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RESEARCH ARTICLE

Swimming in unsteady water flows: is turning in a changing flow an energetically expensive endeavor for fish?

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ABSTRACT

Unsteady, dynamic flow regimes commonly found in shallow marine ecosystems such as coral reefs pose an energetic challenge for mobile organisms that typically depend on station-holding for fitness-related activities. The majority of experimental studies, however, have measured energetic costs of locomotion at steady speeds, with only a few studies measuring the effects of oscillatory flows. In this study, we used a bidirectional swimming respirometer to create six oscillatory water flow regimes consisting of three frequency and amplitude combinations for both unidirectional and bidirectional oscillatory flows. Using the goldring surgeonfish, *Ctenochaetus strigosus*, a pectoral-fin (labriform) swimmer, we quantified the net cost of swimming (swimming metabolic rate minus standard metabolic rate) associated with station-holding under these various conditions. We determined that the swimming costs of station-holding in the bidirectional flow regime increased by 2-fold compared with costs based on swimming over the same range of speeds at steady velocities. Furthermore, as we found minimal differences in energetic costs associated with station-holding in the unidirectional, oscillating flow compared with that predicted from steady swimming costs, we conclude that the added acceleration costs are minimal, while the act of turning is an energetically expensive endeavor for this reef fish species.

KEY WORDS: Wave surge, Costs of swimming, Labriform, Respirometry

INTRODUCTION

Shallow-water, wave-swept ecosystems, such as coral and rocky reefs and other nearshore habitats, are subject to dynamic, unsteady water flows (bidirectional oscillatory wave surge) that influence the distribution, behavioral and physical attributes of organisms that live there (Webb, 1989; Dunson and Travis, 1991). For fishes, swimming in a wave-swept habitat represents a challenge as they are often site-attached, maintaining access to specific feeding or refuge sites, which requires them to hold station within the water column (Liao, 2007; Heatwole and Fulton, 2013). Station-holding behaviors in these dynamic water flows entail continuous adjustments to changes in the flow direction and speed and are therefore expected to be energetically expensive (Fulton and

Bellwood, 2005; Liao, 2007). Coral reefs, in particular, are commonly exposed to a range of wave-surge water flows, while also having extremely diverse communities of fish species. It may be that, in part, coral reef fishes have evolved a spectrum of different swimming modes and morphologies to reduce energy expenditure and improve swimming performance in these environments (Blake, 1983; Langerhans and Reznick, 2010; Pink and Fulton, 2014).

As waves approach shore, and water depth decreases to less than one-half the wavelength, the circular water motion of the wave is compressed into a longitudinal wave surge, oscillating parallel to the seafloor (Fulton and Bellwood, 2005; Denny, 2006). In shallow, wave-swept habitats, station-holding fishes have to continuously accelerate and decelerate to match the changing water speeds, which can increase the amount of energy used relative to steady-state swimming (Liao, 2007; Roche et al., 2014). Furthermore, the adjustment of swimming speeds is coupled with changes in water flow direction, requiring backward swimming or a whole-body rotation to face the flow (Marcoux and Korsmeyer, 2019). Alternatively, the fishes can seek refuge to ease or escape the challenge of dealing with the water flows. Complex structures and holes in the substratum can be used to reduce the water flow experienced by the fish by up to 100% (Johansen et al., 2007, 2008). Furthermore, fish are able to avoid high water flows by strategically placing themselves deeper in the water column as well as taking advantage of the boundary layer effect, where water flows are lower near the substratum (Fulton et al., 2001). However, utilizing these refuge strategies means the fishes have restricted use of the water column, which constrains their use of available resources. Efficient and competent swimmers that do not need to use such strategies could therefore have a competitive advantage. Despite our limited understanding of the energetic challenges that wave-swept habitats impose on fishes, the vast majority of experimental studies on the swimming performance of fishes have been conducted using constant, steady water flows (Brett, 1964, 1972; Steffensen et al., 1984; Korsmeyer et al., 2002; Svendsen et al., 2010). More recently, however, studies of swimming in unsteady water flows have incorporated changes in swimming direction and oscillatory speeds more typical of wave-swept habitats (i.e. unidirectional or bidirectional oscillatory water flow) (Roche et al., 2014; Marcoux and Korsmeyer, 2019).

There is a strong association between the intensity of wave exposure and the swimming modes among assemblages of coral reef fishes (Fulton and Bellwood, 2005). In particular, labriform swimming, using movements of the pectoral fins while keeping the body rigid and straight (Sfakiotakis et al., 1999), has shown to be the dominant swimming mode utilized in coral reefs (~60%) and is positively correlated in relative abundance with increasing wave exposure (Fulton and Bellwood, 2005; Fulton et al., 2005). This correlation suggests that labriform swimming is an effective way of swimming and navigating in wave-surge type flows compared with other swimming forms. Labriform swimming has been shown to

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provide a high degree of stability and maneuverability or be effective for high-speed swimming, although there may be a trade-off between these two capabilities (Drucker and Lauder, 2000; Walker and Westneat, 2002b). Furthermore, studies of the cost of swimming in steady flows show that some labriform swimmers, particularly those with long, tapered (i.e. high aspect-ratio) pectoral fins, may be very efficient at high speeds (Korsmeyer et al., 2002; Fulton et al., 2013). A study on a temperate, estuarine labriform swimmer, the surfperch *Cymatogaster aggregata*, found that a unidirectional, unsteady flow simulating a wave surge without the turning component increased the energetic cost of swimming by 25% compared with swimming in a steady flow, in part owing to added energetic costs of acceleration and deceleration (Roche et al., 2014). A recent study by Marcoux and Korsmeyer (2019) examined station-holding swimming performance and behavior of several coral reef fish species in a simulated wave surge. They found that median and paired fin (MPF) swimmers, such as labriform swimmers, were more cost-efficient as wave frequency increased than body caudal fin (BCF) swimmers, and MPF swimmers more frequently swam backwards instead of turning despite an increase in pectoral fin beat frequency associated with backward swimming. These results suggest that turning is an expensive behavior. However, owing to flow limitations in the experimental setup, the study was only able to simulate wave surge at the lower end of the conditions found naturally in terms of wave amplitudes and water flow velocities, and could not make direct comparisons with steady swimming costs (Fulton and Bellwood, 2005; Marcoux and Korsmeyer, 2019). Thus, the extent of the added costs for station-holding, and what proportion is due to the potential costs of acceleration or turning, is unclear. Together, these previous studies support the hypothesis that it is energetically expensive to swim in a bidirectional oscillating flow and that both the acceleration and turning component of swimming in a wave surge have their individual energetic costs compared with swimming in a steady flow. As metabolic requirements of locomotion and swimming performance are crucial for constructing energy budgets in fishes, understanding how wave action shapes the energetics of fishes in shallow, wave-swept habitats will improve predictions of how fish populations will respond to environmental changes (Enders et al., 2003; Fulton and Bellwood, 2005; Liao, 2007; Roche et al., 2014).

