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

Authors: Sybille Hess\textsuperscript{1,2}, Bridie J. M. Allan\textsuperscript{3}, Andrew S. Hoey\textsuperscript{1}, Michael D. Jarrold\textsuperscript{1,2}, Amelia S. Wenger\textsuperscript{4} & Jodie L. Rummer\textsuperscript{1}

Affiliations:
\textsuperscript{1}ARC Centre of Excellence for Coral Reef Studies, 4811, Australia
\textsuperscript{2}College of Science and Engineering, James Cook University, 4811, Australia
\textsuperscript{3}Department of Marine Science, University of Otago, 9016, New Zealand
\textsuperscript{4}School of Earth and Environmental Sciences, University of Queensland, 4072, Australia

Corresponding author:
Sybille Hess, email: sybille.hess@my.jcu.edu.au

Abstract
Declining water quality, in particular elevated suspended sediments, pose a significant threat to coastal coral reefs. We exposed juvenile anemonefish (Amphiprion melanopus) to two suspended sediment concentrations (0 or 180 mgL\textsuperscript{-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\textsuperscript{-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.

Keywords: suspended solids, sub-lethal effects, predator-prey interactions, turbidity, fish health
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 (Hess et al. 2017); 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, we exposed juvenile cinnamon clownfish (*Amphiprion melanopus*) 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.
Materials and methods

Suspension sediment exposure

*Amphiprion melanopus* larvae were sourced from five captive breeding pairs maintained at 28.5 ± 0.5°C and reared following standard protocols (electronic supplemental material, ESM). 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 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). Sediments were maintained in suspension using a series of pumps in external sumps (ESM).

Fast start performance and anti-predator behaviour

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 (ESM). 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. S1). 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 (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. S2), 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.
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 (see ESM for more details).

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).

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) (ESM).

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.
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. 1a), 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. 1b), 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. 1c), 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. 1d) 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 (Fig. 1e and f, see ESM for details).

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. 1f) 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 (ESM). 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).

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. 2a) 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. 2b) 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 (ESM). 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* (Hess et al. 2017), 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).

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 we 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.

**Fig. 1** 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 response, e) maximum escape speed, and f) maximum acceleration.

**Fig. 2** 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.

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**Data accessibility**

**Conflict of interest statement**
On behalf of all authors, the corresponding author states that there is no conflict of interest.

**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).
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Figure 1
Figure 2
Supplementary methods

Suspended sediment exposure

Sediment was suspended in external 200 L sumps fitted with submersible pumps. Each sump delivered water at a rate of $210 \pm 30$ 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 $28.5 \pm 0.5^\circ C$ 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.

Larval rearing and exposure to experiments

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 $28.5 \pm 0.5^\circ C$ 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.

Fast starts

The following variables were measured:
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 occurred).

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.

Thigmotaxis

Thigmotaxis, or “wall hugging” is a proxy for the shyness of an individual in a novel environment (Schnörr et al. 2012). As suggested by Schnörr et al. (2012), we used the proportion of time an individual spent within 2.5 body lengths from the wall to determine thigmotaxis.

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, we initially included distance to the stimulus 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.
Fig. S1. Experimental set up. 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.
Fig. S2. Photograph of the experimental arena filled with clear water from the control a) and water from the suspended sediment treatment b). The walls of the aquarium were opaque, while the bottom was transparent.

2. Supplementary results

Juvenile *A. melanopus* exposed to suspended sediments for 7 days did not show any changes in maximum acceleration ($\chi^2(1) = 0.26, p = 0.60$) or maximum escape speeds ($\chi^2(1) = 0.93, p = 0.33$) compared to control fish. Acute exposure (i.e. suspended sediments present in the experimental arena) influenced maximum acceleration but not any other fast start variable, i.e. response latency ($n = 65, \chi^2(1) = 2.57, p = 0.11$), turning rate ($\chi^2(1) = 2.19, p = 0.13$), average escape speed ($\chi^2(1) = 0.15, p = 0.70$), maximum escape speed ($\chi^2(1) = 0.60, p = 0.44$), and distance travelled within the first 24 ms ($\chi^2(1) = 0.22, p = 0.64$). There were no significant interactions between 7-day exposure and acute exposure, i.e. response latency ($\chi^2(1) = 0.09, p = 0.75$), turning rate ($\chi^2(1) = 0.31, p = 0.57$), maximum acceleration ($\chi^2(1) = 1.70, p = 0.19$), average escape speed ($\chi^2(1) = 0.18, p = 0.67$), maximum escape speed ($\chi^2(1) = 0.15, p = 0.69$), and distance travelled ($\chi^2(1) = 0.12, p = 0.72$).

Activity and thigmotaxis were influenced by suspended sediment concentrations in the test arena, but not by 7-day exposure to suspended sediments, i.e. activity ($n = 78, \chi^2(1) = 1.88, p = 0.17$) and thigmotaxis ($\chi^2(1) = 0.64, p = 0.42$). There were also no significant interactions between 7-day exposure and acute exposure, i.e. activity ($\chi^2(1) = 0.00, p = 0.94$), and thigmotaxis ($\chi^2(1) = 2.11, p = 0.14$).