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Disc starts: the pectoral disc of stingrays promotes omnidirectional fast starts across the substrate

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Disc starts: the pectoral disc of stingrays promotes omnidirectional fast starts across the substrate

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Abstract

We explored how the flattened and rounded pectoral disc of the ocellate river stingray (*Potamotrygon motoro*, Müller & Henle 1841) enables them to utilize the benthic plane during fast-start escape. Escape responses were elicited via prodding different locations around the pectoral disc, and recorded using video. Modulation of pectoral fin movements that power swimming enabled omnidirectional escape across the substrate, with similar performance in all directions of escape. Hence, translation of the body did not necessarily have to follow the orientation of the head, overcoming the constraint of a rigid body-axis. An increase in prod speed was associated with an increase in initial translational speed and acceleration away from the prod. As prod location shifted towards the snout, yaw rotation increased, eventually reorienting the fish into a forward swimming position away from the prod. Further, stingrays yawed with essentially zero turning radius, allowing reorientation of the head with simultaneous rapid translation away from the prod, and yaw rate during escape was substantially greater than reported during routine swimming for stingrays. We conclude that stingrays employ a distinctive approach to escape along the substrate, which we have termed disc starts, that results in effective manoeuvrability across the benthic environment despite limited longitudinal flexibility of the body, and challenges the concept of manoeuvrability typically used for fishes.

Keywords: *Potamotrygon motoro*, ocellate river stingray, stingrays, fast starts, escape responses, benthic, manoeuvrability, rigid-body axis

Introduction

Fast starts are high-performance manoeuvres, involving high accelerations and speeds from rest or routine locomotion, employed during predator-prey interactions such as in attack and escape responses (Domenici and Blake 1997). Some of the most impressive manoeuvres in biology occur in predator-prey interactions. Hence, fast starts during escape and attack may reveal diversity in the capacity for locomotor performance promoted by physiology and morphology (Harper and Blake 1991; Sharp 1997; Tucker 1998; Martin et al. 2005; Higham et al. 2017), and accordingly, may provide valuable insight into form, function, and behaviour that is effective for survival in different environments (Higham et al. 2016). Body form will have a large impact on swimming mechanics during fast starts. Fishes are often categorized as either body and caudal fin (BCF) or median and paired fin (MPF) swimmers, based on the propulsive surfaces that are used to power routine swimming (Breder 1926; Webb 1974, 1984*a*, 1984*b*; Sfakiotakis et al. 1999; Blake 2004). In BCF swimmers, contractions along the axial-musculature power swimming, passing a propulsive wave through the body and caudal fin (Gray 1933; Bone 1966; Altringham and Ellerby 1999; Donley 2003). In these fishes, two types of fast-start behaviour are exhibited, C-starts and S-starts, which are characterized by the axial deformation of the body that occurs during the event. During a C-start, a unilateral contraction yaws the animal into a C-shape, changing the angular orientation of the head (Webb 1981; Domenici 2004; Borazjani et al. 2012), followed by a return sweep of the tail, which accelerates the fish in the new orientation away from the threat. S-starts involve simultaneous contractions on both sides of the axial musculature, bending the animal into an S-shape (Webb 1976; Hale 2002; Schrieffer and Hale 2004), followed by contralateral contractions, as the fish accelerates forwards with little angular displacement. Hence, manoeuvrability in BCF powered fast starts is dominated by initial yaw rotation and then forward translation. MPF swimmers encompass a diverse range of fishes that may use pectoral, dorsal, anal, and pelvic fins, or a combination of these median and paired fins, to power swimming (Rosenberger 2001; Korsmeyer et al. 2002; Walker and

Westneat 2002; Blake and Chan 2011; Jagnandan and Sanford 2013; Ruiz-Torres et al. 2014). Many MPF swimmers display a decoupled locomotor strategy, whereby they switch from MPF swimming during routine activity to BCF swimming during fast starts (Domenici and Blake 1993; Kasapi et al. 1993; Westneat et al. 1998; Borazjani et al. 2012). This strategy appears common to MPF swimmers with body shapes that closely resemble the profiles of BCF swimmers. Whether all MPF swimmers rely on a decoupled locomotor strategy for fast starts is not clear, yet this is unlikely due to the substantial diversity in morphology in these fishes.

