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Friday Harbor Laboratories- Fish Swimming course 2017- Final Report
Course instructors: Paolo Domenici, John F. Steffensen, Jacob J Johansen

The effects of experimental diurnal timing on the exercise physiology of juvenile pile perch (*Rhacochilus vacca*)

Shannon McMahon and Adam Downie

Abstract

The circadian rhythms of an animal highlight important activity patterns that an individual exhibits over a 24-hour period, which can vary greatly between species even within the same family. Activity and performance of a species can vary over their circadian cycle, however many studies conduct data collection during times which suit human circadian rhythms and can overlook the study species circadian rhythms. This has the potential to misrepresent the study species performance and could create unreliable results. The pile perch *Rhacochilus vacca* has shown to be crepuscular, having heightened activity during twilight hours, and tending to be also more nocturnally active, with its lowest rest activity during daylight hours. In this study we aimed to test the difference between the performance of pile perch (*Rhacochilus vacca*) during the morning (0600) and evening (1800) using critical swimming speed (UCrit) and aerobic scope. We found that there were no significant differences in the UCrit ($p=0.84$) or aerobic scope ($p=0.94$) between morning or evening. Through this study we have seen that the UCrit and aerobic scope of a nocturnal fish species with crepuscular activity peaks does not change

between morning and evening assays. This indicates that although a species may be tested at markedly different points in its circadian rhythm it will not significantly affect its performance.

Introduction

Circadian rhythm is a common characteristic among living organisms, and can be defined as any form of entrained activity that occurs over a 24-hour cycle (Sanchez-Vazquez et al 1995, Whitmore et al 2000). Species-specific patterns of animal behavior are exhibited during certain times of an animal's 24 hour 'biological clock'. For example, animals such as hyenas are nocturnal foragers (Kolowski et al 2007), whereas elephants forage during the daytime (diurnal foragers; Guy 1976), and wallabies forage during twilight hours (crepuscular foragers; dawn/dusk; Loudon and Curlewis 1987). An important characteristic of a circadian rhythm is that it can be adjusted (entrained) by external stimuli, such as photoperiod and temperature (Sanchez-Vazquez 1995). For this reason, any species may exhibit daily, and/or seasonal variations in behaviour. Specifically, fish have circadian rhythms that revolve primarily around swimming activity, feeding, reproduction, and migration (Hinch and Collins 1991).

Fish behaviour can be impacted by factors including light (surface irradiance mediated by photoperiod), oxygen concentration (Kramer 1987), and prey behaviour (Craig 1977). Specifically, photoperiod has profound impacts on the ability for fish to search for food, avoid predators, and perform vertical and/or horizontal migrations (Denbinski 1971). Thus, photoperiod implies an optimal light intensity for activity, and light values above or below a fish's optimal light intensity inhibits activity (Craig 1977). Therefore, fish behaviourally regulate when they are most active, generally, when they feed, which is mostly specific to certain times within their circadian rhythm (e.g. diurnal, nocturnal, and/or crepuscular foragers; Hobson 1965, Noeske and Spieler 1984, Løkkeborg 1998). Daily and seasonal activity patterns have been

investigated across many fish groups and in many different environments. Keast and Welsh (1968) investigated the feeding habits of freshwater fish in a temperate Canadian lake and found variation among species with respect to when fish were active: yellow perch (*Perca flavescens*) are diurnal forgers, whereas rock bass (*Ambloplites rupestris*) exhibit diurnal and nocturnal foraging habits. In contrast, Hobson (1965) found preference for activity time at the family level on tropical reefs: Labridae, Chaetodontidae, and Pomacentridae showed diurnal activity patterns, Lutjanidae, Apogonidae, and Holocentridae showed nocturnal activity patterns, and Serranidae and Carangidae were active both during the day and night. In addition to daily patterns of activity, many fish species exhibit seasonal variation in activity. Fraser et al (1993) found that Atlantic Salmon (*Salmo salar*) have higher diurnal activity during the spring and autumn, and higher nocturnal foraging during the winter. Pike (*Esox Lucius*) were monitored with ultra-sonic transmitters, and exhibited slower swimming speeds during the winter and maximum activity during the summer (Diana 1980). Studies investigating circadian rhythm in relation to daily and seasonal activity patterns note the importance of the many external factors (e.g. daily/seasonal changes in temperature and light) that impact activity, and to correlate activity with metrics of metabolic performance.

Lowe (2001) has suggested that the metabolic cost of activity is largest and most variable portion of an organism's daily energy budget. The variability in oxygen consumption ($\dot{M}O_2$) among fishes has been examined, investigating the impact of standard dynamic action (SDA) over a circadian cycle (Keast and Welsh 1968), how $\dot{M}O_2$ changes over different habitat structure (Fischer 2000), and seasonal variations in $\dot{M}O_2$ (Dickson and Kramer 1971, Lowe 2002). For example, the $\dot{M}O_2$ of rainbow trout (*Salmo gairdneri*) was found to be lowest during autumn and highest during February and April (Dickson and Kramer 1971). Additionally, Lowe (2002) used

biotelemetry to monitor daily movements of juvenile scalloped hammerhead sharks (*Sphyroa lewini*), and found that $\dot{M}O_2$ is correlated with season: juvenile sharks were more active and consumed more oxygen during the warmer summer months than the cooler winter months. This finding was complimented by increased activity at night, as the sharks move onto the reef to hunt (Lowe 2002). Taken together, there are important impacts regarding the interactive effect of changes in daily and seasonal photoperiod and temperatures have on the behaviour and physiology of fishes. While many studies have investigated patterns in circadian rhythm in-situ (telemetry or direct observations), fewer laboratory studies have taken into consideration the natural/seasonal photoperiod of the study fish species in the experimental design. The role of this study is to investigate whether there exist changes in critical swimming speed (Ucrit) and oxygen consumption ($\dot{M}O_2$) in the morning and evening of a juvenile temperate fish, the pile perch (*Rhacochilus vacca*), which are markedly different points in the species circadian rhythm, being essentially the start (1800) and the end (0600) of their active period.

Methods

I. Animal Husbandry

Juvenile pile perch (*Rhacochilus vacca*) were captured during mid-July 2017 at Jackson Beach (San Juan Islands, Washington State, United States) via beach seining. Fish were held in outdoor flow-through aquaria (salinity: 34ppt, average day-time temperature: 13.6°C, average night-time temperature: 11.9°C) at the University of Washington's Friday Harbor Laboratories. They were exposed to the natural photoperiod of San Juan Islands during the summer months (14 hr light: 10 hr dark). Fish used for critical swimming speed and respirometry were kept in a tank

(196 L; height: 27cm, length: 122cm, width: 59.5cm) that was divided into ten sections to separate each fish for easy identification. Daily, fish were fed API Marine fish flakes, however, individuals were fasted for 24 hours prior to experimentation (Niimi & Beamish 1974). Tanks were cleaned every other day to prevent algal build-up and eliminate food/feces. All experimental protocols were approved by the University of Washington and abided by the institution's standards outline by the University of Washington Animal Care and Use Committee.

II. Experimental Protocol

To evaluate whether there are differences in performance between morning (0600) and evening (1800), critical swimming speed (UCrit), and intermittent flow respirometry were used. Juvenile *R. vacca* (6.63±0.054 cm standard length; 5.6±0.5 g) used for UCrit experiments and respirometry followed a repeated measures design (n=8), to correlate swimming speed with aerobic scope. Therefore, each individual fish performed a morning and evening UCrit test, and aerobic scope was estimated during the morning and evening. Each individual was given a minimum of 24 hours of rest in between experiments, and all experiments were randomized to eliminate any biases, training effects, and to mitigate fatigue/stress.

III. Critical Swimming Speed Experiments

Experimental Flume

A linear swimming flume (length: 241cm, width: 38cm, height: 44cm, volume: 350L) was used for critical swimming speed (UCrit) experiments. The dimensions of the swimming area in the flume were 41cm length, 15cm width, 11.5 cm height of the water. Upstream flow straighteners ensured near-laminar flow in the swimming area. A heat exchanger (1230T VWR Scientific, Shel Lab, Sheldon Manufacturing Inc, Cornelius, Oregon) maintained water temperature at 14±0.03°C. The flume's speed was controlled by regulation the voltage input to the motor using an analog dial and a volt meter (8060 A True R MS Multimeter, Fluke Corporation, Eindhoven, Netherlands). The flume was calibrated, and a flow profile was determined, using a TAD W30 flow meter (Höntzsch, Waiblingen, Germany) to measure the water velocity (cm sec^{-1}). Flows were measured at nine points along the bottom of the swimming area (~2cm off the bottom) and nine points near the surface (~2cm from the surface) at ~ 7 cm sec^{-1} increments, which were logged against the voltage input. Average water velocity (cm sec^{-1}) at each speed increment was plotted against volts to determine velocity increments for the UCrit tests. Water was completely drained from the flume and refilled with fully oxygenated seawater after every second fish to maintain water oxygen levels. All swimming experiments were filmed (frame rate: 25 frames sec^{-1}; resolution: 800 X 600) with a Logitech camera (HD Webcam C270) positioned 35cm away from the front of the tank.

Critical Swimming Speed Protocol

A single fish was placed in the swimming area of the flume, and allowed to acclimate to the experimental conditions/recover from handing stress, at ~0.5 body lengths $second^{-1}$ (BL s^{-1}) for 30 minutes. Thirty minutes was a deemed adequate acclimation time, as no fish showed further signs of stress (e.g. sporadic movements) after this period. Following this acclimation

period, the water velocity was increased by 7 cm sec^{-1} (~1 BL sec^{-1}) every 30 minutes until the fish fatigued, as evident by impingement on the downstream barrier (>5 seconds). Final water velocity (cm sec^{-1}) and time at the final speed (minutes) were recorded. Critical swimming speed (UCrit) was calculated using the following formula:

UCrit (cm sec^{-1}) = Vf + (T/t * dv)

where Vf is the water velocity of last completed speed interval (cm sec^{-1}), T is the time swum at the final water velocity (minutes), t is the time increment (30 minutes) and dv is the speed increment (7 cm sec^{-1}; ~1 BL sec^{-1}) (Brett 1964). Each fish (n=8) was swum starting at 0600 and 1800, in a randomized order, with at least 24 hours of rest in between experiments.

IV. Intermittent Flow Respirometry

Experimental Design

Oxygen consumption ($\dot{M}O_2$; mg O_2 kg^{-1} h^{-1}) was measured using two, 240ml closed loop, recirculating, acrylic intermittent flow respirometers. The respirometers were submerged in a 109L tank (legth: 71cm, width: 45cm, height: 34cm) that was aerated, and kept at 14°C using a series of chilling units (RE106 ecoline, Lauda-Brinkmann, Delran, New Jersey, and a RM20, Lauda-Brinkmann, Delran, New Jersey), and a temperature controller (Willhi, WH1436A Digital Temperature Controller, Shenzen-Bayite Technology Co. Ltd, Shenzen City, China). A black tarp was placed over the tank to mitigate stress from visual stimuli. The change in dissolved oxygen concentration in each respirometer was measured (0.5 Hz) using a four channel Firesting Optical Oxygen Meter (Pyro Science, e.k. Aachen, Germany), connected with fibre-optic cables. Oxygen sensitive REDFLASH dye was adhered to each fibre-optic cable, to measure $\dot{M}O_2$. The

fibre-optic cables were calibrated to 100% oxygen saturation prior to each experiment (aerated seawater; 14°C) and calibrated to 0% oxygen experiment every 2 days, using sodium sulfite and borox. A digital temperature controller (Willhi WH1436A) was connected to the flush pumps, so the system would replenish water oxygen levels for 4 minutes following each measurement period. Four minutes was enough time for the chambers to be replenished to 100% oxygen saturation. The measurement period was 5 minutes, and was long enough to provide sufficient number of $\dot{M}O_2$ measurements, before oxygen levels dropped below 80% oxygen saturation. Background measurements of microbial oxygen consumption was performed for 20 minutes prior to the start and at the end of each experiment. Average background respiration was subtracted from all $\dot{M}O_2$ measurements. At the end of each experiment, the respirometry chambers and temperature tank were cleaned with a 6% bleach solution, followed by two additional rinses with freshwater to remove any residue.

Experimental protocol

Juvenile *R. vacca* were placed individually in the respirometry chambers 10 hours prior to their morning (0600) or evening (1800) experiment. The measurement periods were automated, using AquaResp 3, and were 10 minutes in duration. Each 10-minute cycle consisted of a 4-minute flush period, 1-minute wait period, and a 5-minute $\dot{M}O_2$ measurement period. While placed in the chambers 10 hours before the trial it only took 4 hours on average for the fish to relax from handling stress. Standard metabolic rate (SMR) was determined by generating a frequency histogram of $\dot{M}O_2$ values during the last 3 hours of the experiment. Standard Metabolic Rate was determined as the mean peak of the left-most normal distribution curve (Chabot et al. 2016). At the end of the ten-hour period (0600 or 1800), each fish was individually

removed from their tank and gently placed in a 78.5L circular tank (radius: 25cm, height: 40cm, maintained at 14°C) and hand-chased to exhaustion over a 3-minute period. The caudal fin was gently poked to stimulate movement. Fatigue was evident when they became unresponsive to being poked, and a reduction in burst swimming during chase. After 3 minutes, fish were removed from the bucket and exposed to air for 1 minute, following procedures by Roche et al. 2013 and Rummer et al. 2016. Following air exposure, fish were quickly (<10 seconds) returned to their respective respirometers. The $\dot{M}O_2$ measurements began within 15 seconds of the fish returning to their chambers, and the highest $\dot{M}O_2$ measurement after chase was taken as maximum metabolic rate (MMR). Aerobic scope was calculated as MMR-SMR. Respirometry was performed on each fish (n=8) at 0600 and 1800, in a randomized order, with at least 24 hours of rest in between experiments.

V. Daily Activity

To measure the daily activity individuals were placed into a circular arena (100cm diameter, 30cm height) with a water depth of 12 cm. The aquaria was connected to a flow through water system using filtered sea water with a daily temperature range of ~11.5-140C (natural daily fluctuations for Friday Harbor, WA). Fish were placed into the tank at either 0600 or 1800 and allowed 12 hours to acclimate. Following the acclimation period individual's movements were then recorded for a 24 hour period using H264WeCam and an infrared camera with additional infrared lighting. The recordings were then analysed using Logger Pro to calculate the time active and distance travelled for each hour of the 24 hour monitoring period.

VI. Statistical Analysis

To determine whether there is a significant difference between morning and evening values we conducted a paired Welch T-Test. Individual tests were conducted for UCrit (both body lengths and centimetres per second), SMR, MMR, and aerobic scope. Test were performed in S+ 8.0.

Results

The UCrit swimming speeds for *R.Vacca* were not significantly different between trials run in the morning or evening in both respect to centimetres per second (t_{12}=0.20, p=0.84) and body lengths per second (t_{12}=0.21, p=0.86). Morning and evening UCrit averaged 4.67 and 4.77 body lengths per second respectively, resulting in only 2% change, however individuals displayed varied changes from 20% increase to 25% decrease in UCrit.

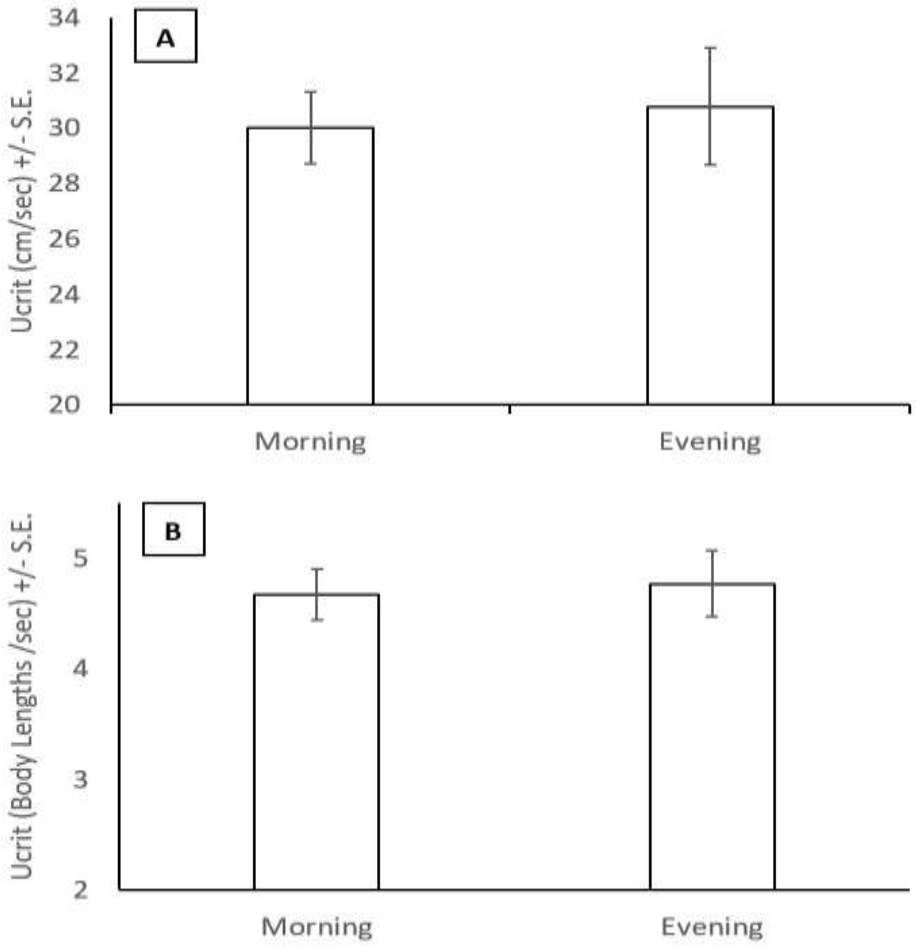

Figure 1 UCrit swimming speeds (+/-SE) for R.Vacca shown as centimetres per second (A) and in Body lengths per second (B). Trials were conducted at either 0600hr (Morning) or 1800hr (Evening).

The standard metabolic rate was not shown to significantly change between morning and evening respiration trials (t_{12}=0.08, p=0.93). The different between morning and evening maximum metabolic rate was also not shown to be significant (t_{13}=0.07, p=0.94). While both of these only saw an average of 3-4% change in the evening trails changes at the individual level ranged from increases up to 106% and decrease as much as 37%.

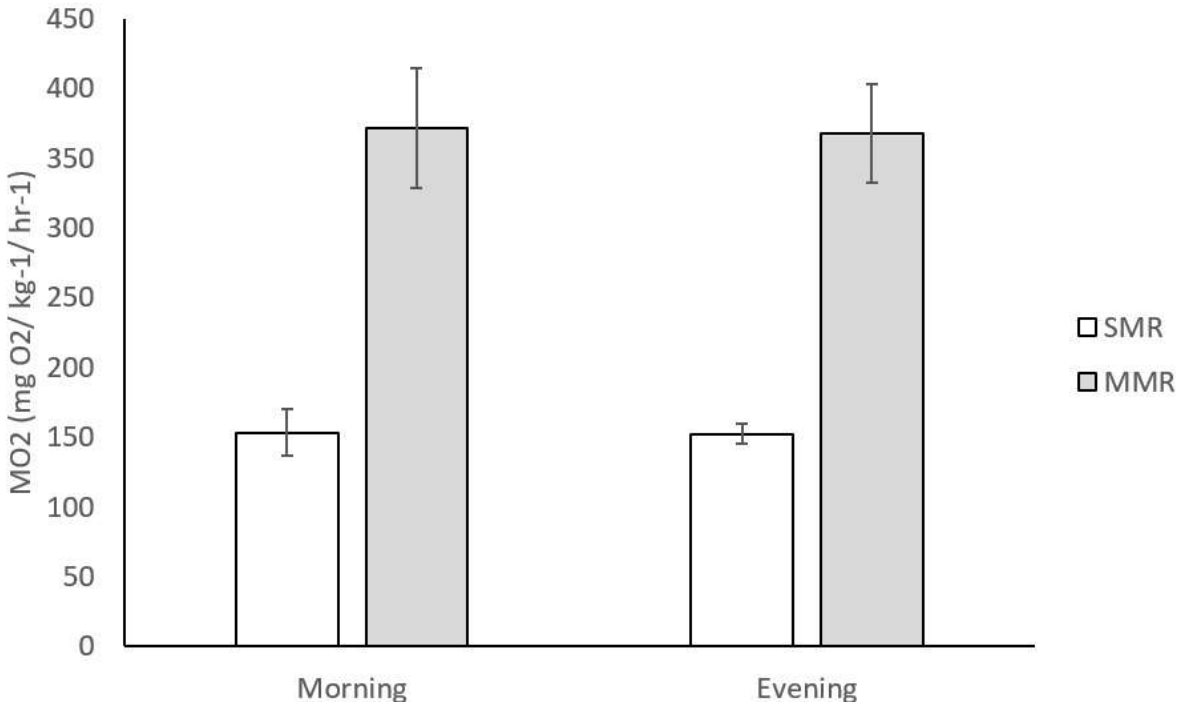

Figure 2 Standard metabolic rate (SMR) and maximum metabolic rate (MMR) for R.Vacca conducted at either 0600hr (Morning) or 1800hr (Evening)

We found no significant difference (t_{13}=0.07, p=0.94) between the morning and evening aerobic scope for *R. Vacca*. The average aerobic scope show less than a 2% change between morning and evening however at the individual level there were vastly different changes with aerobic scope increase up to 160% and decreasing as much as 35%.

