THE EFFECT OF SWIMMING MODES AND MOPHOLOGICAL ATTRIBUTES ON THE ENERGETICS OF CORAL REEF FISHES SWIMMING IN A WAVE-SURGE WATER FLOW

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Abstract

Water flow conditions in shallow-water marine ecosystems are commonly dominated by a wave-surge flow and specifically on coral reefs we observe an immense diversification in morphology and swimming modes of fishes that are linked to their swimming performance. Recently, a few experimental studies have found that in wave-surge conditions the energetic requirements for fishes to station-hold are greatly increased and field studies suggest that fishes ability to occupy these unsteady, dynamic flow regimes is dependent on their swimming mode and morphological attributes. However, previous experimental studies have not examined a combination of diverse morphologies with an adequate range of wave-surge intensities that have been observed in the field. In this study, we used a modified swimming respirometer to create simulated wave-surge flows with variations in frequency and average velocity. The range of simulated wave-surge flows tested was within the range of flow conditions observed on sheltered and exposed reefs in both calm and stormy conditions. This study examined two different aspects of the energetics of swimming in wave-surge flows. In the first part, we quantified the swimming costs associated with station-holding in six levels of both unidirectional and bidirectional wave-surge flows to separate the costs of acceleration and turning using the goldring surgeonfish, *Ctenochaetus strigosus,* a pectoral-fin swimmer. We determined that turning during station-holding increased the cost by up to 2-fold while the added acceleration costs were minimal. In the second study, we quantified the cost of station-holding in steady water flows and simulated bidirectional wave-surge flows for four coral reef fish species, one BCF swimmer and three pectoral fin swimmers, with a variation of body and fin morphologies. We found that, while the BCF swimmer had low costs associated with steady swimming, it had a higher effect of wave frequency on the swimming costs compared to the MPF swimmers. However, the pectoral fin swimmer with a high aspect ratio fins had both low swimming costs in the steady and wave-surge flows. Lastly, the body fineness ratio of the fishes was the largest predictor of NCOS with ratios close to 4.5 resulting in the lowest NCOS in the wave-surge flow.

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This thesis is submitted in partial fulfillment of the requirements for the degree of Master of Science in Marine Science at Hawai'i Pacific University. We the undersigned have examined this document and have found that it is complete and satisfactory in all respects, and all revisions required by the final examining committee have been made.

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Chapter 1: Review of fish swimming and energetics

Introduction

The change in the metabolic rate of fishes, measured as oxygen consumption rate, is widely accepted as a factor relating to overall fitness and can be used to examine the effects of environmental factors on the distribution and physiology of fishes (Fry 1971, Habary et al. 2016). Many studies have examined the swimming performance of fishes under different physical conditions, such as water motion, temperature, and salinity (Liao 2007). Measuring swimming performance and energetics under different environmental conditions is important for understanding the effects of future changes due to anthropogenic environmental factors, such as temperature or wave action, to improve our understanding of fish locomotion and physiology, and for practical applications such as habitat conservation and management (Enders et al. 2003, Fulton & Bellwood 2005, Liao 2007, Roche et al. 2014). Field studies have shown a clear relationship between swimming abilities and habitat use (Binning et al. 2014, Fulton 2007, Fulton et al. 2005, Fulton & Bellwood 2002, Fulton & Bellwood 2005, Fulton et al. 2001). Laboratory studies have long examined the energetics of fish swimming in steady water flows, and more recently, unsteady wave-surge like water flows (Bell & Terhune 1970, Fulton et al. 2013, Korsmeyer et al. 2002, Marcoux & Korsmeyer 2019, Roche et al. 2014, van der Hoop et al. 2018).

Physical factors play an important role in shaping marine community structures, with interactions between the species’ physiology and physical attributes often determining distribution patterns (Dunson & Travis 1991). Studies have linked physical factors, such as temperature, salinity and water motion, to the distribution of species in marine systems, from local to regional biogeographical scales (Bellwood et al. 2002, Fulton & Bellwood 2005, Perry et al. 2005). Reef fish are often highly site-attached, meaning they are dependent on particular feeding and refuge sites (Fulton & Bellwood 2005, Turgeon et al. 2010). It is therefore crucial for their survival to maintain a specific position on the reef, which represents a challenge in wave-swept habitats (Heatwole & Fulton 2013).

As waves enter shallow water and travel along the reef flat, they create surge water flow, in a bidirectional oscillatory motion, affecting the swimming performance and energetics of coral reef fishes (Webb 2004). This could have significant ecological consequences for mobile organisms and influence their foraging activities and habitat use. The energetic costs of a fish maintaining a position in shallow, wave affected waters, compared with calm or open-water conditions, will likely be higher. However, several different swimming modes have evolved with behavioral and morphological adaptions yielding swimming abilities ranging from high maneuverability at low speeds to rapid bursts of linear acceleration (Blake 2004). These different swimming modes may result in differences in the energetic cost of swimming in wave-surge habitats.

This review aims to provide an overview of the energetics and performance of different physiological and morphological swimming adaptations among reef fishes, and the potential effects of wave-surge water flow. Several different swimming modes have been observed for coral reef fishes (Liao 2007). A review of some of the most prominent swimming modes will be provided as well as an explanation for the physical properties of wave-surge water-flows in shallow waters. In addition, the method and theory that is used to measure the energetics of fish and how that relates to swimming performance will briefly be mentioned. Lastly, how swimming modes relate to the wave action in shallow water, will be examined from several laboratory studies on their energetic efficiency and field observations looking at distributional patterns in relation to the intensity of wave-surge flows.

Waves

The majority of the ocean's waves are produced by frictional forces between the surface of the sea and the air movement from winds. Waves consist of a crest and trough, where the wavelength is the distance between two crests and the wave period is the time it takes for two crests to travel one wavelength. However, the physical properties can differ between waves depending on the water depth and the wavelength of the waves, and are generally categorized into three groups: Deep water waves, transitional waves and shallow water waves (Constantin et al. 2008, Constantin & Villari 2008). For deep water waves, the depth of the water must be greater than one-half of the wavelength. The motion of the water under the surface, that is the particle motion of a deep water wave is mostly circular with little forward movement, which degenerates with depth (Constantin et al. 2008). As waves travel towards the coast the depth of the water column decreases. This change in depth creates some physical changes to the particle motion of the waves.

When the water depth decreases to one-half or less of the wavelength, the wave becomes a transitional wave and the particle motion becomes more vertically compressed resulting in a more elliptical trajectory of the particle motion (Constantin & Villari 2008). When the depth further decreases to less than 1/20 of the wavelength, the wave becomes a shallow-water wave. These waves are commonly experienced on shallow-water reefs and the particle motion is now almost strictly horizontal (Constantin & Villari 2008). This wave-surge water flow is what is categorized as a bidirectional oscillatory wave motion. Furthermore, the complex habitat structure of coral reefs and the breaking of shallow water waves create highly turbulent water flows. This turbulence, in addition to bidirectional oscillatory wave motion, makes it difficult for fish to inhabit coral reefs as the ability to navigate and station-hold in the water column is compromised by the unsteady water flow. Swimming is not only energetically expensive for fish due to the high viscosity and density of the water, but also vital to their survival as it aids in predator avoidance and resource utilization (Blake 1983). However, the challenge of the wave-surge water flow also creates niche constraints to which adaptation would give a fish an advantage (Laland et al. 2000). It is therefore thought that the wave-surge water flow is an important factor in shaping the distribution and evolution of mobile reef fishes (Bellwood et al. 2002, Depczynski & Bellwood 2005).

Swimming modes

Along with the immense diversity of morphologies of reef fishes, a large spectrum of different swimming modes has evolved in the coral reef ecosystem. The different swimming modes in fish have been categorized by the structure and movements employed for propulsion (kinematics and mechanics), and their activity level and duration (continuous vs. transient swimming). These factors are fundamental in describing the diversity of locomotion in fishes (Blake 2004). Breder (1926) described swimming modes of fish based on the taxonomic name of a representative fish followed by the suffix “-iform”. However, the classification implies nothing about taxonomic or evolutionary relationships. For example, anguilliform swimming is derived from the eel *Anguilla*, but other genera can use anguilliform swimming as well. Breder's method of classifying swimming modes has been further developed by Lindsey (1978) and Webb & Blake (1985), where fish swimming modes are divided into groups and sub-groups based on their swimming style. The first major division of swimming modes is based on the structure employed by the fish: Median and paired fin swimming (MPF) or body and caudal fin swimming (BCF), each of which is further divided into subgroups based on how the fins and body are used for propulsion (Blake 2004).

Body and Caudal Fin Swimming

BCF swimming is undulatory movements of the posterior part of the body and caudal fin. This locomotion is the most prevalent propulsive force used by fish, accounting for 85% of fish families using this swimming mode (Videler 1993). However, this locomotion type is much less prevalent in coral reef ecosystems, accounting for less than 15% of observed fishes (Fulton & Bellwood 2005). The swimming performance of BCF swimming is associated with higher speeds and cruise swimming (Webb 1998). Within BCF swimming there is a spectrum of types of swimming categorized by the proportion of body used for propulsion (Sfakiotakis et al. 1999). This spectrum can be looked at from an evolutionary perspective: at the beginning of the spectrum are the ancestral forms, that undulate a large part of their body in a sine wave to produce thrust. Less of the body is used for propulsion towards the other end of the spectrum, where at the extremes it is only the caudal fin that is used for propulsion. Furthermore, fish are generally considered more derived and energetically efficient for high-speed cruise swimming towards this end of the spectrum (Sfakiotakis et al. 1999, Videler 1993).

Anguilliform swimming is at the ancestral end of the spectrum and is observed in most eels, some sharks, and other elongated fish. They swim by using their body and caudal fin, creating a series of waves passing down the length of their body, with a wavelength >1 in the body when viewed dorsally. The large amplitude of the long wavelengths, is met by a large amount of opposing pressure drag, decreasing their thrust efficiency and increasing their cost of transport (Kern & Koumoutsakos 2006). In the middle of the spectrum is subcarangiform, used by cod and trout, and carangiform, named after the Jack family, Carangidae, that has reduced the proportion of the body used for propulsion by only using the posterior part of the body and the caudal fin (Sfakiotakis et al. 1999). This change decreases the wavelength within the body and the opposing pressure drag and consequently their cost of transport. Moreover, several other adaptations have evolved in these groups, such as decreasing the size of their caudal peduncle, and having separated fins as a series of finlets to further decrease the pressure drag (Graham & Dickson 2004). The most derived BCF swimming style is thunniform, which includes tunas that have both morphological and mechanical adaptations to minimize pressure drag and maximize swimming thrust (Graham & Dickson 2004). Their body makes <1/2 of a wavelength in bending and they are specialized for fast-cruise swimming with a streamlined, fusiform body shape, thin caudal peduncle, moveable finlets and a functional “hinge” connecting the tail to the caudal peduncle that keeps the caudal fin parallel to the direction of the motion (Blake 2004).

Median and Paired Fin swimming

MPF swimming is the undulation or oscillation of one or more median (dorsal and anal fins) or paired fins (pectoral fins) to provide propulsion while keeping their bodies rigid and straight. These swimming modes are generally good for slow speeds and high maneuverability and therefore navigating in complex habitats like coral reefs (Sfakiotakis et al. 1999). The fishes that use this type of locomotion often switch to the more energetically costly swimming form of BCF when rapid swimming speed is required (Korsmeyer et al. 2002). MPF swimming has evolved in more derived groups of teleosts with soft flexible fin rays, which makes small, maneuvering adjustments easier. The advantage of high maneuverability derives from the ability to move each fin independently, providing propulsion in all directions around the center of the fish (Webb 1984). Furthermore, these swimming techniques have largely reduced the pressure drag by keeping their body rigid while swimming and limiting the posterior lateral movement of the body. In a bidirectional, oscillatory wave motion, this should be more energetically efficient, as the drag forces are minimized and maneuverability is high enabling them to easier turn to face the water flow coupled with continuous acceleration and deceleration in the unsteady flow, compared to BCF swimmers. Several ways of using MPF swimming have evolved and been described. However, this review focuses on labriform swimming and balistiform swimming as they are the most prevalent and best-studied swimming forms on coral reefs.