Stingrays (suborder Myliobatoidei) are dorsoventrally flattened fishes that employ MPF swimming, but which possess a striking difference in morphology compared with most BCF and MPF swimming fishes (Aschliman et al. 2012; Fontanella et al. 2013; Franklin et al. 2014). The caudal region is fashioned into a whip-like appendage (with a barbed stinger) used primarily for defence, while appearing poorly suited to power swimming due to low surface-area. Stingrays have very large and flexible pectoral fins that join to the head and the body, and together, the head, body and fins are referred to as the pectoral disc (Blevins and Lauder 2012; Fontanella et al. 2013; Franklin et al. 2014). Drag-based undulations of the low aspect-ratio (i.e. rounded) pectoral fins are used to power a rajiform mode of MPF swimming (Rosenberger and Westneat 1999; Rosenberger 2001; Blevins and Lauder 2012; Fontanella et al. 2013). Anterior to the pelvic girdle the body axis is relatively inflexible. Hence, a decoupled locomotor strategy, where stingrays could employ axial undulations and BCF swimming during fast starts, seems unlikely. This morphology has been associated with poor manoeuvrability, apparently due to the rigid body-axis (Parson et al. 2011). That said, stingrays can apparently yaw with next to no turning radius during routine swimming (He and Zhang 2013). Furthermore, stingrays are preyed upon by predators such as crocodilians, sharks and marine mammals (Strong et al. 1990; Cliff and Dudley 1991; Visser 1999; Nifong and Lowers 2017), and so we might expect them to have impressive escape (i.e. manoeuvring) abilities.

Yet there seem to be few studies that explore escape behaviours in stingrays (Semeniuk and Dill 2005, 2006), and none have explored behaviour in the context of morphology and fast-start performance where we might expect to observe a fuller demonstration of the implications of a flattened disc-shape for manoeuvrability.

Whether MPF or BCF, a fish suspended in an aquatic environment has the potential to accelerate along three translational freedoms (i.e. forwards-backwards, side-to-side, and up-down) and three rotational freedoms (i.e. yaw, pitch and roll) (Dudley 2002; Webb 2004). Surprisingly, fishes studied thus far do not seem to utilize all freedoms of motion during fast starts, although whether this is a physical or behavioural limitation, or failure to invoke such responses in the experimental design, is not clear. The abilities of stingrays to exploit these freedoms in a benthic environment is also unclear. In the present study we evoked and described escape responses in the ocellate river stingray (*Potamotrygon motoro*, Müller & Henle 1841). Potamotrygonidae stingrays characterize an extreme form of benthic stingrays, such that the pectoral disc is nearly symmetrical from a dorsal view, with rounded pectoral fins that generate high drag (Figure 1) (Blevins and Lauder 2012; Fontanella et al. 2013; Franklin et al. 2014). It was hypothesized that this morphology may promote flexibility in escape behaviour in a benthic environment, allowing the fish to utilize the entire plane along the benthos for escape from rest.

Materials and methods

Animals and housing

All procedures were approved by the University of Calgary animal care committee, following CCAC guidelines. Four river stingrays (*Potamotrygon motoro*) (pectoral disc width 14.9 ± 0.410 cm S.E.M.; mass 155 ± 10.2 g S.E.M.) were purchased from a licenced supplier and transported to facilities at the

University of Calgary. Stingrays were housed in a cylindrical holding tank (180 cm diameter x 70 cm height, approximately 1400 L) with flow-through freshwater at 27° C, pH of 6.5, bubbled with air. Room lighting was provided with a 12:12h on:off cycle. The stingrays were provided 2.5 cm³ of frozen bloodworms per stingray twice a day, and were monitored to ensure feeding. Non-abrasive substrate (fish tank gravel) was used to cover the bottom of the tank, with a grain size greater than 1 cm to reduce burying behaviours. Stingrays were acclimated for two weeks prior to experiments, water chemistry was measured daily, and the fish had regular veterinary oversight.

Data collection

A separate tank with the same dimensions, water chemistry and substrate as the holding tank was used for experimentation. An individual stingray was transferred to the experimental tank via a rubber mesh net and given 24 hours to acclimate without being fed. The stingray was then lured to the middle of the tank using bloodworms wrapped in a small mesh net. An escape response was then induced by quickly prodding the pectoral fins with a flexible, plastic tube (2.5 mm diameter), with a tip made of soft silicone tube. Stingrays were not prodded on the body or the tail because these areas seem to be more prone to a reflex defence-response by the tail (Campbell 1951). The escape response was recorded by five submerged video cameras (Hero3 Black, GoPro Inc, San Mateo, California, USA) at a frame rate and a video resolution of 240/s and 848x480p, respectively. One camera filmed the dorsal view 60 cm above the stingray to capture movements of the stingray and prod across the substrate, discussed below. Four cameras filmed along the substrate, separated by 90 degrees around the tank and placed approximately 20 cm from the fish, to capture movements of the stingray and prod above the substrate; of these four cameras, images from the camera for which the prod moved perpendicular across the field of view were selected for analysis. A measuring stick was placed in the tank to calibrate the cameras before

experimentation commenced, and was used to confirm the calibration was the same across the field of view in the X and Y axes for the camera filming the dorsal view, and in the Z axis for each camera filming in the horizontal view. A pair of light emitting diodes attached to the prod was used to synchronize the cameras at the onset of recording. Two 1000 W halogen lights were attached to the perimeter of the tank to illuminate the filming area (Southwire Tools, Carrollton, Georgia, USA).

A total of 24 responses were collected. Each of the four animals were prodded, and the escape response recorded, six times (one prodding event = one trial). An effort was made to distribute the prodding events evenly around the pectoral fins for each animal. Furthermore, we exercised a range of prodding speeds to investigate the effect of stimulus intensity on escape performance. The four stingrays were rotated into the experimental tank throughout the study, such that only a single escape response was recorded from an animal before it was placed back into the holding tank and another animal moved into the experimental tank. This provided more than a 96-hour resting period between each trial on a given animal.

