Effects of wave-driven water flow on the fast-start escape response of juvenile coral reef damselfishes

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ABSTRACT

Fish often evade predators with a fast-start escape response. Studies typically examine this behaviour in still water despite water motion being an inherent feature of aquatic ecosystems. In shallow habitats, waves create complex flows that likely influence escape performance, particularly in small fishes with low absolute swimming speeds relative to environmental flows. I examined how wave-driven water flow affects the behaviour and kinematics of escape responses in juveniles of three coral reef damselfishes (Pomacentridae) with different body morphologies. Tropical damselfishes have similar fin and body shapes during early development with the exception of body depth, a trait deemed important for postural control and stability. Wave-driven flow increased response latency in two of the three species tested: fish with a fusiform body responded 2.4 times slower in wave-driven flow than in still water, whereas this difference was less pronounced in fish with an intermediate body depth (1.9 times slower response), and absent in fish with a laterally compressed body. The effect of wave-driven flow on swimming performance (cumulative escape distance and turning rate) was variable and depended on the timing and trajectory of escape responses in relation to the wave phase. Given intense predation pressure on juvenile coral reef fishes during settlement, interspecific differences in how wave-driven flow affects their ability to escape predators could influence the distribution and abundance of species across spatial and temporal scales.

INTRODUCTION

Avoiding and fleeing from predators is one of the most important tasks faced by animals (Ydenberg and Dill, 1986). Therefore, behaviour and locomotion are fundamental to the survival of mobile organisms during predator-prey encounters (Howland, 1974; Domenici et al., 2007; Higham, 2007). Fast-start escape responses are the primary behaviour used by fishes to evade predators (Domenici and Blake, 1997; Domenici, 2011). These rapid accelerations are particularly important for juvenile coral reef fishes (Fisher and Leis, 2010), as predation is a key factor influencing their survival (Almany, 2003; Almany and Webster, 2006; Holmes and McCormick, 2009). Predation on reef fishes is greatest during and shortly

after larvae metamorphose from a planktonic to a demersal life stage as they settle on the reef (Steele and Forrester, 2002; Almany and Webster, 2006). Due to their small size, newly-settled coral reef fishes are targeted by many generalist and piscivorous predators (Stewart and Jones, 2001; Holmes and McCormick, 2010). Estimates suggest that over 50% of juveniles, across a range of taxonomically diverse species, can be eaten within 48 hours of settlement (Almany and Webster, 2006). Consequently, environmental factors that affect performance during escape responses by juveniles might have a substantial influence on the recruitment of coral reef fishes to adult populations (Rice et al., 1997; Fisher and Leis, 2010).

Many environmental parameters fluctuate dramatically in aquatic systems (Abrahams et al., 2007). Recent studies have examined the effect of temperature, dissolved oxygen, turbidity, light and pH on fish escape performance (reviewed in Domenici et al., 2007; Wilson et al., 2010). However, the importance of water motion has been largely overlooked (Higham et al., 2015; but see Anwar et al., 2016; Diamond et al., 2016) . This oversight is surprising given that water flow is a ubiquitous and highly variable physical property of aquatic ecosystems (Denny, 1988; Webb et al., 2010). In shallow marine habitats, wave-driven water motion is an important stressor for both sessile and mobile organisms (Denny, 2006; Denny and Gaylord, 2010; Webb et al., 2010). On coral reefs, for example, complex water flow from waves influences the ability of adult fishes to swim and occupy shallow, windward habitats, which leads to strong patterns of community structuring based on a species' ability to withstand ambient flow conditions (Bellwood and Wainwright, 2001; Bellwood et al., 2002).

Wave-driven water motion is characterized by unsteadiness (changes in flow velocity) and turbulence (vortices) as the water flow interacts with the reef structure (Liao, 2007; Webb et al., 2010). These complex flows can be energetically demanding (e.g. Roche et al., 2014; Maia et al., 2015; Schakmann et al., 2020) or beneficial (Taguchi and Liao, 2011; van der Hoop et al., 2018) for fishes, and can have destabilizing effects on important behaviours, including those associated with predator-prey interactions (Webb, 2002; Webb et al., 2010). Currently, the extent to which wave-driven water flow affects fish escape responses is unknown. Such biophysical interactions could be of considerable ecological importance for coral reef fishes given the importance of predation in shaping their distribution and abundance. In addition, wave intensity and frequency are increasing in ocean basins worldwide as a result of climate change (Young et al., 2011; Rhein et al., 2013), with impacts anticipated on key processes in marine communities (Harley et al., 2006; Byrnes et al., 2011), including predator-prey interactions (Domenici and Seebacher, 2020). Basic knowledge of how waves influence the behaviour and unsteady swimming performance of fishes is essential to improve our understanding and ability to predict how environmental change will affect fish communities – for example, by altering energetic demands and the outcome of predator-prey encounters (Higham et al., 2015; Killen et al., 2016).

Here, I examined whether complex, wave-driven water flow affects behavioural and kinematic components of escape responses in post-settlement juvenile coral reef fishes in the family Pomacentridae. The damselfishes are a species-rich, morphologically diverse group, and are widely distributed throughout temperate and tropical waters around the world (Allen, 1991; Cooper et al., 2009). Many are small (< 5 cm total length) and important prey items for predatory coral reef fishes (Kingsford, 1992; Beukers-Stewart and Jones, 2004). The Pomacentridae are characterized by broad differences in body depth, a trait known to influence fast-start behaviours (Domenici et al., 2007) and also been linked to differences in the sustained swimming performance of juvenile coral reef fishes (Fisher et al., 2005; Fisher and Hogan, 2007). A deep, laterally compressed body is thought to improve fast-start

swimming performance and postural control (Domenici and Blake, 1997; Eidietis et al., 2002; Domenici et al., 2008). Additionally, while suboptimal from a hydrodynamic / energetic perspective due to increased pressure and frictional drag, lateral compression has the advantage of allowing a greater expansion of the dorsal and anal fins (Webb, 2004; Webb, 2006), which also helps reduce rolling (Weihs, 2002; but see Webb, 2004).

I studied three damselfish species and examined whether effects of wave-driven water flow on escape responses varied among species with different body morphologies. I predicted greater negatives effects of wave-driven flow on the response latency and escape kinematics of species with a more fusiform than laterally compressed body due to the increased rolling stability conferred by lateral compression.

MATERIALS AND METHODS

Animals and experimental set-up

Early post-settlement juvenile fishes were collected on SCUBA in March 2012, using Aqui-S solution and hand nets on reefs adjacent to the Lizard Island Research Station on the Northern Great Barrier Reef, Australia (14°40'S; 145°28'E). I caught *Neopomacentrus azysron*, *Chromis viridis* and *Dascyllus reticulatus* (family Pomacentridae); based on their body size, these fishes were less than 2 weeks post-settlement (Kerrigan, 1996; Fisher et al., 2005). These species co-occur on the reef but differ in their body morphology (Fig. 1). The fineness ratio (FR) is a measure of how elongate a fish is relative to its transverse sectional diameter (measured as the average of the maximum body width and maximum body depth) (Fisher and Hogan, 2007; Langerhans and Reznick, 2010): *N. azysron* has a shallow, fusiform body (standard length [SL] = 12.7 ± 0.5 mm, FR = 4.23 ± 0.12; means ± s.d.), *C. viridis* has a body of intermediate depth (SL = 12.4 ± 1.0 mm, FR = 3.52 ± 0.13), and *D. reticulatus* has a deep, laterally compressed body (SL = 12.6 ± 0.9 mm, FR = 2.67 ± 0.11). These differences are apparent in juveniles and maintained throughout adulthood. Beyond body shape, pectoral fins are also important for stability (Drucker and Lauder, 2003; Lauder and Drucker, 2004; Webb, 2004). However, pectoral fin shape is similar in juvenile damselfishes, differentiating only later in development (Fulton and Bellwood, 2002). Additionally, pectoral fins are small and transparent in juvenile reef fishes, which makes them impossible to view on whole photographs and difficult to dissect and pin (Fisher and Hogan, 2007). As such, pectoral fin shape was not considered in this study.

Captured fishes were placed in holding aquaria (40 × 29 x 18 cm; L x W x H) with seawater pumped directly from the reef. The water temperature was 29 ± 1 °C (mean ± actual variation) and fishes were exposed to a natural photoperiod of 12 h for at least three days prior to the experiments. Fishes were fed once a day with commercial pellets (INVE NRD 2/4, Primo aquaculture, Australia) and were not fed on the morning of the experiments. Animals were returned to their site of capture at the end of the study.

Experiments were conducted in two rectangular acrylic tanks (70 x 60 x 35 cm; L x W x H) (Fig. 2). Water depth was maintained at 12 cm. Due to their small size and to facilitate filming, individual fish were placed in a fine nylon mesh enclosure (large net breeder, Aqua One, Australia; 26.5 x 15 x 15.5 cm; 1mm stretched mesh) at the centre of the experimental tanks. Four programmable pumps (Vortech MP10wES, EcoTech Marine, USA) were positioned at the back of each tank and wirelessly synchronized to generate complex flow approximating the orbital flow created by waves (see *Flow visualization*). Under still water

conditions (water velocity < 0.2 cm s^{-1}), the pumps remained on, but the propellers were removed to control for any effects of noise and/or vibrations from the pumps. A mirror was inclined at 45° below the aquarium to film escape responses and avoid image distortion from surface water movements (see Domenici and Blake, 1991). Floodlighting was provided by three 150 W spotlights, 70 cm above the water level. The experimental tank was continuously supplied with recirculating seawater, which kept the water temperature constant (29.0 ± 0.7 °C, mean ± actual variation).

Experimental protocol

Prior to each fast-start trial, a test fish was transferred to the mesh enclosure in an experimental tank and left undisturbed for 30 min. Escape responses were induced by mechano-acoustic stimulation (Dadda et al., 2010; Marras et al., 2011). A 50 ml cylindrical container filled with lead weights was released by an electromagnet 45 cm above the water surface. The stimulus fell inside the mesh enclosure containing the test fish, 1 cm from the mesh wall (Fig. 2). To avoid visual stimulation prior to contact with the water, the stimulus fell inside a PVC tube (11 cm diameter) positioned 1 cm above the water surface (Lefrançois et al., 2005). The stimulus was attached by a string to the stand holding the electro-magnet to prevent it from hitting the bottom of the tank. The exact time of stimulus contact with the water surface could be observed in the mirror below the tank (see Marras et al., 2011). Fish moved around freely in the mesh enclosure and were stimulated when in mid water, at the centre of the mesh enclosure to avoid wall effects. The camera's field of view excluded sections of the mesh enclosure within 1 cm from the walls. The distance of the fish's body (centre of mass) and angle relative to the stimulus were controlled for in the statistical analyses.

Escape responses were recorded at 420 Hz by a camera (Casio Exilim EX-FH100, Casio Computer Co., Tokyo, Japan) mounted on a tripod in front of the aquarium, facing the mirror. Typically, fast-start trials are conducted in shallow water to limit vertical displacement and facilitate kinematic measurements of fish movements in two dimensions (e.g., Langerhans, 2009). Here, a minimum water depth was necessary to create wave-driven flow. I used a second camera (Casio Exilim EX-FH100) to film through the aquarium front wall. If the fish moved a vertical distance greater than its body depth, the trial was excluded from the analyses. This distance was chosen to minimize measurement errors of displacement in the horizontal plane. Few trials ($< 2\%$) were excluded for this reason.

Fourteen fish were tested per species in each of the two flow conditions in a full factorial design. Individual fish were tested at least three times with a rest period of 30 min between trials (see Jornod and Roche, 2015). More than three trials were run (with a maximum of five) when fish made sudden movements before the stimulus hit the water surface, covered a vertical distance greater than their body depth, or exited the camera's field of view at the onset of stage 3 of the escape response (see *Measurements of escape performance)*. For 12 of the 84 fish tested, only two trials could be obtained for analysis. Immediately following experiments, individual fish were sedated by submersion in a cool (5°C) water bath for 3 s and then photographed on wetted, plasticized gridded paper. Photographs were analysed in ImageJ v.1.45 to measure size and FR. FR is calculated by dividing body length by the average of the maximum body width and maximum body depth (Bainbridge, 1960). I used SL as body length because an accurate measure of total length (TL) could not be obtained from photographs for 13 *N. azysron*. SL was measured from the tip of the snout to the narrowest point of the caudal peduncle. I used a value of 1.9 mm for the maximum body

depth of all fishes because the body width of the juvenile damselfishes I examined was similar (1.8-2.0 mm) and difficult to measure accurately without handling and harming these small animals.