Labriform swimming

Labriform or pectoral fin swimming is named after the wrasse family Labridae and involves swimming by movements of the paired, pectoral fins. This method has evolved independently several times among many fish groups, such as surgeonfishes, damselfishes, and wrasses, and is the most prominent swimming form on coral reefs (Fulton & Bellwood 2005, Thorsen & Westneat 2005). On some reefs, at least 60% of the species found there use labriform swimming. Labriform swimming is considered highly maneuverable, which is thought to be the reason this swimming mode dominates on coral reefs (Heatwole & Fulton 2013). Furthermore, these reef fishes display a wide range of shapes and sizes of their pectoral fins and bodies, which further relates to how they produce thrust and decrease drag forces.

Fishes with long, wing-shaped fins (high aspect ratio fins: high AR) produce lift-based thrust by a figure-eight flapping fin motion. Fishes with rounded paddle-shaped fins (low aspect ratio fins: low AR) produce resistance-based thrust by a rowing fin motion (Walker & Westneat 2002). Studies have found that labriform swimmers with wing-like, high AR fins are able to maintain high swimming speed. Furthermore, paddle-shaped, low AR fin labriform swimmers are slower swimmers but have higher power output, acceleration, and maneuverability (Fulton 2007, Wainwright et al. 2002). Generally, low AR labriform swimmers inhabit calm water habitats, sheltered from incident wave energy and storms, while high AR labriform swimmers inhabit reefs areas with variable and extreme water flows generated by direct exposure to wave energy (Fulton 2010, Wainwright et al. 2002).

The shape of the body of a fish affects the drag forces exerted on the fish while it’s moving through the viscous and dense medium of water (Sfakiotakis et al., 1999). The shapes of body forms are most effectively described by the body fineness ratio, which is defined by the measure of how elongated a fish is relative to its transverse sectional diameter (Walker et al., 2013). A body fineness ratio of 4.5 has been found to optimally minimize the drag forces and Walker et al. (2013) found that MPF swimming fishes with a body fineness ratio close to the 4.5 had increased endurance during prolonged maximum swimming. However, this held true only for fishes swimming with rigid bodies, like the MPF swimmers.

Balistiform swimming

Balistiform swimmers, named after the triggerfish family Balistidae, generate thrust by movements of both the anal and dorsal fins. The anal and dorsal fins are enlarged and symmetrical with multiple fin-rays undulating or oscillating in a sinusoidal pattern while keeping the body rigid, creating a forward movement parallel to the base of the fins. Moreover, the median fins are usually inclined towards the caudal fin and their bodies are compressed laterally. This design is associated with high thrust efficiency by reducing the pressure drag that opposes the thrust force (Sfakiotakis et al. 1999).

Lighthill & Blake (1990) developed a theoretical analysis of balistiform swimming and concluded that such a design experiences a threefold increase in thrust efficiency compared to BCF swimming. Furthermore, the large flexible dorsal and anal fin can move independently and provide thrust in many directions and they can use their pectoral fins to maneuver as well (Webb 1998). This provides them with high maneuverability, enables them to hover, swim backward and turn about their own center of mass with little lateral translation of their own body (Blake 2004). Korsmeyer et al. (2002) found that lagoon triggerfish, *Rhinecanthus aculeatus*, was not only highly maneuverable but were also able to sustain high swimming speeds. At high speeds, the triggerfish used body-caudal undulation in addition to the undulations of the dorsal and anal fin and this allowed them to sustain swimming speeds of up to 4 body lengths per second (BL/s). This high-speed swimming ability, while also being highly maneuverable, could be advantageous for the triggerfish to hold station in a strong bidirectional, oscillatory wave motion. However, the use of BCF swimming to support high sustainable swimming speeds leads to a higher cost of transport, as opposed to some labriform swimmers that do not rely on BCF swimming until higher, burst speeds are reached (Fulton et al. 2013).

Oxygen consumption as a measurement for swimming performance

How much energy a fish uses while swimming at different speeds or under different environmental conditions is an effective way of examining their performance and efficiency (Steffensen et al. 1984). However, since it is difficult to directly measure how much energy a fish is using, oxygen consumption rate (MO_2) is used as an approximate instead (Nelson 2016). Since all aerobic activities like locomotion require oxygen, this is the closest approximation available. However, it is important to note that this assumes that there are no anaerobic activities.

The most common and optimal experimental design to measure oxygen consumption under varying water flows is the intermittent-flow respirometer. This experimental design can be used to measure the maximum metabolic rate (MMR) and oxygen consumption at rest (SMR: standard metabolic rate) (Steffensen et al. 1984, Svendsen et al. 2016). SMR represents the minimum oxygen uptake needed for a fish to maintain homeostasis and stay alive (Pörtner & Farrell 2008). The SMR can be determined by measuring oxygen consumption at different swimming speeds and then extrapolating to the oxygen consumption rate at zero speed or measuring the oxygen consumption overnight when the fish is at rest (Svendsen et al. 2016). The two methods of finding SMR can agree (Roche et al. 2013, Schurmann & Steffensen 1997) and sometimes not (Duthie 1982, Roche et al. 2013). The MMR and SMR can then be used to calculate the aerobic scope (MMR – SMR). The aerobic scope is an indication of the energy available for all activities like locomotion and is often used to compare the capacity for energy use among species or individuals (Roche et al. 2014). Lastly, to measure the energetic requirements of an activity, like swimming in a certain flow condition, the net cost of swimming (NCOS) can be calculated by subtracting the SMR from the swimming MO_2.

Swimming in wave-surge habitats

The relationship between swimming modes and wave-surge water flow has been extensively studied on Lizard Island on the Great Barrier Reef. Fulton et al. (2001) examined the extent to which locomotor ability shaped the habitat use of wrasses (family Labridae). They found that high wave energy habitats were mostly occupied by wrasses with a high fin aspect ratio. These wrasses use lift-based locomotion and can achieve high sustained swimming speeds (Fulton et al. 2013). Other studies on labrids have since demonstrated that taxa that dominate high wave energy habitats predominantly use lift-based locomotion (Bellwood et al. 2002, Wainwright et al. 2002). Conversely, wrasses with low fin aspect ratios were more abundant in low wave energy habitats. However, they were also found in low numbers in the high wave energy habitats, but with restricted use of the water column and mostly by using the complexity of the substratum and the boundary layer effect as a refuge. This indicates that a species ability to cope with high or low wave-surge water flow can explain a species small-scale distribution in habitat use.

Fulton et al. (2001) proposed that the wrasses with low AR do not have the ability to cope with high wave energy habitats, as they are not able to obtain high enough swimming speeds to compensate for the high wave-surge water flows. However, no explanation is given for the large absence of the high AR wrasses in the low wave energy habitats. It could be that the high fin AR poses a trade-off where the ability to sustain high swimming speeds is associated with a higher cost of transport at lower speeds. This trade-off seems to be the case in the study by Fulton et al. (2013) where wrasses with low AR fins have a lower cost of swimming at lower speeds, whereas at higher swimming speeds the high AR wrasse has a lower cost of swimming and they can also reach much higher swimming speeds. Wrasses with high AR fins would therefore rather move to a high wave energy habitat where there is less competition and an open niche to exploit.

Fulton & Bellwood (2005) took it further to research the effect of different levels of wave-surge water flow on the distribution of swimming modes in coral reef fishes. This study was also performed on Lizard Island where they quantified the wave-surge water flow and the distribution of many fish families using multiple swimming modes within four exposure regimes commonly found on coral reefs. This study was the first time quantitative gradients of wave-surge water flow were compared with the distribution of functional swimming modes of reef fishes. The four exposure regimes were categorized to represent a spectrum of exposure to the wind, and thus, the wave exposure: exposed reefs (perpendicular to direct wind influence), oblique reefs (angled to the prevalent wind), lagoonal reefs (sheltered to some extent by the exposed reefs), and sheltered reefs (sheltered by the island with no direct wind influence). In each of these regimes, the water motion was quantified, and the flow velocities analyzed, which found that a range of mean velocities between 7.4 to 43.2 cm s^{-1} with wave frequencies between 0.03 to 0.33 Hz. Furthermore, the exposed and oblique reefs experienced much higher temporal variation than lagoonal and sheltered, suggesting that reef fishes in lagoonal and sheltered reefs inhabit a more stable environment. To determine the distribution of coral reef fishes, they categorized each fish to the species level and made detail observations of their swimming modes. They ultimately observed 156 species from seven different families. They then divided them into three groups: pectoral fin swimmers, pectoral-caudal fin swimmers, and BCF swimmers. They found that pectoral fin swimmers were the most prevalent in all exposure regimes. Furthermore, they found that as velocity increased the proportion of species using pectoral fin swimming increased while the proportion of pectoral-caudal and BCF swimmers decreased. This pattern suggests that wave-surge water flow has a large influence on the functional structure of reef fish assemblages. Given that wave-surge habitats have a high rate of flow direction change and pectoral fin swimming was the most prominent in these habitats, a high level of maneuverability should provide a significant advantage in wave-surge exposed habitats.

Swimming performance in unsteady water flows

The majority of the literature that examines the swimming performance and energetics of fish in laboratory conditions use unidirectional linear water flows (Fulton et al. 2013, Korsmeyer et al. 2002). In wild conditions, however, there is often a bidirectional oscillatory water flow that forces the fish to constantly turn and accelerate at varying frequencies and speeds. While some species have been shown to both have a higher swimming efficiency and dominate in the areas of high wave energy, it is unclear how the energy expenditures differ from bidirectional oscillatory wave motion to unidirectional linear flow. However, a few studies have made efforts to fill this knowledge gap.

A recent study by Roche et al. (2014) examined the effect of unsteady flows on the swimming energetics of a labriform swimmer, *Cymatogaster aggregata*. They used intermittent-flow respirometry to measure the critical swimming speed (U_{crit}), oxygen consumption rates (M_{O2}), and use of pectoral fins in a unidirectional, unsteady flow compared to a steady flow. The unsteady flow consisted of a low-amplitude (0.5 BL/s) and high-amplitude (1 BL/s) change in flow velocity, in a 5 second period, around a mean flow velocity. They found that fish in low-amplitude flow performed as well as fish in a steady flow. However, they found that the average cost of swimming was 25.3% higher in the high-amplitude unsteady flow than in the steady flow and consequently decreased their U_{crit}. Furthermore, they found that fish that had a higher variation in pectoral fin beat period were better at holding station and used less oxygen. Thus, those fish who were able to better adjust the fin beat frequency to match the varying flow velocities had a lower cost of transport. This suggests that the cost of swimming in labriform fish in unsteady flow is dependent on the individual's ability to adjust their fin beats to the flow environment. However, while the unsteady flow used in this study is in closer proximity to wild conditions than a steady flow, it is still a unidirectional flow. It therefore only incorporates the increasing need for the fish to accelerate and deceleration, but not the need to turn around with the changing flow direction. The cost of swimming in wild conditions would, therefore, be expected to be even more energetically costly.

Another study by Binning et al. (2013) examined the intraspecific phenotypic variation among a damselfish, *Acanthochromis polyacanthus*, that were taken from six different sites, three of which were in sheltered, low wave energy sites and the other three in exposed, high wave energy sites. They found that the intraspecific phenotypic differences within the *A. polyacanthus*, where similar to differences observed at an interspecific level. The *A. polyacanthus* in the exposed sites had a 13.7% higher pectoral fin AR, 36% larger aerobic scope and 33% greater critical swimming speed than the same species in the sheltered sites. This difference suggests that wave-driven action is not only an important factor in structuring fish assemblages but also important in driving phenotypic divergence and speciation across environmental gradients.

A more recent study by Marcoux & Korsmeyer (2019), examined station-holding swimming performance and behavior of several reef fish species in a simulated wave surge water flow. 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 backward instead of turning despite an increase in pectoral fin beat frequency associated with backward swimming. However, due to flow limitations in the experimental setup, the study was only able to simulate wave surge in the lower end of the conditions found naturally in terms of wave amplitudes and water flow velocities, and could not make direct comparisons to steady swimming costs (Fulton & Bellwood 2005). Thus, the extent of the added costs for station-holding is unclear, and what proportion is due to the potential costs of acceleration or turning.