Data analysis

Since none of the fish were prodded in the same location more than once, all trials from the four fish were treated as independent observations. For each trial, the tip of the prod, the body midline, the centre of the disc, and 11 points (30° intervals) around the periphery of the pectoral fins were digitized and their locations tracked for successive frames, using Image J (bundled with 64-bit Java 1.8.0_112, Wayne Rasband Developers 1997). The location of the tip of the prod was used to quantify the impacts of prod speed and prod location on different aspects of escape behaviour, defined below. The movements of the prod and the stingray were measured in three dimensions; X and Y coordinates were

obtained from the camera filming from the dorsal view, while Z coordinates were obtained from a camera filming along the horizontal view. Digitizing of the prod was initiated from the onset of the thrusting motion towards the fish, and terminated when the plastic tube bent from contact with the animal. Escape movement of the stingrays was minimal in the Z axis (i.e. the stingrays escaped extremely close along the substrate). Hence, only the X and Y coordinates of the centre of the disc were used to assess translational kinematics of the stingray. The line between the centre of the disc and the snout was quantified to assess angular rotation (i.e. yawing) kinematics.

To power escape, stingrays roll their pectoral fins up and over towards the dorsal midline. Thus, points around the pectoral fins, every 30 degrees relative to the centre of the disc (starting from the snout, 0°, and not including the tail base, which do not possess pectoral fin), were digitized to explore activation and termination of the first finbeat of the escape response (Figure 1). Digitizing of the kinematics of the pectoral disc (fins, centre and midline) was initiated at the onset of the prod being thrust towards the animal, and was terminated after the stingray clearly stopped yawing (i.e. the stingray reached its preferred swimming orientation away from the prod). Data was smoothed using a quintic spline (Walker 1998) in R (R Development Core Team 2008), and transferred to excel to calculate escape parameters, discussed below.

Escape responses were divided into phases 1 and 2 (Figure 1). Phase 1 corresponds to the first finbeat of the escape response. Initiation of the first finbeat was defined as the onset of the movement of a peripheral point of the pectoral fin upwards and toward the centre of the disc; onset of movement was defined as a fin edge moving more than 5% of the distance between the centre of the disc and the fin edge at rest. Termination of the first finbeat corresponded to the time that all peripheral points of the

fin involved in the response had reached the shortest distance from the centre of the disc (i.e. maximum fin roll). Phase 2 started with the termination of phase 1, and ended when the fish finished rotating and reached its preferred swimming orientation away from the prod. Of note, phase 1 and 2 of the escape responses in stingrays are not intended to be comparable to stages 1 and 2 of a C- or S-start, which have entirely different kinematics; the phases are only intended to facilitate analysis and interpretation of the behaviour.

Prod location on the pectoral disc was measured as the angle between lines connecting the snout to the centre of the disc, to the location of contact of the prod on the pectoral fins. Relative to the substrate, the prod angle of attack was similar across all recordings (119 ± 0.678 S.E.M.). Prod speed was measured as the speed of the prod upon contact with the animal. This was determined using 3-dimensional Pythagoras Theorem from the displacement of the X, Y (dorsal view camera) and Z coordinates (horizontal view camera) and the duration of successive frames. Translational and yawing speeds of the centre of the disc were measured using the two-dimensional displacement of the centre point and its relationship to the snout during each digitized frame. Translational and yawing accelerations were measured as the change in translation and rotational speeds, respectively, between successive frames. Yaw displacement was measured as the total angle rotated throughout the entire escape response (beginning to end of phase 1 and phase 2). Turning radius typically describes the radius of an arc followed by the path of the body of the fish throughout a response (Blake et al. 1995; Walker 2000). However, this definition can be misleading for a fish that rotates around a fixed point (i.e. zero turning radius) while the body translates linearly along the substrate, which would misleadingly equate to an arc of infinite radius. To provide evidence that stingrays were in fact translating in a straight line while achieving yaw with a very small turning radius, we divided the linear displacement of the body disc by the distance of the path it actually travelled throughout the entire response. Ratios near 1 indicate

the fish is translating in a straight line while the body rotates (yaws about a fixed point with low turning radius), rather than following the arc of a circle to turn. In comparison, if the path of the fish is following the arc of a circle the ratio would be near 0.64. Fast-start trajectory (FST) was measured as the angle between lines connecting the snout to the centre of the disc at rest, to the location of the centre of the disc upon the termination of phase 1, and revealed the ability of the fish to accelerate in different directions relative to orientation of the snout at rest; a FST of 0° , 90° , and 180° define movement directly forwards, sideways and backwards from the initial orientation of the stingray, respectively. Head orientation was measured as the angle between lines connecting the centre of the disc at rest, to the centre of the disc then to the snout at the end of phase 1; head orientation revealed whether the body of the fish followed the orientation of the snout at the end of phase 1, as it does in BCF swimming fishes (see Introduction). For example, 180° indicates that the head is leading the centre of disc at the end of phase 1 (forward swimming), 90° indicates that the head is perpendicular to the motion of the centre of the disc (sideways swimming), and 0° indicates that the head is trailing the centre of the disc (backwards swimming).