To separate the cost of acceleration and turning during station-holding swimming in wave-swept habitats, we produced both bidirectional (see Movie 1) and unidirectional (see Movie 2) oscillatory water flows, and measured the oxygen consumption rates ($\dot{M}_{O_2}$) of station-holding goldring surgeonfish, *Ctenochaetus strigosus*. *Ctenochaetus strigosus* is a labriform swimmer common in wave-exposed coral reef and rocky shoreline habitats around the Hawaiian Islands (Hobson, 1974; Mundy, 2005; Randall and Clements, 2001). It is benthopelagic, feeding on detritus, and, like other grazing surgeon fishes, will punctuate steady swimming with turning and reorientation to target specific feeding sites (Hobson, 1974; Mundy, 2005; Randall and Clements, 2001; Friedman et al., 2016). Using a specialized swim flume respirometer, we measured the cost of swimming at steady velocities, and at three different intensities of both unidirectional and bidirectional oscillatory flows. We hypothesized that the added energetic costs of both accelerations and turning would increase the cost of swimming in the unsteady water flows compared with what would be predicted from the costs at steady swimming velocities. Therefore, we predicted that swimming in bidirectional, oscillatory flows would result in the largest increase in costs of swimming (requiring both accelerations and turning), followed by

unidirectional, oscillatory flows (requiring accelerations only), and lastly the cost predicted from steady velocities alone. The difference between the costs in unidirectional and the bidirectional flows should indicate the cost of turning. However, if the cost of turning is minimal, the bidirectional swimming costs should be similar to unidirectional costs under similar flow conditions.

MATERIALS AND METHODS
Fish
Goldring surgeonfish, *Ctenochaetus strigosus* (Bennet 1828) [*n*=10; total length=8.7 cm (8.1–9.5 cm); mass=15.5 g (12.1–19.9 g); mean (range)], were purchased from a local aquarium fish wholesaler that caught the fish from nearshore Oahu, HI, USA. The fish were held individually in indoor, aerated, flow-through seawater tanks (30–100 liters, 26–28°C, salinity=30–32‰, 12 h:12 h light:dark photoperiod) at the Oceanic Institute of Hawaii Pacific University and fed daily with commercial marine fish pellets and chopped frozen squid. The sex of the fish at these sizes could not be determined. Fish were acclimated in laboratory conditions for at least 3 days and fasted for 24 h prior to testing to ensure they were in a post-absorptive state. Following experiments, the fish were euthanized by an overdose of the fish anesthetic, tricaine methanesulfonate (Tricaine-S, Western Chemical, Inc., Ferndale, WA, USA), in buffered seawater. All fish were handled ethically according to Hawaii Pacific University's Institutional Animal Care and Use Policies.

Experimental setup
Rates of oxygen consumption ($\dot{M}_{O_2}$) were measured in a modified 6.7 liter acrylic Steffensen-type swimming respirometer (Roche et al., 2014) with dual propellers on a common axle driven by a computer-controlled motor capable of generating symmetric bidirectional flows (Fig. 1). The fish were placed in the swimming section (20.3 cm long, 8.9 cm wide, 8.9 cm deep) bounded at each end by honeycomb flow-straighteners (PlasCore Inc., Zeeland, MI, USA) to promote linear flow. The fish were forced to swim against the current to maintain their position in the swim section, and by varying velocities in a continuous sine wave function from forward to reverse, the fish also had to turn around, similar to a station-holding behavior in response to wave-driven flows in the field. A few of the individuals had a total length slightly longer (by less than 7%) than the width of the swim section; however, it was apparent that the space used by the fish during a turn was much smaller than their total length owing to a C-like bend in the longitudinal axis of the body. The sides of the swim section therefore did not hinder the fish in its station-holding behavior. The respirometer was submerged in an external water tank (52.4 liters) connected to an aerated sump (52 liters) thermostatted to 27°C and recirculated through a UV sterilizer (Coralife Turbo Twist 12X UV Sterilizer, 36 W). Oxygen levels in the respirometer were measured every 5 s with a WTW Multi 3430 multimeter (WTW, Weilheim, Germany) with an optical dissolved oxygen probe and a water conductivity probe with temperature sensors to automatically correct oxygen concentration for salinity and temperature. Water velocity, flush cycles and data collection were regulated by custom software written in NI LabView 2017 (National Instruments, Austin, TX, USA) and via an AD–DA converter (USB-1208, Measurement Computing, Norton, MA, USA) that controlled a motor driving the speed and direction of the propellers (Movitrac AC VFD, SEW Eurodrive, Lyman, SC, USA) and a relay (USB-SwitchC, Cleware GMBH, Hollingstedt, Germany) activating a flush pump mounted on the side of the respirometer.
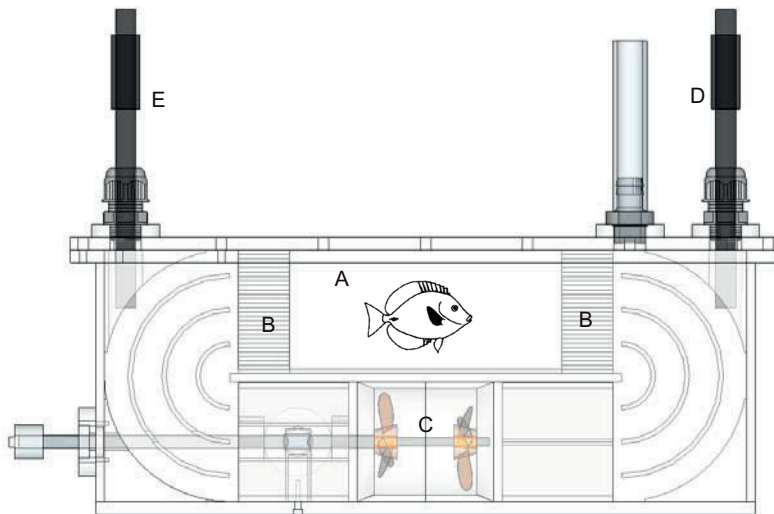
Fig. 1. Schematic of the 6.7 liter intermittent-flow swimming respirometer designed for bidirectional swimming. The fish was kept in the swimming section (A) by two honeycomb flow-straighteners (B) at each end. In the bottom of the tank are two propellers (C) powered by an external motor, able to create water flow in both directions. In the top right and the top left corner, respectively, an optical dissolved oxygen probe (D) and a water conductivity probe with temperature sensor (E) was inserted.