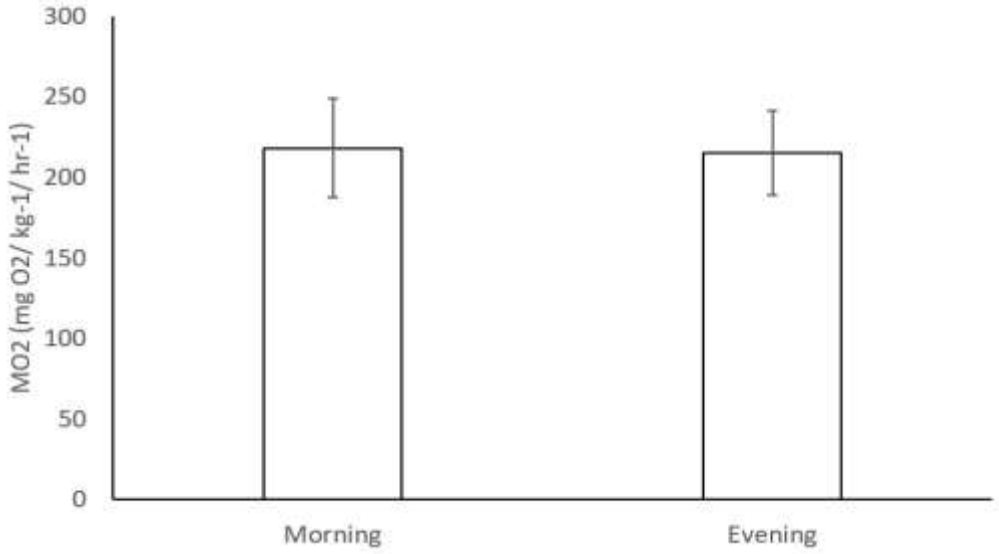

Figure 2 Aerobic scope (+/-SE) for R.Vacca. Trials were conducted at either 0600hr (Morning) or 1800hr (Evening).

The daily activity for this species was shown to peak at 0500 and 1800, with individuals moving 384 and 369 meters respectively. The activity pattern shows that the species were most active during crepuscular periods, however they still shows a strong nocturnal pattern with night hours having almost twice the activity of daylight hours.

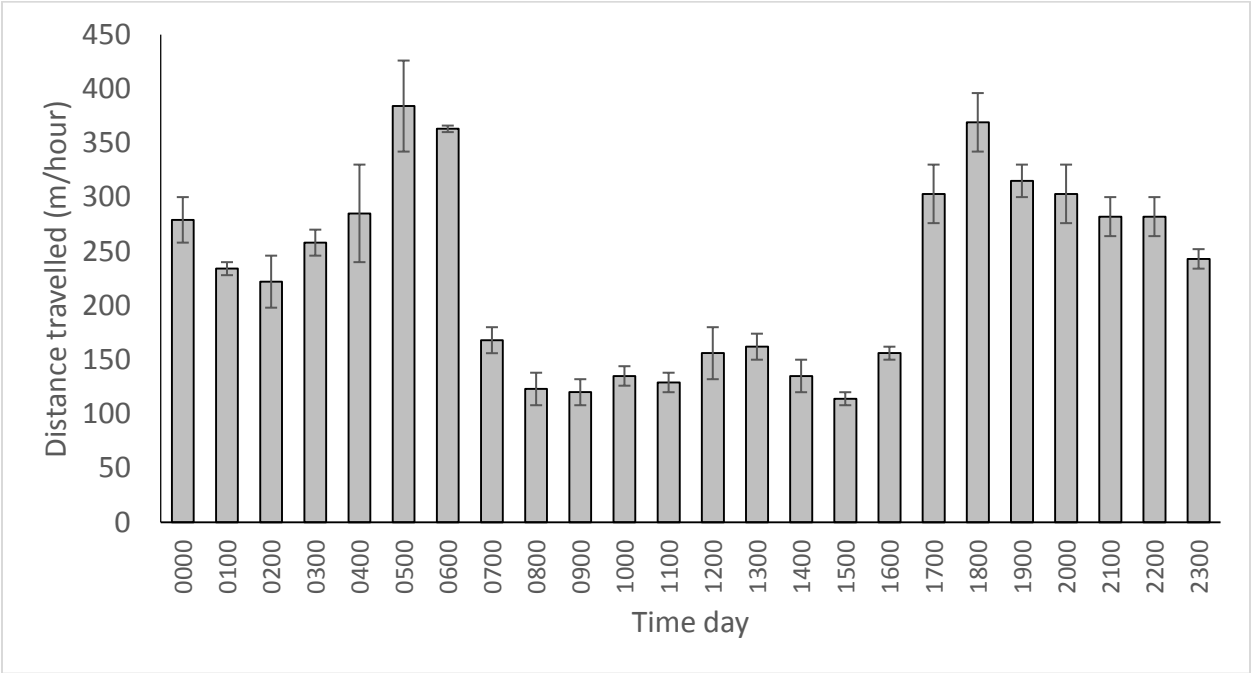

Figure 4 – Daily activity levels (meters per hour) for R.vacca with standard deviation.

Discussion

Understanding when animals are most active have profound influence son understanding aspects of their physiology, behaviour and in general, roles within an ecosystem. From an experimental biology perspective, understanding the circadian rhythm of the study species may have profound results of the experiment. For example, Marais (1978) found that routine $\dot{M}O_2$ of flathead grey mullet (*Mugil cephalus*) was highest during the sunset and lowest during the mid day and midnight. Therefore, measuring the aerobic capacity of this species will be impacted when experiments are conducted. To the knowledge of the authors, no current knowledge on the impacts of photoperiod on physiology and behaviour exist for *R. vacca*. Craig (1977) found seasonal variation in activity of the perch (*Perca flavescens*), and attributed this change in swimming behaviour on low levels of surface level irradiance during autumn, as well as due to less optimal temperatures. Temperature seems to be an important driver impacting measurable physiological and behavioural traits (e.g. oxygen consumption and activity levels) over daily and seasonal cycles. In the current study, water temperature was maintained at 14°C to minimize the confounding impact of temperature on swimming performance and oxygen consumption. Therefore, from an experimental biology perspective, swimming and oxygen consumption trials using *R. vacca* could be performed throughout the day, if temperature is maintained constant. There is very little current literature regarding the impact of photoperiod on UCrit of any fish species, and here we show very little difference in performance at constant temperatures between morning and evening of this diurnal fish species.

Of particular interest is the level of individual variability, particularly in oxygen consumption, between day and night. Løkkeborg (1998) found variability in individual swimming speed of the cod (*Gadus morhua*). Similarly, Leis (2006) emphasised the need for

researchers to indicate individual variability in any research involving swimming speeds of fish, particularly fastest and slowest performers. This highlights the important role condition of fish and genetic diversity, even amongst fish of the same cohort, has on any physiological and behavioural trait. Therefore, it is important for any studies focusing on such parameters to discuss reasons for variability. Outside of genetic diversity, time kept in captivity, water quality in holding tanks, minute differences in length and weight, and motivation to feed may have, in some combination, may have had some influence on the variability in swimming performance and oxygen consumption seen in this study.

Overall, we have shown that as a group the performance is repeatable regardless of the point in their circadian rhythm they are tested, however at the individual level there is evidence that performance differs at different points in the day. Few studies include photoperiod in their methodology, or when their animals are most/least active. From the perspective of *R. vacca,* a diurnal species, swimming and respirometry experiments could be performed either during the morning or afternoon. However, temperature influences many behavioural (e.g. swimming performance) and physiological (e.g. oxygen consumption) processes, and it is recommended that preliminary studies at constant temperature should be performed prior to any experiments to determine i) the impact of seasonal/daily fluctuations in temperature on measured variables and ii) to determine whether photoperiod has any pronounced impacts on the experiment. Additionally, individual variability in performance is a natural consequence of genetic diversity among a population of any given species, and it is important to provide the range by which measured physiological and behavioural variables can be exhibited in any given species.

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The Lazarus effect in Pacific sand lance (*Ammodytes hexapterus*): an examination of response to multiple forms of stressors

Kelly Diamond, Adam Daddino, Louis Penrod

Abstract

The Pacific sand lance, *Ammodytes hexapterus,* is a pelagic schooling species that is capable of burrowing in sandy substrates. When we placed fish in a tank with no substrate, sand lances would appear dead on the bottom of the tank but would reanimate when gently stimulated. We term the behaviour of suspended animation and then reanimation in response to stress, the Lazarus effect, and the state of suspended animation, the Lazarus trance. We were interested in examining how sand lances respond to multiple forms of stressors, and more specifically, which types of stress induce the Lazarus effect. First, we chose a mechanical stressor, to represent the stress of a predator attack, which are singular localized attacks. Our second stressor was a physiological stressor, to represent a low oxygen environment, which are often wide spread and occur over longer periods. We predicted that when given the option, sand lances would prefer to bury when exposed to any form of stressor as this offers the broadest protection. However, in removing the possibility of burying in the sand, we aim to understand the driving forces for burying. We predicted that fish exposed to mechanical stimuli would choose to actively swim more in the absence of a sandy substrate as this would give them a better chance of escaping a predator attack. For fish exposed to hypoxic conditions, we predicted more fish would express a Lazarus trance to conserve energy instead of spending excess energy on swimming as they are less likely to be able to escape from hypoxic water. To test these predictions, we exposed sand lances with access to sandy substrate to mechanical (standard 3-minute chase protocol) and physiological (hypoxia) stressors and recorded their activities. We then removed the substrate and repeated the experiments. Our results generally support our predictions, with more stressed fish choosing to bury when sand is available compared to unstressed fish. More mechanically stimulated fish became active and swam whereas the physiologically stressed fish expressed a Lazarus trance compared to their respective no-substrate treatment. These results suggest sand lances can respond uniquely to different forms of stressors, and that the Lazarus trance may be more critical

for surviving long term stressors, such as hypoxia, compared to shorter term stressors, such as predator attacks.

Introduction

The pacific sand lance (*Ammodytes hexapterus*) plays an important role in the local ecosystems of coastal Alaska, British Columbia and Washington as food for many species of fish, marine mammals and seabirds (Robards et al, 1999). These fish can be found in a benthopelagic habitat, swimming in well-formed schools or buried in in sandy substrate. Species of *Ammodytes* have no swim bladder, making swimming and maintaining their position in the water column energetically expensive (Robards et al., 1999). When not swimming, these fish will bury for winter hibernations, estivation, overnight hibernations, or as an anti-predation response (Wilson et al., 1999; Robards et al., 1999). Sand lances are tolerant of hypoxic conditions and can lower metabolic rate to increase time under the sand (Wilson et al., 1999), or when exposed to air at extreme low tides (Robards et al., 1999). The physiological adaptations that allow for survival under such hypoxic conditions include large brachiostegal membranes and gill cavities with a large volume that can hold and pump interstitial water over the gills to aid in oxygen transfer (Robards et al., 1999).

Our observations of wild caught sand lances revealed a novel behaviour for this fish. When we placed fish in a tank with no substrate, sand lances would appear dead on the bottom of the tank but would reanimate when gently stimulated. We term the behaviour of suspended animation and then reanimation in response to stress, the Lazarus effect, and the state of suspended animation we term the Lazarus trance. To survive in their natural environments, organisms are required to overcome multiple stressors. For some species, this requires having an arsenal of potential behaviours in which to apply to stressor specific interactions. This project aims to understand how behavioural responses, such as the Lazarus effect and burying, may be modified in response to different forms of stress in the Pacific sand lance. The two forms of stress we chose to focus on for the sand lance were those of a mechanical stressor, chosen to represent the stress of a predator attack, and a physiological stressor, to represent a low oxygen environment. These two stressors were chosen as they represent stress that varies over different lengths of time. Predator attacks are singular localized events whereas hypoxia is often wide spread and occurs over a longer period. We predicted that when given the option, sand lances would prefer to bury when exposed to any form of stressor as this offers the broadest protection. However, in removing the possibility of burying in the sand, we aim to understand the driving

forces for burying. If, in the absence of sand, fish still respond to our stressors with a Lazarus trance, it suggests that what mattered was most was conserving energy. On the other hand, fish respond to stressors by actively swimming, this suggest that the driving force for burying was mainly avoiding predators as swimming makes you less vulnerable to predator strikes than lying motionless on the bottom of the habitat. We predicted that fish exposed to mechanical stimuli would choose to actively swim more in the absence of a sandy substrate as this would give them a better chance of escaping a predator attack. For fish exposed to hypoxic conditions, we predicted more fish would elicit a Lazarus trance to conserve energy instead of spending excess energy on swimming as they are less likely to be able to escape from hypoxic water. To test these predictions, we exposed sand lances with access to sandy substrate to mechanical (standard 3-minute chase protocol) and physiological (hypoxia) stressors and recorded the percentage of fish that responded with burying, the Lazarus trance, or continued to swim. We then removed the substrate and repeated the experiments, recoding the percentage of time that fish elicited the Lazarus trance or actively swam.

Methods

Animal collection and holding

Adult sand lances were collected via beach seining between 19 July – 16 August 2017 at Jackson Beach, Friday Harbor, Washington. Collected fish were housed at Friday Harbor Labs in tanks with an ocean water flow through system on a natural light and temperature cycle. Water temperatures ranged from 10.5-16.1℃ C with no more than a 4℃ C difference over a 24-hour period. We collected 526 individuals (standard length ± SE = 7.60±0.04 cm; mass ± SE = 1.60±0.03g). Fish were not used for more than a single trial. Following experiments, fish were released back to the site of capture.

Stress trials

To examine if different forms of stress induce the Lazarus effect, we exposed fish to a mechanical (chase protocol) or physiological (hypoxia) stressor and compared responses to those of unstressed fish which were acclimated overnight (minimum of 8 hours). In each trial, we placed 10 sand lances in 19-liter containers which were set in 208-liter cattle tanks connected to the ocean water flow through to control water temperature and oxygen concentration. Each 19-liter container was filled with 15 cm of sand to allow fish to bury in response to stress. As we were also interested how sand lances would respond to stress in environments where they cannot bury, we

also repeated all treatments (mechanical stressor, physiological stressor, and control) in the absence of sand. For each of the treatments, we conducted eight trials with 10 fish per trial for a total of 80 fish per treatment. All trials were filmed using GoPro cameras (720p, 60 fps) for 30 minutes at a height of 48cm above the height of the water. For our mechanical stressor, we implemented a standard three-minute chase protocol. Following chasing fish were moved to a treatment container and were filmed immediately. For the physiological stressor, fish were acclimated for a minimum of four hours, before nitrogen gas was infused into a custom built circulating system via a solenoid pump. Our pump was connected to an oxygen probe and programmed to turn the nitrogen on and off as need to keep a 20% oxygen saturation. We manually bubbled oxygen into our system as needed. A 30-minute filming period started after our target oxygen saturation was reached.

Analysis

Videos were digitized using the program Kinovia (Charmant, J. V 0.8. 15., 2012). We recorded position of each unburied fish (position of rostrum) and activity category of each fish at one minute intervals for 30 minutes starting with time point 0 for a total of 31 data points per trial. For trials with sand, activity categories included swimming, buried, and Lazarus trance (when the fish was laying on the surface, unburied, with the only visible movement being that of the opercula). As fish could not bury in the absence of sand, activity categories for trials without sand included only swimming or Lazarus trance. We averaged the percentage of fish in each activity category across all treatments for each period for an average activity for each treatment. For the four different treatments (mechanical stressor with and without sand, physiological stressor with and without sand), we compared each activity category between treatment and appropriate control trials using two sample Kolmogorov-Smirnov tests.

Results

Overall, a greater percentage of fish exhibited the Lazarus trance when there was no sand present across all treatments (Table 1). The treatment with the greatest percentage of fish in the Lazarus effect was the physiologically stressed sand lances in the absence of sand (Table 1). When sand was present, sand lances were less likely to burry when mechanically stressed compared to physiologically stressed fish (Table 1).

Table 1. Mean and standard error of the percentage of time fish spent in each activity category averaged over all time periods. Stressors were induced by chase protocol (Mechanical) or 20% hypoxia (Physiological). Substrates were either sandy or not present. For trials without substrates fish did not have the option to bury, so the only options were to swim or exhibit the Lazarus trance.

Treatment	Substrate	Percent swimming	Percent buried	Percent Lazarus trance
Mechanical Stressor	Sand	47.8±1.2	42.7±1.3	8.9±0.6
Mechanical Stressor	None	92.5±0.7	-	7.5±0.7
Physiological Stressor	Sand	6.8±0.4	87.0±0.7	7.6±0.6
Physiological Stressor	None	26.4±1.4	-	73.6±1.4
Control	Sand	13.4±1.4	84.1±1.3	2.5±0.3
Control	None	89.1±0.9	-	11.0±0.9

Mechanical stressor

When we compared activity periods between chased and control treatments in the presence of sand, all three treatments, swimming, buried, & Lazarus trance, were statistically different from our control ($p<0.001$). In the chased with sand treatment, fish swam over 3.5x more and spend 3x as much time in the Lazarus trance above the sand compared to our control (Figure 1, Table 1). In the control with sand treatment, 51% more fish remained buried than in our mechanically stressed with sand treatment (Figure 1, Table 1). In the absence of sand, both treatments, swimming & Lazarus trance, were statistically different from our control ($p<0.001$). In the no sand treatments, 27% more fish exhibited the Lazarus trance in the control treatment, than in the mechanically stressed treatment (Figure 2, Table 1).

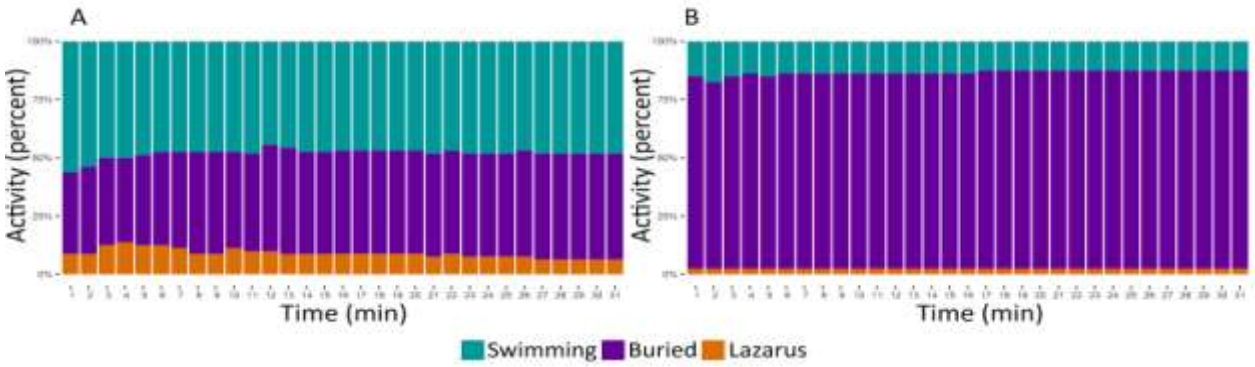

Figure 1. (A) Mechanical stressor with sand substrate and (B) no stress with sand substrate. Bars represent the average percentage of sand lances in each activity period of the trial sampled every minute of the 30 min trial. Trials with mechanically stressed fish (A) had a greater percentage of fish actively swimming (blue) and in the Lazarus trance (orange) compared to control trials (B), which had more fish bury (purple).

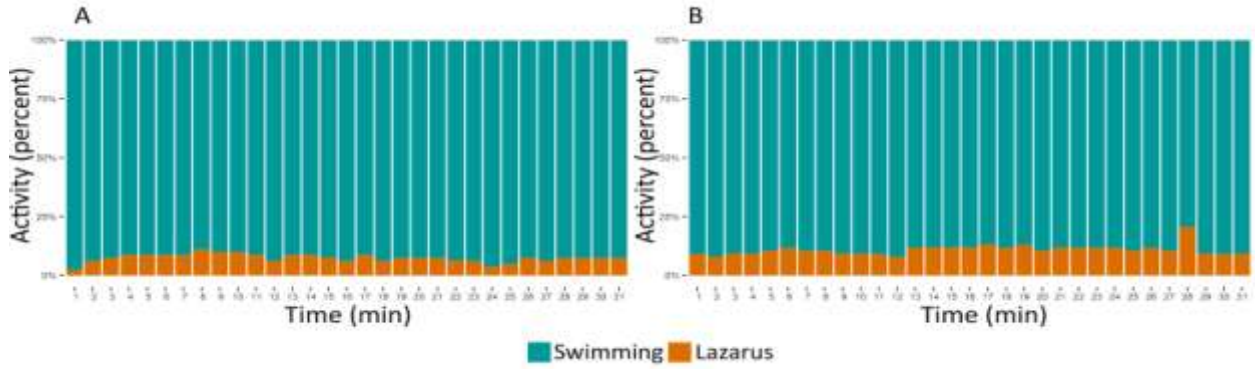

Figure 2. (A) Mechanical stressor without sand substrate and (B) no stress without sand substrate. Bars represent the average percentage of sand lances in each activity period of the trial sampled every minute of the 30 min trial. Trials with mechanically stressed fish (A) had a greater percentage of fish actively swimming (blue) and fewer fish in the Lazarus trance (orange) compared to control trials (B).

Physiological stressor

Our comparison of activity periods between sand lances exposed to 20% hypoxia and control treatments in the presence of sand found that all treatments, swimming, buried, & Lazarus trance, were statistically different from our controls (all three had a $p<0.001$). In the hypoxia treatment with sand, fish spent 54% less time swimming, 63% more time in the Lazarus trance, and 4% more time buried under the sand compared to control treatments (Figure 3, Table1). In the absence of

sand, both treatments, swimming & Lazarus trance, were statistically different from each other ($p<0.001$). In the no sand treatments, on average there were 6.5x as many fish in the Lazarus trance than in the control treatment (Figure 4, Table 1).

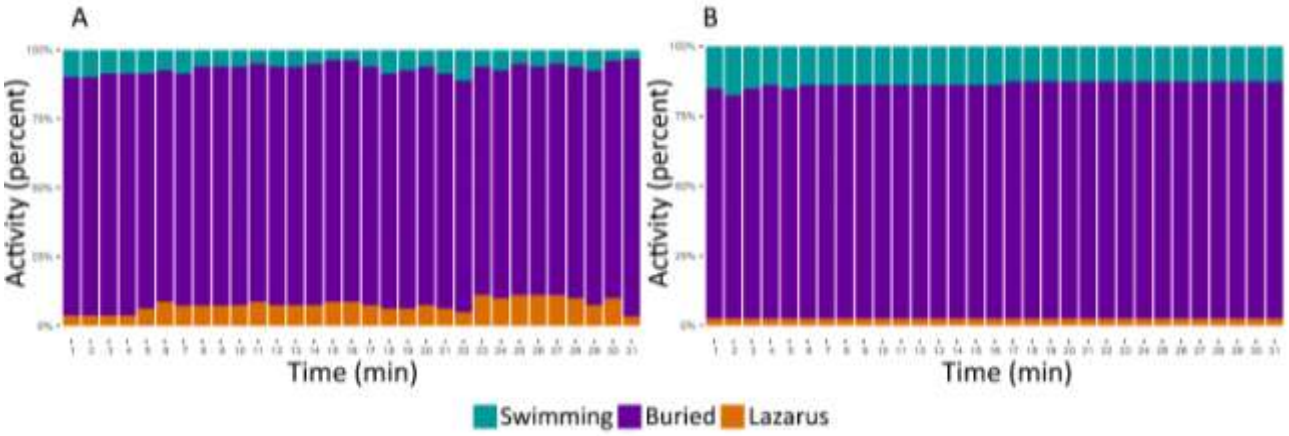

Figure 3. (A) Physiological stressor with sand substrate and (B) no stress with sand substrate. Bars represent the average percentage of sand lances in each activity period of the trial sampled every minute of the 30 min trial. Trials with physiologically stressed fish (A) had a greater average percentage of fish in the Lazarus trance (orange) compared to control trials (B), which had more fish bury (purple) or actively swim (blue).