Summary and unresolved Issues

There is a high diversity of coral reef fishes and many different swimming modes are present in wave-surge habitats like coral reefs. Coral reefs, in general, have variable wave exposure levels and even though the swimming modes distributions usually overlap, specific swimming modes yield different advantages that enable fish to dominate or thrive in certain conditions. Field studies have made comprehensive examination of the wave-surge water flows effect on the functional structure of reef fish assemblages (Fulton & Bellwood 2005, Fulton et al. 2001). However, laboratory studies are lacking to support and give a functional explanation for these observations. Several studies have looked at fishes’ energetic cost of transport under linear, steady flows (Fulton et al. 2013, Korsmeyer et al. 2002) and only two newer studies, under unsteady flows (Marcoux & Korsmeyer 2019, Roche et al. 2014). However, further study is needed in flow conditions that more appropriately approximate wild conditions that incorporate both the acceleration and direction change of the flow ,as well a larger range of flow velocities comparable to what has been observed on coral reefs. While high maneuverability has shown to be an important ability to have when inhabiting high exposure wave-swept areas, the energetic cost of maneuvering in bidirectional oscillatory wave motion has yet to be examined. Quantifying the energetic costs of swimming in conditions that are closer to wild conditions will help us understand the functional structure of reef fish assemblages and enable us to make a better prediction about the impacts of environmental changes.

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Chapter 2: Swimming in unsteady water flows: is turning in a changing flow an energetically expensive endeavor for fish?

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 velocities at steady speeds. 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.

Introduction

Shallow-water, wave-swept ecosystems 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 reef fishes, swimming in a wave-swept habitat represents a challenge as they are often highly site-attached in order to maintain specific feeding or refuge sites (Liao, 2007; Heatwole and Fulton, 2013). Station-holding behavior in these dynamic water flows is therefore expected to be energetically expensive (Fulton and Bellwood, 2005; Liao, 2007). In response, 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).

In wave-exposed habitats, station-holding fishes have to continuously accelerate and decelerate to match the changing water velocities, 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). 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 reef fishes has been conducted using constant, steady water flows (Brett, 1964; Brett, 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 velocity changes more typical of wave-swept habitats (i.e. uni- or bidirectional oscillatory water flow) (Roche et al., 2014; Marcoux and Korsmeyer, 2019; Luongo et al., in prep.).

Previous studies have found a strong association between the intensity of wave-exposure and the swimming modes among assemblages of coral reef fishes (Fulton & Bellwood, 2005). Particularly, 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 on coral reefs (~60% of species) and is positively correlated in relative abundance with increasing wave exposure (Fulton & Bellwood, 2005; Fulton et al., 2005). This correlation suggests labriform swimming is an efficient way of swimming and navigating in a complex and dynamic habitat like coral reefs compared to other swimming forms. 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 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 (Roche et al., 2014). When the turning component was added by measuring oxygen consumption in *C. aggregata* swimming in a bidirectional unsteady flow, metabolic rate was 47% higher than in a unidirectional unsteady flow (Luongo et al., in prep.). These results support the hypothesis that it is energetically expensive to swim in a bidirectional 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. It is important to note, however, that *C. aggregata* mostly inhabits calm, shallow marine waters, bays, and estuaries (NatureServe,

2019) where wave surge is not common. It is therefore uncertain if reef fishes that live in more dynamic flow regimes, have similar energetic requirements to swimming in a bidirectional flow.

A recent study by Marcoux & Korsmeyer (2019), examined station-holding swimming performance and behavior of several 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 backwards swimming. This suggests that turning is an expensive behavior. However, due to flow limitations in the experimental setup, the study was only able to simulate wave surge in the lower end of the conditions found naturally in terms of wave amplitudes and water flow velocities, and could not make direct comparisons to steady swimming costs (Fulton and Bellwood, 2005; Marcoux and Korsmeyer, 2019). Thus, it is unclear the extent of the added costs for station-holding, and what proportion is due to the potential costs of acceleration or turning. As metabolic requirements of locomotion and swimming performance are crucial for constructing energy budgets in fishes, understanding how wave action shapes the energetics of coral reef fishes will improve predictions of responses to environmental changes and increase effectiveness of practical applications such as habitat conservation and management (Enders et al., 2003; Fulton and Bellwood, 2005; Liao, 2007; Roche et al., 2014)

To determine the added costs of turning, we produced both unidirectional (see Movie 1) and bidirectional (see Movie 2) oscillatory water flows, to examine the oxygen consumption associated with station-holding in the goldring surgeonfish, *Ctenochaetus strigosus* (Bennet, 1828), a labriform swimmer frequently found on wave-exposed reef habitats (Hobson, 1974). Using a specialized swim flume respirometer, we measured the cost of swimming at steady speeds, and at different intensities of both unidirectional and bidirectional oscillatory flows. We hypothesize that station-holding in a bidirectional wave surge is more energetically expensive than station-holding in a unidirectional wave surge and relative to swimming at the same speeds in a constant linear flow due to added costs of turning and accelerating with flow direction changes.

Materials & Methods

Fish

The goldring surgeonfish *C. strigosus* (n = 10; total length = 8.74 ± 0.49 cm; mass = 15.24 ± 2.57 g; means ± SD) were obtained from a local wholesale aquarium fish supplier, maintained in flow-through seawater tanks (26 - 28°C, Salinity = 30-32 ‰, 12:12 light-dark photoperiod) at the Oceanic Institute of Hawaii Pacific University and fed daily with commercial marine fish pellets and chopped frozen squid. Fish were acclimated in laboratory conditions for at least three days and fasted for 24 hours prior to testing to ensure they were in a post-absorptive state. All fish were handled ethically according to Hawaii Pacific University's Institutional Animal Care and Use Policies.

Experimental setup

Rates of oxygen consumption were measured in a modified 6.7 L 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 (PlasCore Inc., MI, USA) flow-straighteners to promote linear flow. The fish were forced to swim against the current to maintain its 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 their station-holding behavior in response to wave-driven flows in the field. The respirometer was submerged in an external water tank (52.4 L) connected to an aerated sump (52 L) 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 speed, flush cycles, and data collection were regulated by custom software written in NI LabView 2017 (National Instruments, Austin, TX) and via an AD-DA converter (USB-1208, Measurement Computing, Norton, MA) which controlled a motor driving the speed and direction of the propellors (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. Flow velocities were corrected in real-time for solid blocking effects for each fish (Bell and Terhune, 1970; Korsmeyer et al., 2002).

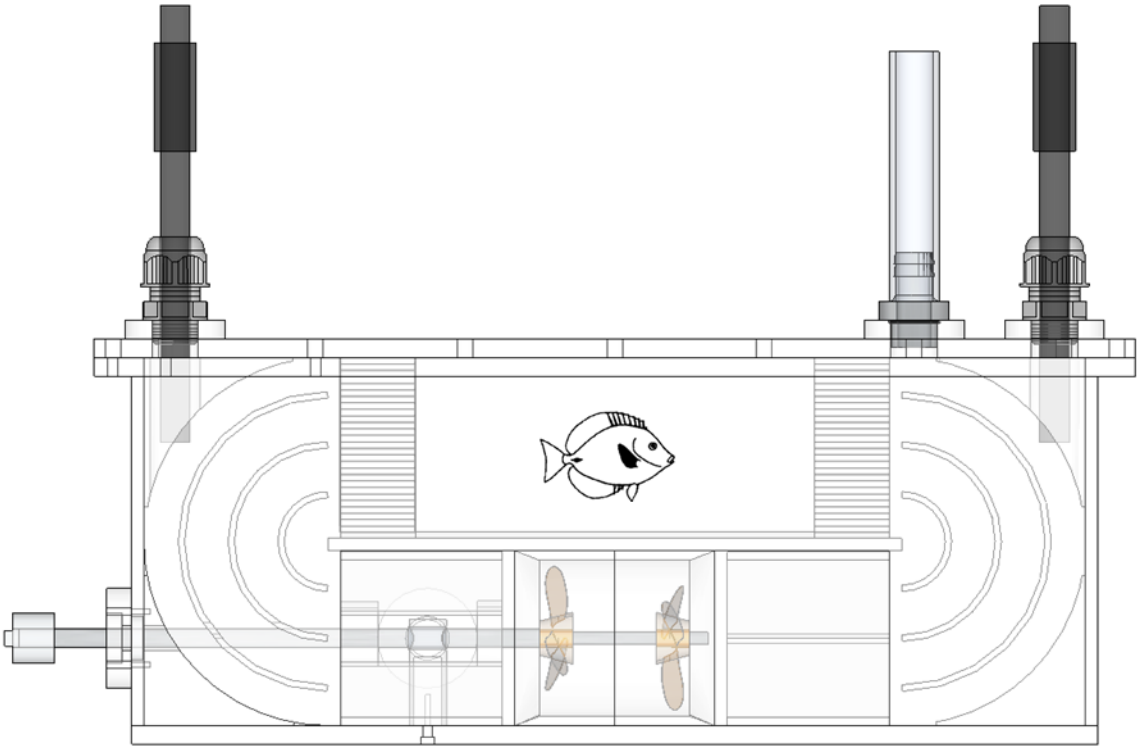

Figure 1: Schematic of the 6.713 L intermittent flow swimming respirometer designed for bidirectional swimming. The fish was kept in the swimming section by two honeycomb flow-straighteners at each end. In the bottom of the tank are two propellers powered by an external motor, able to create water flows in both directions. In the top right and the top left corner, respectively, an optical dissolved oxygen probe and a water conductivity probe with temperature was inserted.

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, USA; 1.005 g cc^{-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 fps, 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 velocities that followed the pattern of a sinusoidal function:

$$U(t) = A \times \sin\left(f \times 2\pi \times t\right) \qquad (1)$$

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

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

The calibration from the steady flow was used set the amplitude, or peak velocities, at each frequency of oscillation, however, a correction was required due to a dampening effect on velocity amplitude in the flume due to inertial effects. Particle tracking tests of actual velocities during the oscillatory flows was used to correct the amplitudes for dampening of the cyclic changes in velocity. With the increased frequency of oscillation, the dampening of the peak velocities 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. Again due to the inertia of the water, the pattern of unidirectional water velocities was different than the intended i.e. identically to the bidirectional water flow but without the water flow changing direction. During the unidirectional oscillatory flows, particle tracking revealed that the velocity did not drop completely to zero, and at the highest frequency peak velocity (U_{peak}) was slightly greater than during bidirectional flows. As a result, the average water flow velocities were higher, but with lower accelerations and wave amplitudes (A), compared with the bidirectional flows (Table 1 & Fig. 2). The unidirectional oscilatory flow, therefore, followed a slightly different function:

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

$$U_{avg} = \frac{2}{\pi} \times A + e \tag{4}$$

$$U_{peak} = A + e \tag{5}$$

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

Table 1. The wave frequency and water flow velocities 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 velocity (BL s^{-1})	Average velocity (BL s^{-1})	Peak velocity (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

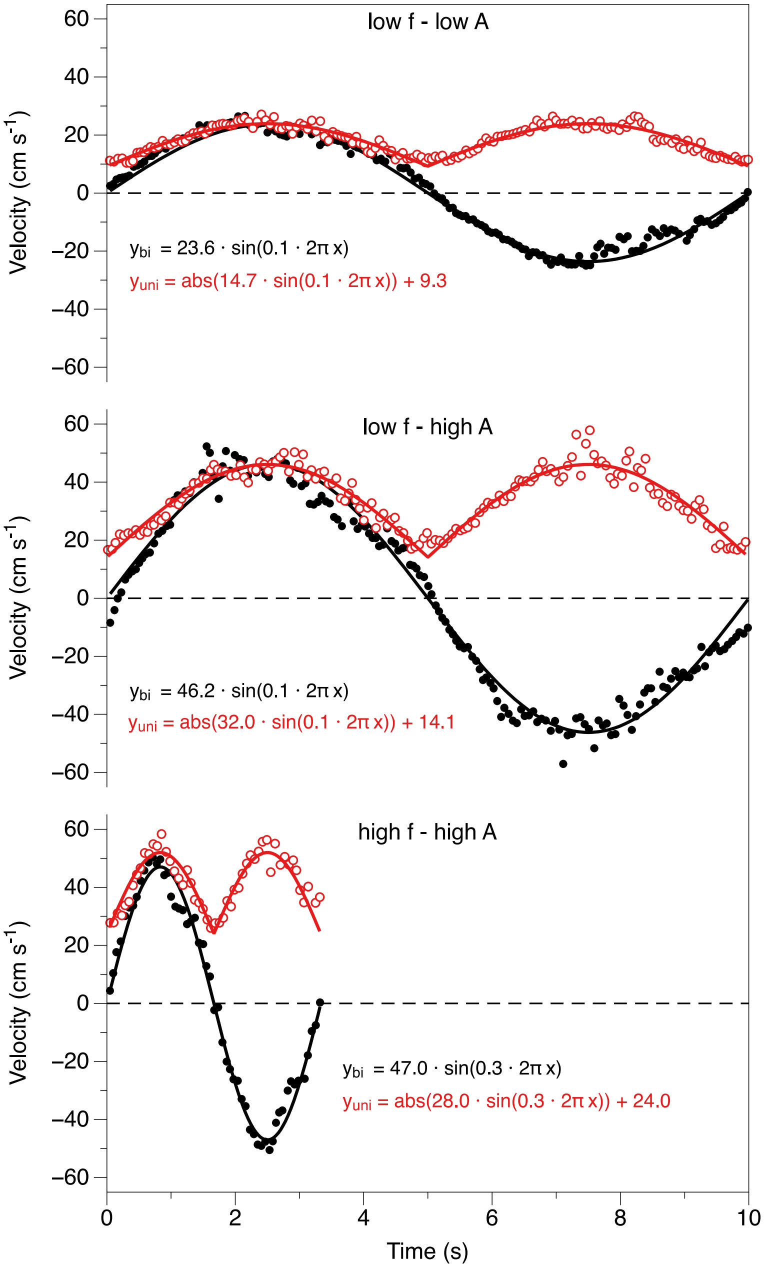

Figure 2: Water flow velocities for the three water flow treatments for both bidirectional (Black, solid circles) and unidirectional (Red, open circles) flow regime. Low and high *f* indicates a frequency of 0.1 and 0.3 Hz, respectively, while low and high *A* indicates the intended peak velocities of approximately 2.36 and 4.71 BL s^{-1}, respectively (see Table 1). Note that for the unidirectional flow, the velocity never reaches zero due to the inertia of the water, and peak velocity is higher at the high frequency oscillation. Lines and equations represent the sine wave functions (see text) for each flow

condition as fit to particle tracking observations of velocity (circles). Each point represents the average from three cycles of oscillation.