Flow speeds were corrected in real-time for solid blocking effects for each fish (Bell and Terhune, 1970; Korsmeyer et al., 2002).

Water flow calibration

The water flow velocity in the swimming section was calibrated to the voltage input of the motor powering the propellers using particle tracking velocimetry. The respirometer was filled with freshwater containing 0.1 g l^{-1} of neutrally buoyant fluorescent red polyethylene microspheres (Cospheric, Santa Barbara, CA, USA; 1.005 g ml^{-1} 212–250 μm), which were illuminated with a 50 mW 523 nm laser with a cylindrical lens to project a thin (1 mm) sheet of light along the center of the swim section parallel to the water flow. Videos (120 frames s^{-1}, GoPro Hero 3+ Black) at each steady flow condition were recorded for subsequent particle tracking analysis (Tracker, version 4.11.0 by Douglas Brown) (Meijering et al., 2012). Water velocity was calibrated over the full range of speeds in both directions.

The bidirectional oscillatory flows had changing speeds that followed the pattern of a sinusoidal function:

$$U(t) = A \times \sin(f \times 2\pi \times t), \quad (1)$$

where U is the speed (cm s^{-1}), A is the amplitude or peak speed (cm s^{-1}), f is the frequency (Hz) and t is time (s). The average speed (U_{avg}) during bidirectional oscillatory flows was calculated as the mean absolute speed through one complete sine-wave cycle, using the equation:

$$U_{avg} = \frac{2}{\pi} \times A. \quad (2)$$

The calibration from the steady flow was used to set the amplitude, or peak speeds, at each frequency of oscillation; however, a correction was required owing to a dampening effect on amplitude in the flume as a result of inertial effects. Particle tracking tests of actual speeds during the oscillatory flows were used to correct the amplitudes for dampening of the cyclic changes in speed. With the increased frequency of oscillation, the dampening of the peak speeds increased, so separate calibrations were made for each experimental frequency.

To create similar oscillations in water flow, but without direction changes, unidirectional oscillatory flows were generated to follow the absolute value of a sine function. Owing to the inertia of the water, the pattern of unidirectional water speeds was different than

what we expected, as the particle tracking revealed that the speed did not drop completely to zero, and at the highest frequency peak speed (U_{peak}) was slightly greater than during bidirectional flows (Fig. 2). As a result, the average water flow speeds were higher, but with lower accelerations and wave amplitudes (A), compared with the bidirectional flows (Table 1, Fig. 2). The unidirectional oscillatory flow, therefore, followed a slightly different function:

$$U(t) = \text{abs}(A \times \sin(f \times 2\pi \times t)) + e, \quad (3)$$

$$U_{avg} = \frac{2}{\pi} \times A + e, \quad (4)$$

$$U_{peak} = A + e, \quad (5)$$

where U is the speed (cm s^{-1}), A is the amplitude (cm s^{-1}), f is the frequency (Hz), t is time (s) and e is the elevation of the waveform above zero, or the minimum speed (cm s^{-1}). Particle tracking was used to determine the values of e for each experimental frequency (Table 1, Fig. 2).

Respirometry protocol

Each of the 10 fish were subject to the same experimental protocol and all experimental treatments to quantify the standard metabolic rate (SMR, the minimum energy required to maintain homeostasis) and swimming costs at steady swimming as well as unsteady swimming in a unidirectional and bidirectional flow. Before being placed in the respirometer, the mass, total body length, depth and width of the fish were quickly measured to allow for solid-blocking correction and calculation of relative speed (body lengths per second, BL s^{-1}). The fish were introduced into the respirometer in the afternoon, to allow several hours of recovery from handling before measurements to determine SMR commenced. Oxygen consumption rates were recorded overnight (~12 h) at a low flow speed (~5 cm s^{-1}) to determine the SMR before swimming metabolism trials were performed the following day. This flow speed was sufficient to provide water mixing but low enough to not induce swimming and allow the fish to rest on the bottom of the swimming section.

The $\dot{M}_{O_2}$ (mg O$_2$ kg^{-1} h^{-1}) was measured using intermittent-flow respirometry (Steffensen et al., 1984; Svendsen et al., 2016) with 12 min cycles consisting of three periods: a 4 min air-equilibrated water flushing period, a 1 min mixing period with the flush pump off to allow the system to equilibrate, and a 7 min closed
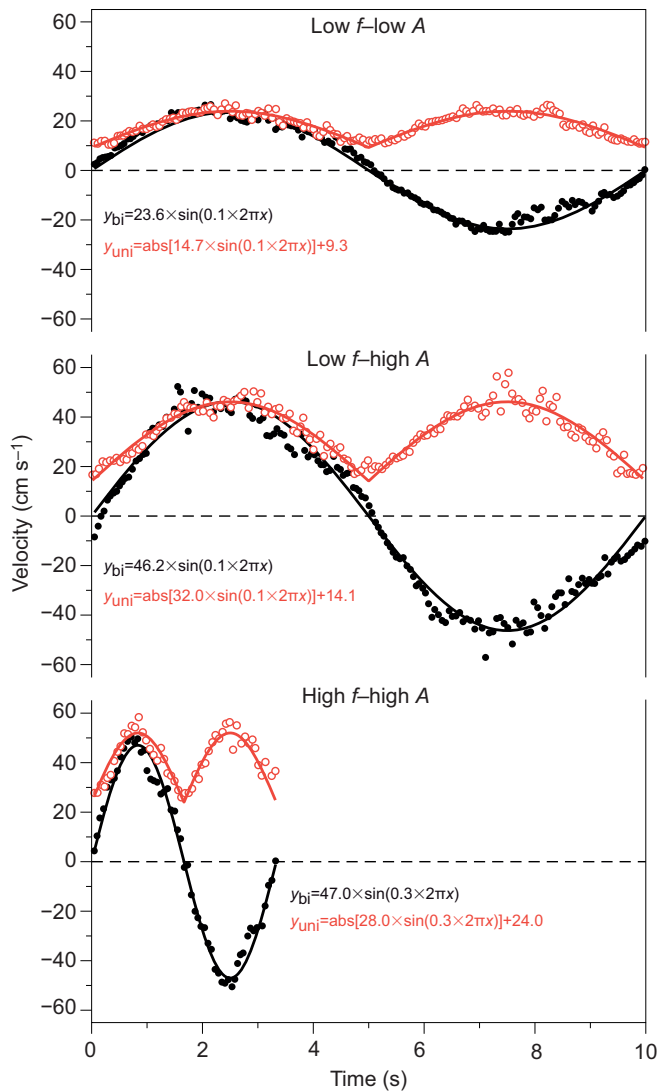
Fig. 2. Water flow velocities for the three water flow treatments for both bidirectional (black, solid circles) and unidirectional (red, open circles) flow regimes. Low and high frequency (f) indicates a frequency of 0.1 and 0.3 Hz, respectively, while low and high amplitude (A) indicates the intended peak speeds of approximately 2.36 and 4.71 BL s^{-1}, respectively (see Table 1). Note that for the unidirectional flow, the speed never reaches zero owing to the inertia of the water, and peak speed is higher at the high frequency oscillation. Lines and equations represent the sine-wave functions for each flow condition as fit to particle tracking observations of velocity (circles). Each point represents the average from three cycles of oscillation.