Flow visualization

I used Particle Image Velocimetry (PIV) to characterize the flow conditions in the wave-driven flow treatment (Fig. 3). Flow velocity and vorticity were estimated by filming neutrally buoyant particles (Fluorescent Green Polyethylene Microspheres 1.025 g cc^{-1} 63-75 μm, Cospheric LLC, Santa Barbara, CA USA) at 30 Hz in high definition with a Casio Exilim EX-FH100 camera. Microspheres were illuminated using a NOVAlaser X100 laser pointer (NOVAlasers Inc., Toronto, Canada - power output 100 mW at 532 nm) fitted with a collimating lens to create a light sheet 2 mm thick (Ryerson and Schwenk, 2012). The light sheet intersected the mesh enclosure in mid-water, 6 cm above the tank bottom. No fish was present at the time of recording. Image sequences were pre-processed in the video editor Avidemux v.2.5.4 (www.avidemux.org) to maximize the contrast between the particles and the black background, and then imported into PIVlab v.1.32 (Thielicke and Stamhuis, 2012). PIVlab estimates the probable shift of particles by cross-correlation between the same interrogation areas in image pairs. Vector maps and velocity or vorticity fields were generated using a 256×256 pixel interrogation area and a 128 pixel step (i.e., the vertical and horizontal distance between the centre of the interrogation areas). The wave frequency was 0.85 ± 0.01 Hz (mean $\pm$ s.d.) and the absolute flow velocity in the experimental arena ranged between 0.5 ± 0.4 and 17.5 ± 2.5 cm s^{-1} (mean $\pm$ s.d.) (Fig. S1, Videos S1 and S2). Wave height was consistent and ranged between 6.2 and 7.1 cm.

Measurements of escape performance

Fast-start escape responses typically consist of a unilateral contraction of the axial musculature (stage 1), which bends the body into a 'C' shape, and a subsequent contralateral contraction, resulting in a half tail-beat (stage 2) (Domenici and Blake 1997, Eaton et al. 2001). Escape sequences were analysed using the software ImageJ v1.45 and the plugin MtrackJ (Meijering et al., 2012). All kinematic measurements are in the environmental frame of reference (i.e., movement relative to the ground) as flow visualisations were carried out without fish in the experimental arena. The two-dimensional X-Y coordinates of the fish's centre of mass (CoM) were plotted every 2.4 ms starting 12 ms before and ending 48 ms after the onset of the stimulus (25 frames in total). The CoM was estimated on four fish per species using frozen specimens; a needle was inserted along the body midline until the point of balance was identified (Domenici et al., 2004). As fish were too small to mark their CoM directly on the body, the CoM was visually determined at a fixed distance from the tip of the snout during video analysis. Measurement error on displacement data from visually estimating the CoM was assessed by digitizing videos from five fish, two times each (Langerhans, 2009). This error was < 4 % for all videos tested. Seven escape performance variables were measured following Lefrançois & Domenici (2006): responsiveness (the percentage of fish that performed an escape response when stimulated); response latency (the time between the moment when the stimulus contacted the water and the first head movement of the fish); cumulative distance travelled (D_{esc}); maximum escape speed (U_{max}); maximum acceleration (A_{max}); stage 1 turning angle (the angle between the straight line joining the tip of the head and the CoM at the onset and end of stage 1); and stage 1 turning rate (stage 1 turning angle divided by stage 1 duration). Distance-time variables (D_{esc}, U_{max}, A_{max}) were evaluated within a fixed time period of 24 ms from the first head movement, corresponding to

the mean duration of stages 1 and 2 for all three species. Measuring distance-time variables within a fixed time period avoids performance biases related to differences in the duration of escape responses (Domenici, 2011). A five-point quadratic polynomial regression (Lanczos, 1956) was used to obtain smoothed values of speed and acceleration, the first and second derivatives of distance (Walker, 1998; Lefrançois and Domenici, 2006).

Statistical analysis

I compared body size (SL) among the three damselfish species with a general linear model (LM) containing three predictors: species, water flow condition, and their interaction. I used a simple LM (one-way ANOVA) to examine differences in the excursion distance of a passive particle and the three fish species in wave-driven water flow. Model assumptions were assessed graphically with diagnostic plots.

I used three general linear mixed-effects models (LMM) to examine how species and water flow condition (still vs. wave-driven) affected three measures of escape performance: response latency (ms), D_{esc} (mm), and turning rate (° ms). Standard length, body angle relative to the stimulus (the angle between the straight line connecting the snout to the CoM of the fish and the tangent to the perimeter of the stimulus), and distance to the stimulus were scaled (i.e., divided by their standard deviation) and included as covariates in each model; an interaction term was included between species and flow condition. The identity of fish was specified as a random factor to account for repeated measurements on individuals. Response latency was log-transformed to normalize model residuals. Differences in U_{max} and A_{max} were not tested to reduce the number of statistical tests (and hence the probability of spurious results), and because U_{max} and A_{max} are less reliable measures of distance-time performance than D_{esc} (Domenici and Blake, 1997). Values of U_{max} and A_{max} for each species and flow treatment are indicated in Table 1. I used the function 'lmer' (Bates et al., 2015) to specify each model as: response.variable ~ scale(SL) + scale(sin(angle.stim)) + scale(dist.stim) + species * flow + (1|individual). I calculated the adjusted repeatability (R_{adj}) of response latency, $D_{esc,}$ and turning rate in still water with the function 'rpt' (Stoffel et al., 2017), specifying species, standard length, body angle relative to the stimulus and distance to the stimulus as fixed predictors.

For fishes in wave-driven flow, I examined how the timing of escape responses in relation to the wave phase affected D_{esc} using a LMM specified as: D_{esc} ~ scale(sin(angle.stim)) + scale(dist.stim) + scale(SL) + species * wave.phase + (1|individual). The factor 'wave phase' was used to distinguish whether fish escaped against the wave path (315-45°), perpendicular to the wave path (46-134° or 226-314°), during a wave trough (water flow speed < 5 cm s^{-1}, escape in any direction), or in the direction of the wave path (135-225°). The direction of the escape response relative to the wave path was measured as the angle between the straight line connecting the fish's CoM at the start of stage 1 and the end of stage 2, and the straight line parallel to the wave path.

The assumptions of mixed-effects models were checked with residual diagnostics using DHARMa (Hartig, 2020). Analyses were done in R v3.6.3 (R Core Team, 2020).

RESULTS

Individuals of all three species in both flow conditions were similar in size (LM: $F_{2,78} = 0.30$, $P = 0.742$, $\eta^2 = 0.007$) (Fig. S2).

All three species moved less (i.e. had a shorter excursion distance) than a passive particle in the wave-driven flow treatment (LM: $F_{3,52} = 60.64$, $P < 0.001$, $\eta^2 = 0.778$). Excursion distance was similar for all three species, with the exception of *D. reticulatus* (laterally compressed body), which was displaced 26% less than *N. azysron* (fusiform body) (Fig. 1, Table S1).

Responsiveness was high in all three species, with fishes responding to the stimulus in 100% of cases in still water and 94% of cases in wave-driven flow. However, fish were slower to respond to the stimulus in wave-driven flow than in still water for two of three species (Tables 1, 2). *N. azysron* (fusiform body) and *C. viridis* (intermediate body depth) were 2.4 and 1.9 times slower at responding to the stimulus in wave-driven flow than in still water. Response latency for *D. reticulatus* (laterally compressed body) was similar in both flow treatments (Tables 1, 3; Fig. 4 A-B).

In still water, response latency ($R_{adj} = 0.49$ [0.35–0.61]) and D_{esc} ($R_{adj} = 0.47$ [0.27–0.65]) were moderately repeatable across all three species, whereas turning rate was not ($R_{adj} = 0.17$ [0.00–0.40]) (Fig. S3).

On average, wave-driven flow did not affect escape kinematics: differences in D_{esc} and turning rate between flow treatments were less than 7% and 12.5%, respectively, for all three species (Tables 1, 2; Fig. 4 C-F). *N. azysron* (fusiform body) and *D. reticulatus* (laterally compressed body) escaped farther than *C. viridis* (intermediate body depth) irrespective of flow conditions (Tables 1, 3). *N. azysron* exhibited an overall higher turning rate than *C. viridis*, but not *D. reticulatus*. *C. viridis* and *D. reticulatus* had similar turning rates (Tables 1, 3).

When considering the timing of a fish's escape response in relation to the wave phase, all species covered a greater D_{esc} and had a faster turning rate when escaping with the water flow rather than perpendicular to or against the water flow (LMM: flow $\chi^2_{(3)} = 35.7$, $P < 0.001$; interaction flow * species ns, $P = 0.174$) (Fig. 6).

Results were qualitatively similar whether I considered all trials per individuals or only an individual's best performance across trials (Table S2), as is sometimes done in studies of fast-start swimming performance.

DISCUSSION

Several biotic and abiotic factors are known to influence the fast-start escape response of fishes, including fin and body morphology, muscle composition, temperature, hypoxia, turbidity, and pollutants, among others (Domenici, 2010a; Wilson et al., 2010). Here, I document differences in escape response among species and water flow conditions.

Interspecific differences in escape performance

There is a dearth of information on the fast-start escape performance of early-stage coral reef fishes (but see Allan et al., 2014; McCormick and Allan, 2017; Allan et al., 2020). Studies over the last two decades have shown that pre- and post-settlement coral reef fish larvae can achieve high sustained swimming speeds of 30 to 50 body lengths s^{-1}, indicating that these larvae are not passive organisms adrift in the plankton (e.g., Stobutzki and Bellwood, 1994;

Leis and Carson-Ewart, 1997; Fisher et al., 2005; Nilsson et al., 2007). The three species tested here achieved high maximum fast-start swimming speeds, occasionally exceeding 100 cm s^{-1} (85 SL s^{-1}; Table 1). In relative terms, these values are almost twice the maximum burst speeds reached by temperate species at similar sizes, including salmonids (Fig. 11.5 in Fisher and Leis, 2010). Fast-start escape responses influence the ability of juvenile coral reef fishes to avoid predation and are therefore directly relevant to their ecology (Fisher and Leis, 2010). The impressive locomotor performance of small pomacentrids is perhaps not surprising given that wild and laboratory measures place them among the fastest swimming coral reef fish larvae (Leis and Carson-Ewart, 1997; Nilsson et al., 2007).

Escape distance (D_{esc}) differed consistently among species in still water and wave-driven flow: *N. azysron* (fusiform body) and *D. reticulatus* (laterally compressed body) exhibited similar performances and escaped farther in a fixed amount of time than *C. viridis* (intermediate body depth), irrespective of flow conditions (Tables 1-2, Fig. 4D). Evidence suggests that differences in fast-start swimming performance result from different, and sometimes opposing, morphological features (Domenici, 2003; Walker, 2004; Langerhans and Reznick, 2010). On one hand, traits such as a shallow, elongated body, a small head and large dorsal and anal fins (such as in pike, *Esox lucius*) are associated with high burst swimming speeds (Webb, 1984; Domenici, 2003; Langerhans et al., 2004). On the other hand, a deep, laterally compressed body has also been shown to enhance burst swimming performance by allowing a lateral profile that increases the mass of water accelerated by body movements (Weihs, 1973; Domenici and Blake, 1991). For example, the deep body of crucian carp (*Carassius carassius*) induced in the presence of predators led to higher burst swimming performance (escape distance, speed, acceleration and turning rate) than that of shallow body morphs found in predator-free habitats (Domenici et al., 2008). These two contrasting profiles roughly correspond to those of the two pomacentrids which escaped the farthest and were at the opposite ends of the body plan spectrum I examined. Turning rate, a measure of agility, mirrored the pattern observed for D_{esc} although differences in performance between *D. reticulatus* and *C. viridis* were less pronounced for turning rate (11.1%) than escape distance (19.3%) (Table 3; Fig 4D,F). This result was unexpected as lateral compression has previously been reported to improve agility (Domenici et al., 2008).

Response latency to the threatening stimulus in still water was distinctly higher for *D. reticulatus* than for the other two species (47% difference; Table 1, Fig. 4A). The deep, laterally compressed body of *D. reticulatus* is a trait frequently associated with reduced vulnerability to gape-limited predators (e.g., Pseudochromidae, Labridae), which are abundant on coral reefs (Rice et al., 1997; Holmes and McCormick, 2010; Domenici, 2011). It is possible that selection pressure for rapid responses in juvenile *D. reticulatus* is less than in the other two species, which have shallower, more fusiform body plans, making them vulnerable to a greater range of piscivorous predators.