Respirometry protocol

Before being placed in the respirometer, the mass, fork length, total length, depth, and width of the fish was measured to allow for solid-blocking correction and calculation of relative velocity (body lengths per second, BL s^{-1}). Each fish was then kept in the respirometer overnight at a low flow velocity (~ 5 cm s^{-1}), as this flow allowed the fish to rest on the bottom while still mixing the water, to determine the standard metabolic rate (SMR, the minimum energy required to maintain homeostasis) before swimming metabolism studies were performed.

The rate of oxygen consumption (MO_2, mg O_2 kg^{-1} h^{-1}) was measured using intermittent-flow respirometry (Steffensen et al., 1984; Svendsen et al., 2016) with 12 minute cycles consisting of three periods: a 4 minute air-equilibrated water flushing period, a 1 minute mixing period with the flush pump off to allow the system to equilibrate, and a 7 minute closed 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:

$$MO_2 = \Delta O_2 V_{resp} M_{fish}^{-1} \tag{6}$$

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. MO_2 measurements were then corrected by subtracting background respiration levels, assuming a linear change in background MO_2 over time.

Standard metabolic rate and net cost of swimming

Standard metabolic rates were calculated from a frequency histogram of MO_2 measurements measured overnight. Two normal curves were fitted to the frequency histogram separating the low MO_2 measurements from the higher MO_2 measurements due to spontaneous activity (Steffensen et al., 1994; Chabot et al., 2016). The SMR was then determined from the mean of the lower distribution of MO_2 measurements for that individual. For all MO_2 measurements during the swimming trials, the net cost of swimming (NCOS) was calculated by subtracting the SMR from MO_2, to represent the increase in metabolic rate due to swimming activity for that individual at each treatment (Korsmeyer et al., 2002).

Steady swimming

After the overnight measurement of SMR, the MO_2 at constant, steady water flows were measured in 1 body lengths per second (BL s^{-1}) increments from 1 to 5 BL s^{-1}. 5 BL s^{-1} was chosen because the intented maximum velocity for the two unsteady water flows was 4.71 BL s^{-1}. Each fish swam for two respirometry cycles (24 minutes) 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 MO_2 as a function of swimming speed was fitted to a hydrodynamics-based power equation (Videler, 1993; Korsmeyer et al., 2002):

$$MO_2 = a + bU^c \tag{7}$$

where *a*, *b* and *c* are constants and *U* is the water flow velocity. This relationship was used to determine the NCOS at steady swimming speeds for each individual. 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.

Oscillatory swimming

The unsteady swimming treatments, designed to simulate a horizontal wave surge of changing water flow velocities, were accomplished by using a computer-generated sine-wave function of a given wave frequency (*f*) and amplitude (*A*) to change the velocity and, for bidirectional flows, the direction of the propeller motor. Immediately after the steady swimming trials and a rest cycle a 0.5 BL s^{1}. Three treatments were performed with both types of water flow regimes (bidirectional and unidirectional oscillatory water flow). The treatments were: a low frequency (*f*) and low amplitude (*A*) oscillation (low *f* – low *A*), a low frequency and high amplitude oscillation (low *f* – high *A*), and high frequency and high amplitude oscillation (high *f* – high *A*) (Table 1.). Both bidirectional and unidirectional conditions were tested at each combination, with the water flow regimes tested in an alternating pattern at each level and 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.

Predicted net cost of swimming

To predict the effect of the non-linear metabolic rate-velocity relationship on costs during oscillatory swimming treatments, the predicted NCOS was calculated by integrating a sinusoidal function based on the variation in water flow velocities during oscillatory swimming (Eq. 1 or 3, Fig. 3) into the equation of oxygen consumption rate (MO_2) as a function of swimming speed (Eq. 4) determined from MO_2 measurements at steady water velocities for each individual fish (Fig. 2) and then subtracting individual SMR (Roche et al. 2014). Thus, the predicted NCOS represents the energetic costs of swimming in oscillating water flow velocities but assumes no additional costs due to acceleration or turning.

Data analysis

Each water flow treatment was categorized by four parameters: oscillation, direction, frequency, and average velocity (Table 1). Oscillation (yes or no) indicated if velocities varied; only steady swimming values were categorized as no oscillation. Direction indicated if 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). Prior to statistical analyses, the NCOS and average velocity were log-transformed to improve normality and linearize the relationship between these two variables (Korsmeyer et al., 2002). Log(velocity) was adjusted to a reference level of 1.5 BL s^{-1} by subtracting log(1.5) from all values. 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 modelled correctly, that the random effects and within residual errors follow a normal distribution and that they had a constant variance. In developing the model, the fit was evaluated using Hurvich and Tsai's Criterion (AICC). As fixed effects, we used oscillation and direction with covariates of frequency and log(velocity), and the interaction between direction and frequency. As random effects, we included intercepts for subjects as well as by-subject random slopes for direction, frequency, and log(velocity), with a covariance structure of variance components. Holm's sequential Bonferroni correction was performed to counteract the problem of multiple comparisons (Holm, 1979). The statistical significance level for this study was $p<0.05$.

Results

During the steady swimming trials, MO_2 and NCOS increased for each fish with increasing velocity (LMM, effect of log(velocity) 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). The steady and predicted NCOS were therefore calculated for each fish at each water flow treatment. Predicted NCOS accounts for the effect of the non-linear relationship between MO_2 and velocity during oscillations in velocity around an average value, and as a result was slightly higher than the steady NCOS at that same average velocity (LMM, Oscillation effect, F(1, 179.5)=16.05, $p<0.001$; Table 2, Figs. 4 & 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 velocity is the same (Fig. 4, low f - high A and high f - high A values).

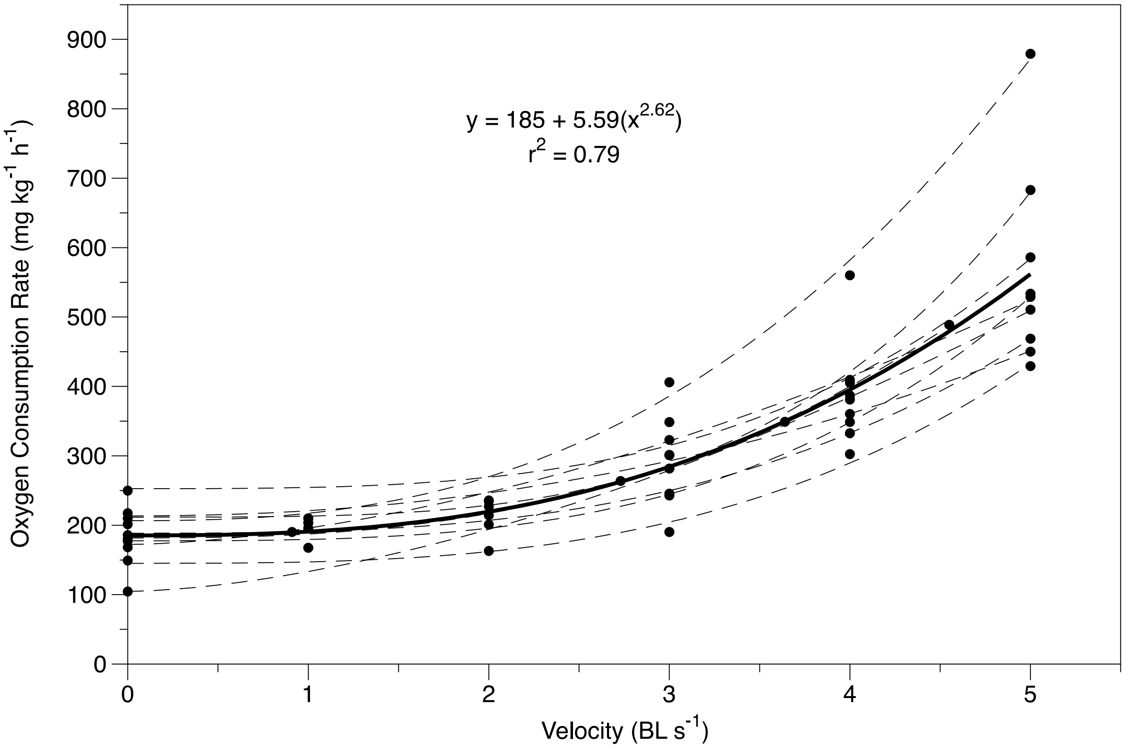

Figure 3: The oxygen consumption rate (*MO_2*), as a function of swimming speed for *Ctenochaetus strigosus* (n=10). The dotted lines are the *MO_2* to velocity (U) relationship (**$y = a+bU^c$**) for each individual fish and the solid line is the combined relationship for all the fish. The power function equation with the r^2 = 0.79 is for the combined *MO_2* to velocity relationship. Each point is the average MO_2 for an individual fish at that velocity.

The measures of NCOS during oscillatory swimming include an effect of wave frequency; however, the strength of this effect varied depending on if 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, SE = 0.149, p=0.83), while frequency had a significant effect during bidirectional oscillatory swimming (Coefficient = 0.436, SE = 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 velocity) (Table 1). The negligible effect of frequency on unidirectional NCOS indicates that the net cost of acceleration was very low; this 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 net 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 velocities 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 effect of velocity (Table 3). These estimates show little effect of acceleration in the differences between the predicted and unidirectional NCOS, but significant differences between uni 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 about 50% of NCOS on average (Table 3). The NCOS attributed to turning at a frequency of 0.1 Hz, increased 5-fold with an increase in average velocity 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 velocity of 3.0 BL s^{-1}) increased the cost of turning by another 50% (Table 3, Fig. 4).