measurement period for recording the decline in oxygen content. Oxygen consumption rates were determined from the linear regression of oxygen content decline in the respirometer as a function of time during the closed measurement period (Steffensen et al., 1984; Svendsen et al., 2016) using the equation:

$$\dot{M}_{O_2} = \frac{\Delta O_2 V_{resp}}{M_{fish}}, \qquad (6)$$

where ΔO_2 is the rate of change in oxygen content (mg O_2 h^{-1}), V_{resp} is the volume of the respirometer minus the volume of the fish (l) and M_{fish} is the mass of the fish (kg). In order to determine the background microbial respiration, oxygen consumption in the respirometer without a fish present was determined in triplicate before and after each experiment. $\dot{M}_{O_2}$ measurements were then

corrected by subtracting background respiration levels, assuming a linear change in background $\dot{M}_{O_2}$ over time.

Standard metabolic rate and net cost of swimming
Standard metabolic rates were calculated from a frequency histogram of $\dot{M}_{O_2}$ measurements measured overnight. Two normal curves were fitted to the frequency histogram separating the lower $\dot{M}_{O_2}$ measurements during periods of rest from the higher $\dot{M}_{O_2}$ measurements owing to spontaneous activity (Steffensen et al., 1994; Chabot et al., 2016). The SMR was then determined from the mean of the lower distribution of $\dot{M}_{O_2}$ measurements for that individual. For all $\dot{M}_{O_2}$ measurements during the swimming trials, the net cost of swimming (NCOS) was calculated by subtracting the SMR from swimming $\dot{M}_{O_2}$, to represent the increase in metabolic rate owing to swimming activity for that individual at each treatment (Korsmeyer et al., 2002).

Steady swimming
After the overnight measurement of SMR, the swimming $\dot{M}_{O_2}$ at constant, steady water flows were measured in 1 BL s^{-1} increments from 1 to 5 BL s^{-1}. The steady swimming protocol stopped at 5 BL s^{-1} because preliminary trials showed that this was below the gait transition and critical swimming speeds for this species, to avoid significant anaerobic metabolism or oxygen debt. Furthermore, this range of steady swimming velocities encompassed the speeds during the oscillatory, simulated wave-surge flows (peak speed of 4.71 BL s^{-1}), allowing calculation of predicted costs of swimming at those flows from the steady swimming $\dot{M}_{O_2}$. Each fish swam for two respirometry cycles (for a total of 24 min) at each velocity. Any trials where the fish was swimming erratically or with significant contact with the walls of the swim section were removed from analyses. The $\dot{M}_{O_2}$ as a function of swimming speed was fitted to a hydrodynamics-based power equation (Videler, 1993; Korsmeyer et al., 2002):

$$\dot{M}_{O_2} = a + bU^c, \qquad (7)$$

where a, b and c are constants and U is the water flow speed. This relationship for each individual was used to determine the NCOS at steady swimming velocities, and predicted NCOS for each oscillatory flow treatment. At the conclusion of the steady water flow swimming protocol, the velocity was reduced to 1 BL s^{-1} for one measurement cycle to confirm the absence of oxygen debt, after which the oscillatory swimming protocol was commenced.

Oscillatory swimming
The unsteady swimming treatments, designed to simulate a horizontal wave surge of changing water flow velocities, were accomplished using a computer-generated sine-wave function of a given wave frequency (f) and amplitude (A) to change the speed and, for bidirectional flows, the direction of the propeller motor. Three treatments were performed with both types of water flow regimes (bidirectional and unidirectional oscillatory water flow). The treatments were: a low frequency and low amplitude oscillation (low f–low A), a low frequency and high amplitude oscillation (low f–high A), and a high frequency and high amplitude oscillation (high f–high A) (Table 1). These simulated water flow conditions are similar to what has been observed and quantified by Fulton and Bellwood (2005), where the low amplitude water flow treatment (low f–low A) is similar to the wave-induced flows in sheltered and lagoonal reefs, and the high amplitude water flow treatments (low f–high A and high f–high A) are more similar to that on oblique

Table 1. The wave frequency and water flow speeds and accelerations of each oscillatory flow treatment (low *f*–low *A*, low *f*–high *A* and high *f*–high *A*, where *f* is frequency and *A* is amplitude) for both bidirectional and unidirectional oscillatory water flows

Water flow regime	Water flow treatment	Frequency (Hz)	Minimum speed (BL s^{-1})	Average speed (BL s^{-1})	Peak speed (BL s^{-1})	Average acceleration (BL s^{-2})	Maximum acceleration (BL s^{-2})
Bidirectional	Low *f*–low *A*	0.1	0.00	1.50	2.36	0.72	1.50
	Low *f*–high *A*	0.1	0.00	3.00	4.71	1.43	3.00
	High *f*–high *A*	0.3	0.00	3.00	4.71	4.29	9.00
Unidirectional	Low *f*–low *A*	0.1	0.93	1.86	2.36	0.55	0.87
	Low *f*–high *A*	0.1	1.41	3.54	4.71	1.29	2.03
	High *f*–high *A*	0.3	2.40	4.22	5.18	3.17	4.99

and exposed reefs. Both bidirectional and unidirectional conditions were tested on each fish at each treatment level, with the order of the water flow regimes (bidirectional or unidirectional) changed at each level and the initial regime alternated between each fish to remove any effect of exposure order. The first water flow treatment was always of the lowest flow condition, low *f*–low *A*, and then increased to low *f*–high *A* and, finally, high *f*–high *A*, after both water flow regimes have been run at each water flow treatment. As with the steady swimming trial, two respirometry cycles (total of 24 min) were conducted at each unsteady swimming treatment. When the last treatment of the oscillatory swimming ended, the speed was reduced to 1 BL s^{-1} at a steady flow for one measurement cycle to confirm the absence of oxygen debt.