Statistical analysis

All statistical analyses were performed using R software (R Development Core Team, 2008). For some analyses, FST was categorized as forwards (0 to $<45^\circ$), sideways (45 to $<135^\circ$), and backwards (135 - 180°); *t*-tests did not show differences between left and right sideways responses for the impacts of prod speed on escape speed and acceleration, so data from left and right sides were grouped together for analysis. For other analyses, FST was treated as a continuous variable. A total of 7 forwards, 9 sideways, and 8 backwards responses were measured. For two of the sideways responses, the prod contacted the substrate rather than the pectoral disc. These trials were not included in analyses that

considered the impact of prod speed on escape kinematics. However, they were included when considering the impact of prod location on FST and yaw displacement since the fish still showed a robust escape response consistent with those with similar prod locations, and there were not differences between statistical results when these data points were included vs not. Linear regressions were employed to test the effects of prod location on FST, FST on head orientation, and prod location on yaw displacement. Linear regressions, accounting for repeated measures, were employed to test the effects of prod speed on translational acceleration and speed, and yaw acceleration and speed. Escape performance (e.g. translational acceleration and speed) was then normalized by prod speed, and a one-way ANOVA was used to test for differences between forwards, backwards and sideways escape. A p -value of 0.05 was the threshold for significance.

Results

As the prod contacted the animal, the initial finbeat (i.e. phase 1) was characterized as a rolling motion of the pectoral fins, up and over towards the dorsal midline of the body, effectively retracting the stingray away from the threat (Figure 1). Phase 1 in forwards and backwards escape were always powered by two fins (left and right sides of the body simultaneously), while sideways escape was mostly powered with one fin contralateral to the prod location (77.8% of responses). Location of the onset and offset of the finbeat around the periphery of the disc were dependent on the prod location, such that finbeats were observed to propagate (i.e. finbeat direction) from anterior to posterior, posterior to anterior, or the entire fin rolled up and over at once (Figure 1). During forwards and backwards responses, finbeat direction was always from anterior to posterior, and posterior to anterior, respectively. During sideways escape, for 72.7% of the total number of finbeats the entire fin rolled up

and over at once, while 18.2% occurred from anterior to posterior, and 9.09% occurred from posterior to anterior.

From rest (phase 1), stingrays displayed the capacity for omnidirectional movement across the substrate. However, the direction of movement was not dependent on the orientation of the head, but rather on the location of the prod. Fast-start trajectory (the trajectory of acceleration of the centre of the disc relative to the resting orientation of the fish, which describes the direction of movement of the body relative to the initial orientation of the head) decreased as prod location moved from the snout toward the tail (Figure 2; $p < 0.001$). Hence, stingrays tended to escape more directly backwards (i.e. FST of 180°) as prod location shifted towards the rostrum, more directly sideways (i.e. FST of 90°) as prod location became more lateral, and more directly forwards (i.e. FST of 0°) as prod location shifted towards the base of the tail. Furthermore, head orientation (the angle between the centre of the disc at rest, and the centre of the disc to the snout at the end of phase 1, which describes the orientation of the head at the end of phase 1 relative to movement of the body) changed from leading the centre of the disc when escaping forwards, to a perpendicular orientation when escaping sideways, to trailing the centre of the disc when escaping backwards. Consequently, as stingrays accelerated away from the prod, the body was not restricted to following the initial or final orientation of the head (Figure 2; $p < 0.001$).

An increase in prod speed evoked a faster phase 1 response, such that for each category of escape direction an increase in prod speed was associated with increased maximum translational speed and acceleration of the disc (Figure 3; speed: forwards $p < 0.001$; sideways $p = 0.00513$; backwards $p < 0.001$; acceleration: forwards $p = 0.0122$; sideways $p = 0.00182$; backwards $p < 0.001$). There were no

differences between categories of escape direction for translational speed ($p = 0.113$) or acceleration ($p = 0.446$) during phase 1. An increase in prod speed was also associated with reduced latency between the time of contact of the prod and initiation of movement of the fins ($p = 0.024$, $r^2 = 0.209$)

Phase 2 was defined as starting at the termination of phase 1 to the time that the animal finished rotating (i.e. yaw rotation) away from the prod (Figure 1). During phase 2, the animal recovered from the initial finbeat by rolling the pectoral fins outwards along the body to their original position. This recovery stroke was then followed either by an additional finbeat (57.1 % of the responses for forward escape, 55.6% for sideways escape, and 87.5% for backwards escape), or by passive gliding, as the animal transitioned into a forward swimming orientation away from the prod. During phase 2, stingrays did not achieve maximum translation accelerations that were greater than measured in phase 1, however, the maximum translational speed of phase 2 was usually greater than that obtained during phase 1 for sideways escape (77.8% of responses) and backwards escape (62.5% of responses), but not for forwards escape (42.9 % of responses), although the average across measurements were all faster for phase 2 (Figure 3; forwards $p = 0.0244$; sideways $p = 0.0309$; backwards $p < 0.001$). Hence, throughout the entire response (for each category of escape direction), maximum translational acceleration always occurred during the first finbeat of phase 1, while maximum translational speed occurred during phase 1 or phase 2 depending on the escape direction and varying between individuals. There were no differences between categories of escape direction for maximum translational speed attained ($p = 0.316$).

