 mgL⁻¹: \( t = 2.61, p = 0.011 \), figure 2a, table S4). 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: \( t = -2.31, p = 0.020 \), figure 2b, table S4). 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 S5 & S6).

To further explore the connection between oxygen consumption rates and changes in gill morphology, we analyzed the relationship between aerobic scope and the squared mean functional lamellar length (as a proxy for gill surface area) of A. melanopus using linear regressions. Separate analyses were used for A. melanopus exposed to 0 and 180 mgL⁻¹ suspended sediments. While there was a significant correlation between aerobic scope and squared mean functional lamellar length in A. melanopus juveniles exposed to control conditions (\( R^2 = 0.34, F_{1,13} = 6.82, p = 0.02 \)), these two variables were not significantly correlated in juveniles exposed to 180 mgL⁻¹ suspended sediments (\( R^2 = 0.00, F_{1,8} = 0.03, R^2 = 0.00, p = 0.85 \)).

4. 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 [26, 28, 46]. 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
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 [61]. 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 [62], 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 [61], 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. $\dot{M}O_{2\text{max}}$). 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 (as a proxy for gill surface area) in control fish, supporting the idea that the reduction in $\dot{M}O_{2\text{max}}$ 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 [35] and may have weakened the relationship between aerobic scope and functional lamellar length. An alternative hypothesis for the drivers of the observed reduction in $\dot{M}O_{2\text{max}}$ could be that suspended sediment exposure may have elicited a stress response, which may have influenced $\dot{M}O_{2\text{max}}$. However, there is limited information available on the effects of stress on $\dot{M}O_{2\text{max}}$.

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 $\dot{M}O_{2\text{max}}$
suggests that oxygen delivery to tissues may be insufficient during activities with elevated oxygen
demand, such as during swimming [43, 63].

Individuals exposed to suspended sediments exhibited increased oxygen consumption at
rest (i.e., $\dot{M}O_2^{\text{rest}}$), which is commonly observed in fish exposed to poor water quality (e.g. [64-66]).
This finding indicates that inhabiting areas with elevated suspended sediments incurs an energetic
cost. The increase in $\dot{M}O_2^{\text{rest}}$ 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 [67, 68]. 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 [69]. A reduction in aerobic scope
may be especially problematic in juvenile fish, which exhibit exceptionally high growth rates [70] but
low survival rates [71]. Factors that reduce growth or survival during early life history stages can
have strong effects on recruitment patterns [72, 73]. While $A.$ melanopus is common on turbid reefs
today [64], 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 (e.g.
reefs further offshore). Any potential habitat shifts by $A.$ melanopus are likely to increase
competition among anemonefish species for host anemones in these habitats, and (depending on
the outcomes) may interfere with the other species’ niche rangers.

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 [3]. Regardless of the underlying mechanism, these findings highlight that
there are inter-specific variations 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 [61]. 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 [24]; 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 [34, 74]; 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 [40, 75]. Not all species perfuse all of the available lamellae, and thus do not use their gill surface area to its full potential [76, 77]. This may allow them to tolerate some gill damage without adversely affecting oxygen uptake [28]. 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 [3, 78, 79]. The reported structural changes induced by suspended sediments, however, are nonspecific [24, 51], 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. [28, 30, 80]). 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 [26]. 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 [25]. 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 [25]. However, when exposed to hypoxia [24, 36], elevated temperatures [37], air [38, 39] and even some pollutants [81], some fish species
are known to actively alter oxygen diffusion distances to regulate oxygen uptake. As suggested by [25], 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 [40, 41, 81]). 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 [41].

5. Conclusion

Up to half of the world’s coral reefs are threatened by poor water quality [82], with continued and rapid expansions of coastal human populations likely to exacerbate the inputs and resuspension of terrestrial sediments [2, 6]. 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 [83, 84]. Our findings suggest that A. melanopus, a species frequently found on turbid reefs today [61], 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 [85]. 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.
Figure captions

**Figure 1.** Effects of suspended sediments on 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 $\alpha < 0.05$.

**Figure 2.** Effects of suspended sediments on oxygen consumption of *A. melanopus*. a) Maximum oxygen consumption rates ($\dot{M}O_{2\text{rest}}$, black circles) and resting oxygen consumption rates ($\dot{M}O_{2\text{rest}}$, 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 $\alpha < 0.05$. 
Figure 1.
Figure 2.
Datasets

Datasets used in this article have been archived in the Research Data Repository (Tropical Data Hub) at James Cook University. Please note that the data is currently under embargo. A DOI has been reserved [86], which will be activated on publication. The datasets can be accessed from the following link:

https://cloudstor.aarnet.edu.au/plus/index.php/s/fsNjcmCyRwDmOXC

Ethics statement

This research has been conducted according to the Australian code for the care and use of animals for scientific purposes and has been approved by the Animal Ethics Committee at James Cook University (animal ethics approval number A2218).

Competing interests

The authors have no competing interests.

Author contributions

SH, ALH, ASW, JLR conceived and designed the study; SM, SH raised fish; SH conducted experiments and performed respirometry; LP analyzed gill morphology; SH carried out the statistical analyses; all authors wrote the manuscript and gave final approval.

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