Predicted net cost of swimming

To predict the effect of the non-linear metabolic rate–speed relationship on costs during oscillatory swimming treatments, the predicted NCOS was calculated by integrating a sinusoidal function based on the variation in water flow speeds during oscillatory swimming (Eqn 1 or 3, Fig. 3) into the equation of $\dot{M}_{O_2}$ as a function of swimming speed (Eqn 4) determined from $\dot{M}_{O_2}$ measurements at steady water velocities for each individual fish (Fig. 2) (Roche et al., 2014). The individual SMR was then subtracted from this value to determine the NCOS. Thus, the predicted NCOS represents the energetic costs of swimming in oscillating water flow speeds but assumes no additional costs owing to acceleration or turning.

Data analysis

Each water flow treatment was categorized by four parameters: oscillation, direction, frequency and average speed (Table 1). Oscillation (yes or no) indicated whether velocities varied; only steady swimming values were categorized as no oscillation. Direction indicated whether the flow was unidirectional or bidirectional. Only the bidirectional oscillatory flows were categorized as such to indicate the fish had to turn around with direction changes. The predicted NCOS was also included as a treatment and categorized as 'yes' for oscillation, but given a frequency of zero and direction of unidirectional, to indicate that no acceleration or turning cost was included, and the predicted NCOS does not change with wave frequency or direction (only amplitude). We used IBS SPSS Statistics (v.26) to perform a linear mixed model (LMM) analysis of the relationship between log(NCOS) and treatment parameters to account for the repeated-measures design (West et al., 2014). The LMM assumed that the dependency structure was modeled correctly, that the random effects and within residual errors follow a normal distribution and that they had a constant variance. Prior to statistical analyses, the NCOS and average speed were log-transformed to improve normality and linearize the relationship between these two variables (Korsmeyer et al., 2002). Log(speed) was adjusted to a reference level of 1.5 BL s^{-1} by subtracting log(1.5) from all values. In developing the model, the fit was evaluated using Hurvich and Tsai's criterion (AIC$_C$). As fixed effects, we used oscillation and direction with covariates of frequency and log(speed), and the interaction between
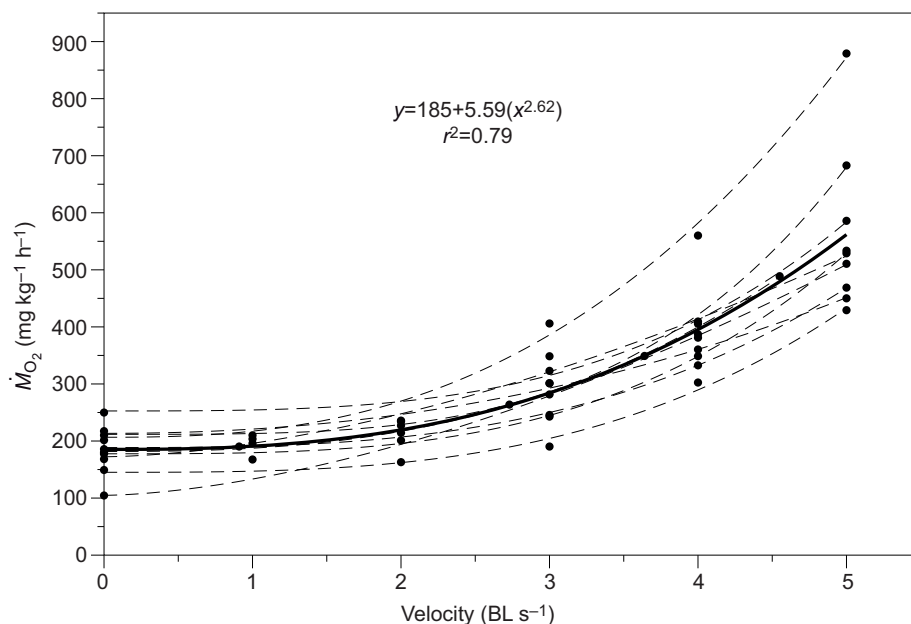

$$y = 185 + 5.59(x^{2.62})$$
$$r^2 = 0.79$$

Fig. 3. Oxygen consumption rate ($\dot{M}_{O_2}$) as a function of swimming velocity for *Ctenochaetus strigosus* (*n*=10). The dashed lines are the $\dot{M}_{O_2}$–speed (*U*) relationships ($y=a+bU^c$) for each individual fish and the solid line is the combined relationship for all the fish. The power function equation is for the combined $\dot{M}_{O_2}$–speed relationship. Each point is the average $\dot{M}_{O_2}$ for an individual fish at that velocity. Values at zero velocity are the standard metabolic rates (SMR) determined from overnight measurements with each fish. BL, body lengths.

Table 2. Estimates of fixed effects from a linear mixed model of log(NCOS) for *Ctenochaetus strigosus* with subject as a random effect

Parameter	Coefficient	s.e.	P-value
Intercept	1.24	0.10	<0.001
Oscillation	0.0982	0.0245	<0.001
Direction	0.243	0.059	0.001
Frequency	0.0321	0.149	0.832
Log(speed)	2.35	0.23	<0.001
Direction×frequency	0.404	0.204	0.049

Log(NCOS) is log-transformed net cost of swimming (mg O_2 kg^{-1} h^{-1}). Oscillation as 'yes' or 'no' (reference level). Direction as 'bidirectional' or 'unidirectional' (reference level). Frequency in Hz. Log(speed) is log-transformed average swimming speed (BL s^{-1}) centered at log(1.5)=0.176.

direction and frequency. As random effects, we included intercepts for subjects as well as by-subject random slopes for direction, frequency and log(speed), with a covariance structure of variance components. Visual inspection of residual plots did not reveal extensive deviations from homoscedasticity or normality. Holm's sequential Bonferroni correction was performed to correct for multiple comparisons (Holm, 1979). The statistical significance level for this study was $P<0.05$.

RESULTS

During both the steady and unsteady swimming trials, all the fish behaved similarly, swimming primarily with their pectoral fins throughout the experiment with no gait transition, backwards swimming or exhaustion. Typical swimming behaviors during the oscillatory wave surge flows during the high frequency and amplitude treatment can be seen in Movie 1 (bidirectional) and Movie 2 (unidirectional). Turning during the bidirectional oscillatory flows involved axial bending and extension of the pelvic, dorsal and anal fins that was not observed in the unidirectional flow oscillations.