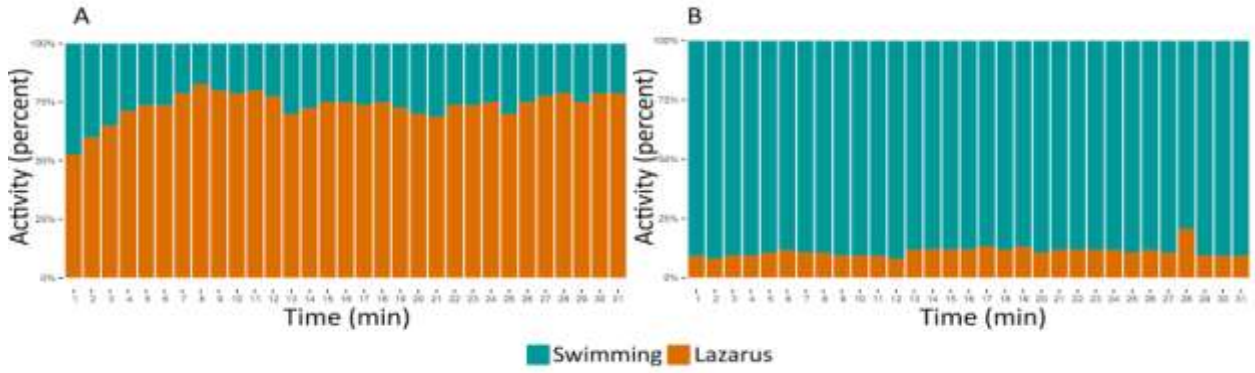

Figure 4. (A) Physiological stressor without sand substrate and (B) no stress without sand substrate. Bars represent the average percentage of sand lances in each activity period of the trial sampled every minute of the 30 min trial. Trials with physiologically stressed fish (A) had a lower percentage of fish actively swimming (blue) and greater fish in the Lazarus trance (orange) compared to control trials (B).

Discussion

Our results show that across all activity types, there are behavioural differences when fish are exposed to stress. Furthermore, we show that the type of stressor and the ambient environment (substrate) can impact behavioural responses. When a sandy substrate was present, we predicted that fish would bury more when exposed to either of our stressors as burying should allow these fish to both hide from predators (mechanical stressor) and minimize energy usage in response to a physiological stressor. As predation is a localized and short-term event, we predicted that fish would respond to our mechanical stressor with a higher percentage of fish swimming instead of laying on the bottom in a Lazarus trance waiting to be eaten. In contrast, hypoxia is often widespread and nearly impossible to escape, so here the best strategy would be to conserve as much energy as possible. Hence, we predicted that in the absence of sand, sand lances exposed to our physiological stressor should use the Lazarus trance to conserve energy instead of swimming.

In mechanically stimulated trials, 43% of fish responded to stimuli by burying under the substrate. When we removed the sand, removing burying as an option, we found that a larger portion of fish responded to the mechanical stimuli by actively swimming (93%) instead of inducing the Lazarus trance (7%). This makes sense as our mechanical stimuli represented the stress induced from predator attacks. If a fish is actively swimming they can evade a predator strike via an escape response, whereas lying motionless on the substrate would make predator evasion very difficult. In contrast, nearly twice as many fish responded by burying (87%) to our physiological stressor compared to the mechanical stressor. Fish that did not bury we about equally likely to actively swim or induce a Lazarus trance in the hypoxia treatment (table 1). When we removed sand, most of the sand lances (89%) responded to hypoxia via a Lazarus trance with only 11% actively swimming throughout these trials. Since hypoxic conditions are often wide spread and last longer then single predation events, if follows that fish would respond to this stressor by trying to conserve as much energy as possible instead of trying to escape from the hypoxic waters.

Organisms do not evolve in a vacuum. To attain maximal fitness, they must respond to multiple stressors that can vary through space and time. Previously, sand lances were thought to either bury as a response to low food or oxygen abundance (Robards et al., 1999), and as an anti-predator response (Hobson, 1986). Our results support both previous observations. By incorporating multiple substrates and multiple forms of stressors we gain a better understanding of how fish respond to different forms of stress faced in their natural environment.

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Bold fish flee first: impacts of bold-shy personality on escape responses of schooling shiner surfperch, *Cymatogaster aggregata*

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Abstract

The influence of individual personality has been linked to several behaviours of fish, such as foraging, migration and social preferences. This study investigated the influence of personality along the bold-shy axis on escape responses of schooling shiner perch, *Cymatogaster aggregata*. Fish were classified as bold or shy using an emergence test before being placed in schools of five bold or five shy individuals. Escape responses were elicited using a mechanical stimulus and filmed with high speed cameras to analyse latency of responses of individual fish within bold or shy schools as well as the polarity of these schools across the escape response. Latencies of escape responses were significantly reduced in schools of bold individuals when compared to schools of shy individuals. Additionally, shy individuals showed a significant reduction in school polarity between 200-600 ms post stimulus when compared with polarity before stimulation, which did not occur among bold schools. Reduced latency and non-significant changes in polarity of bold schools may indicate increased performance of bold fish during predator-prey interactions and may confer a fitness advantage over shy fish.

Introduction

Many fish species exhibit schooling behaviors which has been demonstrated to offer both ecological and physiological benefits for fishes. These include faster location of food, reducing locomotive costs associated with drag and fin beat frequency as well as avoiding predation (Pitcher et al., 1982, Morgan and Godin, 1985, Johansen et al., 2010). Recently, research has documented significant impacts of individual personality of fish on ecologically relevant processes such as foraging, social behaviour, dispersal and predator response (Conrad et al., 2011). However, for schooling species of fish, observations of individual personality differences do not represent wild conditions in which fish swim as a school. While schooling, activities like feeding and predator avoidance are a result of the interactions between multiple individuals, as such it is not known if individual differences in personality affect school behaviour. While previous research has indicated that personality differences between fish along the shy-bold axis influence school positioning and shoal association of individual fish (Ward et al., 2004), few studies have examined the impact of personality on the behavioural performance of the school as a whole (Toms et al., 2010).

School behaviour is particularly important during predator-prey interactions as it is often utilised as an anti-predator tactic. As predation pressure has been linked to the bold-shy axis of personality (Brown et al., 2007), it may be that relative boldness of individuals impacts upon anti-predator responses of a school. Response to an outside stimulus has been used to simulate the escape response of fishes to a potential predator and allows for an examination of schooling dynamics that occur during a predation event. Other behavioural determinants have been shown to influence escape performance of fish, such as lateralization (Dadda et al., 2010), but to date the influence of personality on escape performance of schools has not been examined. This study examines the influence of the

bold-shy personality axis on escape performance on schools of shiner surfperch (*Cymatogaster aggregata*). In particular the latency of escape response (the reaction time of a fish to a stimulus) and polarity (the degree to which fish in a school are facing the same direction) of bold and shy schools of shiner surfperch were analysed after onset of a mechanical stimulus. It is hypothesized that personality differences between schools along the bold-shy axis of behavior influences the escape response of schooling surfperch.

Methodology

Fish Capture

Approximately 150 shiner perch (*Cymatogaster aggregata*) of indiscriminate gender were collected using beach seines at Jackson Beach on San Juan Island, Washington, U.S.A. (48°31´ ; 123°01´ W) during July and August of 2017. Fish were moved to Friday Harbor Laboratories (FHL) and kept in holding tanks (120 cm × 56 cm × 12 cm, length × width × depth) under natural light conditions supplied with natural flow-through, filtered seawater at ambient temperatures (11.2-16.1^{o}C). All fish were given a minimum of 24 hours to acclimate to laboratory conditions prior to use in any experimental trials.

Shy, Bold selection

To classify between shy and bold fish, 132 individuals were randomly selected and transferred to a shelter in an oval tank (measuring 125 cm × 60 cm × 25 cm, length × width × depth). Fish were captured from holding tanks using a small plastic bucket and gently poured into shelters to minimize stress of capture and transfer. The shelter, similar in design to those used by Brown et al. (2005), was composed of a vertical tube (14.5 cm diameter, water depth 22 cm) with an exit in one side (6 cm x 8 cm, width x height) covered by an external door. Immediately after fish were transferred a lid was placed on top of the shelter and fish were held for five minutes. After this time, the external door was gently removed to allow fish to exit the shelter and time until first emergence was recorded (emergence classified as the moment that the snout of the fish first left the shelter). Trials were filmed using Casio Exilim digital cameras (30 fps, f1/3.2, 1/30, ISO 3200) mounted directly above the shelter to identify the moment each fish emerged. Four fish were tested simultaneously in each trial in separate tanks. Trials were conducted in a covered outdoor area under artificial light to prevent changing light level and position influencing fish behavior. Trials were observed from a location that fish were unable to see until after they had fully left the shelter. Each trial lasted 30 minutes from time of door opening. After the trials the fish were classified according to the time of first emergence (0-5 min, 5-7.5min, 7.5-10 min, 10-15 min, 15-30min, >30 min) and transferred to outdoor holding tanks supplied with the same natural flow through, filtered seawater with a natural light regime. Following all trials bold fish (classified as those that first emerged in < 10 min) and shy fish (classified as those that first emerged in > 15 min, or never emerged from the shelter) were pooled to form a single tank of bold fish and a single tank of shy fish.

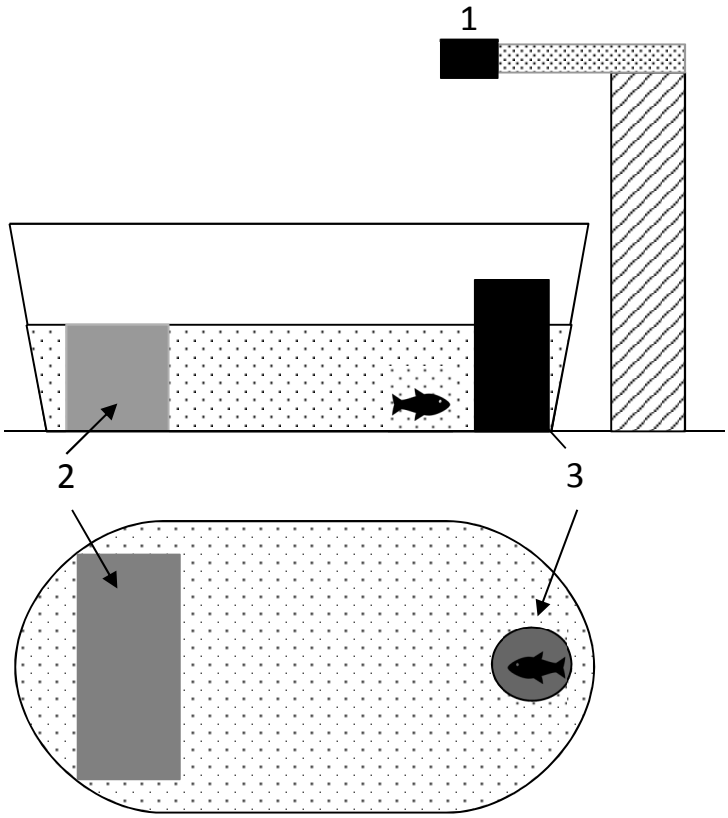

Figure 1: Experimental chamber used for Bold-shy classification, 1. = Casio Exilim camera, 2 = brick to weight down oval tank, 3 = Shelter in which fish was placed.

Schooling test

In order to evaluate the effect of personality (boldness vs shyness) on schooling characteristics, eight groups of five individuals were chosen at random from either fish classified as shy (i.e. emergence time >15 minutes) or bold (i.e. emergence time 0-10 minutes) to create schools of five bold or five shy individuals. Schools were then transferred to a circular experimental tank (380 cm diameter, 22cm water depth) supplied with natural flow through, filtered seawater. Inflow seawater produced a clockwise flow of 4-6 cm/sec at a distance of 5 cm from the edge of the tank and there was no flow in the center of the tank. After transfer to the experimental tank, fish were allowed to acclimate for a period of two hours whilst a HD camera (GoPro Hero 3+), mounted 4 m above the center of the tank, was used to film group behavior.

Following two hours of acclimation, schools were repeatedly startled, over a period of one hour, by dropping a weighted plastic cylinder onto the water surface to create a sudden stimulus and elicit an escape response. Post stimulus fish were allowed a period of 3 minutes to recover before subsequent stimulus were triggered. Stimuli were mounted on the experimental tank approximately 10cm from the outer circumference. Escape responses were filmed using high speed cameras (Casio Exilim, 240 fps, F1/3.2, 1/250, ISO 3200) mounted to one side of the stimulus covering an area of 0.88 m² (125 cm x 71 cm, length x width). A mirror was mounted on the opposite side of the stimulus to the camera, positioned so that the camera could record the moment that the plastic cylinder first made contact with the water surface (Figure 2).

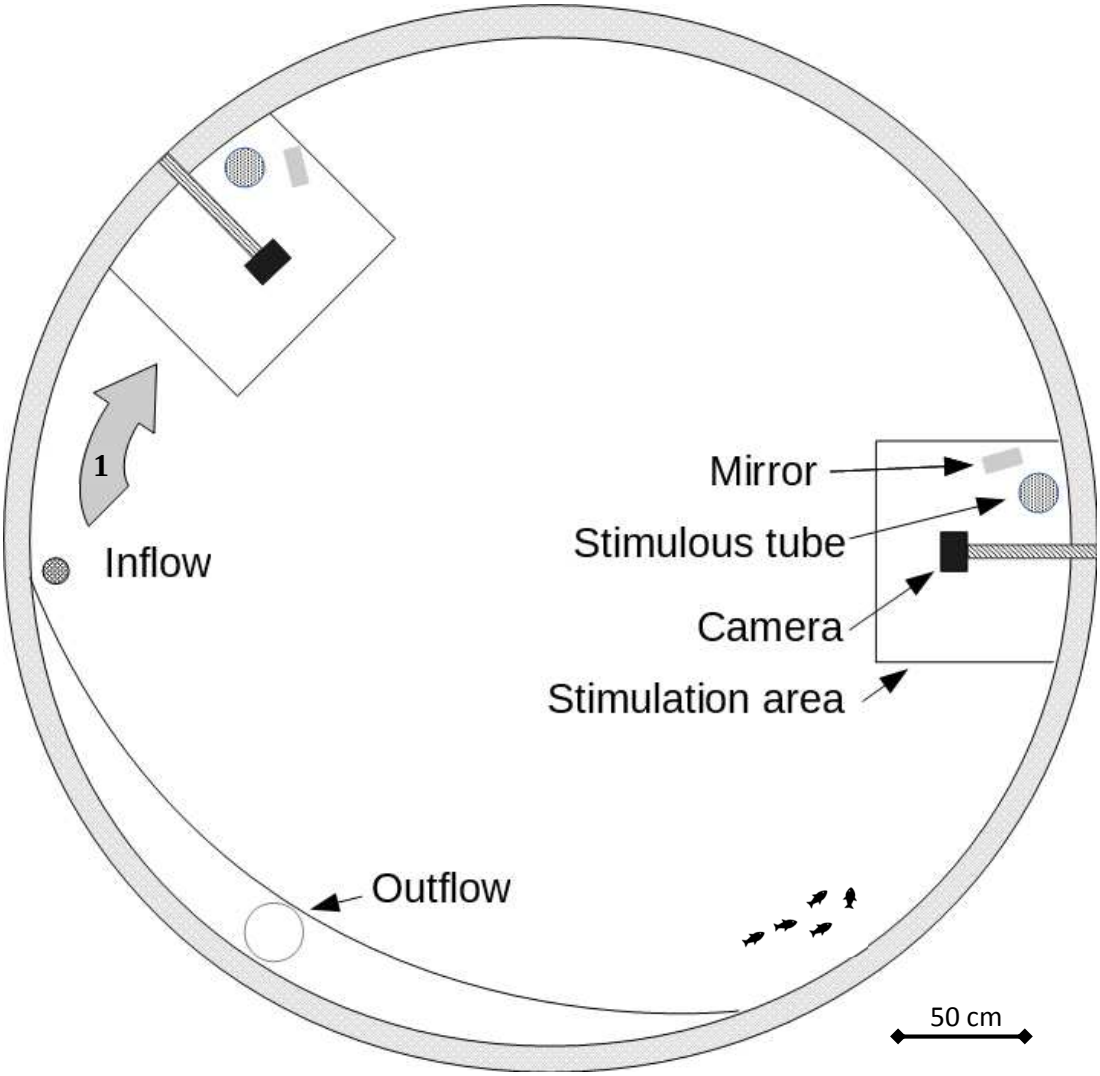

Figure 2: Diagram of behavioural experiment tank, 1 = direction of flow within tank. Maximum flow occurred along circumference of tank and did not exceed 6 cm/s.

Stimulus were triggered, remotely by an observer watching the position of fish in real time via wireless connection to the overhead gopro, when all fish were within the area covered by the high speed camera and swimming towards the stimulus location. Stimulus in which these conditions were not met were excluded from subsequent analysis. All stimulus could be reloaded from a position external from the experimental area to avoid fish being startled by the presence of an observer. Once trials were completed total length and mass of each fish were measured and fish moved to a separate holding tank. No fish were used in more than one trial. All fish were released at the end of the trials.

Data analysis

Escape Response

Escape responses of schools were analyzed with Kinovea video analysis software. Latency was assessed as the first frame after the stimulus impacted the water surface in which each individual fish visibly responded. Additionally distance of each fish to stimulus was estimated as the distance from the tip of each individual's snout to the closest part of the stimulus apparatus. Generalised Linear Mixed Models (GLMM) in R with package lme4 (Bates et al. 2015) were used to assess the effect of personality on latency of school escape responses. Linear Mixed Models included distance from individual to stimulus as a random effect. Model selection was based on comparisons between

models on removal of term using ANOVA in conjunction with Akaike's Information Criteria (AIC).

In addition to latency polarity of fish schools was assessed at the point of stimulus and 100, 200, 300, 400, 500, 600, 800 and 1000ms after stimulus. Polarity of the school was calculated by measuring the angle of the line intersecting the dorsal fin and snout of each fish. The lead fish in each school was assigned as the reference angle (0^o) from which other fish were compared. Angle of each individual was imported to Oriana (Version 4.02 www.kovcomp.com) before the polarity (defined as length of mean vector, r) was calculated for each school. Polarity at each timepoint for shy and bold schools was assessed for normality before a Kruskal-Wallis test was used to test for significant changes in polarity across time following stimulus. Post-hoc analysis then determined which time points showed significant differences in polarity using a Dunn test in package FSA (Ogle, 2017).

Results

Shy, bold selection

Of 132 fish tested, 50 were classified as having a bold personality (emerged in <10 minutes) and 74 were classified as having a shy personality (emerged in >15 minutes) (Figure 2), with the 8 fish emerging between 10 and 15 minutes being removed from trials. There was no significant difference in either length (Kruskal-Wallis, Chi-squared = 0.26291, df =1, P > 0.05) or weight (Kruskal-Wallis, Chi-squared = 0.0088349, df = 1, P > 0.05) between shy and bold fish.

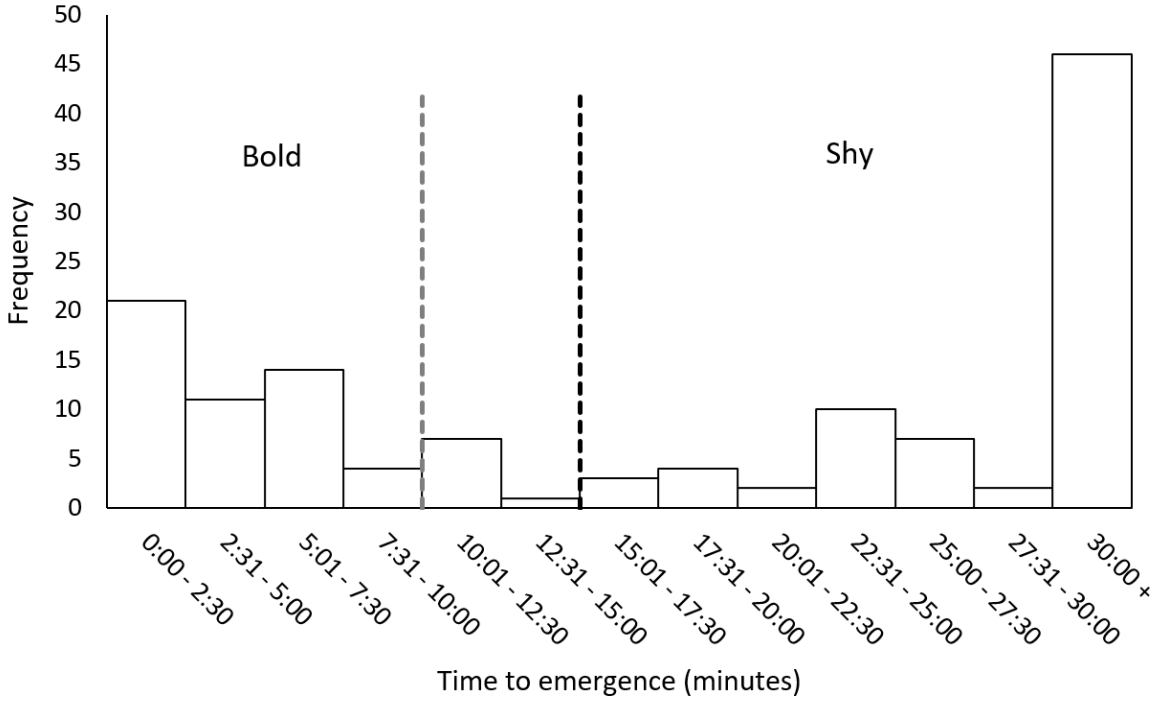

Figure 3: Time for 132 *Cymatogaster aggregata* to emerge from a shelter in a novel environment. Fish that emerged in under 10 minutes were classified as having a bold personality while fish that emerged in over 15 minutes were classified as shy. Fish that emerged between 10 and 15 minutes were not included in schooling trials.