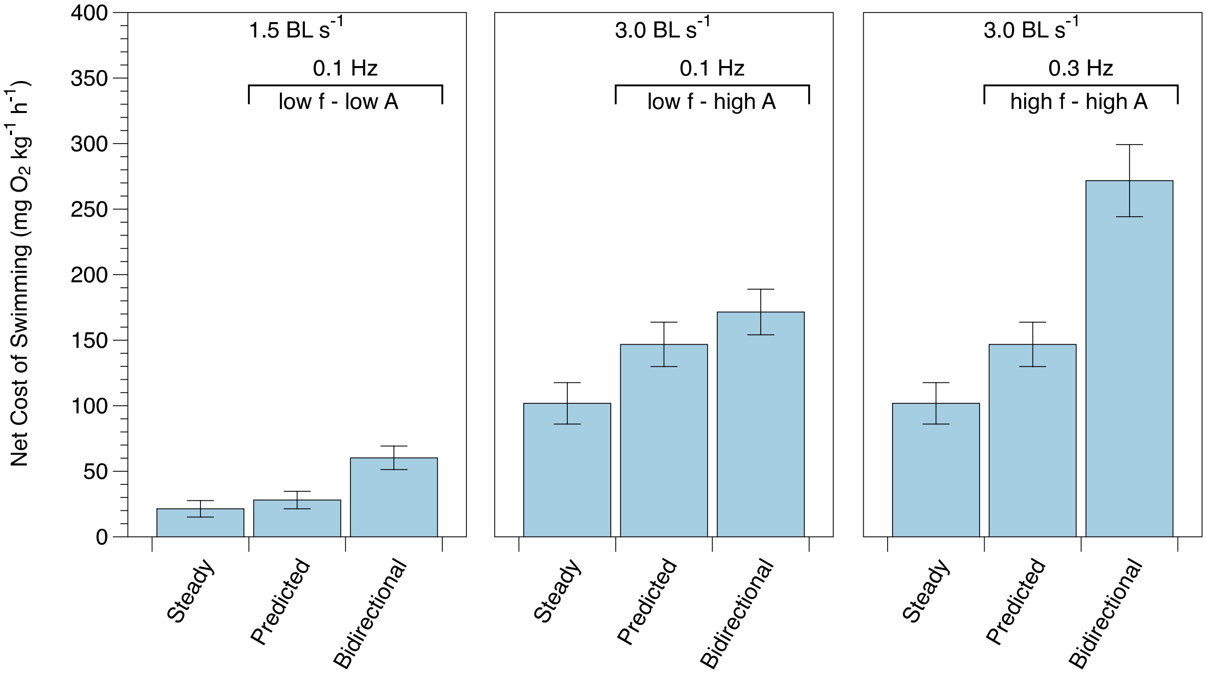

Figure 4: Net cost of swimming (NCOS) in steady and bidirectional oscillatory water flows at two different average swimming speeds (BL s^{-1}) and two different wave frequencies (Hz) for *Ctenochaetus strigosus* (n=10). Each bar is the mean±s.e.m. NCOS for the same 10 fish at a given water flow treatment. The average swimming speeds (1.5 BL s^{-1} and 3.0 BL s^{-1}) are indicated at the top of each box, and the brackets within the boxes indicate the wave frequencies (0.1 Hz 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 bars are measurements of the NCOS at steady speeds and the predicted bars are estimates of the NCOS in oscillatory water flow due 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 bars are the NCOS measured in bidirectional oscillatory water flow.

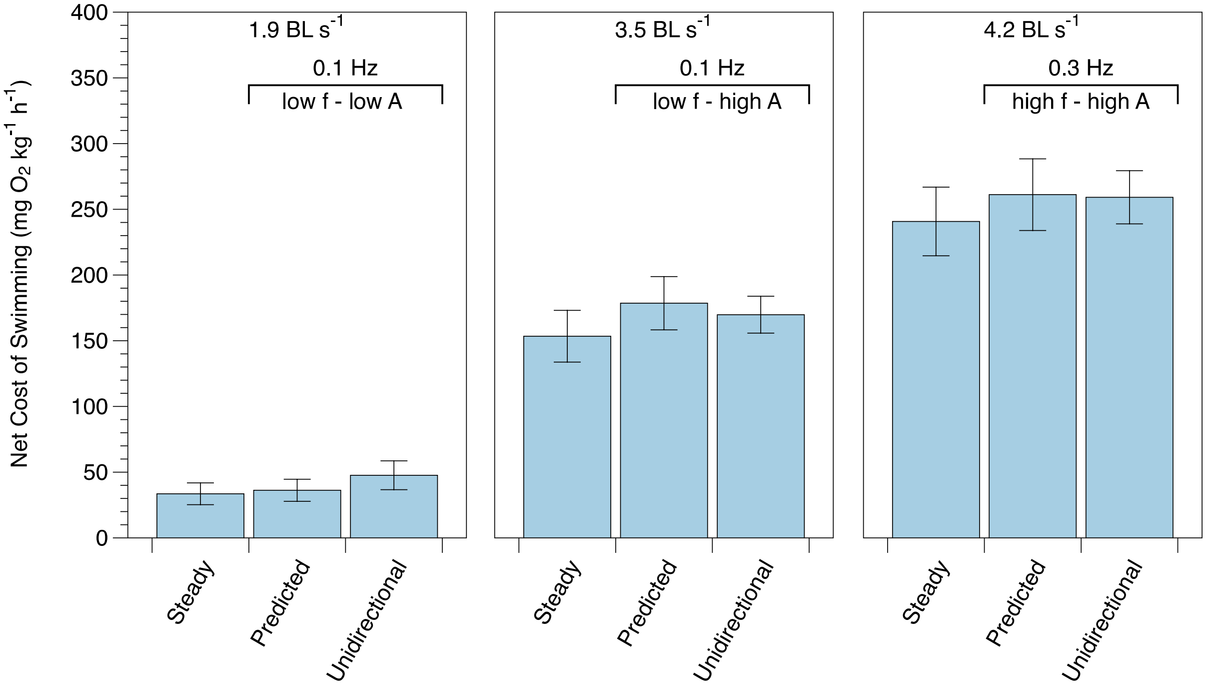

Figure 5: Net cost of swimming (NCOS) in steady and unidirectional oscillatory water flow at three different average swimming speed (BL s^{-1}) and two different wave frequencies (Hz) for *Ctenochaetus strigosus* (n=10). Each bar is the mean±s.e.m. NCOS for the same 10 fish at a given water flow treatment. The average swimming speeds (1.9 BL s^{-1}, 3.5 BL s^{-1} and 4.2 BL s^{-1}) are indicated at the top of each box, and the brackets within the boxes indicate the wave frequencies (0.1 Hz 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 bars are measurements of the NCOS at steady speeds and the predicted bars are estimates of the NCOS in oscillatory water flow due to the non-linear relationship between NCOS and swimming speed. The unidirectional bars are the NCOS measured in unidirectional oscillatory water flow.

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	SE	*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(velocity)	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'. Direction as 'Bidirectional' or 'Unidirectional' (reference level). Frequency in Hz. Log(velocity) is log-transformed average swimming velocity (BL s^{-1}) centered at log(1.5)=0.176.

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

Frequency (Hz)	Average Velocity (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

Discussion

By using a modified swimming respirometer (Fig. 1) we were able to measure net costs of swimming 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 average water flow velocities (15 – 40 cm s^{-1}) 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 velocity 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 & 5, Table 3). Our results show that most of the additional costs of station-holding in wave-surge type flows are due to significant costs of turning to face the flow.

It is possible that the costs of acceleration were underestimated in this 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 velocity profiles (Fig. 2). The unidirectional flows had higher minimum and maximum velocities 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 velocities alone (Fig. 4). Roche et al. (2014) reported a 14% increase in total swimming MO_2 (NCOS + SMR), over predicted values, with unidirectional oscillation flow (1 BL s^{-1} amplitude) for the labriform swimming shiner surfperch, *Cymatogaster aggregata*, which are presumably due to costs of repeated accelerations and decelerations. The goldring surgeonfish used in this study are common in wave-exposed areas of coral and rocky reefs (Hobson, 1974) and 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 a body of water with a propeller as done in this study. Thus, the consistent turning may be a consequence of the higher velocities, 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 suggest that turning is more costly than swimming backwards, at least at the lower velocities used in that study. As average velocity 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 rate of turning (i.e. frequency) by 3-fold did not result in a 3-fold increase in costs of turning, but only ~50% (Table 3). This suggests that there is not a consistent cost per turn, but it varies with velocity 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.

Similar to the present study, Luongo et al. (in prep.) found that for *C. aggregata* swimming MO_2 during station-holding in a bidirectional flow was 53% greater than the predicted MO_2, while little additional cost was found in a unidirectional oscillatory flow. Together, 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 coral reefs 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). Reef fishes that are often highly site attached, 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 to the predicted cost, suggest that mobile organisms living in wave-swept habitats face higher energetic challenges than previously thought (Roche et al., 2014; Marcoux and Korsmeyer, 2019; Luongo et al., in prep.). These additional energetic constraints are likely due to the fishes having to continuously accelerate, decelerate and turn to match the changing flows of the water.

Fish species that have the ability to resist displacement and maintain their position in a wave-dominated habitat like coral reefs could increase their food encounter rate, decrease predation risks and/or protect their territory compared to a conspecific 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. Since *C. strigosus* feed on the substrate, station holding would allow them not to be displaced relative to their food source. Therefore, while they do face higher energetic challenges in such environments, they may then face a higher trade-off 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). 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. On the other hand, 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). *C. strigosus*, used in this 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 like *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 in the changes flow speeds. 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.

This study demonstrates that station-holding in a wave-swept habitat like coral reefs is associated with additional energetic costs, compared to swimming at steady speeds. Furthermore, the turning component of the station-holding behavior appears to be an expensive endeavor, which may have led to the immense diverse adaptations of more maneuverable swimming modes and morphologies present on coral reefs. Reef fishes exposed to bidirectional flow regimes face a constant tradeoff 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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Chapter 3: The effect of swimming mode and body morphology on the energetics of swimming in a wave-surge water flow

Abstract

Coral reef fishes have evolved an immense diversity of body morphologies and swimming modes that are linked to their swimming performance and ability to cope with the unsteady flows of wave-swept habitats. Experimental studies have found that swimming modes and the shape of both fins and body have profound effects on fishes' ability to swim in different flow conditions, while field studies have observed distinct functional groups and distributional patterns that correspond to these morphological differences. In this study, we used a swimming respirometer to create and simulate six levels of wave-surge water flows, within the natural range on coral reefs. We quantified the net cost of swimming (NCOS) associated with station-holding in a steady and wave-surge water flow for four reef fish species: A BCF swimmer, the Hawaiian flagtail, *Kuhlia xenura*, and three pectoral fin swimmers, the Kole tang, *Ctenochaetus strigosus,* the saddle wrasse, *Thalassoma duperrey*, and the Indo-Pacific sergeant major, *Abudefduf vaigiensis*, that have variation in fin and body morphologies. We found that the BCF swimmer, *K. xenura*, had the lowest NCOS during steady swimming but also the highest effect on NCOS with increasing wave frequency in the wave-surge water flow. However, *C. strigosus*, which had the highest pectoral fin aspect ratio (AR), had both a low NCOS in steady swimming as well as in the wave-surge water flow. Lastly, among the pectoral-fin swimmers, the body

fineness ratio of the fishes was the best predictor of NCOS with ratios close to 4.5 resulting in the lowest NCOS in all levels of the wave-surge water flow.

Introduction

Physical environmental factors play an important role in shaping the structure of marine communities, with interactions between the species' physiology and physical attributes often determining distribution patterns (Dunson and Travis, 1991). Studies have linked physical factors, such as temperature, salinity and water motion, to the distribution of species in marine systems, from local to regional biogeographical scales (Bellwood et al., 2002; Fulton and Bellwood, 2005; Perry et al., 2005). In particular, wave activity on coral reefs has been shown to shape small scale community structures and assemblages of reef fishes, as well as interspecific and intraspecific variation in body form and function (Binning et al., 2014; Fulton and Bellwood, 2005). Wave activity is, therefore, thought to be one of the primary drivers of the immense diversification of body and fin forms, and modes of locomotion found on coral reefs (Fulton, 2010).

Reef fishes are often highly site-attached, meaning they depend on specific feeding and/or refuge sites, and must rely on station-holding behaviors to maintain their position on the reefs (Fulton and Bellwood, 2005). Since the majority of coral reefs are subject to unsteady water flows like shallow-water wave surges, station-holding behaviors become a challenging and expensive endeavor as concluded in the previous chapter (Heatwole and Fulton, 2013). As such, station-holding fishes will have to continuously accelerate and decelerate to match the changing water flow velocities, which can increase the amount of energy used relative to maintaining a constant swimming speed (Liao, 2007; Roche et al., 2014). Furthermore, the adjustment of swimming speeds is coupled with direction changes, requiring backward swimming or a whole-body rotation to face the direction of the water flow (Marcoux and Korsmeyer, 2019).

Within these dynamic, unsteady water flows on coral reefs, fishes have evolved a spectrum of different swimming modes and morphologies, and these may be an adaptation to decrease their energy expenditure and improve swimming performance in these environments (Blake, 1983b; Langerhans and Reznick, 2010). The majority of fishes use the body and caudal fin (BCF) for propulsion, which is mostly associated with cruise swimming, ability to achieve high swimming speeds, and pelagic fishes. On coral reefs, however, the majority of fishes use their median and paired fins (MPF) for propulsion, and specifically, pectoral fin swimming is the most predominant with at least 60% of species utilizing this swimming mode (Fulton and Bellwood, 2005). This MPF mode of swimming uses the movement of the paired, pectoral fins to provide propulsion while keeping the bodies rigid and straight (Videler, 1993). This type of swimming is thought to be good for slow swimming speeds, high maneuverability and stability, and therefore an adaptation to complex habitats like coral reefs (Sfakiotakis et al., 1999).