During the steady swimming trials, $\dot{M}_{O_2}$ and NCOS increased for each fish with increasing speed [LMM, effect of log(speed) on log(NCOS), $F_{1,9.1}$=106.32, $P<0.001$; Table 2]; however, the rate of increase differed slightly between each fish (Fig. 3). As a result, the steady and predicted NCOS were calculated for each fish separately at each water flow treatment. Predicted NCOS accounts for the effect of the non-linear relationship between $\dot{M}_{O_2}$ and speed during oscillations in speed around an average value, and as a result was slightly higher than the steady NCOS at that same average speed (LMM, oscillation effect, $F_{1,179.5}$=16.05, $P<0.001$; Table 2, Figs 4 and 5). This predicted value was based only on the steady swimming NCOS and does not include any costs of acceleration or turning during oscillatory swimming. Predicted NCOS, therefore, does not change with increasing oscillation frequency if average speed is the same (e.g. Fig. 4, low *f*–high *A* and high *f*–high *A* values).

The measures of NCOS during oscillatory swimming include an effect of wave frequency; however, the strength of this effect varied depending on whether the oscillation was unidirectional or bidirectional (LMM, direction×frequency interaction, $F_{1,179.69}$=3.91, $P=0.049$; Table 2). For unidirectional oscillatory swimming, the effect of frequency on log(NCOS) was minimal (coefficient=0.0321, s.e.=0.149, $P=0.83$), while frequency had a significant effect during bidirectional oscillatory swimming (coefficient=0.436, s.e.=0.197, $P=0.032$). Compared with predicted NCOS, measured NCOS during unidirectional swimming includes the added acceleration and deceleration required during each cycle of flow and acceleration increases with both frequency and amplitude (average speed) (Table 1). The negligible effect of frequency on unidirectional NCOS indicates that the net cost of acceleration was very low; this
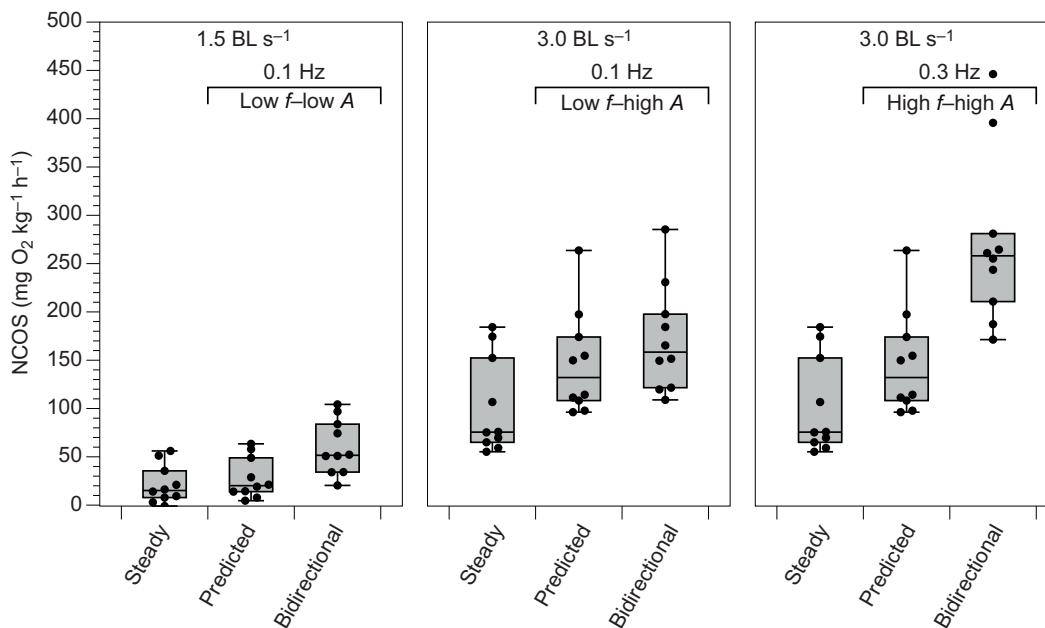

Fig. 4. Net cost of swimming (NCOS) in steady and bidirectional oscillatory water flows at two different average swimming speeds (BL s^{-1}) and two different wave frequencies (Hz) for *Ctenochaetus strigosus* (*n*=10). The average swimming speeds (1.5 and 3.0 BL s^{-1}) are indicated at the top of each panel, and the brackets within the panels indicate the wave frequencies (0.1 and 0.3 Hz) of the bidirectional oscillatory water flow. The text below the brackets indicates the water flow treatment, where *f* is frequency and *A* is amplitude. The steady values are measurements of the NCOS at steady speeds and the predicted values are estimates of the NCOS in oscillatory water flow owing to the non-linear relationship between NCOS and swimming speed. Note that the steady and predicted values in the center and right most plots are identical because the average swimming speeds are identical. The bidirectional values are the NCOS measured in bidirectional oscillatory water flow. Points represent the average values from individual fish.
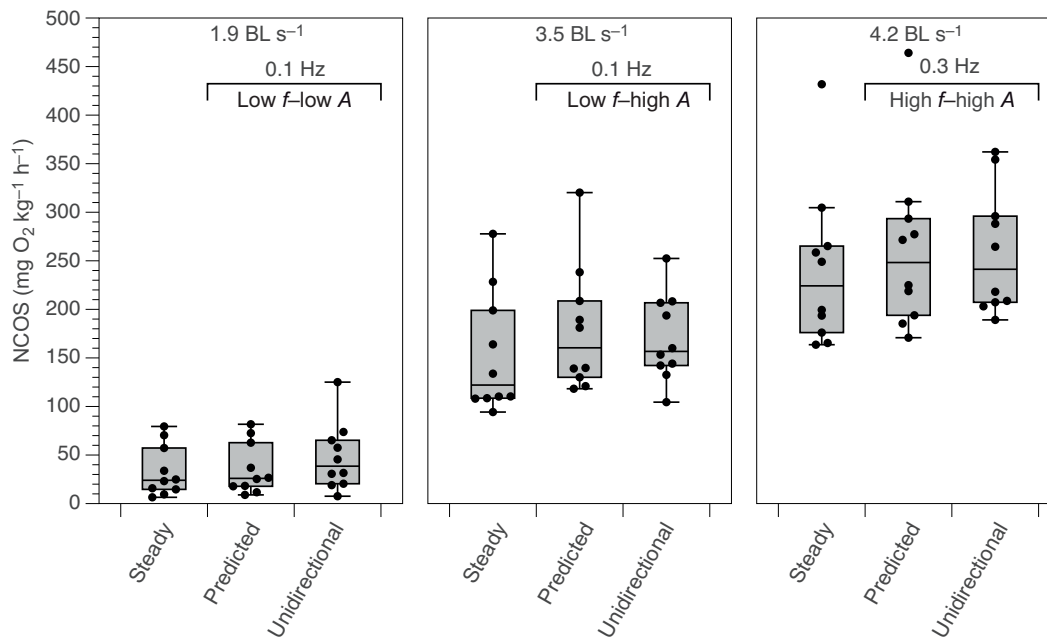
Fig. 5. NCOS in steady and unidirectional oscillatory water flow at three different average swimming speeds (BL s^{-1}) and two different wave frequencies (Hz) for *Ctenochaetus strigosus* (*n*=10). The average swimming speeds (1.9, 3.5 and 4.2 BL s^{-1}) are indicated at the top of each panel, and the brackets within the panels indicate the wave frequencies (0.1 and 0.3 Hz) of the unidirectional oscillatory water flow. The text below the brackets indicates the water flow treatment, where *f* is frequency and *A* is amplitude. The steady values are measurements of the NCOS at steady speeds and the predicted values are estimates of the NCOS in oscillatory water flow owing to the non-linear relationship between NCOS and swimming speed. The unidirectional values are the NCOS measured in unidirectional oscillatory water flow. Points represent the average values from individual fish.