Escape response

Latency of escape response of bold fish was more frequent between 25-50 ms post stimulus whereas latency of shy fish was most frequent between 50-75 ms post stimulus (Figure 4).

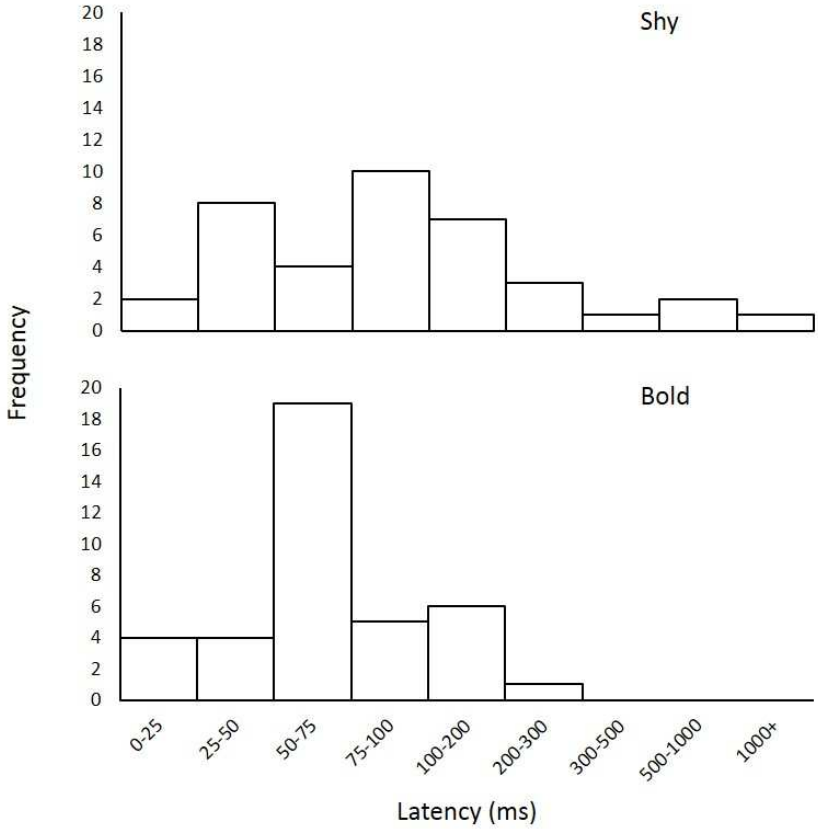

Figure 4: Latency of escape response for individuals within schools of five shy or five bold *Cymatogaster aggregata*.

Of the nine trials conducted for bold and shy fish, two of each were discarded as a result of fish being absent from the field of vision when the first stimulus was triggered. Personality had a signification effect on the latency of escape responses from the first stimulus in schools of bold fish (mean ± S.E. = 73.32 ± 8.38 ms) and schools of shy fish (mean ± S.E. = 175.33 ± 21.83 ms) (Figure 5).

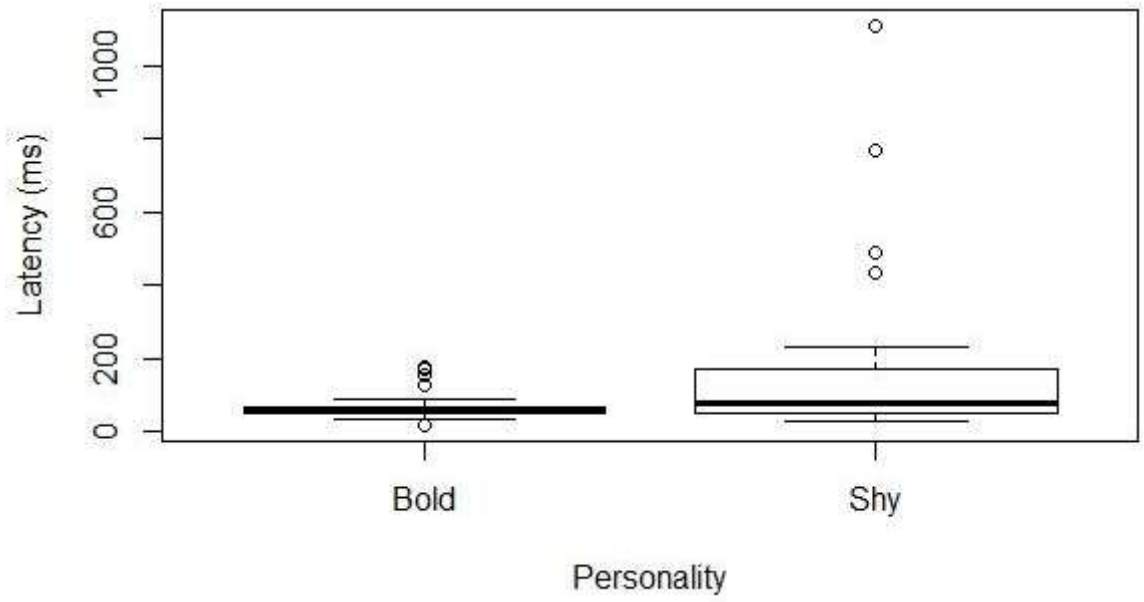

Figure 5: Influence of personality on latency of escape response of seven schools of five *Cymatogaster aggregata* from a novel stimulus.

Table 1: Model outputs from GLMM investigating the difference in escape latency in schools of bold and shy shiner perch. Variance is reported as standard error for fixed effects and standard deviation for random terms (displayed in italics). GLMM = Latency ~ Personality + (1|Distance to Stimulus).

Fixed effect	**df**	**P**	**Effect ± variance**
Personality	52.08	0.0372	105.18 ± 49.18
(Intercept)			73.32 ± 36.04
Distance to stimulus			32427.54 ± 180.08

Polarity of bold schools showed no significant changes from the time of stimulus to 1000 ms post stimulus (Kruskal Wallis with Dunn Test, Chi-squared 10.785, df = 8, P = 0.214, Figure 4a), in contrast shy schools showed significant declines in polarity at time points between 200 and 600 ms post stimulus (Kruskal Wallis with Dunn Test, Chi-squared = 21.315, df = 8, P < 0.01, Figure 4b).

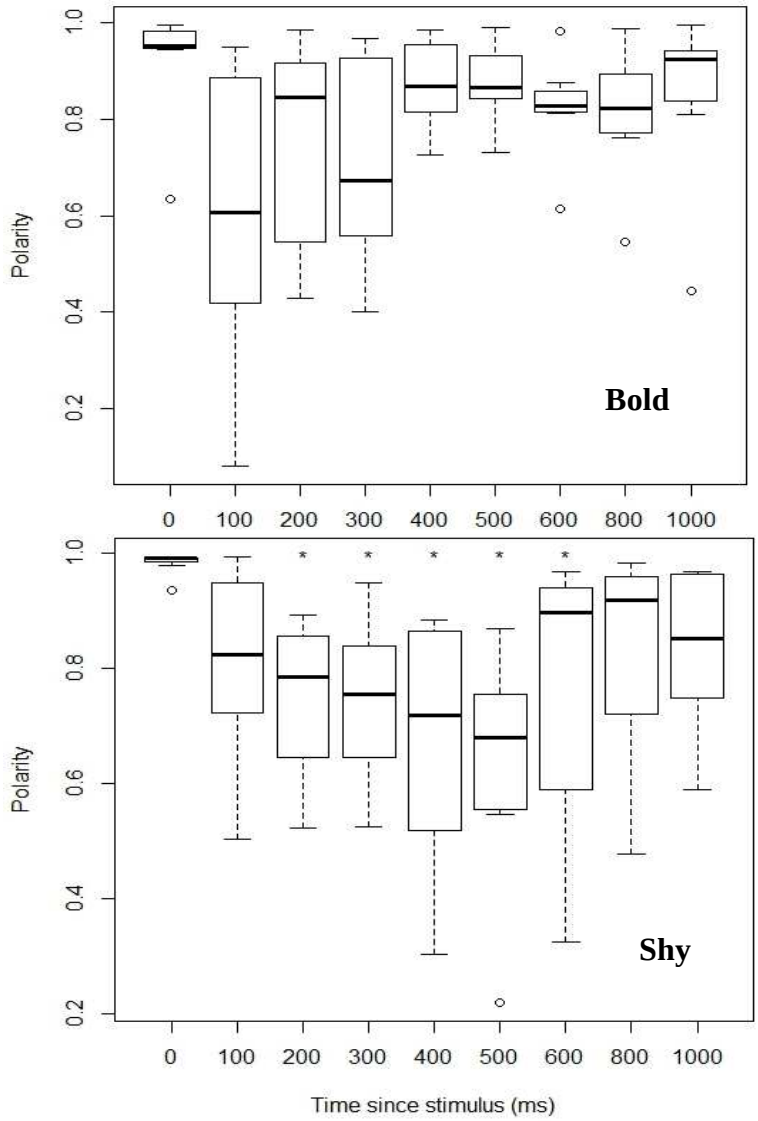

Figure 6: Polarity of bold and shy schools of fish at 0, 100, 200, 300, 400, 500, 600, 800 and 1000 ms post stimulus. Stars indicate significant difference from 0 ms post stimulus. 0 ms post stimulus represents polarity of school prior to stimulus.

Discussion

Personality of fish significantly impacted upon both latency of response and polarity during escape response of shiner surfperch in the current study. Decreased latency time and the lack of a significant reduction in school polarity across escape response may indicate that schools of shiner surfperch which display a bold personality have a performance advantage over shy shiner surfperch during predator-prey interactions.

The results of the current study contrasts with the findings of Jones & Godin (2010) in which convict cichlids, *Amatitlania nigrofasciata*, which were fast to explore a novel environment (and therefore were suggested to have a bold personality) were also slowest to respond to a simulated predator attack. This may indicate that influence of personality on escape response of fish is species specific. Alternatively, as convict cichlids are a non-schooling species, it may be that improved escape performance of bold fish is related to schooling behavior.

Bold fish are suggested to occupy positions closer to the front of schools (Ward et al., 2004), where

foraging efficiency is higher. However, fish positioned at the front of schools are also exposed to increased predation pressure. If bold shiner perch occupy positions near the front of schools in the wild then improved escape response performance in the current study may be an adaptive response to increased exposure to predation risk. This correlates with research demonstrating that fish positioned near the front of schools exposed to a mechanical stimulus were startled first (Marras and Domenici, 2013). Improved escape response in bold fish may also partially explain results of previous research which demonstrated that fish from areas of high predation pressure are consistently bolder than fish from low predation areas (Brown et al., 2005, Harris et al., 2010), by providing bold fish with a fitness advantage over shy fish.

Significant decreases in polarity in schools of shy fish between 200 and 600 ms post stimulus are likely a result of increased variation in latency of responses compared to bold individuals. Shy schools contained several individuals whose response latency was greater than 200 ms with the largest response latency being over one second. In these cases individual fish's response might not be a result of the mechanical stimulus but instead related to the movement of other fish within the school.

Overall the study provides evidence that schools of bold shiner perch show an improved performance of escape response when exposed to a sudden stimulus. This may confer an adaptive advantage to bolder fish which are more likely to be found in areas of higher predation risk such as frontal positions of schools.

Acknowledgements

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Friday Harbor Laboratories- Fish Swimming course 2017- Final Report
Course instructors: Paolo Domenici, John F. Steffensen, Jacob J Johansen

Take it or leave it. Fast-start modulation in the great sculpin *Myoxocephalus polyacanthocephalus*

Penrod, LM; Daddino, A; Diamond, K

Abstract

Fast-start escape responses are used by fish when dealing with predatory threats that require a quick response with high velocity. These responses are controlled by Mauthner cells (M cells) which cause rapid, stochastic reactions to a threat. Recent work on archerfish has suggested that a more precise fast-start towards prey may also be controlled by M cells. We expect that, given the environment and opportunity, a fish could alter its escape response to move towards a stimulus with speed and precision like the archerfish. Great sculpin (*Myoxocephalus polyacanthocephalus*) were trained to perform a fast-start attack and the kinematics were compared between (1) trained fish's attack on food stimulus (2) escape response of untrained fish from a food stimulus and (3) escape response of untrained fish from a strong stimulus. We found that trained fish had a longer latency than both untrained treatments, but the trained and untrained sculpin had similar turning velocities in response to a food stimulus. Untrained fish were more stochastic with their escape responses than trained fish, and had higher variation in their turning velocities and post-response linear velocities. Our results suggest that, given the opportunity, a fish can alter its escape response to an attack. However, due to the higher latency in the attacks, M cells may not be mediating these responses.

Introduction

Fast-start escape or attack behaviors are used by fish when dealing with predator-prey interactions that require quick bursts of high velocity and acceleration. Fast-starts are classified into three kinematic stages, a preparatory stroke (stage 1), a propulsive stroke (stage 2), and transition back to steady swimming (stage 3; Eaton & Hackett, 1984). The line between stage 1 and stage 2 can vary in definition; notably the onset of stage 2 is defined by the change in turning direction of the anterior body midline (Domenici and Blake, 1997) or the start of the propulsive stroke and forward acceleration (Webb, 1978). Stage 3 is not always present and can vary between continuous swimming or coasting (Wohl and Schuster, 2006; Domenici and Blake, 1997). The neural mechanisms that drive the fast-start escape response are initiated by a single pair of neurons, the Mauthner cells (M Cells), connected to a parallel network of neurons that control unilateral contraction on the opposite side of the body in which as stimuli is perceived (Hale, 2005). Traditionally it has been thought that (1) the Mauthner cell controlled fast-start is stochastic and rapid and (2) a separate fast-start without Mauthner cells can allow for precision but at lower speeds.

Findings by Wohl and Schuster (2006) challenged these thoughts when they found that archerfish would attack falling prey at high speed with precision and kinematics similar to those of the Mauthner cells mediated, fast-start escape response. Archer fish have evolutionarily adapted to shoot a jet of water at insects above the water line. To compete with other archerfish, they must have rapid and precise fast-start responses towards the falling prey item. We hypothesize that, given a similar environment and opportunity as the archerfish, other fishes could also alter fast-start escape responses to move towards a stimulus with speed and precision. By altering and acclimating fish to a vastly different feeding regime than in their natural environment, we can provide that opportunity which may alter their fast-start response. To test this hypothesis, Great sculpin (*Myoxocephalus polyacanthocephalus*) were trained to attack a stimulus dropped into the water and the kinematics were compared to (1) untrained fish with the same stimulus, (2) untrained fish with a stronger stimulus, and (3) the kinematics of non-stimulated fish. Although great sculpin lack the evolutionary pressures to select for rapid and precise responses like the archerfish, we expect that a fast-start for feeding will have kinematics equivalent of a fast-start escape response.

Methods

Fish

Great Sculpin (*Myoxocephalus polyacanthocephalus*; N=36) were collected from Jackson Beach, San Juan, WA via beach seining between 19 July – 16 August 2017. Fish ranging from 12.7 cm to 30.9 cm with a weight of 46.6 g to 553.8 g were housed at Friday Harbor Labs in a 130 cm X 60 cm X 25 cm tank with an ocean water flow through system. The center of mass (CM) was determined for three sculpins. Fish were euthanized with an overdose of MS-222 before being frozen in a stretched straight position and CM was determined using plumblines (Webb, 1975). The CM of sculpins is at the anterior base of the dorsal fin at a distance of 35.8±3.2% of the standard length. 35% was used for consistency during digitization.

Training and testing

To investigate the response of sculpin to a stimulus, a filming chamber matching the size of the holding tank was constructed with a tube to deliver the stimulus. The tube was 10.5 cm in diameter and 110 cm tall. It was placed 1 cm above the water line and 0.5 cm from the edge of the tank. A mirror was placed beside the tube so that latency could be measured as the time between when the stimulus hits the surface and the moment the fish commenced an attack or escape response. Fish were separated into the three treatment groups: untrained fish with a strong stimulus, untrained fish with a food stimulus, and trained fish with a food stimulus. For the strong stimulus treatment (EMS), a 12 cm long, 174.4 g pendulum was dropped from the top of the tube with an electromagnet with 110 cm of line so that only the 12 cm pendulum dropped below the tube opening. The untrained food stimulus treatment (UFS) had a 4 g piece of thawed, store-bought shrimp dropped from the top of the tube. For the trained food stimulus treatment (TFS), the testing procedure is the same as UFS but on trained fish. The fish were trained by

enticing them to consume thawed store-bought shrimp using a feeding device constructed of a de-barbed hook (size = 1) and monofilament fishing line (8 lbs test). After acclimating to feeding on the shrimp, the food items were incrementally dropped into the tank from above until reaching a height similar to the tube. Using this method in combination with the feeding device three times a day (7:45, 12:45, and 18:45), sculpin learned to associate the stimulus with food in 3-7 days.

For testing, sculpin were placed in the filming tank and given 30 minutes to acclimate. The stimulus was dropped from the tube while a high-speed video camera (Casio Exilim ZR100, 240fps) filmed the dorsal view of the behavior. Three responses per fish were gathered with a minimum of 30 minutes rest in between each trial. To ensure the responses gathered were either an escape from, or an attack on a stimulus, routine turns (with no stimulus) were filmed by placing a GoPro hero4 Silver above the holding tank. Ten routine turns were randomly selected for comparisons to the stimulus treatments.

Kinematic analysis

For kinematic analysis, three points along the fish were manually digitized in Kinovea (version 0.8.15). These points corresponded with the snout, approximate CM, and the caudal peduncle. During an escape or an attack, latency, stimulus angle, stimulus distance, turning rate (velocity), post-response linear velocity, and direction of their escape/attack (towards or away) were measured. Latency was calculated as the time from when the stimulus hits the surface of the water (as seen in the mirror attached to the tank wall; T0) to the first frame when movement occurs in the fish. The stimulus angle was measured at T0 from the midline of the fish to the front of the tube. Stimulus distance was also measured at T0 and was the distance from the fish's snout to the front of the tube. Direction of escape/attack was categorized into two classifications, towards or away. The response was classified as towards if the fish angled its head towards the stimulus and swam in a direction that reduced the distance between the fish and the stimulus. In an "away" response, fish increased the distance between themselves and the stimulus. All films recorded fit into these two classifications.

Kruskal-Wallis Rank Sum tests were used to statistically compare the treatments for latency, turning rate, and post-response linear velocity. When significant differences were found, a Dunn post-hoc test was used to reveal which treatments were significantly different.

Results

Overall, our results indicate differences in performance among attack and escape treatment groups (Table 1). When response latency was compared among the strong electromagnet stimulus (EMS), the food stimulus on untrained fish (UFS), and the food stimulus on trained fish (TFS), both treatments with untrained fish had a shorter latency than the trained fish (Figure 1). There was no significant difference in latency between the EMS and the UFS ($z= -0.697$, $p= 0.243$), but the TFS treatment was significantly different from the EMS ($z= -3.079$, $p=0.001$) and UFS ($z= 2.480$, $p=0.007$) treatments with median latencies 7.23 and 2.52 times longer,

respectively. All turning rates were faster than the routine turns (Median ±MAD = 95.56±36.38 degrees s^{-1}; range = 1.186-139.394 degrees s^{-1}) so the fish were responding to the stimulus. The EMS treatment resulted in the fastest turning rates, with a significant difference between both UFS (z= 2.245, p= 0.012) and TFS (z= 2.244, p=0.0124) while the turning rate of both trained and untrained fish to the food stimulus were the same (z= -0.317, p= 0.376; Figure 2A). Fish that received the strong stimulus had a faster linear velocity away from the stimulus than both the UFS (z= 1.753, p= 0.040) and TFS (z= 2.237, p= 0.013; Figure 2B). The post-response linear velocity was the same for both food stimulus treatments (z=-0.691, p= 0.245). The trained fish consistently respond to the stimulus by going towards the source of the stimulus, however, in both untrained treatments, the fish escaped but the direction in reference to the stimulus source changed. In the EMS treatment, 41% of the fish move towards the stimulus when trying to escape and in the UFS treatment the direction was 50% towards and 50% away from the stimulus (Figure 3).

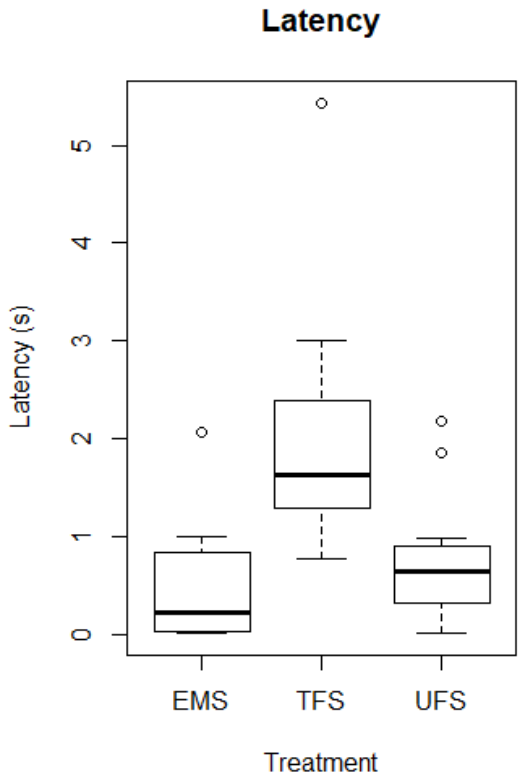

Figure 1. Latency of response to a stimulus.

Table 1. Median ± Median Absolute Deviation and Range (minimum-maximum) of filtering variables (shaded gray) and kinematics for each stimulus treatment.