Effects of wave-driven flow on escape performance

Reef fishes routinely experience a range of flow velocities and vorticity in the wild depending on weather conditions, reef exposure to waves, microhabitat, and depth below the surface. For example, Johansen (2014) recorded flow velocities ranging between 0 and 82 cm s^{-1} on reefs of different depths and wave exposure at Lizard Island, where test fishes were collected. The wave-driven flow treatment in the current study was representative of water flow speeds routinely experienced by fishes under winds of 15 knots and intermediate wave heights on semi-exposed reefs at Lizard Island (Roche D.G. unpublished data; Fulton and Bellwood,

2005). The maximum flow velocity was 66% of the critical swimming speed (26.3 cm s^{-1}) achieved by congeneric juvenile pomacentrids after settlement on the reef (Stobutzki and Bellwood, 1994).

Responsiveness to the stimulus was high for all three species in both flow conditions, and similar to values reported in another juvenile damselfish exposed to the same type of stimulus used here (>97% responsiveness; Allan et al., 2014). This indicates that wave-driven flow of the magnitude examined in the present study (Fig. S1) did not impede the ability of the test fishes to sense the stimulus. Fishes detect approaching predators by sensing water flow through the lateral line (Stewart et al., 2013), and it has been suggested that environmental flows might impede perception (Higham et al., 2015; Diamond et al., 2016). However, fishes also rely on other senses to perceive threats, such as hearing, vision, and smell. It is likely that the mechanosensory, auditory and visual nature of the stimulus and its large size relative to the test fishes produced a signal strong enough to elicit a response even when dampened by wave-driven flow (see Domenici, 2010a). Supporting this interpretation is the indication that a fish's distance (range: 3.0–14.8 cm) and body angle (range: 14.4–179.6°) relative to the stimulus had little influence on behavioural and kinematic measures of escape performance (Table 2).

On average, escape kinematics (D_{esc}, turning rate) were similar in still water and wave-driven flow within species (Table 1, Fig. 4C-F). This pattern is a consequence of the large variation in escape performance by fishes in wave-driven flow, which was driven by variation in the timing of the stimulus (and hence the escape response) relative to the wave phase (Table S3, Fig. 6). Irrespective of body morphology, fish escaping with the flow achieved a greater D_{esc} and faster turning rate than fish escaping perpendicular to or against the flow as well as during a wave trough (Fig. 6). Since maximum muscle power output is unaffected by flow conditions, these patterns support the prediction that differences in fast-start swimming performance between flow conditions should equal differences in water flow velocity. Indeed, maximum flow speeds were approximately 20% of the maximum escape speeds achieved by fishes (Table 1, Fig. S1), and fish escaping in the direction of the water flow achieved a D_{esc} that was, on average, 20.3% greater than that achieved in still water (Table S3). These results both concur and contrast with those of studies on fish escape responses in steady flow: Anwar et al. (2016) found a preference for downstream escapes by bluegill sunfish (*Lepomis macrochirus*), resulting in a greater escape distance and speed than escapes in still water or upstream flow (flow speeds: 9.4 cm s^{-1} and 23.1 cm s^{-1}); conversely, Diamond et al. (2016) found no evidence that flow speed influenced peak escape velocity in the Hawaiian stream goby (*Sicyopterus stimpsoni*) (flow speeds: 15 cm s^{-1} and 30 cm s^{-1}).

Speed (~D_{esc}) and agility (turning rate) are important locomotor determinants of escape responses (Walker et al., 2005); however, escape success also largely depends on behavioural components, namely the responsiveness and response latency of prey to an attacking predator (Domenici, 2010b; Domenici and Hale, 2019). For example, Fuiman et al. (2006) observed that the responsiveness of red drum larvae (*Sciaenops ocellatus*) to a stimulus was the main predictor of survival during a predator-prey encounter and explained 86% of the variation in survivorship. Similarly, zebrafish (*Danio rerio*) larvae are over three times more likely to evade predation if they initiate their escape before instead of after adult conspecifics open their mouth to prey on them (Stewart et al., 2013). I found that wave-driven water flow increased the response latency of some species but not all (Fig. 5B). *N. azysron* (fusiform body) and *C. viridis* (intermediate body depth) were 2.86 and 1.94 times slower in responding

to the stimulus in wave-driven flow than in still water. In contrast, the response latency of *D. reticulatus* (laterally compressed body) was unaffected (Table 1; Fig. 4B).

Slower responses to the stimulus in wave-driven flow could be due to two main factors: first, the destabilizing effect of water motion and the added challenge of maintaining an upright posture and adequate orientation relative to the flow; and second, the challenges of perceiving a threat due to added noise in complex flow versus still water. All else being equal, a more laterally compressed body should improve resistance to rolling disturbances (Eidietis et al., 2002; Weihs, 2002). A deep body plan should also improve mechanoreception by allowing the placement of the lateral line away from moving body parts (i.e., pectoral fins), a trait which has been posited to decrease self-generated noise and increase the signal-to-noise ratio (Dijkgraaf, 1963; Coombs and Braun, 2003). These two considerations might explain why the longer response latency in wave-driven flow was inversely related to body depth in the three species I examined. Notably, *D. reticulatus* tended to orient more directly into the flow and was less displaced by water motion (i.e., had a shorter excursion distance) than the fusiform-shaped *N. azysron* (Fig. 7), although no such difference was apparent with *C. viridis*, which has an intermediate body depth (Fig. 3, Table S1). Given that reactivity to potential threats (i.e., responsiveness, response latency) is a key determinant of escape success, even moderate flow, such as the one tested here, might increase predation risk for juvenile fishes that are more vulnerable to postural disturbances.

Fast-starts in fishes are typically controlled by the Mauthner cells, a pair of large reticulospinal neurons that receive various sensory inputs, such as visual and mechano-acoustic signals (Eaton et al., 2001). High speed neural transmission and processing via these cells allows for rapid responses to imminent threats within 5-20 ms. Interestingly, *N. azysron* (fusiform body) exhibited much greater variation in escape latency in wave-driven flow than the other two species tested (Fig. 5B). The strong positive skew in the distribution of its response latency (Fig. 5A) suggests that some escape responses in wave-driven flow were not Mauthner cell mediated. Other pathways, through different reticulospinal cells, can control escape responses but have longer latencies (Domenici, 2010a; Domenici and Hale, 2019). Slower responses are generally associated with lower performance (e.g., slower turning rates), and are observed both in healthy fish and in fish with an ablated Mauthner system (Domenici, 2011; Hecker et al., 2020). In wave-driven flow, postural disturbances from waves and associated stability control issues seemingly led to some non-Mauthner cell escapes, with longer latencies (>40 ms), in at least one species.

Conclusion

Hydrodynamic stability is advantageous for fishes moving in high energy coral reef environments characterized by turbulence and rapid changes in flow velocity (Bartol et al., 2003). I found variable effects of wave-driven water flow on the escape response of juvenile damselfishes with different body morphologies, increasing the response latency of species with a more fusiform body profile. Juvenile reef fishes are highly sensitive to environmental variables (Leis and McCormick, 2002) and negative effects on their settlement and survival patterns can have important consequences for adult populations (Munday et al., 2008). Wave intensity and frequency is increasing across ocean basins worldwide as winds and severe weather events become more frequent with climate change (Webster et al., 2005; Rhein et al., 2013). Strong winds and waves coinciding with juvenile recruitment pulses could affect not only larval dispersal (e.g., Burgess et al., 2007), but also predator-prey interactions and the survivorship of post-settlement juveniles. Many damselfishes recruit seasonally (Russell et

al., 1977; Williams, 1983) and are initially limited by habitat as they settle on the reef (Sale, 1978). Once the recruitment pulse is over, post-settlement mortality becomes important because juveniles that are predated on can no longer be replaced even if habitat becomes available. Given the present findings, future studies should examine whether relationships exist between wave intensity, predation rates and recruitment patterns in the wild, as well as differences in the effect of wave-driven flow on predators and prey due to size differences (see Abrahams et al., 2007; Domenici et al., 2019).

Animal ethics
Animal collections and experiments were approved by the Great Barrier Reef Marine Park Authority (permit G11/34462.1) and the ANU Animal Experimentation Ethics Committee (protocol A2011_21).

Competing interests
The author declares no competing or financial interests.

Data and code availability
The data and analysis script for this study are publicly accessible on the repository Figshare (Roche 2021) and were made available to the editors and reviewers upon initial submission.

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Table 1. Descriptive statistics: escape responses in still water and wave-driven flow.
Measures of escape performance (mean ± s.d.) for three species of damselfish (Pomacentridae) with different body morphologies: response latency (latency), escape distance (D_{esc}), maximum swimming speed (U_{max}), maximum acceleration (A_{max}), and turning rate (T_{rate}).

Variable	*N. azysron*		*C. viridis*		*D. reticulatus*	
	still	wave-driven	still	wave-driven	still	wave-driven
Latency (ms)	9.9 ± 5.4	28.3 ± 28.0	10.1 ± 4.7	19.6 ± 8.6	14.7 ± 8.0	17.1 ± 7.8
D_{esc} (mm)	14.9 ± 3.7	15.9 ± 4.20	11.6 ± 2.6	11.6 ± 3.4	13.9 ± 2.5	14.5 ± 2.4
U_{max} (cm s^{-1})	88.1 ± 22.2	92.5 ± 28.0	74.7 ± 16.8	76.7 ± 24.0	83.4 ± 14.6	92.0 ± 18.2
A_{max} (m s^{-2})	67.8 ± 17.1	65.9 ± 22.8	47.8 ± 10.7	52.6 ± 22.9	55.4 ± 10.6	61.6 ± 16.8
T_{rate} (° ms^{-1})	11.8 ± 2.8	11.2 ± 3.14	9.3 ± 2.4	9.6 ± 2.5	9.9 ± 2.4	11.1 ± 2.8

Table 2. Inferential statistics: escape responses in still water and wave-driven flow.
Effect of body angle, distance to stimulus, body size, species, and water flow condition on three measures of escape performance. Escape performance was measured as response latency, escape distance (D_{esc}), and turning rate (T_{rate}) in three species of damselfish (Pomacentridae) with different body morphologies. R^2_m = marginal r-squared; R^2_c = conditional r-squared.

Variable	Predictor	χ^2	df	P	R^2_m	R^2_c
Latency	sin(angle)	2.65	1	0.103	0.277	0.499
	distance	1.09	1	0.297		
	body size	0.43	1	0.513		
	species	6.12	2	**0.047**		
	flow	32.83	1	**<0.001**		
	species * flow	8.93	2	**0.012**		
D_{esc}	sin(angle)	2.34	1	0.126	0.233	0.665
	distance	0.26	1	0.611		
	body size	3.82	1	0.051		
	species	7.76	2	**0.021**		
	flow	1.20	1	0.273		
	species * flow	0.71	2	0.700		
T_{rate}	sin(angle)	0.12	1	0.726	0.108	0.203
	distance	0.08	1	0.778		
	body size	0.002	1	0.989		
	species	14.99	2	**<0.001**		
	flow	0.83	1	0.362		
	species * flow	4.15	2	0.129		

Table 3. Group comparisons of escape performance. Post-hoc comparisons and effect sizes for response latency, escape distance (D_{esc}) and turning rate (T_{rate}) in three species of damselfish (Pomacentridae) with different body morphologies. Since wave-driven water flow had no overall effect on D_{esc} and T_{rate} (see Table 2), interspecific comparisons are presented for these two variables.