effect can also be seen by comparing predicted and unidirectional NCOS values at all flow intensities, which were very similar (Fig. 5).

In contrast with the unidirectional flows, swimming in bidirectional oscillations requires both acceleration–deceleration and turning around to face the change in flow direction. Because of the negligible effect of acceleration in unidirectional oscillations, the increases with frequency in bidirectional swimming NCOS appear to have been primarily due to the costs of turning with direction changes (Fig. 4). Although we were unable to match the same speeds in both unidirectional and bidirectional treatments (Table 1, Fig. 2), we can use the LMM (Table 2) to calculate estimated marginal means of NCOS at each bidirectional flow treatment, correcting for the effect of speed (Table 3). These estimates show little effect of acceleration in the differences between the predicted and unidirectional NCOS, but significant differences between unidirectional and bidirectional NCOS at all three flow intensities ($P \leq 0.001$). From this analysis, the costs of turning in bidirectional oscillatory flows accounted for approximately 50% of the NCOS on average, or a 2-fold increase from predicted values (Table 3). The NCOS attributed to turning at a frequency of 0.1 Hz

increased 5-fold with an increase in average speed from 1.5 to 3.0 BL s^{-1}. A 3-fold increase in the frequency of turning (0.1 to 0.3 Hz at an average speed of 3.0 BL s^{-1}) increased the cost of turning by another 50% (Table 3, Fig. 4).

DISCUSSION

By using a modified swimming respirometer (Fig. 1) we were able to measure NCOS during steady velocities and during station-holding in both unidirectional and bidirectional oscillatory flows similar to shallow, wave-surge flows. The wave frequencies (0.1 and 0.3 Hz, or wave periods of 10 and 3.3 s, respectively) and water flow speeds (up to 45 cm s^{-1} for a 9 cm fish) were consistent with the range of flows experienced by fishes on shallow-water coral reefs (Fulton and Bellwood, 2005; Denny, 2006; Webb et al., 2010) (Fig. 2). Comparing the two unsteady flow regimes, bidirectional and unidirectional, allowed us to separate the energetic challenges of speed changes (accelerations and decelerations) from the cost of turning to face the direction of the water flow. When the fish had to continuously turn and accelerate to station-hold in the bidirectional flow they used, on average, twice as much energy for swimming (NCOS) when compared with the insignificant change in swimming costs when they only had to accelerate and decelerate in the unidirectional flow (Figs 4 and 5, Table 3). Our results show that most of the additional costs of station-holding in wave-surge type flows for the labriform swimming *C. strigosus* are due to significant costs of turning to face the flow.

Although we expected the swimming costs to increase the most in the bidirectional oscillatory flow, we also expected some significant effect of the accelerations required in the unidirectional oscillatory flow, which was not found. Roche et al. (2014) reported a 14% increase in total $\dot{M}_{O_2}$, over predicted values, with unidirectional oscillatory flows (1 BL s^{-1} amplitude) for the labriform swimming shiner surfperch, *Cymatogaster aggregata*, which are presumably

Table 3. Estimated marginal means from the linear mixed model of net cost of swimming (NCOS) for *Ctenochaetus strigosus* at three levels of frequency (*f*) and amplitude (*A*) of oscillatory swimming (low *f*–low *A*, low *f*–high *A* and high *f*–high *A*)

Frequency (Hz)	Average speed (BL s^{-1})	NCOS (mg O$_2$ kg^{-1} h^{-1})		
		Predicted	Unidirectional	Bidirectional
0.1	1.5	21.6	21.8	41.9*
0.1	3.0	110	111	213*
0.3	3.0	110	113	261*

*Significant difference from unidirectional NCOS at *P*<0.001.

due to costs of repeated accelerations and decelerations. Unlike the species in the present study, *C. aggregata* mostly inhabits calm shallow marine waters, bays and estuaries (NatureServe, 2019), where wave surge is not common. Therefore, *C. aggregata* may be less adapted to the energetic requirements of swimming in unsteady flows. It also is possible that the costs of acceleration were underestimated in the present study. The unidirectional oscillatory flows created by the same sinusoidal function driving the flow within the flume as the bidirectional water flow were not identical in speed profiles (Fig. 2). The unidirectional flows had higher minimum and maximum speeds but a lower amplitude, resulting in lower rates of acceleration (Table 1). Nevertheless, substantial accelerations were still required in the high *f*–high *A* unidirectional treatment, with no apparent increases in NCOS from predicted values based on speeds alone (Fig. 4). The goldring surgeonfish used in this study are common in wave-exposed areas of coral and rocky reefs (Hobson, 1974) and, therefore, may be more efficient at acceleration. It is also possible that the added costs of accelerating with each wave cycle are offset with energy savings during a passive deceleration phase (Marcoux and Korsmeyer, 2019).