As the stingray translocated away from the prod, they also yawed with almost no turning radius (i.e. were able to spin without having to swim in an arc), seemingly in an effort to achieve a forward

swimming orientation away from the prod while simultaneously moving directly away from the prod. This was demonstrated by the ratio of the direct-line displacement of the centre of the disc from start to end of the escape being nearly equivalent to the measured distance it travelled (mean $0.953 \pm \text{S.D. } 0.00038$), where a value of 1 would indicate travel in a straight line, compared to a value near 0.64 if the fish were following an arc of a circle while yawing. Initiation of yaw rotation tended to begin during the latter part of phase 1 (83% of total trials) or during phase 2 (8.3% of total trials), however, in a few instances initiation of yaw rotation occurred simultaneously with the initiation of translational movement at the onset of phase 1 (8.3%). Throughout the entire escape response (i.e. phase 1 and 2), total yaw displacement increased as prod location became oriented more towards the head (Figure 4; $p < 0.001$), where fish tended to rotate so that the head was eventually oriented away from the prod location by the end of phase 2 (Figure 1). Although prod speed did not influence the yawing speed or acceleration in forwards and sideways escape, both increased with prod speed in backwards escape (Figure 4; speed: forwards $p = 0.627$; sideways $p = 0.277$; backwards $p = 0.00716$, acceleration: forwards $p = 0.968$; sideways $p = 0.0864$; backwards $p = 0.00116$). Maximum yawing speed tended to occur during phase 2 of the response (86.4% of observations), consistent with observations that initiation of yaw often occurred either late in phase 1 or during phase 2.

Discussion

Disc-start behaviours

We define fast-start escapes in stingrays with rounded pectoral discs as disc starts, functionally distinct from the more commonly described C- and S-starts employed by BCF swimming fishes, whereby the body is not restricted to following the orientation of the head, allowing the fish to escape in all directions across the substrate. A disc start is thus a unique type of fast start, with rapid, brief, and

unsteady swimming at much greater speeds than during routine swimming (see Introduction), used as a response to avoid or escape from predators or other threats. While the performances we observed in the present study were not necessarily maximal, at least in all cases, they are notably greater than routine swimming and thus can be considered as fast-start behaviour. It is not clear if disc starts involve Mauthner-cell mediated activation, as are commonly associated with escape behaviour in many fishes (Eaton et al. 1977, 2001). This distinction, between a maximal, Mauthner-cell mediated response, and an unsteady swimming manoeuvre to rapidly retreat from a threat, may have implications for the kinetics and kinematics of swimming during the response, and hence interpretation of how different types of fast starts are accomplished.

The ocellate river stingray (*Potamotrygon motoro*) is a dorsoventrally flattened fish that uses high-drag and flexible pectoral fins to power MPF swimming. This mode of swimming, along with a relatively rigid body-axis, have been proposed to lead to relatively poor manoeuvrability performance compared with most BCF swimmers (Parson et al. 2011). However, the swimming and manoeuvring abilities of these fish have not been studied under circumstances where the fish might be expected to exert greater efforts than during routine swimming, and thus, if they might exceed these expectations of modest performance. It was found that the morphology of the ocellate river stingray promotes omnidirectional fast-start performance (i.e. acceleration and speed) along the benthos (i.e. disc starts), including the ability to move in all available freedoms of motion across the substrate: forward-backwards, side-to-side and yaw (Figure 2 and 4). These stingrays demonstrated similar translational performance regardless of the orientation of the head relative to the direction of the prod (Figure 3). Furthermore, as these fish retracted away from the prod they simultaneously rotated in the yaw (i.e. left/right) freedom with next to no turning radius, quickly achieving a forward swimming orientation away from the prod, with a maximum yaw rate that was up to 27 fold greater than what was previously described for benthic

stingrays during routine swimming manoeuvres (Parson et al. 2011) (Figure 4). We thus conclude that the morphology of stingrays affords a unique tactic of fast-start swimming that allows impressive flexibility in escape behaviour in a benthic environment, in spite of the apparent constraints of a rigid-body axis (Parson et al. 2011).