Variable	comparison	t	df	*P*	effect size (95% CI)
Latency	*N. azysron*: still – wave-driven	-5.73	76.8	**<0.001**	**-1.96 (-2.71, -1.22)**
	C. viridis: still – wave-driven	-4.42	76.0	**<0.001**	**-1.51 (-2.22, -0.79)**
	D. reticulatus: still – wave-driven	-1.55	79.7	0.125	-0.54 (-1.23, 0.16)
D_{esc}	*N. azysron* – *C. viridis*	4.68	83.3	**<0.001**	**1.64 (0.90, 2.38)**
	N. azysron – *D. reticulatus*	1.56	80.1	0.270	0.53 (-0.15, 1.22)
	C. viridis – *D. reticulatus*	-3.17	83.1	**0.006**	**-1.11 (-1.82, -0.39)**
T_{rate}	*N. azysron* – *C. viridis*	**4.10**	**85.7**	**<0.001**	**0.81 (0.40, 1.22)**
	N. azysron – *D. reticulatus*	2.10	80.3	0.097	0.40 (0.02, 0.78)
	C. viridis – *D. reticulatus*	-2.11	84.5	0.095	-0.41 (-0.81, -0.02)

Fig. 1. Test species. Indo-Pacific damselfishes (Pomacentridae) with different body plans: *Neopomacentrus azysron*, *Chromis viridis*, *Dascyllus reticulatus*. FR = fineness ratio. Scale bar = 10 mm.
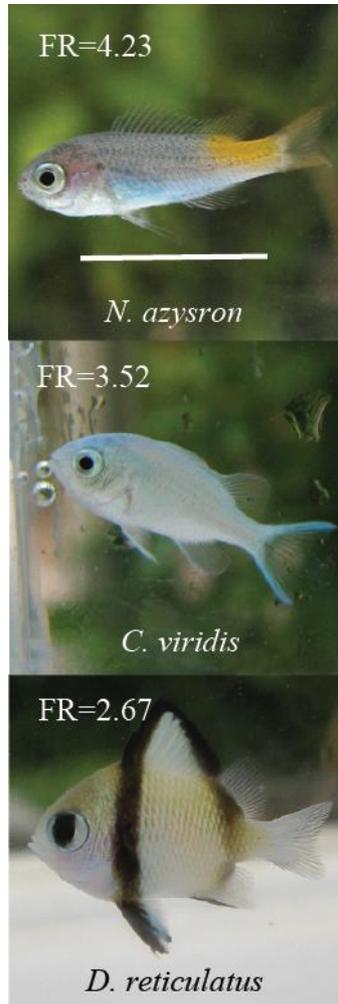
Fig. 2. Experimental setup and particle image velocimetry (PIV). (A) Four programmable pumps (Vortech MP10wES, EcoTech Marine, USA) on the back wall of the experimental tank created wave-driven flow that travelled back and forth in the aquarium at a frequency of 0.85 ± 0.01 Hz (mean ± s.d.). Juvenile fish were placed in a mesh enclosure at the centre of the tank and startled with a remotely operated mechano-acoustic stimulus. PIV analysis showing flow velocity (black arrows) in the mesh enclosure in wave-driven flow conditions during (B) flow direction change and (C) at the wave crest. The colour map represents the magnitude of flow velocity in m s^{-1}. White vectors are values interpolated by the software due to spurious or missing data points. Scale bar for vectors = 0.20 m s^{-1}. For frame sequences of velocity vector and vorticity fields, see Video S1 and Video S2.
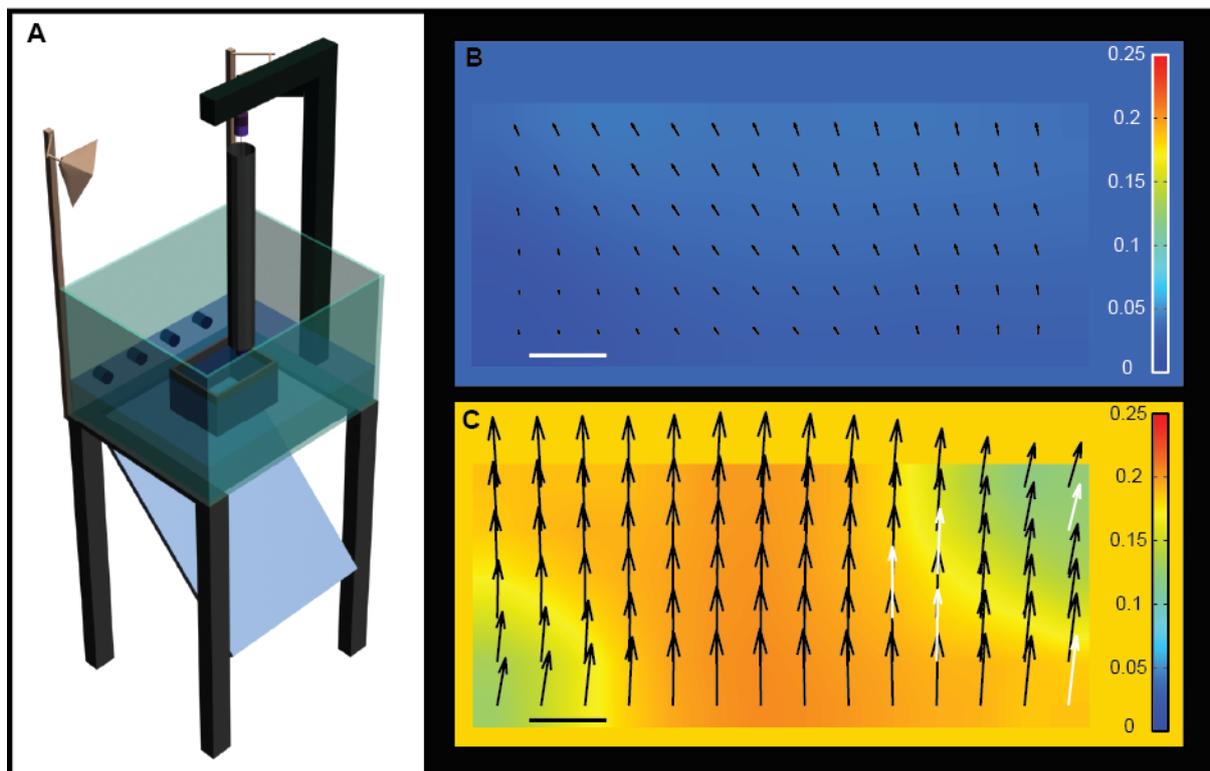
Fig. 3. Excursion distance of a passive particle and three species of Pomacentridae in wave-driven flow. Red circles are individual data points (n = 14 for each group); black dots are means; error bars are one s.e.m. Box plots show the median and interquartile range (IQR); whiskers are 1.5 times the IQR.
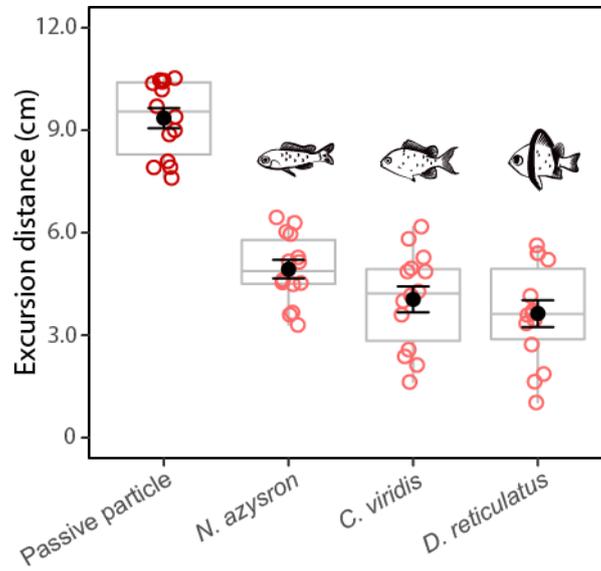
Fig. 4. Response latency, escape distance (D_{esc}), and turning rate for three species of damselfish (Pomacentridae) in still water (turquoise) and wave-driven flow (blue). Left panels (A, C, E) show the *raw data*: circles are the mean for each individual (n=14 individuals per species); black dots are group means; error bars are one s.e.m. Box plots show the median and interquartile range (IQR); whiskers are 1.5 times the IQR. Right panels (B, D, F) are marginal effects plots showing partial residuals (circles) an estimated marginal means for each group (black dots); red dots are estimated marginal means for each species (n=28 individuals) when there was no effect of flow condition; error bars are 95% confidence intervals. Estimated marginal means and partial residuals account for the fixed and random effects included in the statistical model.
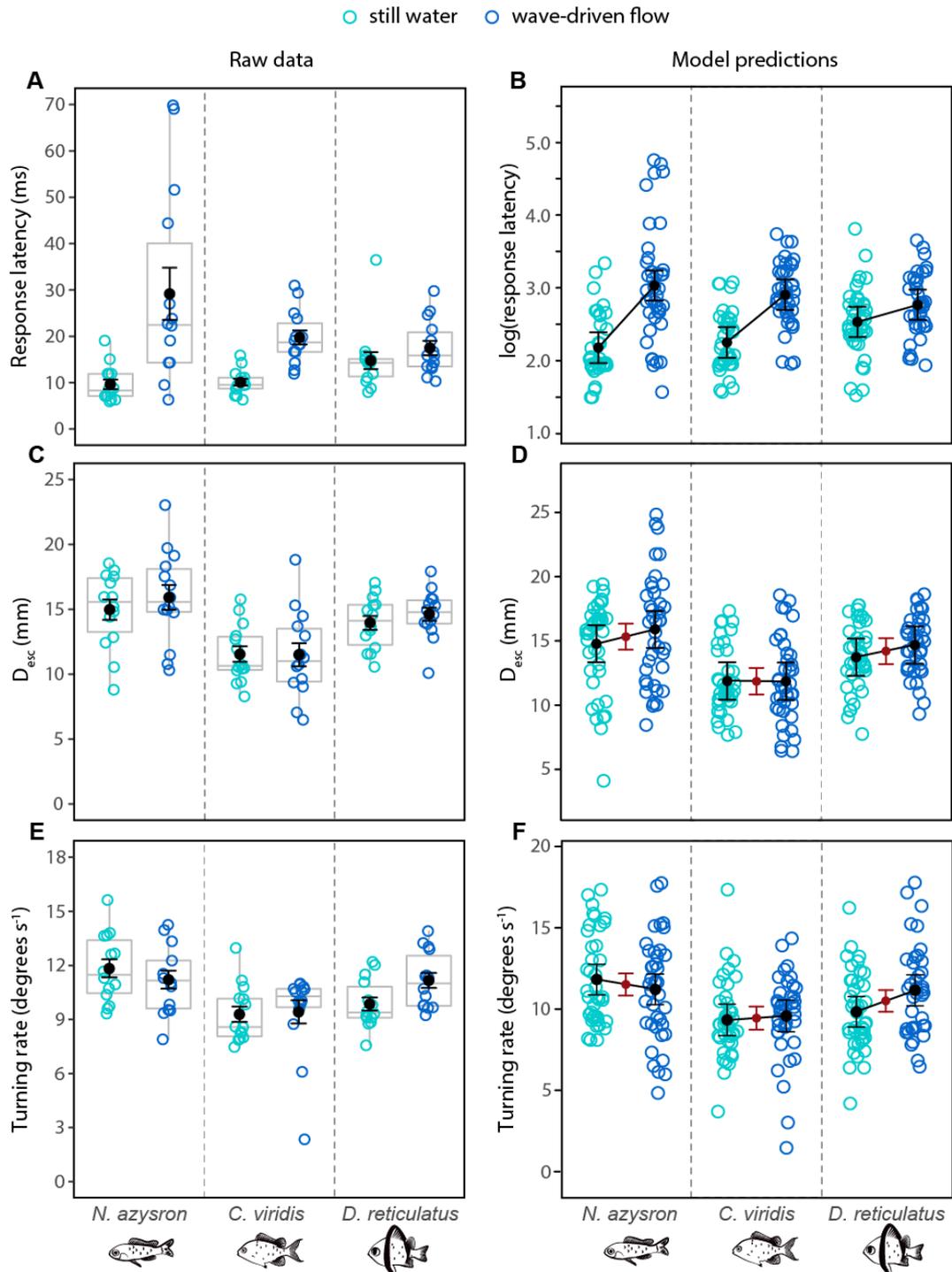
Fig. 5. Response latency in still water and wave-driven flow. Kernel density plots showing the distribution of response latency (ms) for (A) *Neopomacentrus azysron*, (B) *Chromis viridis*, and (C) *Dascyllus reticulatus* in still water (turquoise) and wave-driven flow (blue).
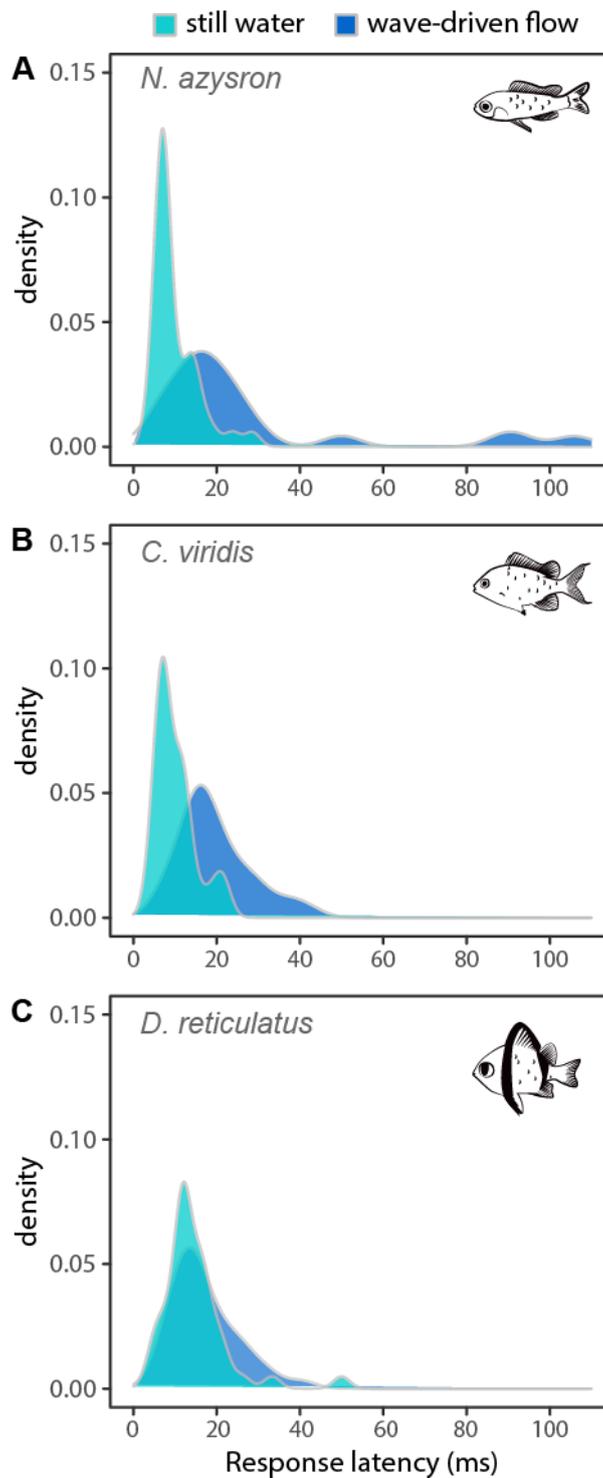
Fig. 6 Escape distance (D_{esc}) in a fixed time interval (24 ms) for three species of damselfishes (Pomacentridae) in wave-driven flow conditions. Fish escaped against the wave path, perpendicular to the wave path, during a wave trough (flow speed < 5 cm s^{-1}), or in the direction of the wave path (*with*). The top panel (A) shows the raw data for each of the three species (sample sizes in Table S3); black dots are group means; error bars are one s.e.m. Box plots show the median and interquartile range (IQR); whiskers are 1.5 times the IQR. The bottom panel (B) is a marginal effects plots showing partial residuals (circles) and estimated marginal means (black dots) across all three species; error bars are 95% confidence intervals.
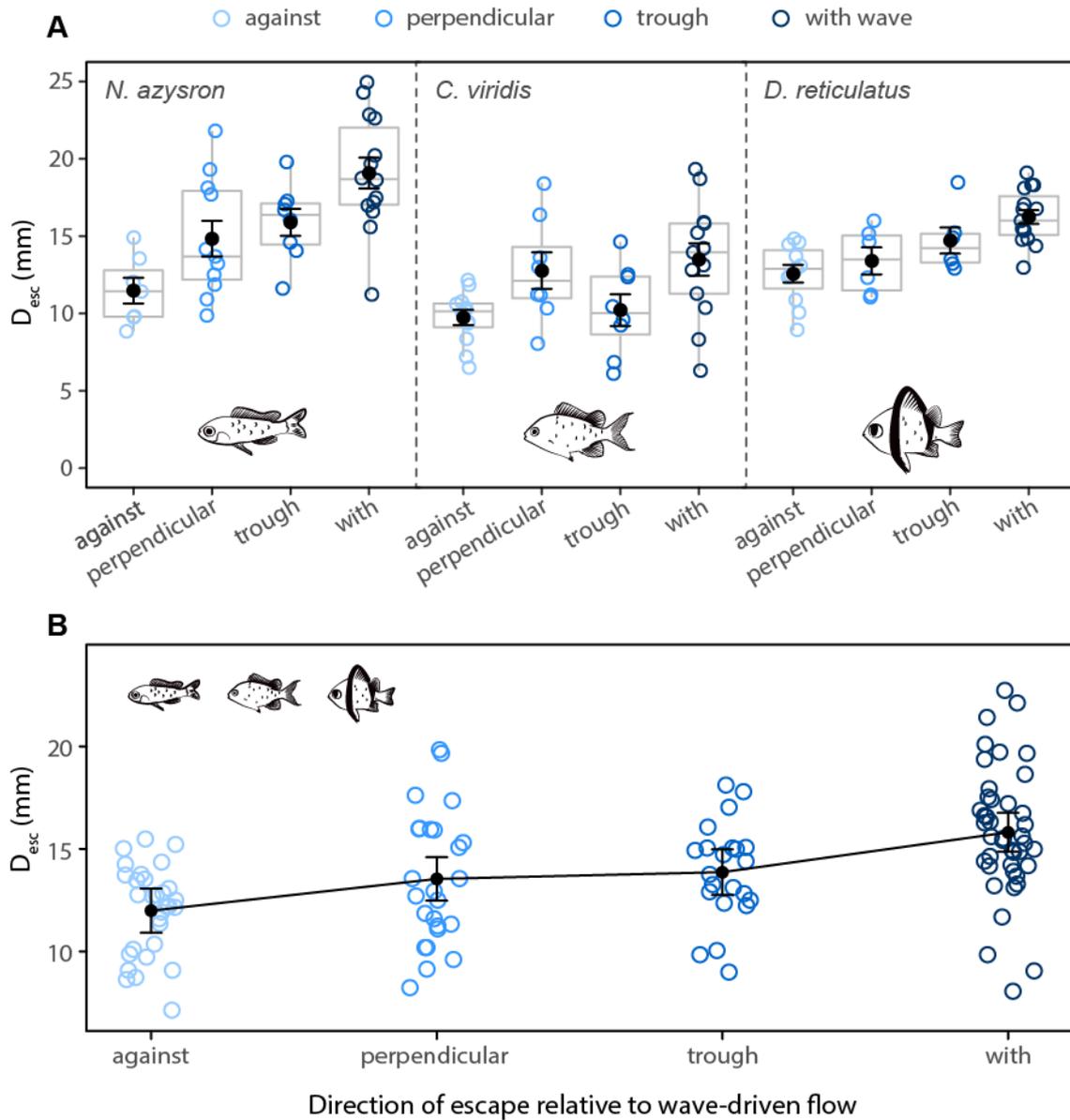
Effects of wave-driven water flow on the fast-start escape response of juvenile coral reef damselfishes