For pectoral fin swimmers, the primary thrust production comes from the pectoral fins, and the shape of the fin can therefore greatly influence their swimming performance. The aspect ratio (AR) of the fins is the most commonly used measurement that describes the ratio between the length of the leading edge and the surface area of the fin (Blake, 2004). Coral reef fishes with wing-like, long tapered fins (high fin AR) produce lift-based thrust in a figure-eight motion, tend to be fast swimmers and more efficient at prolonged swimming at higher speeds; conversely fish with paddle-shaped pectoral fins (low AR fin) produce a resistance-based thrust via a rowing motion that is effective for high thrust production at lower speeds and maneuvering (Fulton, 2007; Walker and Westneat, 2002a).

The opposing force to thrust is drag and in a viscous and dense medium such as water, drag is high and limiting to the swimming performance of fishes (Sfakiotakis et al., 1999). Especially in wave-surge water flows the drag force is changing constantly in magnitude (Liao, 2007). However, the drag forces exerted on the body of the fish is not only dependent on the velocity of swimming but also the shape and size of the fish, with more streamlined bodies decreasing the drag forces (Hoerner, 1965). The body of coral reef fishes ranges from laterally compressed discs to elongated, fusiform hulls. This range of body forms is effectively quantified by the body fineness ratio, which is defined by the measure of how elongated a fish is relative to its transverse sectional diameter (Walker et al., 2013). Elongated fish, such as wrasses, therefore, have a high body fineness. Blake (1983) found that an optimum body fineness ratio exists at 4.5 where the drag forces are at a minimum. Walker et al. (2013) found that MPF swimming fishes with a body fineness ratio close to the 4.5 had an increased endurance-swimming performance. However, it is uncertain how the body fineness relates to the drag forces exerted on the body while the fish is turning in the wave-surge water flow as the drag forces are no longer parallel to the long axis of the fish.

Marcoux & Korsmeyer (2019) examined the swimming performance of several species of reef fish with various swimming modes in a simulated wave-surge flow. In that study the BCF swimmer had a surprisingly low net cost of swimming compared to the MPF swimmers, however, the BCF swimmer had a 24 % increase in costs with a 50% increase in wave frequency whereas two MPF species showed little to no change. This result indicates a higher cost to changing direction (turning) in wave-surge flows for the BCF swimmer. Due to limitations in their experimental setup, they were only able to create a simulated wave surge velocities in the lower range of what has been observed on coral reefs (Fulton and Bellwood, 2005). Fulton & Bellwood (2005) measured the water flow conditions and quantified the wave-surge flow across four exposure regimes in both calm and stormy conditions and found the flow direction change to range between 0.03 to 0.33 Hz and average velocities between 7.4 to 43.2 cm s^{-1}. The previous chapter was able to measure the swimming performance of *Ctenochaetus strigosus* in more intense simulated wave-surge, that better represents the full spectrum of wave-surge flows on coral reefs according to Fulton & Bellwood (2005). In that study, we found that the net cost of swimming in a simulated wave-surge water flow increased by up to 115 % compared to swimming at constant steady speeds.

In present study we simulated a bidirectional, oscillatory water flows (wave surge water flows) that were within the range of natural conditions on coral reefs and examined how the swimming modes, as well as morphological attributes of body fineness and fin aspect ratio influenced the energetic swimming performance under both constant, steady flow and six levels of simulated wave-surge flows. We hypothesize that while the BCF swimmer will be the most efficient in the steady water flows it will have the greatest increase in the net cost of swimming with wave frequencies due to the cost of turning and maneuvering. Furthermore, among the MPF swimmers, a higher AR pectoral fin should result in higher efficiencies at steady water flows, especially at the higher velocities, since high AR pectoral fins are associated with efficient high-speed swimming. However, since low AR pectoral fins are thought to be better suited for high thrust production at lower speeds and maneuvering, they might have lower energetic costs in the simulated wave surge flows. It is also likely that the efficiency of each species relative to each other depends on the velocity and frequency of the wave-surge flow, with low AR fish being more efficient at lower velocities and frequencies and vice versa for high AR fish. Lastly, the effect of body fineness will be the most significant factor affecting the cost of swimming, as it is the factor directly related to drag forces (Hoerner, 1965) and as the water flow velocities and direction are constantly changing, it is the force that varies the most. These results could further explain how the swimming techniques and morphologies of coral reef fishes affect their distribution as observed in field and provide evidence to support the notion that the complex, unsteady water flow and structure of coral reefs shaped much of the morphological adaptations in reef teleosts during their evolution (Binning et al., 2014; Fulton, 2007; Fulton and Bellwood, 2002; Fulton et al., 2001; Wainwright et al., 2002).

Methods

Fish

The species of fish were chosen based on their availability, swimming modes, and morphology. The body and caudal fin swimmer were the Hawaiian flagtail, *Kuhlia xenura* (Jordan and Gilbert, 1882) otherwise categorized as a subcarangiform swimmers. The pectoral fin swimmers included the Kole tang, *Ctenochaetus strigosus* (Bennet, 1828), the Saddle wrasse, *Thalassoma duperrey* (Quoy & Gaimard, 1824)*,* and the Indo-Pacific sergeant, *Abudefduf vaigiensis* (Quoy & Gaimard, 1825). All species were found in Hawaiian waters on exposed reefs or in tidepools as juveniles, which made them ideal candidates experiencing the same environmental conditions. Individuals were either collected by hand net, cast net, caught by hook and line or purchased through aquarium dealers. The fish were held in flow-through seawater tanks (26 - 28°C, Salinity = 30-32 ‰, 12:12 light-dark photoperiod) at the Oceanic Institute of Hawaii Pacific University and fed daily with commercial marine fish pellets and chopped frozen squid. The metabolic rates of seven fish of each species (32 fish in total) were measured in different velocities of steady flow and different velocities and frequencies of flow direction change in a bidirectional oscillatory water flow, designed to mimic the wave action on coral reefs. All fish were handled ethically according to Hawaii Pacific University's Institutional Animal Care and Use Policies.

Fish morphometrics

To measure the morphometrics of the fishes, including aspect ratio and body fineness ratio, fish were anesthetized using 100 mg L^{-1} MS-222 buffered with an equal mass of SeaChem Marine Buffer in seawater. The mass, total length, body width, and depth were measured. They were then digitally photographed with the pectoral fin spread out on waterproof a graph paper (Binning and Fulton, 2011). The photographs were analyzed in the software ImageJ (http://imagej.nih.gov/ij) to measure the length of the leading edge and the fin surface area. The pectoral fin aspect ratios were calculated by the following formula:

$$AR = \frac{(fin\ leading\ edge)^2}{surface\ area\ of\ fin}$$

The body fineness ratio is a measure of the body length relative to the cross-section diameter and is calculated by the following formula:

$$Body\ fineness\ ratio = \frac{length}{\sqrt{depth * width}}$$

Experimental setup

A swimming respirometer was used to measure the fishes' oxygen consumption rate at the different treatments of steady and simulated wave-surge water flows (Steffensen et al., 1984). The swimming respirometer, made of transparent acrylic, consisted of a 6.713 L rectangular recirculating loop with flow created by two propellers driven by an external motor. The fish was kept in the swim section (20.3 cm long, 8.9 cm wide, 8.9 cm deep) separated at each end from the rest of the rectangular loop by two honeycomb flow-straighteners to promote linear flow. This setup forced the fish to swim against the current and hold its relative position similar to station-holding behavior on coral reefs. This experimental setup was the same as in chapter 2.

Water flow calibration

The water flow was calibrated using DPIV with the software Tracker (version 4.11.0) by Douglas Brown to manually track the particles, identical to the calibration performed in the previous chapter.

Respirometry protocol

An intermittent-flow swimming respirometer was used to provide a measurement of oxygen consumption as an approximation for the fishes' metabolic rates (Nelson, 2016). The measurements of the oxygen consumption were acquired in 12 min cycles. One cycle consisted of three timing periods: a 4 min open flush period where there was a continuous flow of fresh, oxygenated water through the respirometer, followed by a short 1 min closed mixing period to allow the water in the chamber to mix well and the oxygen content to begin to decline linearly before the 7 min closed measurement period. Prior to and after each experiment, three blank cycles with no fish in the respirometer were run to measure the oxygen consumption rates of any microbial activity in the respirometer. MO_2 was then corrected by subtracting the background respiration, assuming a linear relationship over time. Each fish was fasted for at least 36 hours prior to the experiment to avoid any elevation in metabolic rates associated with digestion. The fish was weighed, and the fork length, total length, depth, and width was measured, to allow for solid-blocking correction (Bell and Terhune, 1970) and calculation of relative velocity (body lengths per second, BL s^{-1}). The fish remained in the respirometer overnight with a low flow velocity (~ 5 cm/s) to keep the water circulating. This enabled the determination of the standard metabolic rate (SMR, the minimum energy required to maintain homeostasis) as the fish would be expected to have the least activity during the night, as well as acclimating the fish to the experimental conditions. The next morning the swimming experiment proceeded.

Standard metabolic rate and net cost of swimming

The standard metabolic rate (SMR) was calculated with two normal curves fitted to the frequency histogram of MO_2 measurements measured overnight, to separate the low MO_2 that occur during rest from the higher MO_2 measurements that are due to spontaneous swimming, as was done in the previous chapter (Chabot et al., 2016). The net cost of swimming (NCOS) was calculated by subtracting the SMR from the swimming MO_2 to represent the energetic cost of swimming at a given flow treatment (Korsmeyer et al., 2002).

Steady swimming

Metabolic rates during steady swimming were measured, starting at 1 body lengths/s (BL s^{-1}) and increasing at 1 BL s^{-1} increments, with two cycles run at each velocity, until 5 BL s^{-1} was reached, as in chapter 1. The MO_2 measurements at the five velocities were fitted to a hydrodynamic-based power function (Korsmeyer et al., 2002; Videler, 1993)

$$MO_2 = a + bU^c$$

where a, b and c are constants and U is the water flow velocity. After the steady water flow protocol, the velocity was reduced to 1 BL s^{-1} to allow the fish to rest and ensure that there was no oxygen debt present.

Unsteady swimming

The metabolic rates in the simulated wave-surge flows were measured during six treatments with a combination of two average velocities, 2 and 3 BL s^{-1}, and three frequencies of flow direction changes, 0.1, 0.2 and 0.3 Hz. The experiments were run at 2 BL s^{-1} average velocity at each frequency followed by 3 BL s^{-1} at each frequency, with a rest cycle at 1 BL s^{-1} in between the increase in velocity. This sequence ensured that the fish always experienced an increase in wave surge intensity to prevent high metabolic rates that might have led to an oxygen debt. Each treatment was run with two measurement cycles for a total of 24 min.

Predicted net cost of swimming

An predicted net cost of swimming was calculated by integrating a sinusoidal function based on the variation in water flow velocities used in the experiment (sine waves with amplitude of 3.14 BL

s^{-1} and 4.71 BL s^{-1} for a mean velocity of 2 BL s^{-1} and 3 BL s^{-1}, respectively) into the equation of oxygen consumption as a function of swimming speed derived from steady flows, as was done in chapter 2. The predicted net cost of swimming was calculated from the variation in water flow velocities as in a wave surge flow but does not include any costs of turning or acceleration/deceleration, therefore it was designated as an oscillating flow, but with a frequency of 0 Hz.

Data analysis

The morphological variables of pectoral fin AR and body fineness ratio were compared among species with a one-way ANOVA followed by a post-hoc comparison and a Holm's sequential Bonferroni correction to counteract the problem of multiple comparisons (Holm, 1979).