Benthic stingrays can accelerate along the forward-backwards, side-to-side, and yaw freedoms, such that the body is not restricted to following the orientation of the head, and this range of manoeuvrability appears to be unique to disc starts (Figure 1 and 2). Some MPF swimming fishes can swim backwards, but it is unclear if they are capable of fast-start performance in this direction, particularly with the capacity to simultaneously rotate within a low turning-radius (Lannoo and Lannoo 1993; Webb and Fairchild 2001). Most fishes use BCF swimming to power fast starts, performing C-starts and S-starts for escape (Domenici 2010a, 2010b; Domenici and Blake 1997; Wakeling 2001). During these fast starts, the body is restricted to following the orientation of the head. This would appear to restrict BCF swimming fishes in the extent of omnidirectional movement they can attain, at least over a small distance of travel. Although most BCF swimming fishes are likely not capable of fast starting in the sideways or backwards freedoms, it is possible that omnidirectional manoeuvrability (across the substrate) could still be achieved via forward swimming and high yaw displacement and rates within low turning-radii. This type of rotational performance may be promoted by extremely flexible bodies (e.g. eel-like fishes) performing C-start behaviours. However, C-starts and S-starts tend to be powered by a preparatory stroke followed by a main propulsive stroke, so that BCF swimming fishes would seemingly require two BCF motions to displace their entire body away from the initial orientation. In contrast, stingrays can readily retract the entire pectoral disc directly away from a prod via the first beat of the pectoral fins. Some BCF swimming fishes, with elongated and flexible bodies (e.g. eel-like fishes) living in structurally complex environments, can retract their head toward the body

in one motion, often pulling their head or tail into a tunnel-like feature, defined as a withdrawal response (Bierman et al. 2004; Ward and Azizi 2004). Nevertheless, apparently these withdrawal responses often do not involve a propulsive component (i.e. acceleration) of the body.

A flattened and rounded pectoral disc likely promotes the capacity for omnidirectional disc starts across the substrate. The large and flexible pectoral fins can create propulsive forces in any direction across the substrate, while the dorsoventrally flattened shape of stingrays will reduce the mass of water that must be displaced as the fish accelerates along the anteroposterior and lateral axes (Webb 1988). The latter should decrease the drag forces retarding acceleration, providing stingrays with the ability to effectively slice through the water and obtain comparable fast-start performance regardless of direction across the substrate. Stingrays were never observed to escape upwards. While they were never prodded from below or directly lateral on the edge of the disc to intentionally invoke an upwards escape, the lack of upwards escape is more likely due to the high profile-drag that would be experienced by the fish during acceleration in the vertical axis (relative to its resting position), and the relatively substantial mass of water that must be displaced to move upwards due to the large surface area of the dorsal surface of the flattened pectoral disc. Further, even when a threat is moving along the substrate, stingrays maintain escape along the substrate and not up (Seamone personal observation).

Pleuronectiformes (i.e. flatfish such as sole and flounder) are also flattened in the same plane as the substrate; these fishes are laterally flattened and flipped on their side (Friedman 2008). Yet rather than performing disc starts, flatfish perform BCF escape responses, undulating their longitudinal body-axis and power C-starts perpendicular to the ground (Webb 1981; Brainerd et al. 1997). Whether these fishes have the capacity to disc start backwards and sideways is not clear, however, it is highly unlikely due to the asymmetrical shape from anterior to posterior and the reliance on BCF propulsion. Hence, it is not only the flattened profile in stingrays that is responsible for the range in manoeuvrability across

the substrate; the flexible high-drag pectoral fins that wrap around the head and body seem key in displacing water in different directions to promote disc-start behaviours.

Drag-based propulsion

The flexible and low aspect-ratio (i.e. rounded) pectoral fins roll up and over during the disc start, forming a large propulsive surface that is dragged through the water along the anteroposterior and lateral axes (Figure 1). This presents a large surface to exert drag-based forces on water and displace water for powering movement. In sideways escape, the entire fin on one side of the fish tends to roll up and over towards the dorsal midline at once. During forwards and backwards escape, rolling of the fin is initiated along the anterior or posterior portion of the pectoral fins, respectively, and is propagated along the anteroposterior axis. These fin movements would generate momentum in the water, in different directions around the body depending on the direction of fin movement, to effectively accelerate the fish across the substrate in the counter direction to the fin beat (Drucker and Lauder 2002). The flexible and rounded fins of benthic stingrays are important for promoting drag-based propulsion, and would appear to restrict disc starts to benthic stingrays, and possibly skates, that possess this rounded morphology. As such, although it has not yet been explored, it is unlikely that pelagic stingrays can effectively perform disc starts with their high aspect-ratio triangular shaped fins that tend to produce lift rather than generate drag (Lighthill 1969; Fish 1996; Rosenberger 2001; Fontanella et al. 2013), even when swimming near the benthos during foraging behaviours.