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Table S1. Group comparisons of excursion distance in wave-driven flow. Group (post-hoc) comparisons and effect sizes (analogous to Cohen's *d*) of excursion distance for a passive particle and three damselfish species (Pomacentridae) in wave-driven flow.

comparison	t	df	*P*	effect size (95% CI)
Passive particle – N. azysron	9.25	52	**<0.001**	**3.50 (2.47, 4.52)**
Passive particle – C. viridis	11.10	52	**<0.001**	**4.19 (3.07, 5.31)**
Passive particle – D. reticulatus	11.98	52	**<0.001**	**4.53 (3.36, 5.70)**
N. azysron – C. viridis	1.85	52	0.264	0.70 (-0.07, 1.47)
N. azysron – D. reticulatus	2.73	52	**0.042**	**1.03 (0.25, 1.82)**
C. viridis – D. reticulatus	0.882	52	0.814	0.33 (-0.43, 1.09)

Table S2. Descriptive statistics: best values of escape performance in still water and wave-driven flow. Best values of escape performance (mean ± s.d.) across trials for three damselfish species (Pomacentridae) with different body morphologies: response latency (latency), escape distance (D_{esc}), maximum swimming speed (U_{max}), maximum acceleration (A_{max}), and turning rate (T_{rate}).

Variable	*N. azysron*		*C. viridis*		*D. reticulatus*	
	still	wave-driven	still	wave-driven	still	wave-driven
Latency (ms)	7.0 ± 2.4	16.2 ± 11.1	7.1 ± 1.9	13.4 ± 4.6	10.7 ± 5.5	12.1 ± 5.5
D_{esc} (mm)	16.9 ± 2.9	18.1 ± 4.32	12.4 ± 2.5	12.6 ± 3.3	15.4 ± 1.9	16.1 ± 2.2
U_{max} (cm s^{-1})	101.0 ± 18.8	110.0 ± 23.8	80.8 ± 14.2	86.7 ± 29.2	91.5 ± 11.3	101.0 ± 17.5
A_{max} (m s^{-2})	77.3 ± 13.5	80.2 ± 18.6	54.4 ± 11.0	65.3 ± 27.6	62.2 ± 7.8	74.2 ± 14.5
T_{rate} (° ms^{-1})	13.9 ± 2.5	14.0 ± 2.2	10.8 ± 2.4	10.8 ± 2.9	11.9 ± 1.9	13.5 ± 2.4

Table S3. Mean escape distance (D_{esc}, mm) for each species in still water and at different moments of the wave phase in wave-driven flow. Numbers in square brackets are [one standard deviation, sample size]. Note that the sample sizes include repeated measurements on individuals in still water conditions.

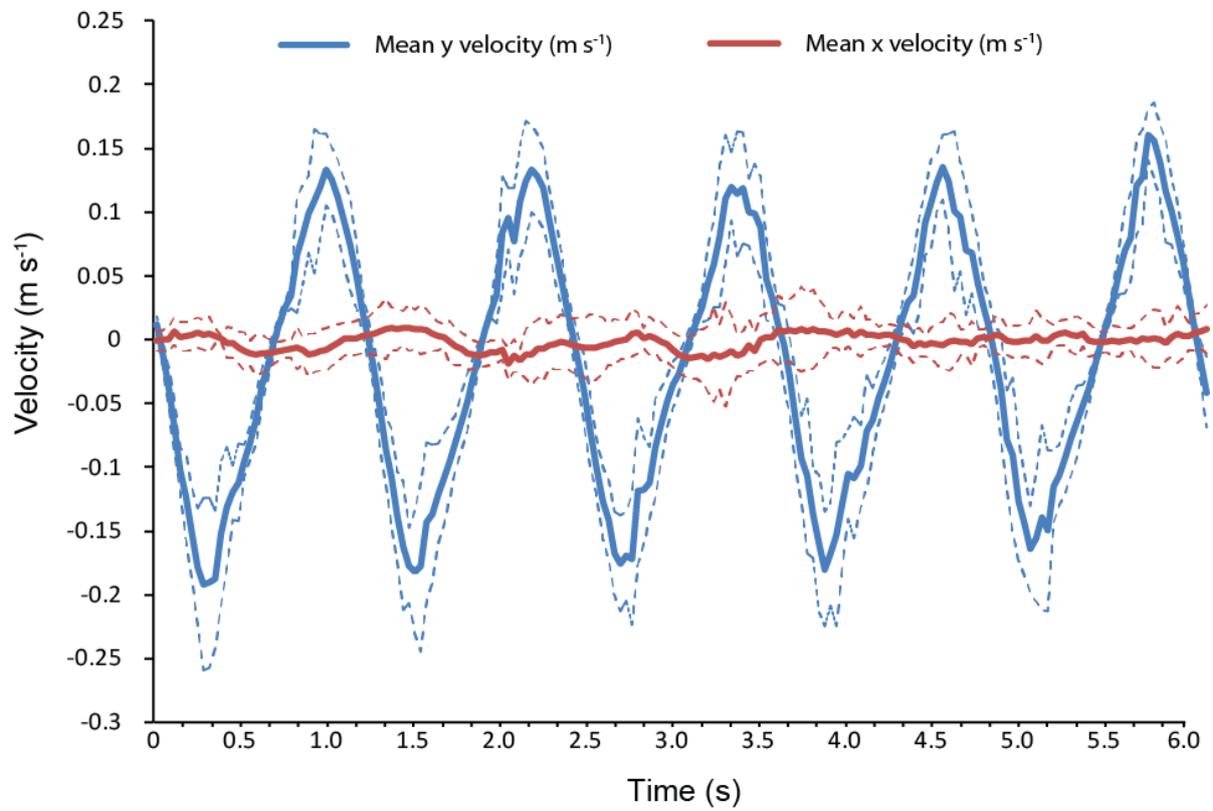|	*N. azysron*	*C. viridis*	*D. reticulatus*
against wave	11.48 [2.20, 7]	9.74 [1.70,12]	12.57 [1.92, 11]
perpendicular	14.83 [3.82, 11]	12.77 [3.35, 8]	13.40 [2.14, 6]
wave trough	15.89 [2.46, 8]	10.22 [2.90, 8]	14.71 [2.06, 6]
with wave	19.08 [3.73, 14]	13.49 [3.76, 13]	16.24 [1.73, 15]
still water	14.90 [3.72, 41]	11.58 [2.59, 39]	13.93 [2.45, 41]
ratio with/still	1.28	1.17	1.17