	EMS		**TFS**		**UFS**	
	Med±MAD	Range	Med±MAD	Range	Med±MAD	Range
Distance from Stimulus	27.89±13.66	5.62-41.43	23.51±1.81	9.16-45.87	23.15±9.12	10.34-37.24
Angle from Stimulus	81±21.50	61-128	49±28.17	5-120	72±14.09	49-104
Length (cm)	16.35±3.11	13.5-30.9	18.1±2.52	13.9-21.6	16.8±5.115	12.7-28.2
Latency (s)	0.23±0.30	0.01-2.07	1.62±0.57	0.77-5.44	0.65±0.38	0.01-2.18
Turning Rate (Degrees/s)	1168.87±916.19	259.26-3062.5	500±172.97	188.48-616.67	451.50±323.60	133.69-2297.30
Linear Velocity (cm/s)	62.21±26.63	33.27-119.40	24.40±9.53	17.97-110.66	40.56±33.25	12.93-117.82

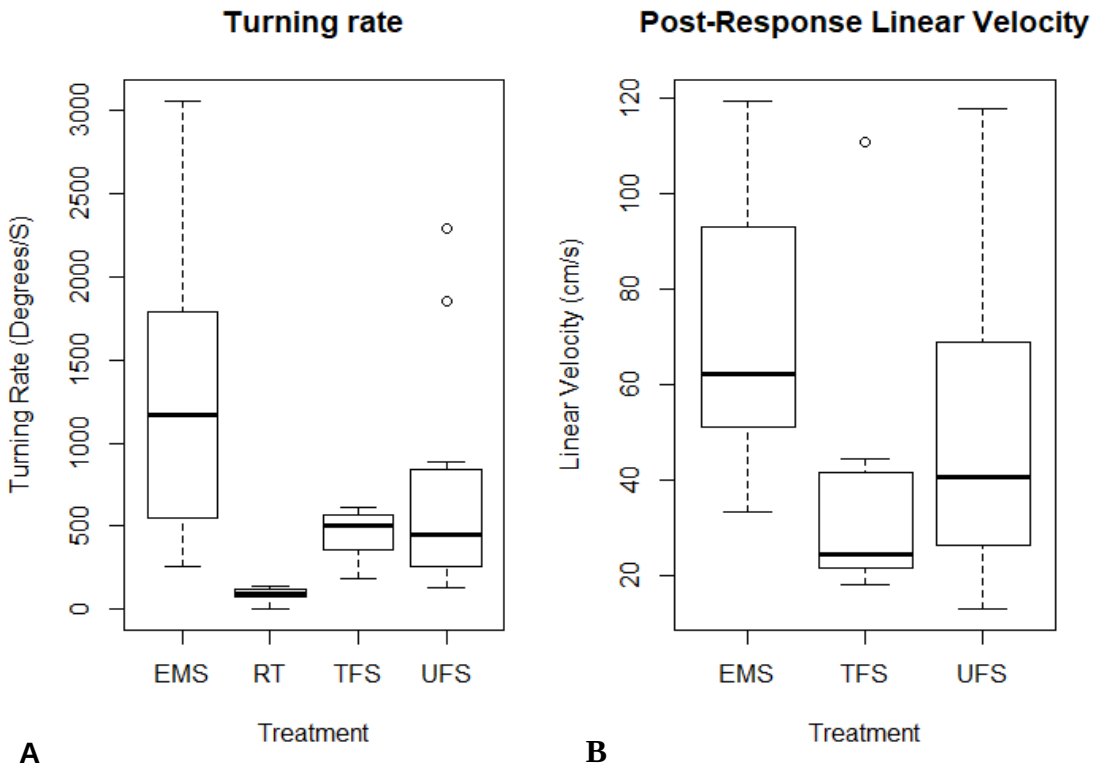

Figure 2. (A) Turning rate and (B) Linear velocity after a stimulus.

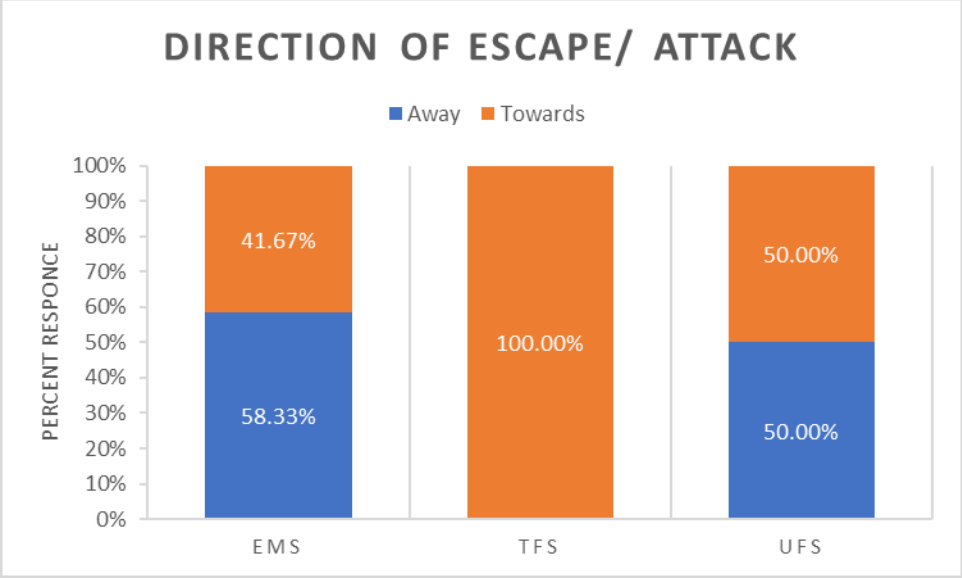

Figure 3. Direction of escape and attack responses. Untrained fish in both stimuli treatments moved either towards or away from the stimulus. Towards is classified as the fish angling its head towards the stimulus and swimming in a direction that reduced the distance between the fish and the stimulus while away responses increase the distance between the fish and the stimulus. The trained fish consistently moved towards the stimulus.

Discussion

We were able to alter the sculpin's behavior with the training protocol which led to differences in attack and escape responses across all kinematic variables. Further, while the magnitude differs among escape stimuli, patterns suggest that the mechanism used for fast-start escape responses away from a stimulus may also be used to attack prey in the trained great sculpin. However, contrary to our prediction, the longer latency for trained fish suggests other mechanisms may also contribute to differences among our treatments.

While we did not directly test the role of mauthner cells in these responses, similarities in the turning rate and linear velocities of trained and untrained fish to the same stimuli suggest similar mechanisms. Furthermore, in our trained treatment, we found reduced variation in both the turning rate and linear velocity compared untrained treatments. This result suggests that Sculpin, like archerfish, may be able to modify their fast-start behavior to increase the precision of prey capture. Finally, in untrained fish, the direction the fish choses to move is stochastic; a burst in a random direction to escape a threat, whereas in the trained fish, they consistently went to the source of the stimulus. Overall, our work supports that of Wohl and Schuster (2007), that stochastic fast-start escape responses may be modified to improve prey capture. However, we show that this modification is not particular to the archerfish and that given the right opportunity, a fish can quickly adjust their escape response to improve prey capture.

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Friday Harbor Laboratories- Fish Swimming course 2017- Final Report
Course instructors: Paolo Domenici, John F. Steffensen, Jacob J Johansen

Bidirectional flow regimes affect energetic budgets for a labriform fish: *Cymatogaster aggregata*

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ABSTRACT

Dynamic flow regimes are common in marine ecosystems, but often overlooked in respirometry studies, which tend to measure energetic costs of marine organisms swimming in a steady, constant direction. While some studies have attempted to measure the energetic costs of fish in unsteady flows, none have measured the additional costs fish may experience if they need to turn in the flow. Here, we demonstrate a novel technique to simulate a surge environment in which fish would have to turn 180º to maintain position. Using Shiner Perch, *Cymatogaster aggregata*, as a model species we measured mean oxygen consumption (MO_2) of fish in constant-speed flow as well as two hydrodynamic flow treatments, unidirectional and bidirectional to quantify the additional costs associated with turning to face the flow.

We determined a theoretical MO_2 ($MO_{2,\ \text{Estimated}}$), based on a modified U_{crit} -protocol by integrating the MO_2 into a sine wave function, allowing us to predict the dynamic respiratory oxygen demand (mean = 175.4 mg O_2 kg^{-1} hr^{-1}) at various water flow velocities (mean = 1.59 BL s^{-1}). Moreover, based on the properties of the sinusoidal wave function, we calculated, on average, a 47% increase in the oxidative respiratory demand for turning orientation in the reversing water flow (bidirectional flow) (255.8 mg O_2 kg^{-1} hr^{-1}) compared to the uniform wave (unidirectional flow) (173.6 mg O_2 kg^{-1} hr^{-1}). We conclude that increased costs associated with dynamic flow could have adverse implications for fish living in high surge ecosystems, potentially intensifying with predicted climate change (e.g. increased turbulence, temperature and altered water oxygen content).

INTRODUCTION

Standard swimming performance experiments are often conducted using constant, steady flows (Beamish 1964; Brett 1964, 1971, 1972; Steffensen 1984; Kramer and McLaughlin 2001; Liao 2007), not taking the energetic costs associated with swimming with variable water flow (Roche *et al*. 2014). Such constant conditions of flow is rarely a true reflection of natural conditions, as aqueous ecosystem are experiencing dynamic, unsteady water exchange (velocity, turbulence, periodicity, gravity, wind, etc.) (Denny 2006; Roche *et al.* 2014). These effects are pronounced in shallow, rocky reefs and shorelines, and hence, may have potentially profound effect on the metabolism of the inherent organisms. Traditionally, these energetic budgets have been determined for aquatic breathers measuring oxygen uptake rates (Nelson 2016); however, using conventional steady-flow methods might underestimate the actual metabolic costs of swimming performance and locomotion, as the hydrodynamical properties of the waterbody is neglected *in vitro* (Denny 2006; Liao 2007; Roche *et al.* 2014, Webb *et al.* 2010).

Water exchange is beneficial for ecosystems, maintaining water quality and food availability, influencing organismal and behavioural routines, and migration of conspecifics etc. Some stationary organisms subjected to unsteady flows are compelled to keep resuspended in the water column to avoid dispersal (Liao 2007). In general, benthic species (i.e., species associated with the seafloor) exhibit morphological and behavioural traits allowing them to reduce exposure to ambient hydrodynamics, by refuging and interact with the substrate (Webb 1989). However, in turn, many pelagic schooling fish utilize the characteristics of the open water profile to forage, and rely on station-holding behaviours on reefs. These species often maintain their spatial position above the substrate, showing high site-fidelity to shelter, and hence, must swim to accommodate daily hydrodynamical fluctuations to avoid dispersal (Webb 1989).

Despite the ecological benefits from residing areas of dynamic water movement, station-holding fishes must continuously adjust to meet the conditions that are presented: For example, as water flow increases velocity, fish must accelerate and increase their swim speed, thus requiring a greater amount of energy relative to maintaining a constant speed (Liao 2007; Roche *et al*. 2017). Adjusting to meet faster and potentially more strenuous conditions

may incur excess metabolic requirements; however, if water flows are more cyclical (i.e., wave surge), then fish may need to continuously match changing water velocities.

Previous work shows that the labriform swimmer, shiner perch (*Cymatogaster aggregata*) exposed to a unsteady flow treatment, consisting of a repeatable, unilateral wave surge, incurred an increase in energetic cost by 25.3 % compared to steady flow treatments (Roche *et al.* 2014). In addition, some species adjust to current changes by making a whole-body rotation, maintaining their spatial orientation relative to the benthos, to face the direction of the current (Hamner et al, 1988). The metabolic requirements for these behaviours is not well-documented in unsteady, bidirectional flows, and is crucial in our understanding of energy budgeting for aquatic breathers when simulating natural conditions (Liao 2007).

Here we mimicked natural wave-driven water movement by simulating two standardized, repeatable, sinusoidal wave functions (*e.g.*, bidirectional- and unidirectional, unsteady flow). Hereby allowing us to reproduce the flow experienced by inshore fish, and determine the energetic costs (MO_2*:* oxygen uptake rates) of swimming and turning in a dynamic flow for the shiner surfperch. Oxygen uptake rates were also measured by intermittent-flow respirometry at various swimming speeds (U) in a steady, laminar flow.

We compare the energetic costs for locomotion in two dynamic flow treatments (bidirectional- and unidirectional flow) with a theoretical, estimated MO_2, in order to investigate the additional oxidative respiratory (MO_2) component associated with maintaining spatial positioning in an altered flow.

MATERIALS AND METHODS

Experimental animals and husbandry

Shiner perch (n = 18; total length = 11.71 ± 0.36 cm; mass =21.33 ± 1.71 g; means ± s.d.) were caught using a beach seine on San Juan Island, Washington, USA (48°32′N 123°05′W), during August 2017. Fish were held in continuously, flow-through tanks (34 ppt) at the Friday Harbor Laboratories at University of Washington, at ambient temperatures (12.79 ± 1.17º C under a natural light:dark-regime (16:8 h). No feeding were provided during husbandry, and fish habituated in husbandry tanks for at least 24 h prior experimental trials to ensure conforming satiation.

Experimental setup

Swimming performance and oxygen consumption was examined by intermittent flow-respirometry in a 8.45 L plexiglas Steffensen-type respirometer (Steffensen *et al.* 1984; Methling *et al*. 2011), with a working section of 9.0 x 26.0 x 10.0 cm (width x length x depth), immersed in an 35.8 L experimental tank, connected to an aerated 81 L water sump. Water was circulated through a UV filter (TetraPond 19520, 120V, 60Hz; 9.8W) to minimize microbial respiration and reduce background noise. Additionally, the chamber was cleaned with a bleach solution every two days to remove microbial growth.

Oxygen was measured with a fiber optic oxygen sensor (Fibox 3, Precision Sensing GmbH, Regensburg, Germany). The zero calibration was done in 1:1 mixture of Borax [sodium borate] and sodium thiosulfate. All oxygen measurements were monitored and logged with AutoResp V1 (Loligo Systems, Copenhagen, Denmark). Temperature was maintained at 13 ± 0.1º C, controlled by a programmable relay (PR-5714D, PR Electronics, Denmark), connecting to a cooling unit (Lauda Brinkman, RM20 refrigerating circulating bath).

Water velocity, amplitude and water direction (unidirectional and bidirectional) were controlled by LabTech Notebook (https://www.omega.com/), operating a motor-unit (Movitrac AC VFD, SEW Eurodrive, Lyman, SC, USA) via a USB-1208 ADDA converter

(USB DAQ, Data Acquisition, Measurement Computing). Solid blocking effects of the trial fishes in the chamber were corrected by AutoResp.

Water velocity calibration

Water velocity (*cm/s*) in a steady, laminar flow was calibrated using a digital flow meter (TAD W30, Hontzsch, Waiblingen, Germany). The digital flow meter was inserted the working section of the swim tunnel at three different levels (to ensure homogenous water velocity throughout the working area). Starting from a static system, the motor voltage (V) was increased and the corresponding flow was recorded every 0.5V.

The water velocity throughout the bidirectional and unidirectional wave cycles was analyzed and calibrated using digital particle image velocimetry (DPIV) to generate a velocity curve over an entire flow period. A 532nm, 50mW laser line module locator (laserland, Besram Technologies) was mounted above the swim flume and illuminated a thin sheet along the working area. Video was captured at 120FPS at 640 x 480 pixels with a Olympus TG-4 (Olympus Australia Pty Ltd, Macquarie Park, NSW).

Artemia cysts (Sander' Permium Great Salt Lake Artemia Cysts) were used as naturally buoyant tracer particles. Artemia were prepared by soaking the cysts for 40 min (PIV; Lauder and Clark, 1984), after which cysts at the surface were removed and the solution of neutrally buoyant cysts were added to the swim tunnel.

Water Velocity analysis

DPIV images were analyzed using LoggerPro (Vernier, Beaverton, OR). Three particles per frame were tracked over three continuous frames (3 frames, 0.025s) over three entire flow period (5s; 600 frames) for each dynamic flow (bidirectional and unidirectional). Velocity was calculated by measuring the average distance the particle travelled over each measurement period. IgorPro(v7.0.4.1; WaveMetrics, Inc. Oregon, WA, USA) was used to calculate the line of best fit for both flows (Fig. 2).

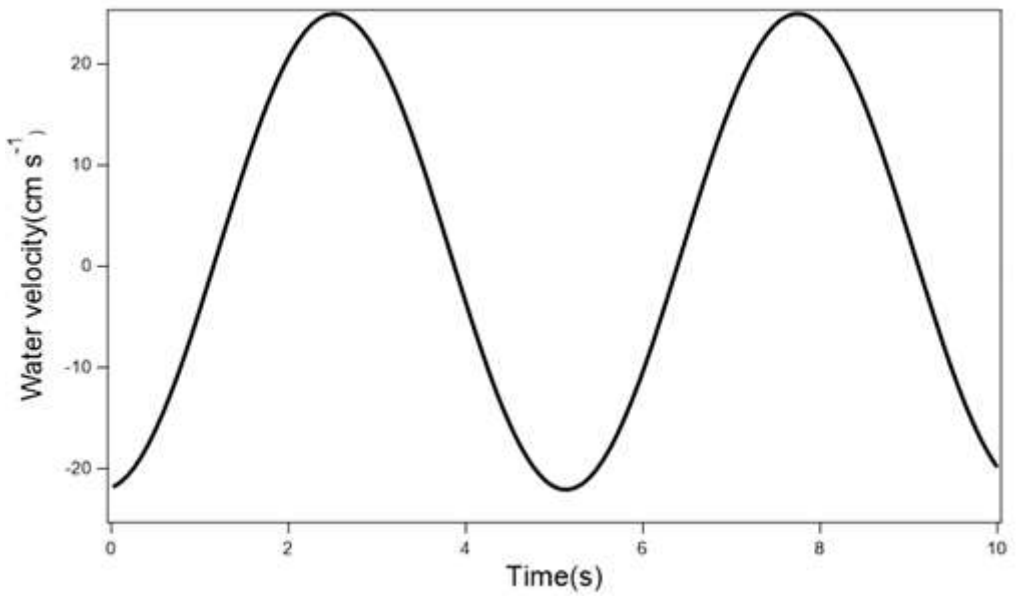

Figure 1: Average water velocity, over 2 wave cycles (5 s) during bidirectional and unidirectional flows calculated using DPIV (water velocity = 1.45018 ± 0.403 + 23.739 ± 0.551 * sin (1.2028 ± 0.00538 * time -1.44185 ± 0.0481).

Oxygen consumption

Oxygen consumption rates (MO_2: mg O_2 kg^{-1} h^{-1}) were used as a proxy for the oxidative metabolism at various swimming speed regimes (Beamish 1964; Chabot *et al*. 2016; Nelson 2016), determined with respect to the linear regression of the decline in oxygen content as function of time (Steffensen *et al.* 1984; Svendsen *et al*. 2016):

$$MO_2 = V_{resp} M_{fish} O_2 \qquad \textbf{\#1}$$

Where O_2 is the slope of the change in oxygen saturation as function of time, V_{resp} is the relative volume of the respirometer subtracted by the volume of the fish (M_{fish}), assuming the density of the water equals the density of the fish (Green and Carrit 1967).

Microbial respiration was determined prior and after each measurement trial, with an extended measurement phase (30 min; $r^2 \geq 0.8$) to ensure linear decline of the oxygen content over time and was subtracted the oxygen consumption.

Experimental protocol

To ensure enough space for turning within the working chamber in the bidirectional flow treatment the dimensions of the respirometer were relatively large; As a result, the mean respirometer:organism volume-ratio was, on average, 398, thereby exceeding the recommendations (<150) for swimming respirometers according to Svendsen *et al*. (2016). Therefore, to ensure linear decline (with an $r^2 \geq 0.8$) of oxygen as function of time, oxygen uptake rates were determined from a series of continuously, intermittent experimental cycles, consisting of a 900 s measurement phase, followed by a 240 flush- and 60 s wait-phase.

Steady swimming protocol

MO_2 (**eq. 2**) at varying unidirectional water flow velocities was established following a modified standard critical swimming speed protocol (U_{crit}) (Brett 1964). Prior to each swimming trial, individual fish (n = 7) were introduced to the respirometer 3-4 h before measurement at a steady speed of 0.5 BL s^{-1}, allowing the fish to habituate and stabilize MO_2 (Chabot *et al.* 2016; Roche *et al*. 2014). Water flow velocity (U) was incrementally increased by 0.5 BL s^{-1} following every third experimental cycle. Measurements were stopped following U = 3.0 BL s^{-1}, and all individuals were transferred to their holding tanks. No fish were observed to fatigue at any of the flow velocities.

A three-parameter, hydrodynamic-based power function was fitted to MO_2 as function of swimming speed (Roche *et al.* 2014) for the steady flow swimming protocol. The relations are described by the following equation:

$$MO_{2Ucrit} = a + b*U^c \quad \#\,\mathbf{2}$$

Where a is equal to the theoretical oxygen consumption at standard metabolic rate at zero speed ($MO_{2,\,@SMR}$), b is the linear coefficient and U is the water flow velocity increasing with its exponent c.

Unsteady swimming protocol

A Computer-generated sine-wave function (**eq. 3**) simulated moderate wave periods (5 s) similar to the San Juan Islands, Washington inshore coasts (Finlayson 2006; Roche et al 2014) (Figure 1). The relations are described by the following equation:

$$U(t) = \alpha \cdot [2/\pi] \text{ \# } \mathbf{3}$$

Where U is the flow velocity as function of time (t), andαequals the amplitude of the sinusoidal wave (2.5 BL s^{-1}) (Figure 1: solid, red line and dashed, blue line).

(1) Applying the sinusoidal wave function properties to the flow regimes, the water direction was reversed every half of a wavelength period (2.5 s) (hereafter referred to as bidirectional flow regime) (Figure 1: dashed red line).
(2) A one-way, uniform wave function (hereby referred to as unidirectional flow regime) equalled a one-half of the wavelength of the sinusoidal wave period (2.5 s), *e.i.* corresponding to the amplitude (α) and velocity (Figure 1: dashed purple line).

Prior to each swimming trial, test subjects (n = 11) were introduced to the respirometer 3-4 h before measurement at a steady speed of 0.5 BL s^{-1}, allowing the fish to habituate and stabilize MO_2 (Chabot *et al*. 2016; Roche *et al.* 2014). For each individual, the unidirectional or bidirectional flow regime were randomly introduced: Data acquisition were collected through three experimental cycles, before returning to the initial steady flow (0.5 BL s^{-1}), allowing them to settle down and restore baseline MO_2 (steady state), before being introduced to the alternative flow regime for an additional three experimental cycles.