Each water flow treatment was categorized by three parameters: oscillation, average velocity, and frequency. Oscillation was a categorical parameter (yes or no) and indicated if the velocities varied; only the steady swimming treatment was categorized as no oscillations. The predicted NCOS was assigned 'yes' for oscillation because it included the effect of variations in velocity, and a frequency of zero because it does not include any costs of acceleration or turning. The NCOS and average velocity were log-transformed to ensure normality and linearize the relationship between the parameters (Korsmeyer et al., 2002). Log(velocity) was subsequently adjusted to a reference level of 2.0 BL s^{-1} by subtracting log(2) from all values. IBS SPSS Statistics (v.26) was then used to perform a linear mixed model (LMM) to analyze the relationship between log(NCOS), species and treatment parameters. This model allowed us to account for the repeated-measures design (West et al., 2014). To evaluate and compare the fit of our model as we built it, we used the Hurvich and Tsai's Criterion (AICC). As fixed effects, we used species and oscillation with covariates of frequency and log(velocity), and the interactions between species, frequency, and log(velocity). Interactions, where the overall, main effects were not significant, were removed from the model. As random effects, we included intercepts for subjects as well as by-subject random slopes for frequency, and log(velocity), with a covariance structure of variance components. Holm's sequential Bonferroni correction was performed to counteract the problem of multiple comparisons (Holm, 1979). The statistical significance level for this study was $p<0.05$.

Results

The three MPF, pectoral fin swimmers have different combinations of morphometrics that allow for comparisons between the effect of pectoral fin AR and body fineness. There was a significant difference in the pectoral fin AR between the species (ANOVA, $F(3,24)=7.7$, $p<0.001$) and more interestingly, *C. strigosus* had a higher pectoral fin AR compared to *A. vaigiensis*; however, they had a similar low body fineness (Table 1). Furthermore, there was also a significant difference between the fishes' body fineness (ANOVA, $F(3,24)=84.4$, $p<0.001$) where *T. duperrey* has a higher body fineness than *A. vaigiensis* but they both have a very similar pectoral fin aspect ratio (Table 1).

Table 1. Primary swimming mode, sample size (N), pectoral fin aspect ratio (AR), body fineness ratio, mass and standard metabolic rate (SMR) for each species.

Species	Swimming mode	N	Pectoral fin AR	Body fineness	Mass (g)	SMR (mg O_2 kg^{-1} h^{-1})
K. xenura	Caudal	7	1.70 ± 0.23^A	5.59 ± 0.22^A	12.7 ± 2.2	166 ± 34
C. strigosus	Pectoral	7	1.63 ± 0.12^A	4.34 ± 0.22^B	16.7 ± 3.3	194 ± 34
T. duperrey	Pectoral	7	1.35 ± 0.16^B	6.43 ± 0.37^C	18.1 ± 3.8	131 ± 39
A. vaigiensis	Pectoral	7	1.32 ± 0.16^B	4.07 ± 0.33^B	16.1 ± 5.6	235 ± 42

All measurements are reported as mean ±S. D. Dissimilar letter indicates significant difference between species with a *p*-value less than 0.05.

A review of the videos taken for each fish at each measurement cycle of each treatment revealed that during the simulated wave surge flow, all the fishes performed a station-holding behavior by turning with the direction changes of the flow with no backward swimming. During the steady swimming trials, *T. duperrey* was the only fish that was reluctant to swim adequately at the lower speeds (1 - 3 BL s^{-1}), which is why there are more measurements at higher water flow velocities (Fig. 1 C). However, this was only evident for *T. duperrey* and only at the steady swimming trials.

MO_2 measurements increased with velocity for each fish, however, the measurements and the rate of increase differed slightly between the species (Fig. 1). *Kuhlia xenura* had the lowest increase in MO_2 during the steady swimming, most noticeably at the higher velocities, however *C. strigosus* had only slightly higher MO_2 measurements. Both species had similar SMR. *Abudefduf vaigiensis* had the highest SMR and higher MO_2 measurements at all speeds than *K. xenura* and *C. strigosus* during steady swimming. Lastly, *T. duperrey* had the lowest SMR but the highest rate of increase and MO_2 measurements at the highest steady swimming speeds.

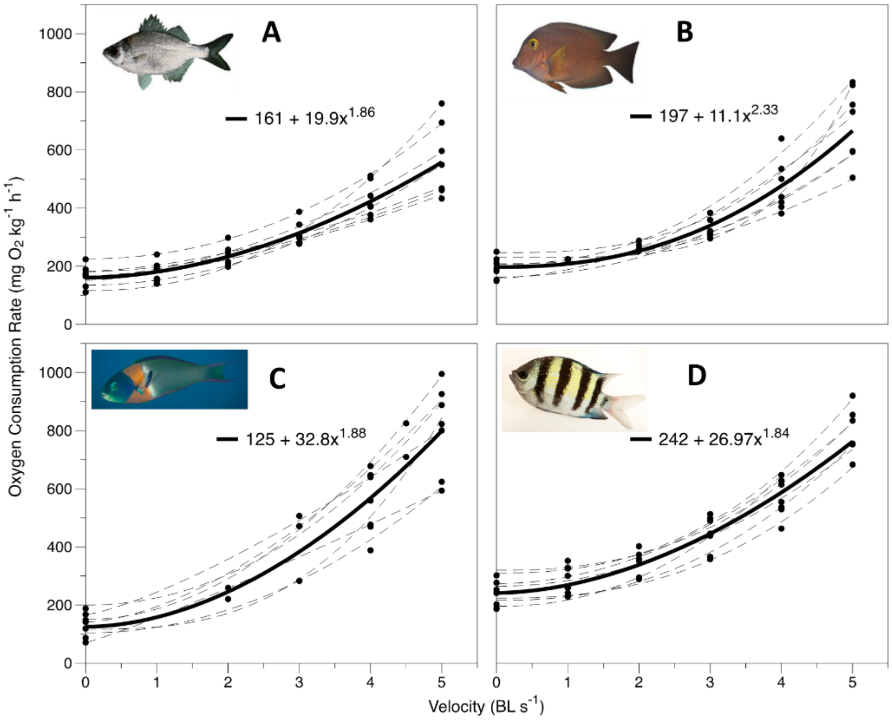

Figure 1: Oxygen consumption rate (MO_2; mg O_2 kg^{-1} h^{1}) with increasing steady water flow velocities (BL s^{-1}) for (A) *K. xenura,* (B) *C. strigosus,* (C) *T. duperrey* and (D) *A. vaigiensis*. The dotted lines are the MO_2 to velocity relationship (**$y = a+bU^c$**) for each individual fish (N = 7 for each species) and the solid lines are the combined relationship for all the fish of that particular species. Each point is the average MO_2 for an individual fish at that velocity. Points at 0 BL s^{-1} are the SMR calculated with two normal curves fitted to the frequency histogram of MO_2 measurements measured overnight.

The overall net cost of swimming (NCOS), calculated by subtracting the SMR from the total MO_2, varied with species (Fig. 2; LMM, species effect on log(NCOS), F(3,25.1)=17.7, $p<0.001$). Comparing NCOS during steady swimming, *K. xenura*, the BCF swimmer, and *C. strigosus*, a pectoral fin swimmer, had the lowest values and were similar to each other (Fig. 2). The next highest NCOS was for *A. vaigiensis* and the highest overall was for *T. duperrey*, with a NCOS more than double that of the two species with the lowest NCOS (Table 2, Fig. 2). Predicted NCOS during oscillatory swimming was also higher than steady swimming at the same average velocity (LMM, oscillation effect, F(1,338), $p<0.001$; Fig. 2). The strength of the effect of both the flow velocity and frequency on NCOS varied with species (LMM, species × log(velocity) interaction, F(3,23.9), $p=0.021$; species × frequency interaction, F(3,24.3), $p=0.016$) and there was a significant interaction between them (LMM, log(velocity) × frequency interaction, F(1,339)=26.2, $p<0.001$). Both frequency and velocity had significant effects on the NCOS of all the species (Table 2 & 3).

The effect of velocity was lowest in *T. duperrey* which increased 99.6% from 2 to 3 BL s^{-1}, while the effect was highest in *C. strigosus* with an increase of 131.6% (Table 3). The effect of frequency on NCOS was, on average, lower for all the pectoral fin swimmers than the BCF swimmer (Table 3). The effect of frequency was, therefore, highest in *K. xenura*, the BCF swimmer, that had a 264% increase in NCOS from 0 Hz to 0.3 Hz at 2 BL s^{s} (Fig. 2 A & Table 3). Conversely, the frequency effect was lowest for *A. vaigiensis* that only had a 56% increase between the same treatments (Fig. 2 D & Table 3). Both *T. duperrey* and *C. strigosus* had an intermediate effect of frequency and were not significantly different from any other species, with a 156% and 101% increase, respectively (Fig. 2 B, C & Table 3).

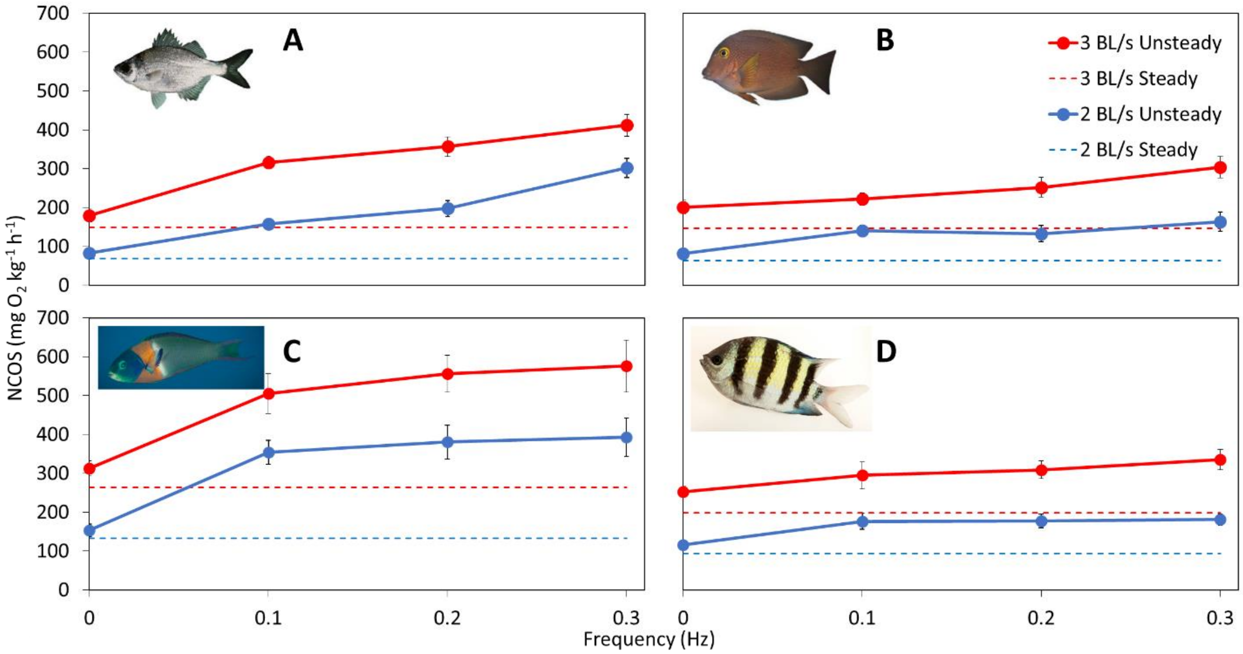

Figure 2: NCOS (mean±s.e.m**) with increasing frequency of simulated wave-surge water flow (unsteady) at two average swimming speeds (2 and 3 BL s^{-1}) for (A) *K. xenura,* (B) *C. strigosus,* (C) *T. duperrey* and (D) *A. vaigiensis.*** The dotted lines are the NCOS at steady swimming speeds, and values at frequency 0 are predicted unsteady NCOS calculated by integrating a sinusoidal velocity function into the equation relating oxygen consumption as a function of steady swimming speeds. N = 7 for each species.

Table 2. Estimates of fixed effects from a linear mixed model of log(NCOS) for all four species, *K. xenura*, *C. strigosus, T. duperrey,* and *A. vaigiensis*, with subject as a random effect.

Parameter	Coefficient	SE	*P*-value
Intercept	1.83	0.0396	<0.001
C. strigosus	-0.0394	0.0541	0.474
T. duperrey	0.318	0.0541	<0.001
A. vaigiensis	0.123	0.0541	0.032
Oscillation	0.167	0.0134	<0.001
Log(velocity)	1.79	0.126	<0.001
Frequency	1.43	0.188	<0.001
Log(velocity) X Frequency	-2.02	0.395	<0.001
C. strigosus X log(velocity)	0.169	0.158	0.294
T. duperrey X log(velocity)	-0.340	0.158	0.041
A. vaigiensis X log(velocity)	0.0460	0.158	0.773
C. strigosus X Frequency	-0.614	0.258	0.026
T. duperrey X Frequency	-0.373	0.258	0.162
A. vaigiensis X Frequency	-0.881	0.258	0.002

The coefficients for *K. xenura* are set to zero as it is used as the reference species. Log(NCOS) is log-transformed net cost of swimming (mg O_2 kg^{-1} h^{-1}). Frequency in Hz. Log(velocity) is log-transformed average swimming velocity (BL s^{-1}) centered at log(2)=0.301. Oscillation as 'yes'(=1) or 'no' (=0).