Fig. S1. Velocity of wave-driven flow through time. Example flow profile showing X and Y components of mean flow velocity in the experimental arena over six seconds. The mean flow velocity at each point in time was computed by averaging all vectors per frame in the software PIVlab v.1.32. Dotted lines represent one standard deviation from the mean.
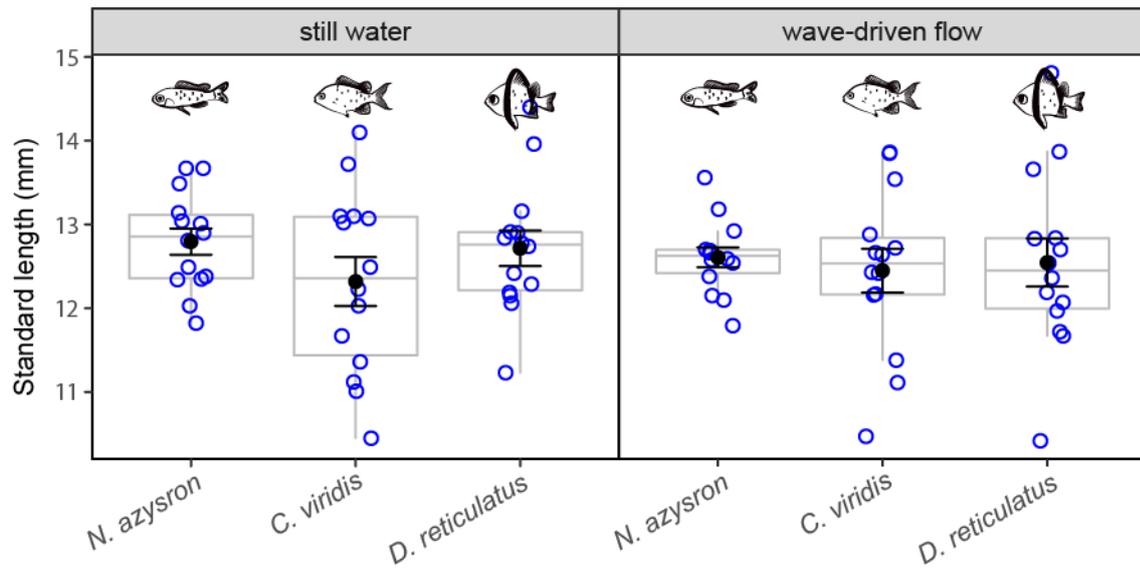
Fig. S2. Body size of test fishes. Standard length (mm) of individuals in three species of damselfish (Pomacentridae) tested in still water and wave-driven flow (n = 14 per group). Blue circles are individuals; black dots are means; error bars are s.e.m. Box plots show the median and interquartile range (IQR); whiskers are 1.5 times the IQR.
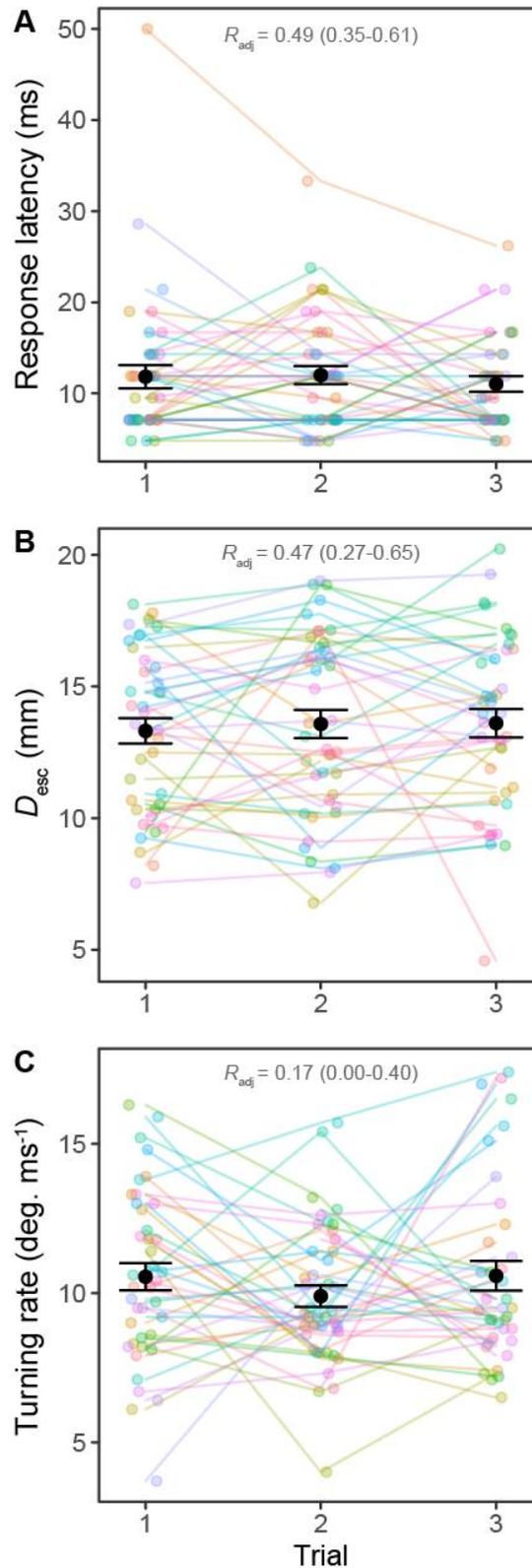
Fig. S3. Reaction norms of escape performance metrics. (A) Response latency, (B) D_{esc}, and (C) turning rate across three repeated trials per individual for three fish species in still water: *Neopomacentrus azysron* (n=14), *Chromis viridis* (n=14), and *Dascyllus reticulatus* (n=14). Mean values and their 95% confidence intervals are indicated by black dots and error bars. Adjusted repeatability (R_{adj}) and its 95% CI are indicated for each species. Data points are jittered along the X axis for presentation purposes.

Movie 1. Examples of escape responses for *Chromis viridis*, *Dascyllus reticulatus*, and *Neopomacentrus azysron* in still water and wave-driven flow conditions (ventral view; recorded at 420 Hz; played at 30 Hz).

Movie 2. Particle movements and frame sequences of velocity vector fields and vorticity fields in the fine mesh enclosure at the center of the experimental aquarium in wave-driven flow (see Fig. 2). Recorded at 30 Hz; played at 30 Hz.

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JEXBIO/2020/234351_R1 Response to reviews

I thank the editor and reviewers for their helpful comments and have revised the manuscript accordingly. Please find detailed responses (in black) to all comments (in grey) below. Line numbers are indicated where changes have been made in the manuscript. I apologize for the slow turnaround in submitting these revisions – I had to deal with childcare duties at home due to intermittent daycare closures over the last three months.

Monitoring Editor Comments for the Author:

As you will see, the reviewers are both generally positive about your study. However, whereas R2 raises several specific but generally minor points to improve the clarify or your paper, R1 raises two key critical points that I agree will require some reanalysis of your kinematics data, as well as the need to do a more in-depth mechanistic assessment related to the response and performance of the three damselfish species. Hopefully, these are issues that you can address upon revision.

If you think that you can deal satisfactorily with the criticisms on revision, I would be pleased to see a revised manuscript. We would then return it to the reviewers.

Response: Thank you for the opportunity to revise the manuscript. Following your advice, I contacted the editorial office on Oct 15, 2020 to ascertain whether my response to Reviewer 1's main comments would be satisfactory. The editorial administrator forwarded my letter to Reviewer 1 and I received the following answer on Nov 4, 2020:

Thank you for following up with this. Here are the comments from the reviewer for you to consider in making revisions and responding to the reviewers:

"Without the analyses relative to the water, the results are still interesting, just harder to interpret. His PIV, even though it's simple, should be enough to get the average flow speed - unless the video wasn't synced to the flow generators. If they weren't synced, then I agree that trying to sync them would introduce errors. A fairly simple analysis would suffice. For example, in Fig 6, is the fact that fish tend to travel farther when they escape with the wave due to the wave motion? Or is there a difference in the escape itself?"

My responses in the letter to Reviewer 1 are pasted below, under "Reviewer 1 Comments for the Author". In response to the question above […] *is the fact that fish tend to travel farther when they escape with the wave due to the wave motion? Or is there a difference in the escape itself?*: I commented on this point in the original manuscript and edited this section for improved clarity following Reviewer 1's minor comment #10. The revised text now reads (L418-424): "Since maximum muscle power output is unaffected by flow conditions, these patterns support the prediction that differences in fast-start swimming performance between flow conditions should

equal differences in water flow velocity. Indeed, maximum flow speeds were approximately 20% of the maximum escape speeds achieved by fishes (Table 1, Fig. S2), and fish escaping in the direction of the water flow achieved a D_{esc} that was, on average, 20.3% greater than that achieved in still water (Table S3)." As pointed out by Reviewer 1, the analysis if fairly simple (i.e., a comparison of values presented in tables and figures) but it convincingly shows (in my opinion) that fish tend to travel farther when escaping with the wave because they are carried by the water motion – not because of differences in the escape response itself.

We are aware that you may currently be unable to access the lab to undertake experimental revisions. If it would be helpful, we encourage you to contact us to discuss your revision in greater detail. Please send us a point-by-point response indicating where you are able to address concerns raised (either experimentally or by changes to the text) and where you will not be able to do so within the normal timeframe of a revision. We will then provide further guidance. Please also note that we are happy to extend revision timeframes as necessary.

Response: Thank you. I appreciated being able to contact the editorial office to discuss the revisions and submit a letter to Reviewer 1 before addressing all of the reviewer comments.

Reviewer 1 Comments for the Author:

This manuscript describes differences in the fast start escape responses in three species of juvenile damselfishes in wave-like flow. The author used a wave tank to study the escape responses of Pomacentrid damselfishes that differ in body depth. Body depth is thought to relate to turning performance, and so the deeper bodied species might be expected to turn more rapidly and be less affected by the wave flow. The largest effect of wave-driven flow was to increase response latency in the two more fusiform of the three species. Other performance metrics generally did not vary significantly among the species.

I have two major criticisms.

1. I think the author should perform all of the analyses relative to the water. As a functional measure, both the fish and their predators would both be in the same overall wave motion, so it does not make much sense to measure escape distance relative to the lab. In particular, D_{esc} seems to vary substantially depending on the wave phase (as shown in Fig. 6). Given the PIV measurements, the author should know the speed of the flow, at least approximately, for any time in the videos.

Response: (*Originally submitted to the editorial office on Oct 15, 2020*) This is a valid suggestion. Unfortunately, the analysis requested by the Reviewer is not possible because I conducted the PIV analyses without the test fishes in the tank, which is noted in the manuscript on L207 and L219-224. In addition, using an approximate estimate of flow speed by trying to match the exact timing of the escape response with the PIV flow measurements would generate considerable measurement error, which I am not comfortable with. While I agree with the reviewer that using the water as a point of reference would produce informative data, I do not see

that using the ground as the frame of reference is problematic if the predator's kinematics are also measured relative to the ground (note that this was not done in my study, which only looked at prey). Ultimately, comparing the kinematics of the prey and predator is informative as long as both are measured in the same frame of reference.

The Reviewer also makes a good point that drawing inferences from kinematic measurements relative to the water relies on the assumption that the prey and predator experience similar water flow. However, this will often not be the case in nature, further complicating the analysis. At the initiation of a predator strike, the predator and prey can be separated by several tens of centimeters, resulting in different flow velocities on shallow coral reefs where wavelengths are short. Additionally, the predator and prey can experience different flows due to flow interactions with the reef microhabitat – for example, if the predator is 30 cm up from the reef, in the water column, and the prey is 5-10 cm above a coral head, closer to the boundary layer. Further examining the effects of complex water flow on predators and prey (of different sizes and in different habitats) is a valuable avenue of research, which I noted in the last sentence of the conclusion (L490-493). Such studies would benefit from state-of-the-art PIV, which was not available to me at the field station where the present study was conducted. Nevertheless, I believe my study makes a valuable contribution as a first examination of how complex flow influences the behavioural and kinematic components of escape responses in three small prey species with different morphologies.