Modulation of escape performance and direction

Given that escape appears to be an energetically expensive event (Jayne and Lauder 1993; Westneat et al. 1998), it is not entirely surprising that fishes do not always employ maximum performance in

response to a threat (Ydenberg and Dill 1986; Seamone et al. 2014). In the present study, contacting the stingray with a greater prod speed evoked a greater magnitude of escape acceleration and speed in all translational directions away from the threat during phase 1 (Figure 3), increased yaw acceleration and speed during backwards escapes (to achieve a forward swimming orientation away from the threat) (Figure 4), and reduced latency between the time of contact of the prod and initiation of fin movement (see Results). Although maximum rates of yaw rotation in forwards and sideways escape were also associated with higher prod speeds, the relationships were not significant, which might have a basis in the relatively small amount of yaw displacement required to achieve a forward swimming orientation away from the prod (i.e. during forward escape the fish is already oriented away from the prod). Nevertheless, stingrays clearly have the capacity to modulate the level of performance relative to the intensity of tactile stimulation, which may be an effective behaviour in a benthic environment. Routinely, benthic stingrays likely contact a range of different objects that are not threatening, especially in turbid environments such as the South American river basins where the ocellate river stingray resides. Always employing maximum performance and escaping away from all tactile interactions would be a waste of energy, and the potential for missing feeding and mating opportunities (Ydenberg and Dill 1986).

Predators of stingrays likely include various types of ambush predators, in addition to some large animals that are very effective at swimming through the water column at high cruising and burst speeds, such as sharks and marine mammals (Strong et al. 1990; Cliff and Dudley 1991; Visser 1999). The ability of stingrays to escape downwards from such predators is restricted due to the substrate, and escaping upwards is likely not an appropriate behaviour in the face of faster predators with the capacity to attack at high speeds, particularly given the aforementioned limitations on moving a disc-shaped body in a direction other than parallel with the substrate. However, larger predators tend to have lesser

rotational manoeuvrability (Domenici 2001). Thus, the ability of stingrays to readily escape in any direction across the substrate, and to quickly yaw to any direction while simultaneously accelerating away from an attack, promotes movement along the benthic plane that least exposes the stingray to the aptitudes of these predators, and perhaps forces the predator to reorient itself toward the escaping stingray, a time-consuming process. Therefore, disc-start behaviours across the substrate would seem to exploit the limitations of a large swimming predator. Remaining close to the substrate, stingrays could then bury when the opportunity arises.

Manoeuvrability

Disc-start behaviours in stingrays challenge the concept of manoeuvrability used to describe fast starts in other fishes. Our current understanding of manoeuvrability in fishes stems from those that undulate their body axis and power C-starts and S-starts (for reviews see Domenici 2010a, 2010b; Domenici and Blake 1997; Wakeling 2001), where the body follows the orientation of the head during the fast starts, and manoeuvrability is achieved via forward thrust in conjunction with angular rotation (predominantly yaw). Thus, manoeuvrability in fishes is most often discussed in the context of turning (i.e. yaw rotation) angle, turning radius, and turning rate, while forward acceleration and speed are often discussed separately as propulsive performance (Domenici and Blake 1997; Domenici 2004, 2010b). However, a manoeuvre defines a change in position (i.e. acceleration) of an object. Thus, fishes suspended in a 3-dimensional environment have the potential to accelerate (i.e. manoeuvre) and escape predators in 6 degrees of freedom of motion: 3 translational freedoms (forwards/backwards, sideways, up/down) and 3 rotational freedoms (pitch, yaw and roll). Although translational acceleration (forwards, backwards and sideways) in stingrays is low relative to maximum forward acceleration in BCF swimmers, where some species can accelerate over 100 m/s^2 (for review see Domenici and Blake 1997), BCF swimmers are

not known to accelerate backwards or sideways to nearly the same extent that stingrays can. Furthermore, although yawing rates of stingrays were relatively low (Figure 4) compared to the maximum measured in BCF swimmers, whereby numerous species can rotate over 2,000 deg/s (for review see Domenici 2001), yawing in BCF swimmers typically occurs first as part of a C-start, followed by forward translation, while stingrays can yaw and translate simultaneously. Thus, while the relatively high yaw-rates of BCF swimmers contribute to a shorter prelude to forward movement, they don't in themselves necessarily contribute to rapid translation toward/away from a prey/predator. A stingray that employs slower yaw rates, but which can be accomplished with simultaneous translation may actually have a faster effective response. As such, high yaw-rates in BCF predators may not be as effective for pursuing an animal using disc starts as they might first appear. Also, stingrays have the capacity to yaw with next to no turning radius (He and Zhang 2013), perhaps zero (spin on the spot), which BCF swimmers cannot accomplish. Thus, interpreting manoeuvrability in fishes may be more complex than simply the ability to yaw at a high rate. We propose that while stingrays may not exhibit rotational and translational accelerations as high as some BCF swimmers numerically, they should still be considered as highly manoeuvrable.

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Figures

Figure 1. Disc starts for forwards (upper row), sideways (middle) and backwards responses (lower).

Movement of eleven points around the periphery of the pectoral fin (white dots) were analysed relative to the centre of the disc to determine the direction of the resultant finbeat powering escape. During escape responses, fins rolled up and over towards the midline (black and grey shades indicate the dorsal and ventral pectoral fin surfaces, respectively). Phase 1 (dotted arrow) corresponds to the first finbeat of the escape response. Phase 2 (solid arrow) was defined as the time from the termination of phase 1 to the time that the fish finished rotating/yawing and reached its preferred swimming orientation away from the prod.