Table 3. Coefficients [95% confidence interval] and p-values for the effect of frequency and log(velocity) for each species from the linear mixed model of log(NCOS) (Table 2), as well as pairwise comparisons of the effects among species.

	Frequency Coefficient		Log(Velocity) Coefficient	
Species	Estimate	*P*-value	Estimate	*P*-value
K. xenura	1.43 [1.05, 1.82]^{A}	<0.001	1.79 [1.54, 2.05]^{AB}	<0.001
C. strigosus	0.821 [0.438, 1.20]^{AB}	<0.001	1.95 [1.71, 2.22]^{A}	<0.001
T. duperrey	1.06 [0.679, 1.45]^{AB}	<0.001	1.45 [1.20, 1.71]^{B}	<0.001
A. vaigiensis	0.553 [0.170, 0.936]^{B}	0.006	1.84 [1.59, 2.09]^{AB}	<0.001

Dissimilar letter indicates significant difference between species with a *p*-value less than 0.05. Frequency coefficients calculated at the reference velocity of 2 BL s^{-1}, and Log(velocity) coefficients at a frequency of 0.0 Hz.

Discussion

By including several species with different swimming modes and morphological features we are now able to further expand our understanding of how wave action on shallow-water coral reefs can shape the immense diversity in fish assemblages and morphological adaptations. Consistent with the conclusion of Chapter 2, it was evident that swimming and station-holding in a wave-surge water flow was an energetically expensive endeavor compared to swimming in steady water flows. All four species showed an increase from the predicted NCOS (0 Hz) to the simulated wave-surge flows (0.1 – 0.3 Hz) at each velocity (2 BL s^{-s} and 3 BL s^{-s}). However, more interesting were the noticeably lower energetic costs of swimming in these wave-surge flows for *K. xenura* and *C. strigosus*, the differences between the two swimming modes, as well as the differences among the three MPF swimmers. The interpretation of these differences indicates that their morphologies play an important role in their habitat selection and niche utilization in relation to wave action. While this has been studied extensively in the field and the laboratory, this study was the first to compare the energetics of species with different swimming modes and morphologies in both a steady and simulated wave-surge flow (Bellwood et al., 2002; Depczynski and Bellwood, 2005; Fulton et al., 2005; Fulton et al., 2013a; Fulton, 2010; Korsmeyer et al., 2002; Marcoux and Korsmeyer, 2019).

Is has been a long-standing hypothesis that BCF swimmers are generally considered specialists for steady, high-speed cruise swimming and that MPF swimmers are better at maneuverability and stability at low speeds and, therefore, more efficient at swimming through structurally complex habitats and station-holding in these dynamic, shallow water environments (Blake, 2004; Fish, 2010; Webb, 1984). However, our study suggests that this generalization is more complex. During steady swimming, the lowest MO_2 measurements and NCOS were found for both the BCF swimmer, *K. xenura*, and the pectoral fin swimmer, *C. strigosus* (Figs. 1 & 2). While this was expected for the BCF swimmer, which is thought to be specialized to such conditions, it was less so for the pectoral fin swimmer. *Ctenochaetus strigosus* possess high AR pectoral fins (Table 1; 1.63), which have been found to be associated with efficient high-speed swimming compared to low AR fins (Fulton et al., 2013b). The range of pectoral fin AR's in fishes generally ranges between 0.7 to as high as 2.2 (Binning and Fulton, 2011; Thorsen and Westneat, 2005). The swimming performance of pectoral fin swimmers has shown a strong correlation between their fin AR and sustained swimming speeds (Wainwright et al., 2002). Field observations have found that species with high AR pectoral fins were more abundant in wave-swept habitats while species with low AR pectoral fins were rare or completely absent in these habitats (Bellwood and Wainwright, 2001; Fulton et al., 2001). It is reasonable to assume that the presence of species with high AR pectoral fins in wave-swept habitats is not only due to their ability to achieve and sustain faster swimming speeds but also because they can do it more efficiently. The high AR pectoral fins for *C. strigosus* could, therefore, explain the low NCOS compared to the two other pectoral fin swimmers, at both the steady swimming speeds as well as in the simulated wave surge flows.

The simulated wave surge can be described as a bidirectional, oscillatory water flow where the velocity and direction of the flow follows the pattern of a sinusoidal function. To station-hold in such a flow, the fishes have to continuously accelerate and decelerate to match the changing water velocities coupled with a whole-body rotation to face the flow. Swimming in such a flow was associated with a higher energetic cost. The cost of turning during the station-holding behavior was found in Chapter 2 to be the most energetically expensive component of this behavior. In the simulated wave-surge flow in this study the NCOS of the BCF swimmer increased the most with frequency compared with the pectoral fin swimmers on average (Table 3: Frequency coefficient = 1.43 compared to 0.811, respectively). The frequency coefficient represents the rate of increase of NCOS with increasing frequency in the wave-surge flow, or the additional costs as turning frequency increases. This result is consistent with that of Marcoux & Korsmeyer (2019), where it was found that the effect of frequency on NCOS was greater for *K. xenura* than *C. strigosus*, supporting the hypothesis that BCF swimmers are less efficient at maneuvering and swimming in a wave-swept habitat. In the present study, *K. xenura* had more than a 2.5-fold increase in NCOS as the frequency of the wave-surge flow increased from 0 to 0.3 Hz, whereas the highest relative increase for the pectoral fin swimmers was 1.5 for *T. duperrey*, and as low as 0.5 for *A. vaigiensis* (Fig. 2). Even though *T. duperrey* had an overall higher NCOS at all the flow treatments than the BCF swimmer, the relative increase in NCOS during increases in frequency of the wave surge flow was lower.

The pectoral fin morphology (i.e., aspect ratio, AR) of labriform swimmers has proved to be a powerful predictor of habitat use with high AR fins associated with the most wave-swept habitats, of coral reefs (Fulton et al., 2005). Similar to the comparisons of BCF with MPF swimming, a tradeoff is suggested to exist between pectoral fin AR and swimming performance, with low AR fins expected to enable a high degree of maneuverability, in contrast to high AR fin permitting efficient high sustaining swimming speeds (Drucker and Lauder, 2000; Wainwright et al., 2002). The differences in pectoral fin AR of *C. strigosus* (high) and *A. vaigiensis* (low), combined with their very similar body fineness ratios (Table 1), allows for a comparison of how fin AR affects swimming performance in the different flows.

Ctenochaetus strigosus, with the high AR fins, matched our predictions by having a lower overall NCOS at the steady swimming speeds and during the simulated wave-surge flow. However, *A. vaigiensis* showed a lower relative increase in costs as turning frequency increased (Fig. 1, Table 3). This difference, although small, is consistent with an increased maneuverability and stability with low AR pectoral fins, possibly allowing these fishes to navigate wave surge and structurally complex habitats. However, this is inconsistent with what field studies suggest, where low AR pectoral fin swimmers are found less frequently in high energy, wave-swept habitats (Bellwood and Wainwright, 2001; Fulton et al., 2001). While lower AR fins might increase effectiveness and ability to maneuver in such environments the higher AR fins might give fish better efficiency. However, these differences were subtle and not statistically significant. The entire range of fin ARs in pectoral fin swimmers is greater than the differences between the species used in this study (Walker and Westneat, 2002b), and comparisons between species with a wider range in pectoral fin AR may be needed to reveal a clear difference in energetic costs.

For a fish moving through a dense and viscous medium such as water, the shape of the body greatly affects the drag forces imposed on them, which in turn should affect the efficiency of their swimming performance (Blake, 2004). While steady, prolonged swimming is theoretically dealing with a constant drag force at a given speed, it is much more complex in an unsteady wave surge flow as the drag force is constantly changing with the flow speed and the orientation of the fish during turns. While a high body fineness is thought to increase the maximum prolonged swimming speeds and the efficiency of such (Walker et al., 2013), it is uncertain how it affects the ability to efficiently turn and maneuver. By comparing *T. duperrey* with a high body fineness and *A. vaigiensis* with a low body fineness but both with very similar fin AR (Table 1), it is evident that NCOS is much higher for *T. duperrey* at all the flow treatments (Fig. 1). The relatively deeper and shorter body of *A. vaigiensis* might allow the fish to better utilize the drag forces to its benefit during turning. During a turn, the caudal fin is an important part of the maneuver (Weihs, 1972) and even the MPF swimmers utilize body and caudal fin propulsion during a turn to turn faster (Liu and Hu, 2005). The videos from this study showed that when the fish turns it performed a lateral bend in the body with extended pelvic fins, likely to take advantage of the drag to aid the turn. In *A. vaigiensis,* which has a greater surface area and pelvic fins closer to the center of mass, it would also have greater drag forces closer to the center of mass as the body rotates perpendicular to the axis of deceleration. Increased drag forces closer to the center of mass might assist in rotating the body during the turn, allowing the fish to utilize fewer muscle fibers in the 180 degrees turn as well as decreasing the turning rate and radius, ultimately decreasing the cost of turning and thereby increasing the efficiency of maneuvering and station-holding in an unsteady wave surge flow. However, it was expected that the high body fineness of *T. duperrey* should enable it to have a lower NCOS at the steady swimming speeds as fish with high body fineness have been found to better obtain and sustain high swimming speeds compared to low body fineness fish, allowing them to inhabit and exploit reefs and reef zones with high water flows (Walker et al., 2013). The elevated NCOS could be because the *T. duperrey* is more efficient at higher speeds than used in this study. There may be a tradeoff between energy consumption and unique niche utilization. While *T. duperrey* has a high overall NCOS, it might be able to subsidize these energetic costs by exploiting high-energy habitats. Furthermore, the slim and long body could allow *T. duperrey* to better utilize and navigate in and through the complex coral reef habitat, giving further advantage in its niche utilization. A recent study found that specifically the genus *Thalassoma*, had achieved global ecological success due to their ability to achieve and maintain high speed swimming combined with trophic versatility (maximization of exploitation of rich resources) in coral reefs habitats around the world with high wave-surge flows (Fulton et al., 2017).

Traditional drag-based models have found that the optimum body fineness ratio for efficient swimming is 4.5 (Blake, 1983a). The body fineness of both *A. vaigiensis* and *C. strigosus*, the two most efficient swimmers in the unsteady water flow, is very close to this optimum, which supports the hypothesis that body fineness is important for efficient navigation and station-holding in shallow water habitats dominated by wave surge flows (Table 1). Lastly, if body fineness is the only factor examined, the two species that are closest to the 4.5 optima have the lowest overall NCOS in unsteady flows, and as the body fineness increases for *K. xenura* and lastly for *T. duperrey* so does the NCOS at all the simulated wave surge flows, regardless of swimming mode and fin aspect ratio.

This study further demonstrates that station-holding in a wave swept habitat like coral reefs is an expensive endeavor and that the swimming mode as well as the morphological attributes, body fineness and fin aspect ratio, greatly influences these energetic costs. The general hypothesis that BCF swimmers are efficient steady swimmers and MPF swimmers are efficient at maneuvering were shown to be much more complex. The BCF swimmer, *K. xenura* did have one of the lowest NCOS at steady swimming but it was very similar and not statistically different from the high AR pectoral fin swimmer, *C. strigosus*. However, while *T. duperrey* had an overall higher NCOS than the BCF swimmer, *K. xenura* had the largest relative increases in NCOS with increasing wave surge frequency, supporting the hypothesis to some extent. While the effect of pectoral fin AR was very subtle and insignificant, it was evident that body fineness might play a large role in the efficiency of station-holding in a wave surge flow, as the two pectoral fin swimmers, *C. strigosus* and *A. vaigiensis*, closest to the 4.5 optima had the lowest NCOS increases in the wave-surge flows. These results suggest that reef fishes have evolved a spectrum of different swimming modes as well as morphologies to reduce energy expenditure and improve swimming performance in these environments. However, because the morphological diversification is so immense and morphologies that are inefficient have not been outcompeted, the adaptations that decrease energy expenditure are likely in a constant tradeoff with niche utilization and energy acquisition.

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