2. I would like to see some more mechanistic analysis, or even speculation, about why the fish have different performance. For example, since the escape distance is measured at 24ms, does that include the latency period? If so, could the differences in latency also explain the differences in escape distance? Are the differences in turning rate due to some kinematic difference? Relatedly, how was the location of the stimulus relative to the fish controlled? Could that explain some of the variation?

Response: (*Originally submitted to the editorial office on Oct 15, 2020*) Measurements of D_{esc} did not include the period associated with a fish's response latency, as is standard practice in studies of fish escape responses. I will specify this explicitly in the revised manuscript to avoid any confusion. Differences in latency among species therefore do not explain observed differences in D_{esc}. Each test fish's distance to the stimulus and angle relative to the stimulus was measured from the videos and controlled for in the statistical model (see L294-295). Neither affected D_{esc} or T_{rate} (see Table 2), likely because I tried within reason to standardize the position of the test fish relative to the stimulus when it was startled.

In the section of the discussion titled *Interspecific differences in escape performance*, I discuss possible reasons for differences in swimming performance among the three species (L364-384):

"Escape distance (D_{esc}) differed consistently among species in still water and wave-driven flow: *N. azysron* (fusiform body) and *D. reticulatus* (laterally compressed body) exhibited similar performances and escaped farther in a fixed amount of time than *C. viridis* (intermediate body depth), irrespective of flow conditions (Tables 1-2, Fig. 4D). Evidence suggests that differences in fast-start swimming performance result from different, and sometimes opposing, morphological features (Domenici, 2003; Walker, 2004; Langerhans and Reznick, 2010). On one

hand, traits such as a shallow, elongated body, a small head and large dorsal and anal fins (such as in pike, *Esox lucius*) are associated with high burst swimming speeds (Webb, 1984; Domenici, 2003; Langerhans et al., 2004). On the other hand, a deep, laterally compressed body has also been shown to enhance burst swimming performance by allowing a lateral profile that increases the mass of water accelerated by body movements (Weihs, 1973; Domenici and Blake, 1991). For example, the deep body of crucian carp (*Carassius carassius*) induced in the presence of predators led to higher burst swimming performance (escape distance, speed, acceleration and turning rate) than that of shallow body morphs found in predator-free habitats (Domenici et al., 2008). These two contrasting profiles roughly correspond to those of the two pomacentrids which escaped the farthest and were at the opposite ends of the body plan spectrum I examined. Turning rate, a measure of agility, mirrored the pattern observed for D_{esc} although differences in performance between *D. reticulatus* and *C. viridis* were less pronounced for turning rate (11.1%) than escape distance (19.3%) (Table 3; Fig 4D,F). This result was unexpected as lateral compression has previously been reported to improve agility (Domenici et al., 2008)."

I agree with the reviewer that a more mechanistic analysis using higher resolution PIV would yield valuable insights. I noted this on L219-224 of the manuscript: "The affordable (and hence relatively unsophisticated) DPIV method I used to characterise the experimental flows at the field station (LIRS) prevented me from investigating the specific forces acting on the fishes' body surfaces during their escape responses. State-of-the-art DPIV setups allow such measurements (e.g., Tytell and Lauder, 2008) but constrain the sample size that can be achieved and directly expose animals to laser light and seeding particles, which could affect natural behaviour, particularly in the small fishes tested here." Please note that this text has now been removed from the revised manuscript following Reviewer 2's comment that it was unnecessary.

As noted above, the current study was a first attempt at examining the effect of complex flow from waves on the escape response of small prey fishes. I believe the study produced useful insights that will be of interest to the readership of *JEB* while acknowledging that future studies are needed that use state-of-the art PIV to further understand how complex flows interact with fish surfaces to influence their fast-start swimming kinematics.

Please note that *JEB* publishes fast-start escape response studies that are relevant to comparative physiologists and biomechaniscists but do not provide an in-depth mechanistic analysis of the patterns observed (for a recent example, see Allan et al., 2020). I believe that my study resulted in an acceptable compromise by providing an adequate (albeit not state-of-the-art) PIV analysis combined with a well-executed and statistically sound examination of the escape kinematics in three species of prey fish across two flow conditions. This approach resulted in 240 individual measurements of escape responses, which is a large sample size for a comparative study of this kind: 3 species x 2 flow conditions x 14 individuals x 3 repeated measurements per individual – note that only 2 repeated measurements could be obtained for 12 individuals. I also believe that making my study's data and analysis script public provides value added by allowing others to readily examine and build on my results.

Minor comments

1. ln. 117. I was confused here by what "transverse sectional diameter" meant until the author

defined the fineness ratio calculation in lns. 189-196. Maybe briefly define here also?

Response: Done. I added in parenthesis "transverse sectional diameter *(measured as the average of the maximum body width and maximum body depth)*"

2. ln. 257. Is D_{esc} genuinely the "cumulative" distance traveled? i.e., the total path length along the curved path of the center of mass? Or is it just the straight-line distance from the starting point to the location of COM 24ms later? Please clarify.

Response: D_{esc} is genuinely the cumulative distance travelled, as typically measured in detailed kinematic analyses of fast-starts in fishes. I specified in the original manuscript "Seven escape performance variables were measured following Lefrançois & Domenici (2006): […]" and I have now added the word *cumulative* before escape distance in the abstract for clarity.

3. ln. 261. As mentioned above, is this time from the moment of the stimulus, or from the first movement?

Response: I have added "from the first head movement" to clarify that this time period does not include the response latency, following Lefrançois & Domenici (2006). As stated in the second half of this sentence, the 24 ms period corresponds to the mean duration of stages 1 and 2 [of the fast-start escape response] for all three species. Stage 1 begins with the first head movement of the test fish in response to the startle stimulus (Domenici and Blake, 1997; Domenici, 2011).

4. ln. 286-289. Please explain the statistical notation here. Not everyone is familiar with how R codes statistical models. In particular, dose "scale(standard.length)" indicate that standard length is included in the model as a quantitative predictor (ie, a covariate)?

Response: Please note that the models are described in words three sentences above in this paragraph: "Standard length, body angle relative to the stimulus, and distance to the stimulus were scaled (i.e., divided by their standard deviation) and included as covariates in each model; an interaction term was included between species and flow condition. The identity of fish was specified as a random factor to account for repeated measurements on individuals." I added the R syntax for extra clarity as R is now a widely used statistical software and some readers appreciate seeing models spelled out both ways. Please note that the word *covariate* is included to describe standard length in the text description of the models; I added "*divided by their standard deviation*" after the word 'scaled' to explain exactly what this term means (L264). Scaling is recommended to facilitate comparisons/interpretation when a model contains covariates that were not measured on the same scale (as was the case here).

5. ln. 288. Why was sin(angle.stim) used, as opposed to just angle.stim? What was the stimulus angle measured relative to?

Response: Sine transforming angles (0-360°) is common practice in circular statistics (also directional statistics) to account for directionality: angles between 1-180 are positive and angles between 181-360 are negative. I have clarified how the angle of the test fish relative to the stimulus was measured by adding "the angle between the straight line connecting the snout to the

CoM of the fish and the tangent to the perimeter of the stimulus" in parenthesis after body angle relative to the stimulus (L284-286).

Response: Thank you for noting this. I now use SL consistently.

Response: R_{adj} is defined in the statistical analysis section of the methods: "I calculated the adjusted repeatability (R_{adj}) of response latency, D_{esc}, and turning rate in still water with the function 'rpt' (Stoffel et al., 2017) […]." *JEB* instructions to authors specify that abbreviations should be used consistently after first use, so I have left the text unchanged.

Response: I have replaced "burst swimming speeds" with "maximum fast-start swimming speeds".

Response: I have added the following text to the methods on L280-286: "The factor 'wave phase' was used to distinguish whether fish escaped against the wave path (315-45°), perpendicular to the wave path (46-134° or 226-314°), during a wave trough (water flow speed < 5 cm·s^{-1}, escape in any direction), or in the direction of the wave path (135-225°). The direction of the escape response relative to the wave path was measured as the angle between the straight line connecting the fish's CoM at the start of stage 1 and the end of stage 2, and the straight line parallel to the wave path."

Response: Indeed, this sentence is important, so thank you for noting that it was unclear. The sentence is meant to explain that water flow velocity, not muscle power output, is responsible for the greater cumulative escape distance achieved by fish escaping in the direction of the wave path rather than against or perpendicular to it. I have attempted to clarify this statement as follows: "Since maximum muscle power output is unaffected by flow conditions, these patterns support the prediction that differences in fast-start swimming performance between flow conditions should equal differences in water flow velocity. Indeed, maximum flow speeds were approximately 20% of the maximum escape speeds achieved by fishes (Table 1, Fig. S2), and fish escaping in the direction of the water flow achieved a D_{esc} that was, on average, 20.3% greater than that achieved in still water (Table S3)."

Response: Yes, thank you. Fixed.

12. ln. 468. More recent studies have found that the Mauthner cell is indeed required for fast starts. See (Hecker et al., 2020).

Response: Thank you for bringing this recent paper to my attention. I read it carefully and it does indeed provide very convincing evidence that ablating the Mauthner cell soma and neuron significantly reduces responsiveness, increases escape latency, and reduces the angular speed of escape responses. Figs 1 and 2 in the paper show that the response latency of fish with ablated Mauthner cells increases from ~ 4-10 ms to ~ 30-80 ms, which is consistent with the longer response latencies I observed for *N. azysron* in wave-driven flow (Fig. 5A). Hecker et al. (2020) concluded that Mauthner cells are required for high-speed, short-latency escapes, but their data show that slower escape responses still occur without Mauthner cells. Therefore, I now cite the paper in the following sentence on L472-474: "Slower responses are generally associated with lower performance (e.g., slower turning rates), and are observed both in healthy fish and in fish with an ablated Mauthner system (Domenici, 2011; Hecker et al., 2020)."

13. Fig. 4. The presentation of raw data here is quite confusing. The left panel shows raw means for each individual, and the right one shows each individual trial, correct? I was initially confused by why there were more points on the right than the left. Does the right panel marginal means, accounting for differences in size, stimulus angle and distance? I don't think the label "model predictions" at the top captures that idea properly. Also, are the red points between the still water and wave-driven flow groups meant to indicate that there is not a significant difference between the groups? It might be clearer to use an asterisk in panel B, instead. The color difference between still and wave flow is also quite subtle. Even though they are graphically separated, I would encourage you to choose some more easily distinguished colors.

Response: I apologize for the confusion. I have clarified the caption for this figure (L842-851) and for Fig. 6. The left panels do indeed show the raw data and the right panels, the estimated marginal means (i.e., least-squares means in the context of traditional regression models) and the partial residuals. These marginally-averaged predictions are more informative than the raw data for drawing inferences because they account for the covariates/fixed factors and random effects included in the model. As specified in the revised legend, the red dots are estimated marginal means for each species (n=28 individuals) when there was no effect of flow condition; differences between species are described in the text and can be inferred from the lack of overlap between the error bars representing 95% confidence intervals around the means. Colours: the turquoise and blue dots representing still water and wave-driven flow appear distinct on my computer screen and when I print the graphs. Unless the reviewers/editor have a strong preference for different colours, I would prefer to keep them as is since they are also used in Fig. 5.

14. Fig. 6 and methods. Please explain what "pendicular" and "trough" mean in the legend, and how they were defined (see comment 9). Is "pendicular" a typo?

Response: 'Pendicular' is indeed a typo and has been fixed. Thank you for picking this up. I now explain how these categories were determined on L280-286 of the methods (please also see my answer to point 9 above) and have added the following text to the figure caption: "Fish escaped against the wave path, perpendicular to the wave path, during a wave trough (flow speed < 5 cm s^{-1}), or in the direction of the wave path (*with*)."

Reviewer 2 Comments for the Author:

This manuscript looks at an ecologically crucial behavior - escape responses - in juvenile coral reef fishes under relevant environmental conditions of still and wave-driven water flow. The manuscript is well curated and the results are overall supported by the data. My main concern is that the lack of pronounced effects in the kinematics variables could be drive by methodological aspects. There are also a couple of issues that I would like to see clarified mainly within the materials and methods and the presentation of some of the data.

Response:

Major comments:

Introduction

Page 3 lines 104-106 - While I appreciate the predictions, how is this supposed to translate in terms of the variables measured?