In the study by Marcoux and Korsmeyer (2019), *C. strigosus* frequently swam backwards for one-half of a wave cycle, instead of turning around. However, no individual in our study exhibited this behavior at any time. This difference could be due to variations in the experimental setup, as Marcoux and Korsmeyer (2019) used lower amplitudes to simulate the bidirectional wave surge and because the swim section in their respirometer was moving in a body of still water instead of moving in a body of water with a propeller as done in the present study. Thus, the consistent turning may be a consequence of the higher speeds, microturbulent flows and a greater amount of space afforded in the swimming flume (Fig. 1). The cost of direction changes in bidirectional flow in the present study was also greater than what was found by Marcoux and Korsmeyer (2019), which suggests that turning is more costly than swimming backwards, at least at the lower speeds used in that study. As average speed increased from 1.5 to 3.0 BL s^{-1}, with the same frequency, or rate of turning, the costs associated with turning increased dramatically from 20 to 100 mg O$_2$ kg^{-1} h^{-1} (Table 3) indicating that turning at higher speeds may require greater postural control to maintain stability. Conversely, increasing the frequency of turning by 3-fold did not result in a 3-fold increase in costs of turning, but only a 1.5-fold increase (Table 3). This suggests that there is not a consistent cost per turn; instead, it varies with speed and rates of acceleration into and out of the turn. Fish species with other swimming modes (e.g. BCF) have shown greater increases in cost with frequency of oscillation than the labriform swimmer (Marcoux and Korsmeyer, 2019), and future studies should examine the costs of turning and acceleration as it relates to swimming mode.

These studies show that turning around during routine swimming is an energetically expensive behavior and a significant factor in the energy budgets of fishes in habitats with dynamic, wave-induced water flows. Shallow-water, marine habitats may experience more bidirectional oscillatory water flows from incoming wave activity than prolonged periods of steady flow. Measuring the costs of swimming in steady flows to understand the energetic requirements of fish in these dynamic conditions may, therefore, underestimate the actual cost of swimming in the field (Webb, 1989; Roche et al., 2014). For example, coral reef fishes are often highly site attached and constantly experience the energetic challenge of station-holding in the face of changing water flow driven by wave surge. The additional cost of swimming in a bidirectional flow compared with the predicted cost suggests that mobile organisms living in wave-

swept habitats face higher energetic challenges than previously thought (Roche et al., 2014; Marcoux and Korsmeyer, 2019). The costs of turning may be related to added recruitment of body and fin muscles during the maneuver. For labriform swimmers, steady swimming involves only the pectoral fins, but during turning, other fins, such as the pelvic, dorsal, anal and caudal, are recruited, in addition to bending of the entire body (Gerstner, 1999; Danos and Lauder, 2007; Pink and Fulton, 2014). In the present study, *C. strigosus* extended its pelvic, dorsal and anal fins during turns, which were not otherwise used (see Movie 1). Furthermore, a study by Drucker and Lauder (2001) examined the hydrodynamics of turning maneuvers of the labriform swimming bluegill sunfish, *Lepomis macrochirus*, and found that during a turn, the force that needs to be produced by the pectoral fins is functionally distinct and significantly higher compared with fin strokes during steady swimming. The pectoral fin on the outside of the turn initiated the turn and produced a lateral force four times higher than that of a steady swimming stroke, in order to rotate the body. Moreover, the pectoral fin on the inside of the turn created a posteriorly oriented thrust with a force nine times higher than a steady swimming stroke, to move the body linearly into the direction of the turn. The requirements to recruit additional muscle as well as producing higher stroke forces could explain why turning is such an expensive behavior.

In our experimental setup, the fish did not have the option to seek refuge, which is a common behavioral strategy used by fishes occupying areas of high water flow (Fulton et al., 2001; Johansen et al., 2007, 2008). Complex structures in the water column, such as corals, and holes in the substratum can serve as flow refuges with diminished water speeds that enable energy savings (Johansen et al., 2007, 2008). In addition, fish can strategically place themselves lower in the water column or use the boundary layer to escape high levels of water motion (Fulton et al., 2001). However, fish that are able to more effectively occupy areas of high water motion could better utilize the available resources that may be out of reach for less efficient swimmers. Fish that have the ability to resist displacement and maintain their position in a wave-dominated habitat could increase their food encounter rate, decrease predation risks and/or better protect their territory compared with others that are unable to adequately cope with the flow conditions (Fulton, 2010). Planktonic feeders can increase their food encounter rates by station-holding and letting a greater amount of water pass by them, compared with being displaced with the water mass. *Ctenochaetus strigosus* feed off the substrate, and in that case, while the fish would also station-hold in order not to be displaced relative to their food source, they will more frequently be lower in the water column and near potential refuges that attenuate the flow. Therefore, different niche utilization strategies create different energetic challenges and trade-offs in fitness-related benefits to maintaining their position (Liao, 2007). On coral reefs, this could have created niches and a selective pressure to minimize energy expenditure in such habitats. This could help explain the high biomechanical and morphological diversity we observe in coral reef fishes while shaping the long-term structure of communities and assemblages (Fulton et al., 2005). Binning et al. (2014) reported that individuals of a labriform swimming damselfish (*Acanthochromis polyacanthus*) exposed to high wave-surge flows compared with more sheltered individuals of the same species had acquired physiological as well as morphological adaptations that aided them in dealing with such conditions. Specifically, for labriform swimmers, the shape and motion of the pectoral fins can result in large differences in maneuvering performance and swimming energetics, which have

led to inferences about their behavior and distributions (Walker and Westneat, 2002b). For instance, labriform swimmers with tapered, high-aspect-ratio fins that use a flapping fin motion are able to maintain higher swimming speeds and lower energetic costs. In contrast, fishes with paddle-shaped, low-aspect-ratio fins that use a rowing fin motion have a higher thrust production and maneuverability at lower speeds but quickly suffer higher energetic costs at elevated swimming speeds (Walker and Westneat, 2002a). *Ctenochaetus strigosus*, used in the present study, has a tapered, high-aspect-ratio pectoral fin (1.9; Marcoux, 2016), and uses a flapping fin motion to provide propulsion. Therefore, for a fish with high-aspect-ratio fins such as *C. strigosus*, accelerating, following each turn, to higher swimming speeds in the bidirectional flow might be a more energetically expensive endeavor when compared with a fish with low-aspect-ratio fins. If true, this should also apply to the unidirectional oscillatory flow as the fish is frequently required to accelerate during the changes in flow speed. However, we did not observe this in our study. It would be interesting to compare the cost of swimming and turning of fishes with low-aspect-ratio fins that use a rowing motion with those with high-aspect-ratio fins using a flapping motion. In addition, we did not select our experimental fish from a particular habitat of a given wave exposure, and individuals of *C. strigosus* may show phenotypic differences in swimming ability based on their local habitat (Binning et al., 2014).

This study demonstrated that station-holding in a wave-swept habitat is associated with additional energetic costs, compared with swimming at steady speeds. Furthermore, the turning component of the station-holding behavior, at least in a labriform swimming coral reef fish, appears to be the most expensive component, and may have resulted in adaptations of more maneuverable swimming modes and morphologies present in diverse shallow-water habitats such as coral reefs. Fishes exposed to bidirectional flow regimes face a constant trade-off between energetic constraints and maximization of fitness-related activities, a balance that might change with changes in wave activity and coastal exposure.

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