Estimated oxygen uptake rates

We estimated MO_2 ($MO_{2,\text{ Estimated}}$) (Figure 1: Solid green line) as a reference-profile in relation to the nonlinear oxygen consumption properties (Roche *et al*. 2014), associated with the effects of maintaining spatial position in sinusoidal varying water flow velocities (Roche *et al*. 2014). This was achieved by integrating the MO_2 (**eq. 2**) obtained in the steady,

unidirectional swimming protocol at varying flow velocities into into the sinusoidal function (**eq. 3**). The relations are summarized by the following equation:

$$MO_{2,\,Estimated} = a + b * (\alpha \cdot [2/\pi])^{c} \qquad \#\ \mathbf{4}$$

According to the sinusoidal water flow direction (Figure 1. solid, red line), negative sign of flow velocities indicate reversed flow direction, hence, average water velocity (1.59 body length s^{-1} [BL s^{-1}]) was calculated as the average of the positive-half of the sine-wave function ($\alpha \cdot [2/\pi]$, α = 2.5 BL s^{-1} [maximum water velocity]) (corresponding to 70% of U_{crit} for *C. aggregata*; (Roche *et al*. 2014)).

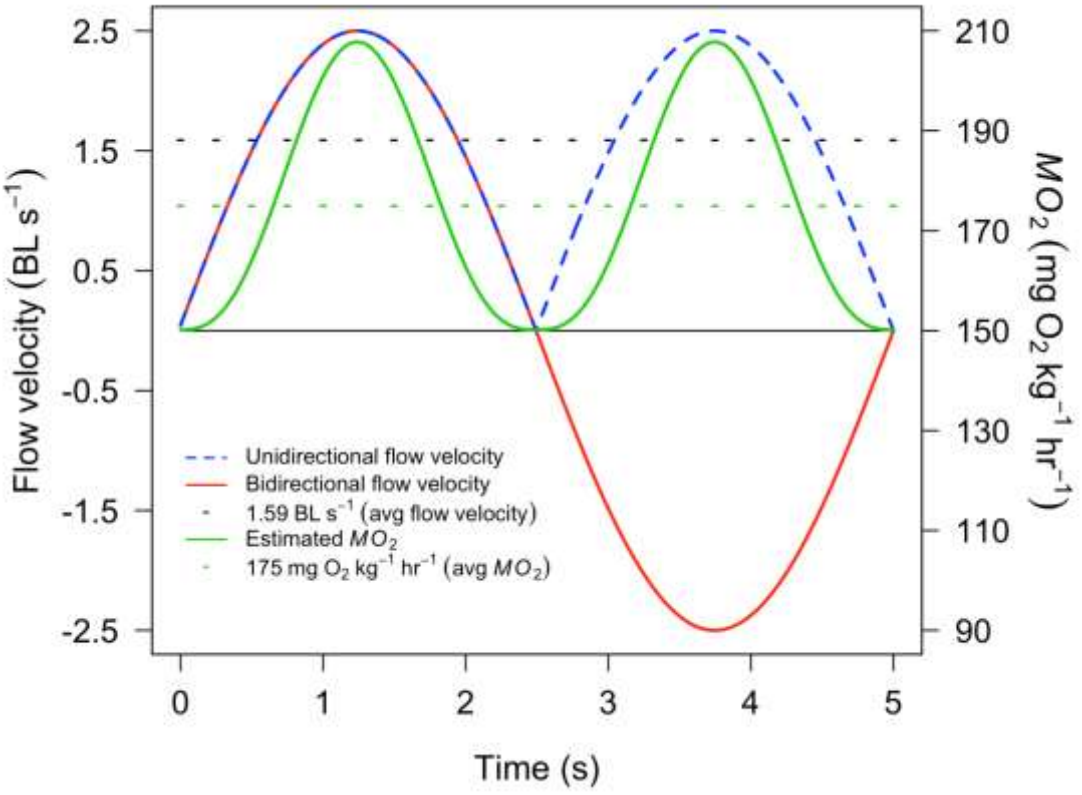

Figure 2: Computer generated wave functions for uni- and bidirectional flow regimes: Conceptualization of the bidirectional (solid red line) and unidirectional (dashed blue line) wave flow (double wavelength period = 5 s; α= 2.5 BL s^{-1}), both treatments had an average flow velocity of 1.59 BL s^{-1} (dashed black line). $MO_{2,\,Estimated}$ (solid green line) and mean $MO_{2,\,Estimated}$ (dashed green line) is based on the integration of MO_2 (**eq. 2**) into a sinusoidal function (**eq. 3**), hereby predicting the dynamic, nonlinear oxygen uptake rates associated with the varying velocities for the opposing flow regimes (**eq. 4**).

Laterality

Swim tunnel turning behavior

To investigate the degree of lateralization in the respirometer, we monitored full-length bidirectional flow-treatments (n = 9) using a Panasonic MiniDV camcorder (10 FPS, 640 x 420). The turning chosen to either the right- or left side was recorded for five consecutive time periods (each 1 min) (to a total of 120 turns). Time periods were selected at least five minutes following the learning period, which we defined as time the test subject used to perform ten unimpeded, consecutive turns in the respirometer, without being compromised by the respirometer walls. This was conducted, as the embedded adjustment to the respirometer conditions comprised increased respiratory oxygen demands, not being equivalent to perceived natural conditions. Following the trial, fish were placed into individual recovery tanks.

To investigate lateralization and turning preference of *C. aggregata* each test subject (n = 9) was transferred from the recovery tank to the center of a double T-maze runway (127 x 60 x 25 cm, length x width x height; center runway: 76 x 17 cm, length x width) (Figure 3), to undergo a detour test at least 4 hours following MO_2 measurements.

Following habituation (10 m) in the center of the test arena, the detour to the left - or right for each test subject was observed from a total of ten trials: For each trial the relative lateralization (L_R) was indexed from -100 to +100 based on the priority of turning right or left, respectively, quantifying the direction and degree of lateralization (Bisazza *et al*. 1998; Dadda *et al*. 2010)

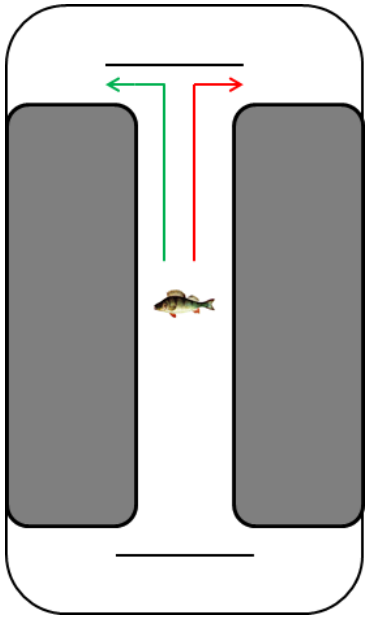

Figure 3: Double T-maze for detouring test setup. The preference of left- or right turning for individual test subject was recorded using a relative lateralization index, according to Bisazza et al. (2010). See text for details.

Data analysis

An analysis of variance (ANOVA), along with a Tukey's post-hoc test was used to compare MO_2 for bidirectional, unidirectional and mean $MO_{2,\ Estimated}$ from our power-function (Fig. 2).

RESULTS

Intermittent-flow respirometry

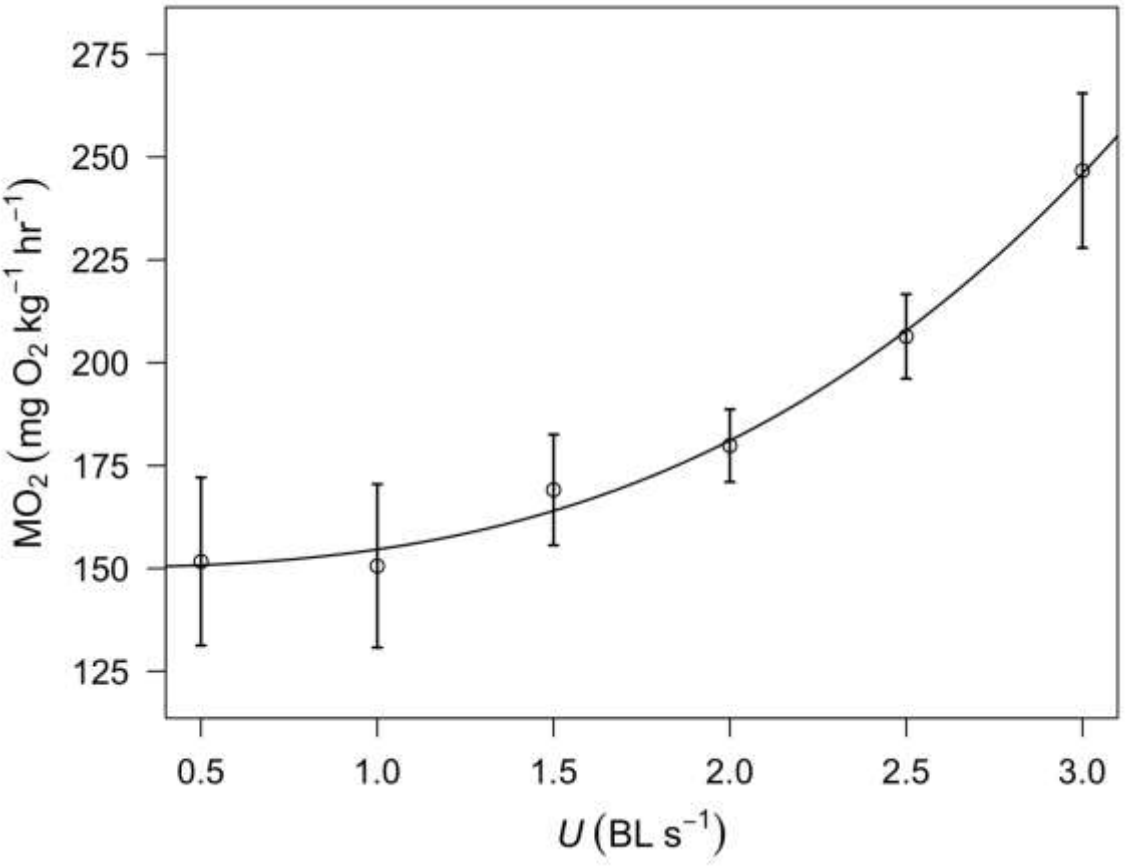

Figure 4: Oxygen consumption rate (MO_2: mg O_2 kg^{-1} hr^{-1}) in relation to swimming speed for *Cymatogaster aggregata* (n = 7) in laminar flow (mean ± S.D.), fitted to a power function: MO_2 = 150.16±3.38 + 4.47 ± $1.74U^{2.79\pm0.34}$. r^2 = 0.99. Swimming speeds were selected below gait-transitioning ($U_{p\text{-}c}$) to ensure fish were demonstrating aerobic swimming (Roche et al. 2014).

A three-parameter hydrodynamic based power function was fitted to the oxygen uptake (MO_2) as function of the swimming speed (U), according to the following equation (**eq. 5**) (Fig. 4) (Roche et al. 2014):

$$MO_2 = 150.16\pm3.38 + 4.47\pm1.74U^{2.79\pm0.34} \qquad \textbf{\# 5}$$

MO_2 increased consistently with swimming speed (U) through the entire water velocity range (0.5 - 3 BL s^{-1}), hence, on average, $MO_{2,\ Estimated}$ was determined to be 175.4 $mgO_2\ kg^{-1}\ hr^{-1}$, calculated as the integrated area under the curve (Figure 1: solid green line) (mean $MO_{2,\ Estimated}$) of the sinusoidal function (cf. **eq. 3**), corresponding to an average water velocity of 1.59 BL s^{-1})

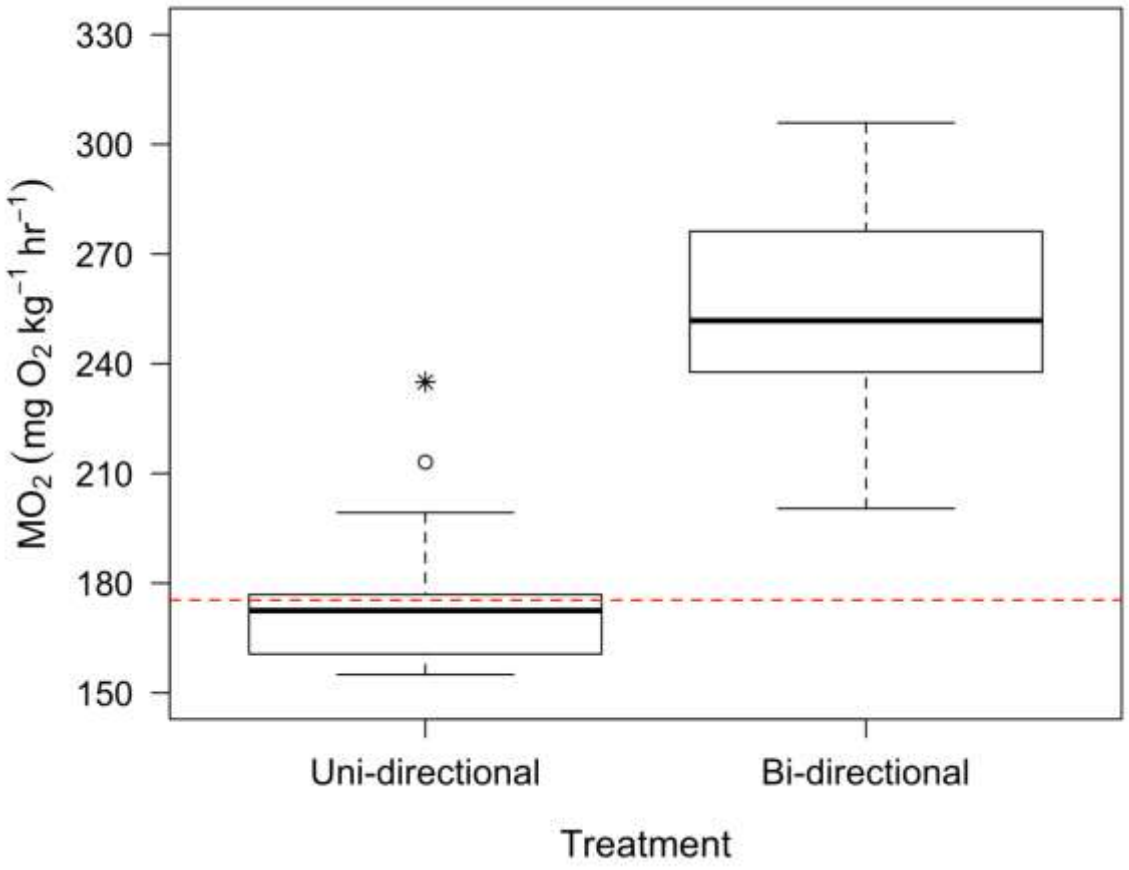

Figure 5: Oxygen consumption rate (MO_2: mg O_2 kg^{-1} hr^{-1}) for unidirectional and bidirectional dynamic flow treatments (n = 11). Boxes represent the first- and third quartiles; bold lines indicating median values. Whiskers denote variability outside of the quartiles and open circles represent outliers. Asterisk indicates $p < 0.001$. The red, dashed line indicates the mean $MO_{2,\ Estimated}$ (175.4 $mgO_2\ kg^{-1}\ hr^{-1}$), derived from the nonlinear relationship between MO_2 and sustained swimming in dynamic flow velocities (**eq. 4**).

There was a significant difference in MO_2, on average, between the unidirectional (173.6 mg O_2 kg^{-1} hr^{-1})- and bidirectional (255.8 mg O_2 kg^{-1} hr^{-1}) flow treatments (ANOVA; $F_{(2,18)} = 29.02$, $p < 0.001$) (Fig. 5). The bidirectional flow treatment demonstrates a 47% increase in mean MO_2 compared to the unidirectional treatment. No statistical significance (Tukey's HSD, $p > 0.5$) was revealed between mean $MO_{2,\ Estimated}$ (175.4 mg O_2 kg^{-1} hr^{-1}) (cf.

eq. 4) and MO_2 of the unidirectional flow treatment (173.6 mg O_2 kg^{-1} hr^{-1}). MO_2, on average, of bidirectional treatments (255.8 mg O_2 kg^{-1} hr^{-1}) was significantly different from mean MO_2 of the unidirectional treatments (Tukey's HSD; $p = 0.008$ and $p < 0.001$).

Quantifying laterality

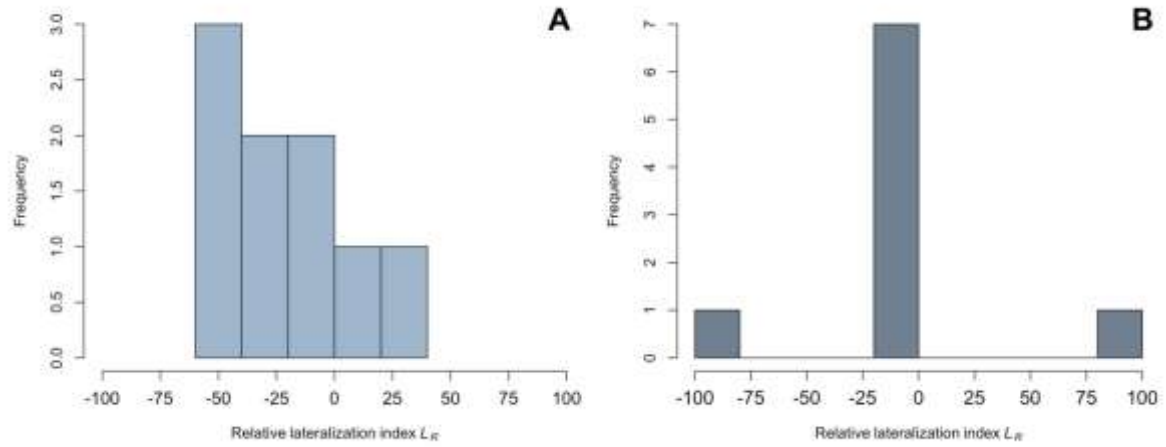

Figure 6: Relative lateralization index of each fish (n = 9) for both the detour-test (A) and in the swim tunnel (B).

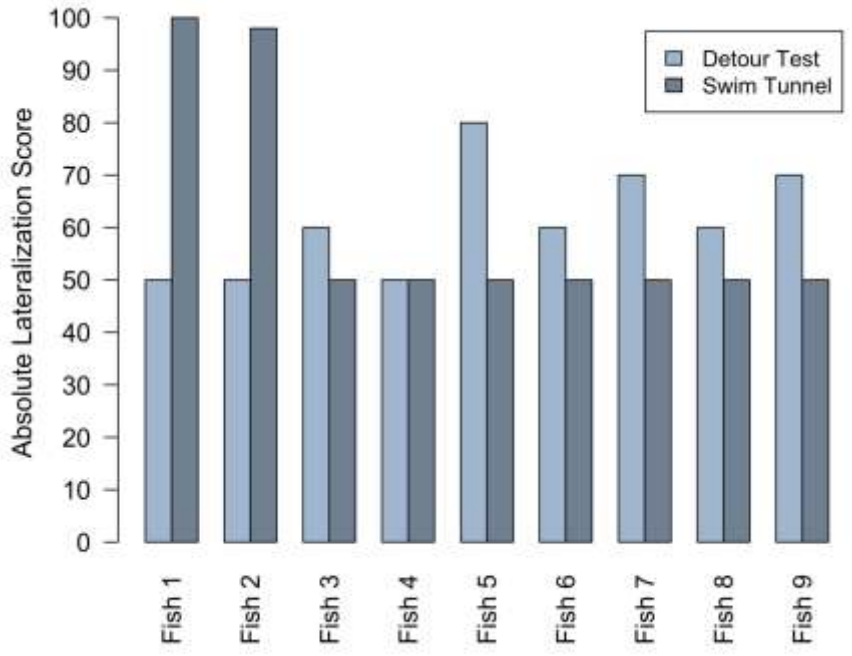

Figure 7: Absolute lateralization score of each fish for the detour-test and in the swim tunnel (n = 9).

Shiner perch took an average of 286 ± 199 s (mean ± S.D.) to adjust to the bidirectional flow. There was no overall relative lateralization of fish in either the detour-test

or the swim tunnel. Fish displayed no absolute lateralization in both the detour-test (61.1 ± 10.5) or the swim tunnel (60.9 ± 21.6) (n = 9) (Fig. 7). There was no significant difference in the absolute lateralization score for the detour-test and in the swim tunnel (t-test; t = 0.027, *df* = 11.6, p > 0.5) (Fig. 5). Of the fish tested only two of the nine demonstrated laterality in the swim tunnel, while only one test subject of the nine demonstrated laterality during the detour-test (Fig. 3). There was no correlation between lateralization and learning time or MO_2.

DISCUSSION

Unsteady water dynamics are ubiquitous throughout the natural world and assuming that fish, and other marine organisms, swim in steady flows highly underestimates the true costs of swimming. Shiner perch had an increased oxygen uptake rate by 47% (82.2 mg O_2 kg^{-1} hr^{-1}) when swimming in the bi-directional flow, while swimming in unsteady, unidirectional flow showed no overall change in oxygen uptake compared with estimated oxygen uptake rates from steady swimming measurements. The excess energetic costs from the bi-directional flow, relative to the unidirectional flow treatment, is the cost required for fish to turn as water directions change. Fish that station-hold must turn regularly to resist displacement, maintain their position, and/or maximise food encounter rate; therefore fish must make trade-offs between energy consumption (foraging) and energy expenditure (swimming and turning).

Station-holding is a common behaviour seen in fish dependent on currents for foraging, mating, and/or site attached fish (Webb 1989). While station-holding can be beneficial (e.g., increased prey encounter rates) if the velocity of the current is too high, fish may face a tradeoff between maintaining position and increased energetic requirements associated with turning. If fish in ecosystems experiencing frequent surge, are unable to contend with the energy required to maintain their position, they must seek out refuges or areas of lower flow, at the cost of decreased foraging (Webb 1989), or else risk being displaced to potentially deleterious habitats (e.g., onto shore or into pelagic zones) (Carlson and Lauder 2010).