Response: Good point. I revised this sentence as follows (L107-110): "I predicted greater negatives effects of wave-driven flow on the response latency and escape kinematics of species with a more fusiform than laterally compressed body due to the increased rolling stability conferred by lateral compression."

Materials and methods

Page 3, lines 112-114, I know the size of the fish is reported but it would help to have a time frame of how late in terms of post-settlement are we talking about.

Response: Based on published values for the body size of recently settled juveniles and light-trapped larvae of coral reef fishes (Kerrigan, 1996; Vigliola and Meekan, 2002; Fisher et al., 2005), the fishes I examined were less than two weeks post-settlement. I now indicate this and cite the two most relevant papers on L118-120.

page 3, lines 123-128 This seems out of place here and not necessary

Response: This text was included in the manuscript because several previous studies (cited in the manuscript) have identified that pectoral fins are important for stability. Since a fish's ability to maintain an upright posture in complex flow could influence its response latency and fast-start

swimming performance, I felt it was important to explain that pectoral fin shape is similar in the juvenile fishes I examined, differentiating only later in their development. I would prefer to retain this text in the manuscript unless the editor strongly opposes it.

Response: The breeder net was 1 mm stretched mesh. I have added the following information to L144-145: "fine *nylon* mesh enclosure (large net breeder, Aqua One, Australia; 26.5 x 15 x 15.5 cm; *1mm stretched mesh*). Unfortunately, I did not record flow velocities in the tank without the mesh enclosure to assess how the net dampened the waves. The flow velocities presented in the text and in Fig. S2 are from measurements with the mesh enclosure present as this was the setup used for the experiments. The net would indeed have had a dampening effect on the waves as Reviewer 2 points out, but the resulting flow was nonetheless adequate and relevant. Unfortunately, I am no longer in Australia and cannot return to the laboratory facilities at the Lizard Island Research Station to carry out further measurements with this setup.

Page 5 lines 226-233 - This seems out of place here and would be better in the results or discussion sections

Response: I have moved this short paragraph to the discussion as suggested.

Page 5, lines 244-245, I am not sure how the fact that flow visualization was carried out without the fish affects the frame of reference...

Response: Since the flow visualization was carried out without the experimental fish present, the kinematic measurements are in the environmental frame of reference; that is to say, relative to the ground rather than the water. I made sure this was explicit in the manuscript. Please refer to a discussion of this point in my responses to Reviewer 1.

Page 5, line 247, is the 25 frames referring to the whole trial that was analyzed? This seems like very little resolution especially considering that you are fitting a 5 point quadratic polynomial regression to filter the data... Could this be driving the lack of kinematic differences? This is even more striking if you are only looking at the 24ms referred to in lines 260-262 of page 6, which would mean that the information on kinematic variables is all obtained in 10 frames. I read this in the Supplementary material but I am not sure it is properly addressed in the methods, why are there more trials for each fish under still water?

Response: The polynomial regression was used over 25 frames (60 ms) to obtain smoothed values of swimming speed and acceleration, not cumulative escape distance (L265 of the original manuscript). Smoothing is routinely used for measures of speed and acceleration since these metrics are highly variable, being the first and second derivative of distance, respectfully. On L282-285 of the original manuscript, I specified "Differences in U_{max} and A_{max} were not tested to reduce the number of statistical tests (and hence the probability of spurious results), and because U_{max} and A_{max} are less reliable measures of distance-time performance than D_{esc} (Domenici and

Blake, 1997)." Nevertheless, U_{max} and A_{max} values for the three species and two flow conditions are presented in Tables 1 and S2 for reference purposes.

Kinematic differences were identified among species (see Tables 2 and 3, Fig. 4) as well as within species, across the wave phase in wave-driven flow (Fig. 6). I explain the likely reason for a lack of difference in kinematics between the still water and wave-driven flow treatments on L412-416: "On average, escape kinematics (D_{esc}, turning rate) were similar in still water and wave-driven flow within species (Table 1, Fig. 4C-F). This pattern is a consequence of the large variation in escape performance by fishes in wave-driven flow, which was driven by variation in the timing of the stimulus (and hence the escape response) relative to the wave phase (Table S3, Fig. 6)."

In Table S3, the sample size in wave-driven flow is broken down according to different points of the wave phase, potentially explaining why Reviewer 2 thought the sample size was larger in still water. The number of trials was roughly the same for fishes in both experimental conditions – this can be gleaned from the archived data (https://figshare.com/s/206ab8277dda47d9812f). The sample size is also detailed on L184-190 of the main text: "Fourteen fish were tested per species in each of the two flow conditions in a full factorial design. Individual fish were tested at least three times with a rest period of 30 min between trials (see Jornod and Roche, 2015). More than three trials were run (with a maximum of five) when fish made sudden movements before the stimulus hit the water surface, covered a vertical distance greater than their body depth, or exited the camera's field of view at the onset of stage 3 of the escape response (see *Measurements of escape performance)*. For 12 of the 84 fish tested, only two trials could be obtained for analysis."

Results

Page 7, line 332, I am not sure what rather refers to here.

Response: I have clarified the text as follows (L323-324): "when escaping with the water flow rather than perpendicular to or against the water flow".

Discussion

Page 8, lines 358-359 While some types of fast starts can be involved in prey capture, this sentence is misleading because the type of fast starts analyzed in the paper are not feeding related.

Response: I have modified this sentence as follows: "Fast-start escape responses influence the ability of juvenile coral reef fishes to avoid predation and are therefore directly relevant to their ecology (Fisher and Leis, 2010)."

Page 9, lines 445-447 and related sentences. It would be helpful to explore further what mechanisms might be causing the increase in latency and how they could be related to the body morphology. For example, how would body compression affect lateral line filtering?

Response: Thank you for bringing this to my attention. In researching the topic, I found that Dijkgraaf (1963) postulated that the signal-to-noise ratio can be increased when the lateral line is positioned further from the pectoral fins, which is more easily achieved in deep-bodied than slender fishes. I know mention this on L445-455:

"Slower responses to the stimulus in wave-driven flow could be due to two main factors: first, the destabilizing effect of water motion and the added challenge of maintaining an upright posture and adequate orientation relative to the flow; and second, the challenges of perceiving a threat due to added noise in complex flow versus still water. All else being equal, a more laterally compressed body should improve resistance to rolling disturbances (Eidietis et al., 2002; Weihs, 2002). A deep body plan should also improve mechanoreception by allowing the placement of the lateral line away from moving body parts (i.e., pectoral fins), a trait which has been posited to decrease self-generated noise and increase the signal-to-noise ratio (Dijkgraaf, 1963; Coombs and Braun, 2003). These two considerations might explain why the longer response latency in wave-driven flow was inversely related to body depth in the three species I examined."

I could not find more recent references or empirical studies examining how body morphology affects lateral line filtering but would be happy to expand on this topic if the Reviewer can suggest other references.

Conclusion

The conclusion should focus more on the findings of this study and less on vague implications related to anthropogenic pressures. I was particularly confusing by the reference to winds that has not been made anywhere else in the manuscript.

Response: I understand the Reviewer's perspective and re-read the conclusion carefully. I agree that tenuous links to climate change often undermine rather than strengthen comparative physiology studies. However, I do not think this is the case here and note that the importance of wind for wave generation is a well-understood phenomenon, which I mentioned several times in the manuscript. For example:

L67-70 of the introduction: "On coral reefs, for example, complex water flow from waves influences the ability of adult fishes to swim and occupy shallow, windward habitats, which leads to strong patterns of community structuring based on a species' ability to withstand ambient flow conditions (Bellwood and Wainwright, 2001; Bellwood et al., 2002)."

L79-88 of the introduction: "Such biophysical interactions could be of considerable ecological importance for coral reef fishes given the importance of predation in shaping their distribution and abundance. In addition, wave intensity and frequency are increasing in ocean basins worldwide as a result of climate change (Young et al., 2011; Rhein et al., 2013), with impacts anticipated on key processes in marine communities (Harley et al., 2006; Byrnes et al., 2011), including predator-prey interactions (Domenici and Seebacher, 2020). Basic knowledge of how waves influence the behaviour and unsteady swimming performance of fishes is essential to improve our understanding and ability to predict how environmental change will affect fish

communities – for example, by altering energetic demands and the outcome of predator-prey encounters (Higham et al., 2015; Killen et al., 2016)."

L390-395 of the discussion: "The wave-driven flow treatment in the current study was representative of water flow speeds routinely experienced by fishes under winds of 15 knots and intermediate wave heights on semi-exposed reefs at Lizard Island (Roche D.G. unpublished data; Fulton and Bellwood, 2005)."

Conclusions are often a matter of personal preference and, in this case, I aimed to highlight the broader ecological relevance of the study's results given my background in ecophysiology and what I considered to be relevant questions arising from the findings. I do feel that the text of the conclusion is justified and relevant – for example, in the last sentence, I highlight a key topic of interest to comparative physiologists and biomechanicists in the context of environmental change: the importance of assessing potential differences in the effect of wave-driven flow on predator and prey locomotion due to size differences. See Abrahams et al. (2007) and Domenici et al. (2019) for discussions of this topic. I hope the Reviewer will agree and support my request to retain the current conclusion.

Acknowledgements

Is the author referring to the experimental setup CAD models? I was confused.

Response: I have changed this sentence to "3D CAD models of the experimental setup were kindly made by H.F. Zhu". Thank you.

Figures

Figure 2, The second (A) and (B) should read (B) and (C)

Response: Fixed. Thank you!

Figure 4, looking at this figure, I wonder if the author looked into the coefficient of variation and how that changes from still to wave-driven.

Response: Thank you for this suggestion. I did look at the CV and it was apparent that there was often more variation in wave-driven flow than in still water. This is what led to Fig. 5 and the analysis of kinematic differences in wave-driven flow presented in Fig. 6.

Figure 5, I would've loved to see a bit more discussion on the findings of this figure. I do think it might actually help interpret the mechanisms that underline the changes in performance under wave-driven conditions in some species but not others.

Response: On L445-455, I now discuss the potential for lateral compression to improve mechanoreception by decreasing self-generated noise arising from movements of the pectoral fins in complex flow. As mentioned above, I thank the Reviewer for this excellent suggestion and would welcome suggestions of more recent references if they exist.

Response: Panel B displays the model predictions (i.e., the estimated marginal means), which account for the covariates included in the model and the repeated measurements on individuals. For this reason, it is not possible to draw inferences from simply looking at the raw data presented in panel A. As such, I strongly believe that presenting the raw data and the model 'output' as two panels in the same figure is the most honest and transparent means of presenting the results. It also satisfies two types of potential readers: those who wish to see the raw data and those of wish to focus on the model output (or both).

Supplementary Material

Fig S1 is not really necessary.

Response: I have removed this figure as suggested and renumbered the supplementary figures accordingly.

Fig S3 is also not necessary, especially because the findings are stated in the results.

Response: I agree but some readers appreciate being able to visualize the distribution of the data points rather than only having access to the means and a measure of variation. For this reason, I would prefer to retain this figure in the supplementary material if possible.

Fig S4 is also not necessary.

Response: Agreed. This figure is not essential, but it will be of interest to researchers studying the repeatability of behavioural traits (response latency) and performance measurements (cumulative escape distance, turning rate). For this reason, I opted to include this figure as supplementary material rather than in the main text.

Instead of the two videos provided, it would've been more relevant to show the raw PIV video and some fish escape behaviors.

Response: I am working remotely at the moment because of covid-19 restrictions but I will have access to the hard drive containing these videos in mid-January. I will upload these videos then and notify the editorial office as soon as it is done.

Minor comments:

Page 2, lines 83-85, this is not a very strong sentence and it should be reworded for focus

Response: I have added "for example, by altering energetic demands and the outcome of predator-prey encounters", two important points noted in Higham et al. (2015) and Killen et al. (2016).

Page 3, line 100, not just that, the plain larger body depth will reduce roll

Response: Absolutely. For clarity, I have added the word "also" to this sentence since I mention in the earlier sentence that larger body depth is beneficial for postural control (i.e., reduces rolling).

Page 5, line 200, remove Digital and the D from DPIV.

Response: Done. Here and elsewhere.

Throughout the manuscript make sure that the units are showing properly, this is I believe a MAC issue.

Response: Done. Thank you.

Page 5, lines 219-224 In my opinion, this is not needed, however if a reviewer told you to include it, by all means keep it.

Response: I have removed this text from the revised manuscript.

Page 8, line 363, in still AND wave driven flow? I think the and is missing here

Response: Fixed. Thank you.

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