Figure 2. Stingrays can fast start away from a prod in all directions across the substrate, and the body is not restricted to following the orientation of the head.

A) The impacts of prod location on the fast-start trajectory (FST) measured upon completion of phase 1 (linear regression $r^2 = 0.942$, $p < 0.001$, $y = -1.13x + 180$). Prod location of 0° is the head and 180° is the tail. A FST of 0° indicates the stingray moves in the direction its head was initially oriented, 90° directly sideways (left or right) to initial head orientation, and 180° directly backwards. The dashed line represents a line of unity. B) Circular plot showing the range of fast-start trajectories exercised by the stingrays from rest. Zero degrees indicates a fast start directly forwards, 90 degrees directly sideways, and 180 degrees directly backwards. Solid lines indicate escape that included leftward trajectory, broken lines rightward. C) The relationship between FST and head orientation (HO) of the escape response ($r^2 = 0.936$, $p < 0.001$, $y = -0.886x + 176$). During phase 1 the body is not restricted to following the orientation of the head, whereby fish that move in the direction of the snout at the start of phase 1 (0° FST) are still moving in the direction of the snout at the end of phase 1 (HO near 180°), fish that move laterally relative to the orientation of the

snout at the start of phase 1 (90° FST) are still moving laterally relative to the snout at the end of phase 1 (HO near 90°), and fish that move backwards at the start of phase 1 (180° FST) are still moving backwards at the end of phase 1 (HO near 0°). If the fish first rotated to follow the head during phase 1 when escaping away from the prod, HO would remain near 180° throughout the full range of FST, which it does not. The dashed line represents a line of unity.

Figure 3. The impacts of prod speed on maximum translational performance of the centre of the disc during forwards, backwards and sideways escapes. Triangles and dotted lines represent forward escape (0 to <45° FST), squares and dashed lines represent sideways escape (45 to <135° FST), and circles and solid lines represent backwards escape (135-180° FST). A) An increase in prod speed was associated with increased maximum translational acceleration of the centre of the disc measured during phase 1 in all escape directions (forwards: $r^2 = 0.695$, $p = 0.0123$, $y = 34.9x - 1190$; sideways: $r^2 = 0.854$, $p = 0.00182$, $y = 28.4x - 408$; backwards: $r^2 = 0.916$, $p < 0.001$, $y = 18.7x + 110$). There were no differences in acceleration between the three escape directions ($p = 0.446$). B) An increase in prod speed was associated with increased maximum translational speed of the centre of the disc measured during phase 1 in all directions (forwards: $r^2 = 0.902$, $p < 0.001$, $y = 0.952x + 12.8$; sideways: $r^2 = 0.782$, $p = 0.00513$, $y = 0.971x - 0.626$; backwards: $r^2 = 0.937$, $p < 0.001$, $y = 0.813x + 23.7$). There were no differences in speed between the three escape directions ($p = 0.113$). C) An increase in prod speed was associated with increased maximum translational speed of the centre of the disc measured during phase 2 of the escape response in all directions (forwards: $r^2 = 0.604$, $p = 0.0244$, $y = 1.33x - 5.22$; sideways: $r^2 = 0.567$, $p = 0.0309$, $y = 0.930x + 23.1$; backwards: $r^2 = 0.933$, $p < 0.001$, $y = 0.775x + 27.7$). There were no differences in speed between the three escape directions ($p = 0.316$).

Figure 4. Yaw rotational performance during stingray escape. Triangles and dotted lines represent forward escape (0 to <45° FST), squares and dashed lines represent sideways escape (45 to <135° FST), and circles and solid lines represent backwards escape (135-180° FST). A) The impact of prod location on the magnitude of yaw rotation measured from the start of phase 1 to the end of phase 2 ($r^2 = 0.583$, $p < 0.001$, $y = -0.661x + 132$). As prod location became more anterior (i.e. 0° snout, 180° tail), stingrays rotated a greater amount toward a forward swimming orientation away from the prod, but did not always swim directly away from the prod as evidenced by the scatter around the regression. The dashed line represents a line of unity. B) An increase in prod speed was associated with increased maximum yaw speed, measured throughout the entire response, during backwards escape, while there was no significant effect of prod speed on yaw speed in forward and sideways escape (forwards: $r^2 = -0.139$, $p = 0.627$, $y = 1.032x + 255$; sideways: $r^2 = 0.0752$, $p = 0.277$, $y = 3.46x + 260$; backwards: $r^2 = 0.681$, $p = 0.00716$, $y = 3.70x + 307$). C) An increase in prod speed was associated with increased maximum yaw acceleration achieved throughout the entire response in backwards escape, while there was no significant effect of prod speed on yaw acceleration in forward and sideways escape (forwards: $r^2 = -0.200$, $p = 0.968$, $y = -4.05x + 804$; sideways: $r^2 = 0.371$, $p = 0.0864$, $y = 95.9x + 3850$; backwards: $r^2 = 0.823$, $p = 0.00116$, $y = 193x - 875$).