Most open shorelines rarely experience prolonged periods of steady flows; therefore, to understand the energetic requirements of fish (and other organisms) it is necessary to simulate natural conditions. Failing to account for unsteady flows, such as wave surge, leads to underestimating the cost of swimming for fish in these ecosystems (Roche et al. 2014). These additional costs associated with unsteady environments are often overlooked due to the difficulty in determining their true value (Liao 2007). While Roche et al. (2014) found a 25.3% increase in MO_2 for fish in unsteady water flow compared to steady, we show that swimming and turning in a bidirectional surge was 47% more costly than when swimming in a uni-directional unsteady flow. Furthermore, this cost was 53% greater than the costs predicted by integrating the cost of swimming in a steady flow along the generated wave.

Overall this species did not show any relative or absolute lateralization in both the swim tunnel and the detour test. This is consistent with previous findings that have been

shown for this species to be non-lateralized (Dadda *et al*. 2010). Two fish showed lateralization in the swim tunnel and not the detour test, which could be due to individual variation, or indicative of lateralization for a certain behavior, which could be beneficial as fish that are lateralized for certain behaviors have been shown to be good at multitasking (Vallortigara and Rogers 2005). However, there was no correlation between MO_2 and laterality.

Further studies should begin to incorporate other factors (*i.e.* environmental conditions) in conjunction with variable hydrodynamic conditions, for example temperature, which is a key driver known to affect fish performance (Johansen and Jones 2011). Hydrodynamic conditions are unavoidable for fish in most ecosystems, while these conditions can be beneficial, fish may be faced with constant energetic trade-offs to maximize fitness.

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Friday Harbor Laboratories- Fish Swimming course 2017- Final Report
Course instructors: Paolo Domenici, John F. Steffensen, Jacob J Johansen

The Effects of Unsteady Flow on the Kinematics of Pectoral Fin Swimming in the Tube-Snout (Aulorhynchus flavidus)

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Abstract

Fish regularly experience unsteady flows in their environments, however most studies on fish swimming are conducted in steady flows. Here we examine how regular changes in water velocity effect the swimming kinematics of the Tube-Snout *Aulorhynchus flavidus*. We measured fin beat frequency, amplitude, and duty factor as *A. flavidus* swam in an unsteady flow and steady flows of different velocities. The unsteady flow consisted of changes in water velocity from 5 to 20 cm/s every 2.5 seconds. Similar to steady flow swimming kinematics, tube-snouts swimming in an unsteady flow increased their fin beat frequency and duty factor, while fin beat period decreased, and fin beat amplitude remained unchanged. However, where fin beat frequency peaked at the highest steady flows, it peaked just before highest water velocity in the unsteady flows. These indicate that fish adjust fin beat kinematics throughout a wave and attempt to minimize displacement.

Introduction

Most studies of fish swimming are conducted in a simplified hydrodynamic environment with a steady flow. However, many fish live in environments with highly dynamic flow which can perturb fish swimming, requiring them to quickly adjust swimming kinematics

in order to maintain their trajectory, velocity, and/or position (Lupandin, 2005). Dynamic flows, similar to what a fish might experience in nature, can be created in a laboratory setting by altering a steady flow. An altered flow can be turbulent or unsteady. Turbulent flows are composed of chaotic vortical flows of multiple strengths and sizes superimposed onto a mean flow, similar to conditions that might be found in a high energy stream. An unsteady flow is one where fluid velocity varies with respect to time for a given point in space (Liao, 2007), but lacks chaotic vortices found in turbulent flows. This flow is similar to wave motion which fish might experience in a near shore environments, such as reefs which are subject to wind and tidally driven waves (Fulton and Bellwood, 2005).

Waves have been found to effect fish energetics, biomechanics, and ecology. Roche et al. (2014) exposed shiner perch (*Cymatogaster aggregate)* to an unsteady flow composed of a unidirectional flow with cyclical changes in velocity. They found that individuals swimming in a high amplitude unsteady flow (velocity amplitudes of 1 BL/s, period of 5 s) had higher swimming costs than would be estimated using steady speed swimming costs. They also found that individuals who had greater variation in fin beat period were better at holding their position in an unsteady flow than fish with a low variation in fin beat period. Flow can also effect ecology by influencing which habitats a fish can effectively occupy given its swimming ability. Fulton *et al.* (2001) found that labrids with fins with high aspect-ratio residuals (fish capable of achieving high swimming speeds) were found in shallow high wave energy habitats, where fish with low aspect ratio fins were found in abundance in low wave energy, deeper water habitats. Fulton *et al.* (2005) followed up with a similar study examining 111 species

across 3 families and found similar results, that wave energy influenced assemblage on the reef through interactions with morphology and swimming ability.

In these shallow wave swept reef environments pectoral fin swimming is the most prevalent swimming mode (Fulton and Bellwood, 2005). Use of the pectoral fins for locomotion is adaptation to living in these complex environment (Gosline, 1994). However, there are multiple forms of pectoral fin swimming. One is lift based swimming where the fish generates a propulsive force on the upstroke and down stroke of a fin beat. Fish can also swim with drag based swimming. Drag based swimming, sometimes called rowing, involves a distinct propulsive power stroke phase and a separate recovery phase which does not generate a propulsive force. Both swimming types can have a refractory period, a portion of the stroke where the fin is held against the body and the fish “glides” through the water. It has been shown that in a steady flow, lift based swimmers can achieve higher sustained swimming speeds than rowing swimmers and that rowing is better for stopping, starting, and yaw turning behaviors (Walker and Westneat, 2001). Roche et al. 2014 focused on the kinematics of a lift based swimmer, *Cymatogaster aggregata* swimming in an unsteady flow. Whilst previous studies have explored the kinematics of body-caudal fin and lift-based pectoral fin (Roche *et al*., 2014) swimming in unsteady flow regimes, to our knowledge, the kinematics of drag-based pectoral fin swimming is yet to be characterized in an unsteady flow.

Here we investigate the swimming kinematics of the drag-based pectoral fin swimmer tube-snout, *Aulorhynchus flavidus* in an unsteady, wave-like flow. *A. flavidus* is a planktivorous, coastal, shallow-water and schooling species native to the north-western

coast of North America. We predict that *A. flavidus* will (i) dynamically adjust their fin beat frequency as water velocity changes over the course of a wave; (ii) fish that exhibit more consistent patterns in fin beat frequency over successive wave cycles will experience a lower spatial displacement.

Materials and Methods:

Fish Husbandry:

Adult *Aulorhynchus flavidus* were collected between the 15th of July and the 11th of August 2017 on the San Juan Islands, Washington, USA, at two sites – Jackson's Beach and Friday Harbour Laboratories. The fish were held at Friday Harbour Laboratories in a 61x15x128cm tank supplied by a flow-through seawater system, with temperature ranging between 10.5 -15.4ºC. The constant seawater flow provided all the necessary nutrition for the planktivorous *A. flavidus*. Trials began within two weeks of fish collection. The experimental protocol was approved by the University of Washington.

Flow Characterization:

Flow speed in the steady flow regimes was measured using a Höntzsch TAD W30 flow-meter. Point measurements were taken at widths of 2cm, 7.5cm, and 13cm, and depths of 2cm, 5cm, and 10 cms, within the centre of the working section (Fig. 1). Each set of point measurements was carried out at 1v increments from 0v – 10v. The results are

displayed in table 1. As flow speed varied across the width and depth of the flow tank, the average speed was used during data analysis.

A unidirectional unsteady flow was created by varying the voltage applied to the propeller motor in the swim tunnel, as in Roche et al. 2014. We used a computer-generated sine function (amplitude = 4.5 volts, mean = 5.5 volts) to manipulate the voltage input to the motor, allowing a unidirectional longitudinal wave to be generated. This wave had a period of 2.5 or 5 seconds (only the wave with a period of 2.5 seconds is reported here) and the velocity ranged from 5 to 20 cm/s. Water velocity data generated from a wave are non-linear. We describe events occurring at times in the wave as happening at either a percentage of the way through a wave or as a number of degrees. 0% (or 0˚) is the “start” of our wave and coincides with peak water velocity. Water decelerates to minimum velocity at 50% (or 180˚) through the wave. Then water accelerates to maximum speed at 100% (or 360˚) to “end” the wave. Since water speed was changed using a sine wave, peak deceleration and acceleration of the water should occur at 25% (90˚) and 75%(270˚) of the way through the wave, respectively. The unsteady flow velocities over the course of a wave were characterized using digital particle image velocimetry (DPIV). *Artemia* cysts were saturated with seawater by soaking them for 40 minutes at an approximate density of $40gL^{-1}$. Positively buoyant cysts were skimmed and discarded, allowing the approximately neutrally buoyant particles to be collected from the water column. The collected particles were used to seed the flume. The vertical midplane of the working area was illuminated with a 1 watt laser of wavelength 445nm, and the setup was recorded with a Megaspeed X4 Pro at 180 FPS and 1280x1024 pixels.

Analysis of the DPIV was performed using the DLT package in MatLab 9.2. The flume was divided into thirds based on depth, allowing the path of particles in the top, middle, and bottom of the water column to be characterized separately. Three particles were used per depth and per frame, giving a total of 9 particles per frame. The velocity was determined at each depth by averaging the distance travelled by each of the three particles over 3 consecutive frames, and doing this throughout 3 wave-cycles for each unsteady flow regime. Resulting velocity changes were found to closely fit (r^2 value of 0.9865) a sin wave function with the following formula: F(x) = 7.00376*sin(2π*1/150*x-0.314138)+12.5 (Fig. 2).

Swimming Trials:

Individual fish were swum in a custom-built plexiglass swim flume (34cm L x 17cm W x 19cm H working section, 11cm water depth) illuminated by two 120 watt lamps and sheltered from exogenous disturbances. Fish were acclimated to the flume for a minimum of 30 minutes prior to starting the trials. Each fish performed three trials in a randomized order: steady flow, low frequency unsteady flow, and high frequency unsteady flow.

Steady flow trails consisted of a 15-minute acclimation to, and then 1 minute of filming at, speeds of 0.045, 0.097, 0.145, and 0.200m s^{-1}. Low frequency unsteady flow trails used a 0.2Hz wave, whilst high frequency unsteady flow trails operated at 0.4Hz. Lateral and ventral views were recorded simultaneously using a MegaSpeed X4 Pro (60 FPS, 1280x1024) and a Logitech HD920 (30 FPS, 1920x1080) respectively, allowing the fish's X, Y, and Z position to be tracked.

Presented here are kinematic data from 12 seconds of video for four steady flow speeds, and one unsteady flow (wave period of 2.5 seconds) from three tube snouts, mean length 8.65cm. We measured displacement, fin beat frequency, fin beat period, fin beat amplitude, and duty factor (the ratio of time spent in the powerstroke as compared to the time of the whole stroke).

Results

Steady Flows

First we looked at how *A. flavidus* changed their swimming kinematics at different steady flow velocities. The kinematics of *A. flavidus* changed with velocity during the steady flow trails which ranged from 4.48 cm/s to 19.99 cm/s. Fin beat frequency increased significantly from a mean of 1.86 Hz at the lowest steady flows speed to 3.04 Hz at the highest speed steady flows (Fig. 3 a). Fin beat amplitude did not change significantly across steady flow speeds (Fig. 3 b). Mean fin beat period decreased significantly from 0.57 s at the lowest steady flows speed to 0.35 s at the highest speed steady flows (Fig. 3 c). Additionally, mean duty factor decreased significantly from 0.32 at the lowest steady flows speed to 0.26 at the highest speed steady flows (Fig. 3 d). Mean displacement per fin beat increases significantly from a mean displacement of 84.78 mm at the lowest steady flow speed to 148.7 mm at the highest steady flow speed.

Unsteady Flow

To analyze kinematics in an unsteady flow we looked at fin displacement, beat amplitude, duty factor, frequency, and period. These variables were plotted for each fin beat against when during a wave that fin beat took place. Results indicate that in an unsteady flow fish changed their fin beat kinematics dynamically. Duty factor ($p<0.01$), fin beat frequency ($p<0.01$), and fin beat period ($p<0.01$) varied significantly with wave speed. Fin beat amplitude did not change throughout the course of a wave (Fig. 4a). Duty factor was lowest after peak wave velocity and highest just after minimum wave velocity (Fig. 4b). As water velocity decreased fish decreased their fin beat frequency, then as water velocity over the course of the second half of the wave fin beat frequency increased (Fig. 4c). Fin beat period showed the inverse trend with fish taking the quickest fin beat before peak acceleration (Fig. 4d). We looked at the relative time spent in the powerstroke phase, refractory period, and recovery period (Fig 5.) In a circular-linear correlation, the powerstroke and refractory periods were found to correlate with the wave cycle ($p<0.01$). However, recovery period did not significantly change over the course of a wave.

To quantify which fin beat pattern minimized displacement while the fish was swimming through a wave. Fin beat pattern was summarized as the mean vector in a polar plot of all the fin beats taken throughout the course of a wave (Fig 6). The relationship between displacement and mean vector is significant ($p<0.01$), with fish with vector values closer to 360° displacing more and vectors closer to 270° displacing less throughout the wave.

Discussion

We exposed *A. flavidus* to a wave-like unsteady flow similar to flows it might experience in its natural environment. We assessed the how a fish with a drag-based swimming mode responds to a wave-like unsteady flow. *A. flavidus* fish dynamically changed their fin beat frequency and duty factor over the course of a wave in order to maintain position in the water column (Fig. 4). The fish also dynamically changed the portion of time they spent in the powerstroke and refractory periods during a fin beat throughout the course of a wave. Time spent in the refractory periods increased as the water decelerated. This, combined with the fact that fish had an extended fin beat period during this time indicates that the fish are coasting through this portion of the wave, letting drag forces passively slow them down. Fish did exhibit behaviors that would increase drag: lateral extension of the pectoral fins perpendicular to the flow during a noticeably longer recovery period of the fin beat. The anal and dorsal fins were also raised in coordination. Extension of the anal and dorsal fins occurred at several points within a wave – however, extension of the pectoral fins perpendicular to the flow was observed only during portions of the wave where the water velocity was decreasing, suggesting a use in braking. On average however, the relative portion of time spent in the recovery period was not significantly longer during the decelerative portion of the wave. This consistent recovery period suggests that the flaring out of all fins to increase drag is more of an “emergency brake” than it is a behavior used regularly for deceleration.

Similar to findings by Roche *et al.*, *A. flavidus* actively adjust fin beat period, duty factor, and frequency varied through the course of a wave-like unsteady flow. *C.agregatta* and *A. flavidus* use these changes in kinematics to match the surrounding water speed.

Importantly, neither species exhibits static kinematics or swim at a static speed which would cause them to drift back as water speed increases above mean speed and drifting forward as the water velocity deceases below the mean speed, to a greater extent than if they dynamically adjust their kinematics. This is despite the fact that "going with the flow," or swimming at a static speed as in a steady flow is potentially less energetically costly. The attempts by these fishes to change kinetics to match water speed and hold station despite potential increased energetic costs indicates that there may be other benefits to hold a position when experiencing an unsteady wave-like flow. For a planktivorous fish like a tube-snout this may include staying in a section of flow with a high prey density.

It remains unclear if there is a fin beat pattern for minimizing displacement. Fish that had a mean fin beat closer to 270 degrees displaced less than fish whose mean fin beat was closer to 360 degrees (Fig. 6). 270 degrees corresponds to the moment in our unsteady flow when acceleration was highest, where 360 degrees corresponds to the moment when velocity was highest. This may indicate that taking more fin beats while navigating accelerating water is an important factor in minimizing displacement, however more data analysis must be conducted. Additionally, we still intend to look into how other fin beat kinematics from steady flows compare to unsteady flows, most specifically, duty factor and the relationship between power stroke, refractory, and recovery periods. We also intend to look at how these changes in kinematics compare to those in a low frequency wave.

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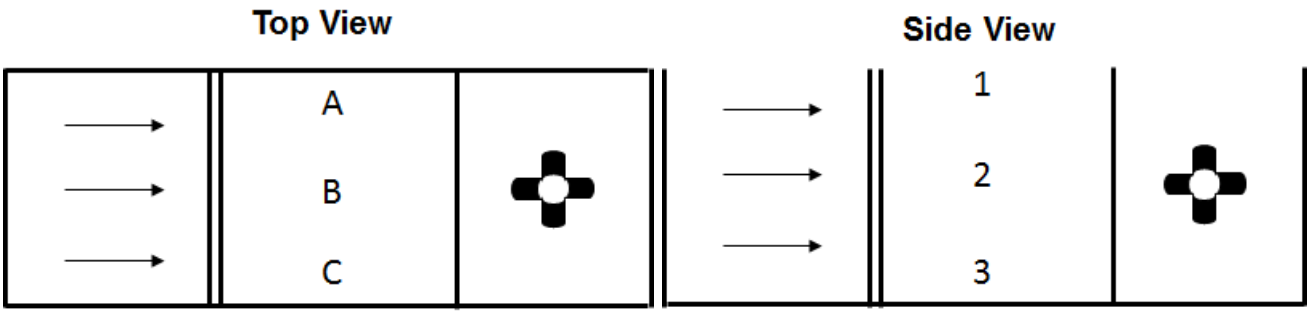

Figure 1. Tank set up. Flow direction is indicated by arrows. Fish swam in the middle section of the tank. Steady flow speed measurements were taken at 3 points across the width of the tank (A, B, and C) and 3 depths(1, 2, and 3).

Voltage	A1	A2	A3	B1	B2	B3	C1	C2	C3
1	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
2	0.002	0.001	0.002	0.002	0.001	0.002	0.002	0.002	0.002
3	0.040	0.040	0.040	0.040	0.035	0.040	0.050	0.040	0.050
4	0.065	0.065	0.065	0.065	0.060	0.065	0.070	0.065	0.065
5	0.090	0.075	0.090	0.090	0.075	0.090	0.090	0.080	0.100
6	0.105	0.105	0.105	0.110	0.100	0.110	0.120	0.105	0.120
7	0.135	0.130	0.135	0.135	0.120	0.135	0.140	0.130	0.145
8	0.160	0.145	0.160	0.145	0.140	0.160	0.160	0.145	0.160
9	0.180	0.175	0.180	0.175	0.160	0.175	0.180	0.170	0.190
10	0.205	0.190	0.205	0.205	0.175	0.205	0.205	0.190	0.205

Table 1. Flow speed measurement results (in m/s). A voltage from 1 to 10 volts was applied to the motor and flow speeds were measured as described in Fig 1.

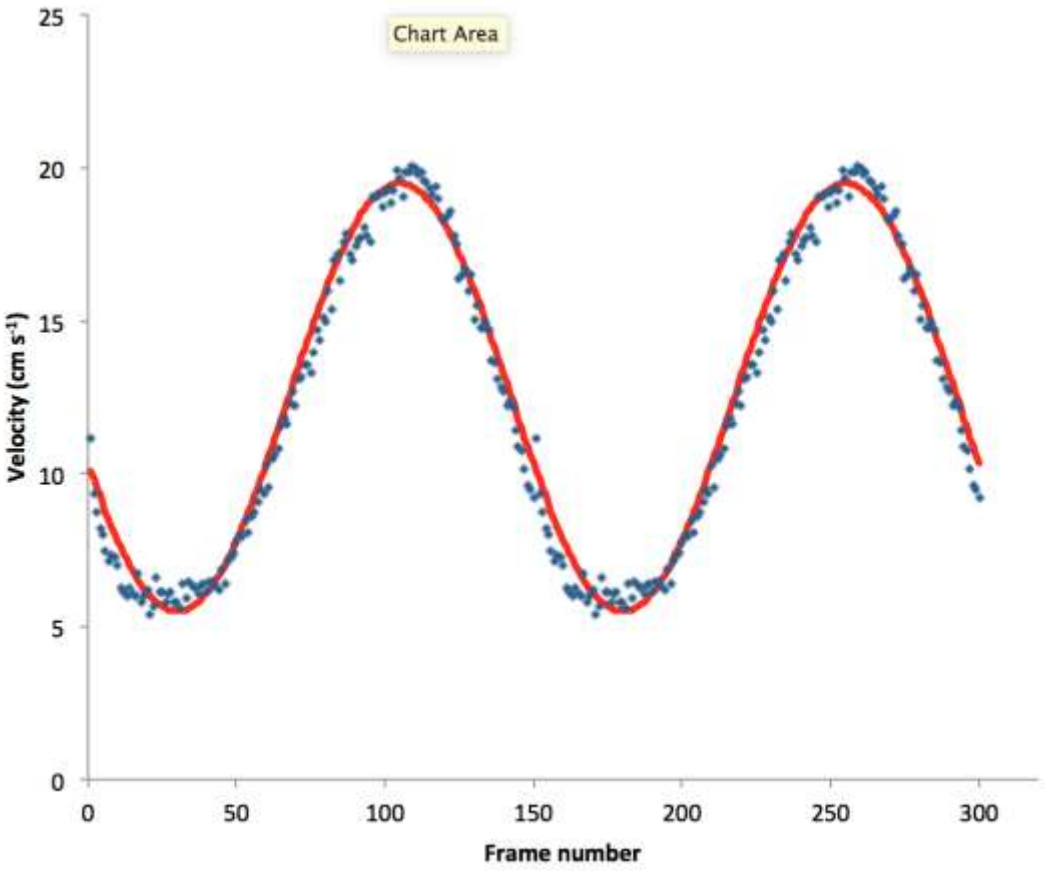

*Figure 2. High frequency flow characterization. Actual water velocity during calibration are plotted as blue points. Sine wave used to estimate water velocity in trials is plotted as a red line. Equation: Y=+-7.00376*SIN((X+0.314138)/1) +12.5.*

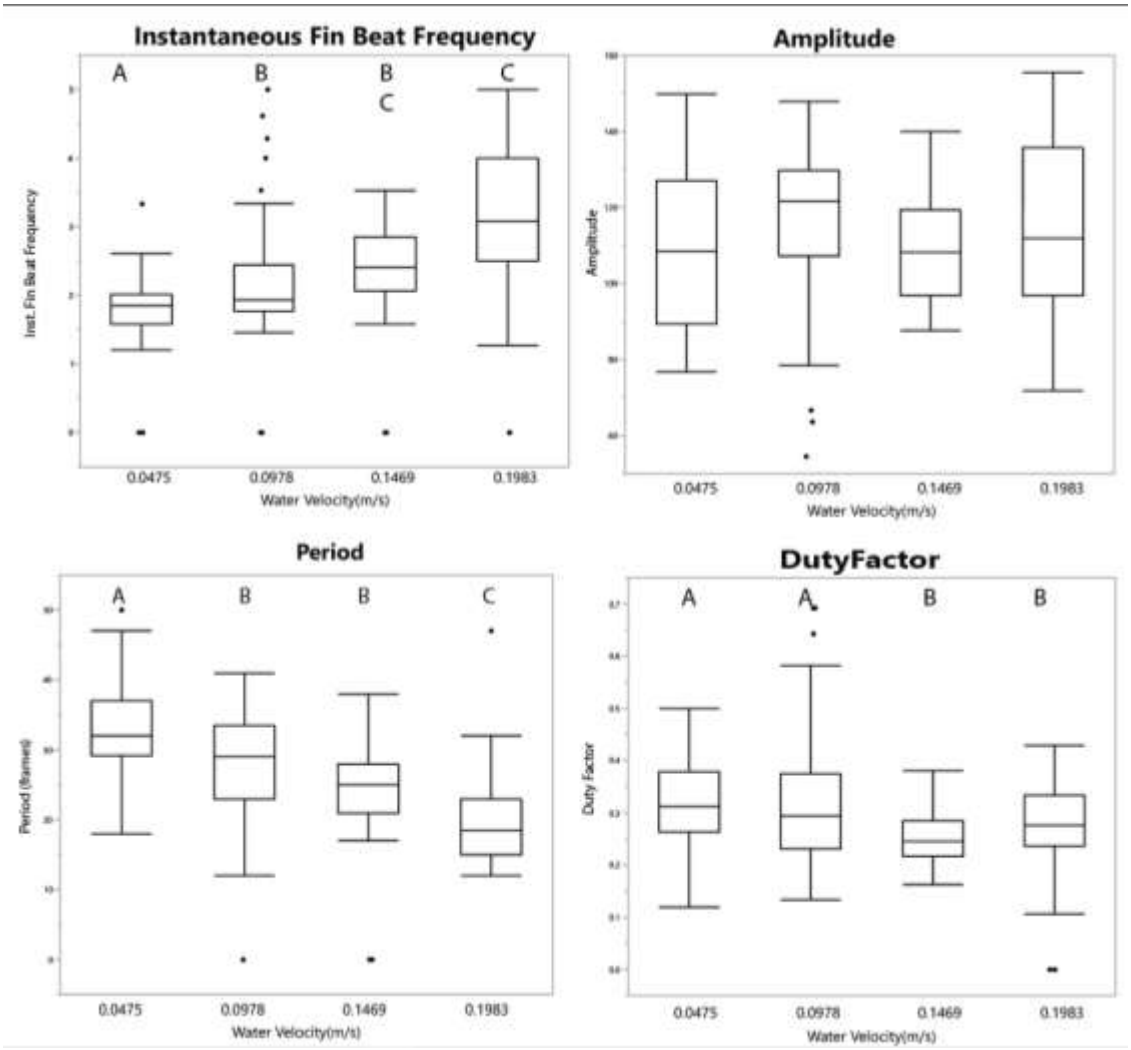

Figure 3. Fin beat kinematics at four steady speed flows.

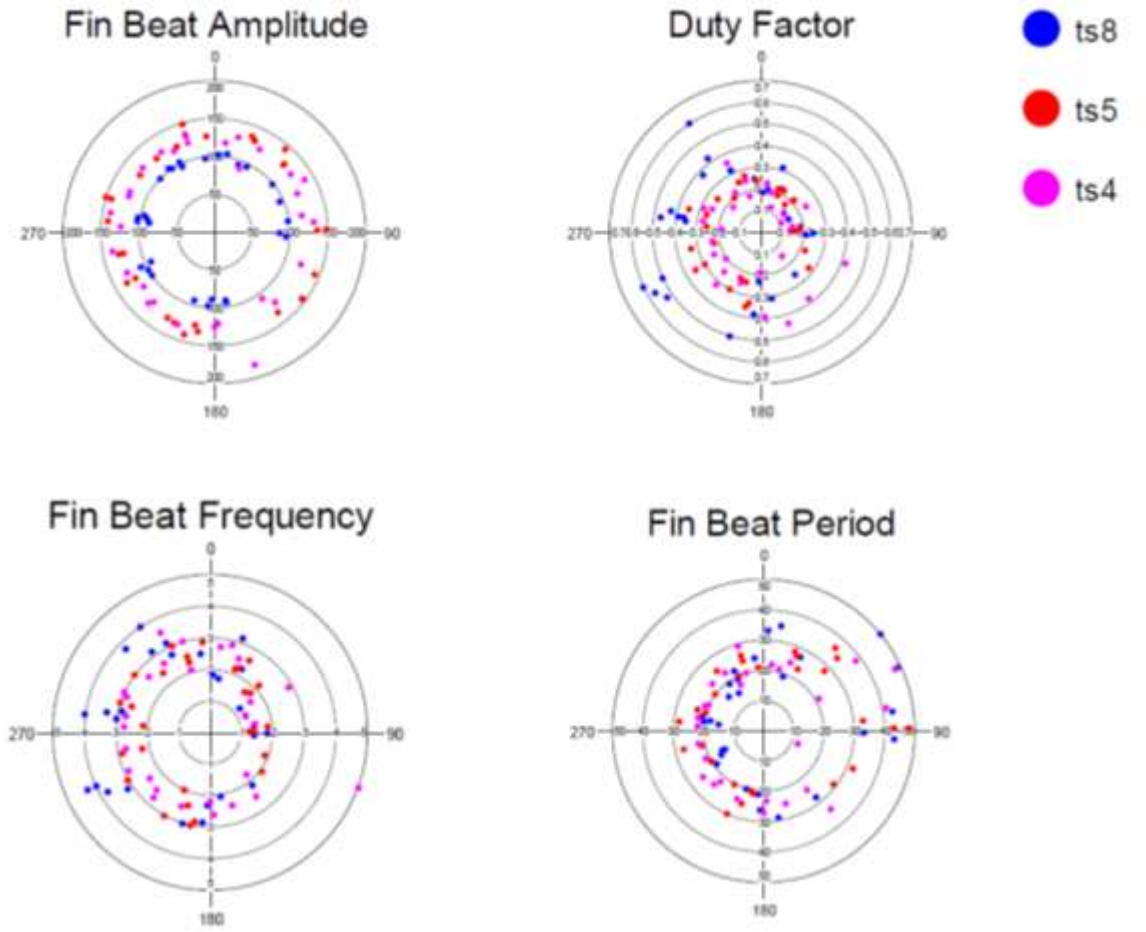

Figure 4. Fin beat kinematics in a high frequency flow. 0(also 360)˚ corresponds to maximum flow velocity, 90˚ is maximum deceleration, 180˚ is minimum velocity, and 270˚ is maximum acceleration.

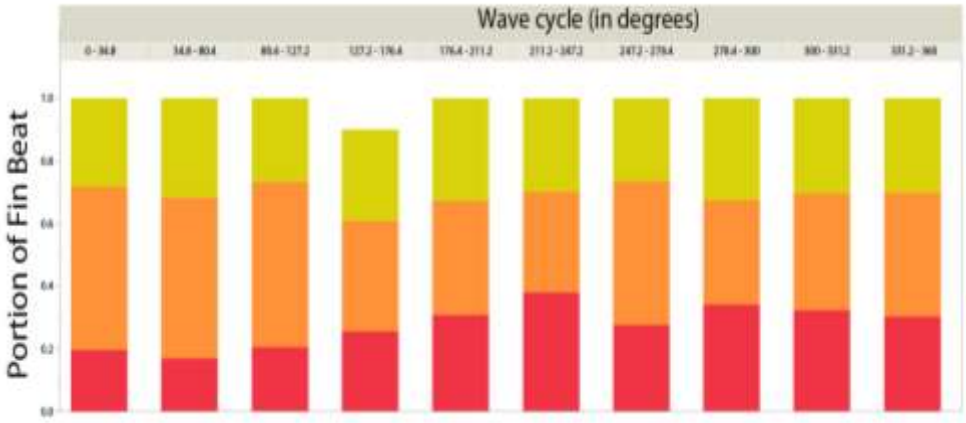

Figure 5. Powerstroke, refractory, and recovery periods expressed as a portion of an entire fin beat over the course of a wave. Powerstroke is in red. Refeactory period is in orange. Recovery period is in Yellow. 0(also 360) ˚ corresponds to maximum flow velocity, 90˚ is maximum deceleration, 180˚ is minimum velocity, and 270˚ is maximum acceleration.

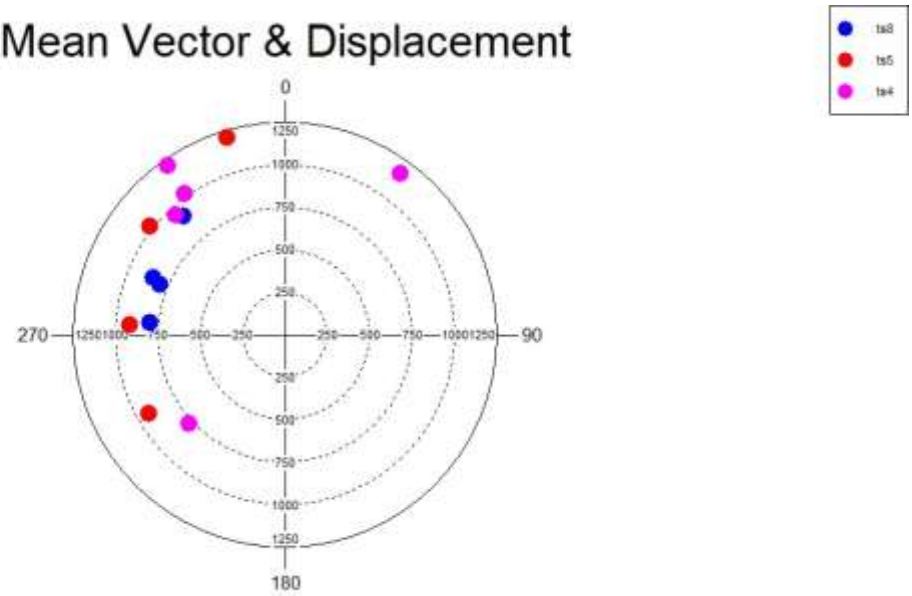

Figure 6. Fin beat pattern in a wave (as summarized by a mean vector) vs. displacement. The angle a point is located at indicates its mean fin beat pattern throughout the wave. Distance from the center indicates displacement.

Friday Harbor Laboratories- Fish Swimming course 2017- Final Report
Course instructors: Paolo Domenici, John F. Steffensen, Jacob J Johansen

Bidirectional flow regimes affect energetic budgets for a labriform fish: *Cymatogaster aggregata*

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ABSTRACT

Dynamic flow regimes are common in marine ecosystems, but often overlooked in respirometry studies, which tend to measure energetic costs of marine organisms swimming in a steady, constant direction. While some studies have attempted to measure the energetic costs of fish in unsteady flows, none have measured the additional costs fish may experience if they need to turn in the flow. Here, we demonstrate a novel technique to simulate a surge environment in which fish would have to turn 180º to maintain position. Using Shiner Perch, *Cymatogaster aggregata*, as a model species we measured mean oxygen consumption (MO_2) of fish in constant-speed flow as well as two hydrodynamic flow treatments, unidirectional and bidirectional to quantify the additional costs associated with turning to face the flow.

We determined a theoretical MO_2 ($MO_{2,\ \text{Estimated}}$), based on a modified U_{crit} -protocol by integrating the MO_2 into a sine wave function, allowing us to predict the dynamic respiratory oxygen demand (mean = 175.4 mg O_2 kg^{-1} hr^{-1}) at various water flow velocities (mean = 1.59 BL s^{-1}). Moreover, based on the properties of the sinusoidal wave function, we calculated, on average, a 47% increase in the oxidative respiratory demand for turning orientation in the reversing water flow (bidirectional flow) (255.8 mg O_2 kg^{-1} hr^{-1}) compared to the uniform wave (unidirectional flow) (173.6 mg O_2 kg^{-1} hr^{-1}). We conclude that increased costs associated with dynamic flow could have adverse implications for fish living in high surge ecosystems, potentially intensifying with predicted climate change (e.g. increased turbulence, temperature and altered water oxygen content).

INTRODUCTION

Standard swimming performance experiments are often conducted using constant, steady flows (Beamish 1964; Brett 1964, 1971, 1972; Steffensen 1984; Kramer and McLaughlin 2001; Liao 2007), not taking the energetic costs associated with swimming with variable water flow (Roche *et al*. 2014). Such constant conditions of flow is rarely a true reflection of natural conditions, as aqueous ecosystem are experiencing dynamic, unsteady water exchange (velocity, turbulence, periodicity, gravity, wind, etc.) (Denny 2006; Roche *et al.* 2014). These effects are pronounced in shallow, rocky reefs and shorelines, and hence, may have potentially profound effect on the metabolism of the inherent organisms. Traditionally, these energetic budgets have been determined for aquatic breathers measuring oxygen uptake rates (Nelson 2016); however, using conventional steady-flow methods might underestimate the actual metabolic costs of swimming performance and locomotion, as the hydrodynamical properties of the waterbody is neglected *in vitro* (Denny 2006; Liao 2007; Roche *et al.* 2014, Webb *et al.* 2010).

Water exchange is beneficial for ecosystems, maintaining water quality and food availability, influencing organismal and behavioural routines, and migration of conspecifics etc. Some stationary organisms subjected to unsteady flows are compelled to keep resuspended in the water column to avoid dispersal (Liao 2007). In general, benthic species (i.e., species associated with the seafloor) exhibit morphological and behavioural traits allowing them to reduce exposure to ambient hydrodynamics, by refuging and interact with the substrate (Webb 1989). However, in turn, many pelagic schooling fish utilize the characteristics of the open water profile to forage, and rely on station-holding behaviours on reefs. These species often maintain their spatial position above the substrate, showing high site-fidelity to shelter, and hence, must swim to accommodate daily hydrodynamical fluctuations to avoid dispersal (Webb 1989).

Despite the ecological benefits from residing areas of dynamic water movement, station-holding fishes must continuously adjust to meet the conditions that are presented: For example, as water flow increases velocity, fish must accelerate and increase their swim speed, thus requiring a greater amount of energy relative to maintaining a constant speed (Liao 2007; Roche *et al*. 2017). Adjusting to meet faster and potentially more strenuous conditions

may incur excess metabolic requirements; however, if water flows are more cyclical (i.e., wave surge), then fish may need to continuously match changing water velocities.

Previous work shows that the labriform swimmer, shiner perch (*Cymatogaster aggregata*) exposed to a unsteady flow treatment, consisting of a repeatable, unilateral wave surge, incurred an increase in energetic cost by 25.3 % compared to steady flow treatments (Roche *et al.* 2014). In addition, some species adjust to current changes by making a whole-body rotation, maintaining their spatial orientation relative to the benthos, to face the direction of the current (Hamner et al, 1988). The metabolic requirements for these behaviours is not well-documented in unsteady, bidirectional flows, and is crucial in our understanding of energy budgeting for aquatic breathers when simulating natural conditions (Liao 2007).

Here we mimicked natural wave-driven water movement by simulating two standardized, repeatable, sinusoidal wave functions (*e.g.*, bidirectional- and unidirectional, unsteady flow). Hereby allowing us to reproduce the flow experienced by inshore fish, and determine the energetic costs (MO_2*:* oxygen uptake rates) of swimming and turning in a dynamic flow for the shiner surfperch. Oxygen uptake rates were also measured by intermittent-flow respirometry at various swimming speeds (U) in a steady, laminar flow.

We compare the energetic costs for locomotion in two dynamic flow treatments (bidirectional- and unidirectional flow) with a theoretical, estimated MO_2, in order to investigate the additional oxidative respiratory (MO_2) component associated with maintaining spatial positioning in an altered flow.

MATERIALS AND METHODS

Experimental animals and husbandry

Shiner perch (n = 18; total length = 11.71 ± 0.36 cm; mass =21.33 ± 1.71 g; means ± s.d.) were caught using a beach seine on San Juan Island, Washington, USA (48°32′N 123°05′W), during August 2017. Fish were held in continuously, flow-through tanks (34 ppt) at the Friday Harbor Laboratories at University of Washington, at ambient temperatures (12.79 ± 1.17º C under a natural light:dark-regime (16:8 h). No feeding were provided during husbandry, and fish habituated in husbandry tanks for at least 24 h prior experimental trials to ensure conforming satiation.

Experimental setup

Swimming performance and oxygen consumption was examined by intermittent flow-respirometry in a 8.45 L plexiglas Steffensen-type respirometer (Steffensen *et al.* 1984; Methling *et al*. 2011), with a working section of 9.0 x 26.0 x 10.0 cm (width x length x depth), immersed in an 35.8 L experimental tank, connected to an aerated 81 L water sump. Water was circulated through a UV filter (TetraPond 19520, 120V, 60Hz; 9.8W) to minimize microbial respiration and reduce background noise. Additionally, the chamber was cleaned with a bleach solution every two days to remove microbial growth.

Oxygen was measured with a fiber optic oxygen sensor (Fibox 3, Precision Sensing GmbH, Regensburg, Germany). The zero calibration was done in 1:1 mixture of Borax [sodium borate] and sodium thiosulfate. All oxygen measurements were monitored and logged with AutoResp V1 (Loligo Systems, Copenhagen, Denmark). Temperature was maintained at 13 ± 0.1º C, controlled by a programmable relay (PR-5714D, PR Electronics, Denmark), connecting to a cooling unit (Lauda Brinkman, RM20 refrigerating circulating bath).

Water velocity, amplitude and water direction (unidirectional and bidirectional) were controlled by LabTech Notebook (https://www.omega.com/), operating a motor-unit (Movitrac AC VFD, SEW Eurodrive, Lyman, SC, USA) via a USB-1208 ADDA converter

(USB DAQ, Data Acquisition, Measurement Computing). Solid blocking effects of the trial fishes in the chamber were corrected by AutoResp.

Water velocity calibration

Water velocity (*cm/s*) in a steady, laminar flow was calibrated using a digital flow meter (TAD W30, Hontzsch, Waiblingen, Germany). The digital flow meter was inserted the working section of the swim tunnel at three different levels (to ensure homogenous water velocity throughout the working area). Starting from a static system, the motor voltage (V) was increased and the corresponding flow was recorded every 0.5V.

The water velocity throughout the bidirectional and unidirectional wave cycles was analyzed and calibrated using digital particle image velocimetry (DPIV) to generate a velocity curve over an entire flow period. A 532nm, 50mW laser line module locator (laserland, Besram Technologies) was mounted above the swim flume and illuminated a thin sheet along the working area. Video was captured at 120FPS at 640 x 480 pixels with a Olympus TG-4 (Olympus Australia Pty Ltd, Macquarie Park, NSW).

Artemia cysts (Sander' Permium Great Salt Lake Artemia Cysts) were used as naturally buoyant tracer particles. Artemia were prepared by soaking the cysts for 40 min (PIV; Lauder and Clark, 1984), after which cysts at the surface were removed and the solution of neutrally buoyant cysts were added to the swim tunnel.

Water Velocity analysis

DPIV images were analyzed using LoggerPro (Vernier, Beaverton, OR). Three particles per frame were tracked over three continuous frames (3 frames, 0.025s) over three entire flow period (5s; 600 frames) for each dynamic flow (bidirectional and unidirectional). Velocity was calculated by measuring the average distance the particle travelled over each measurement period. IgorPro(v7.0.4.1; WaveMetrics, Inc. Oregon, WA, USA) was used to calculate the line of best fit for both flows (Fig. 2).

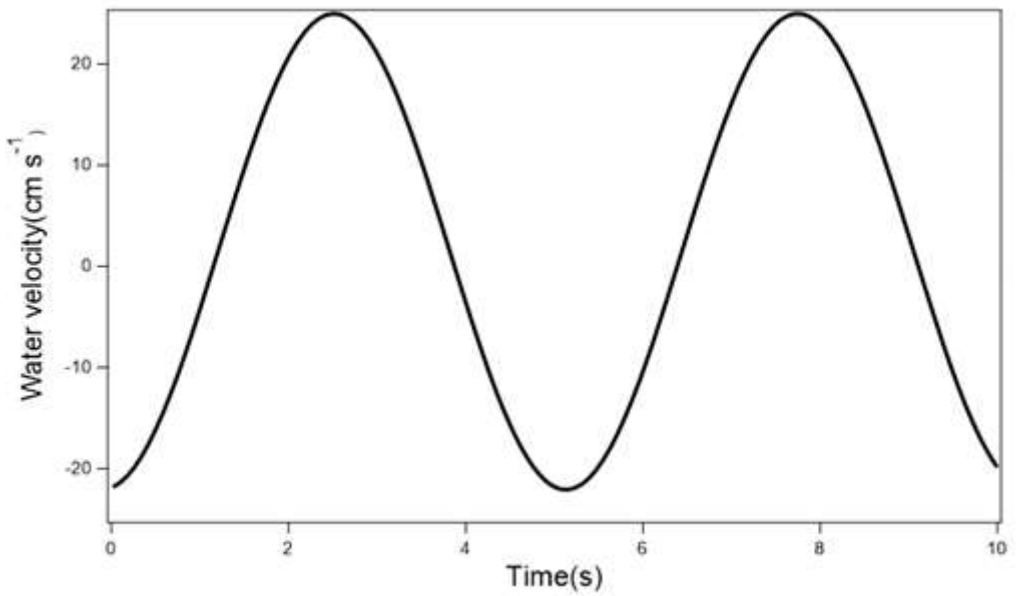

Figure 1: Average water velocity, over 2 wave cycles (5 s) during bidirectional and unidirectional flows calculated using DPIV (water velocity = 1.45018 ± 0.403 + 23.739 ± 0.551 * sin (1.2028 ± 0.00538 * time -1.44185 ± 0.0481).

Oxygen consumption

Oxygen consumption rates (MO_2: mg O_2 kg^{-1} h^{-1}) were used as a proxy for the oxidative metabolism at various swimming speed regimes (Beamish 1964; Chabot *et al*. 2016; Nelson 2016), determined with respect to the linear regression of the decline in oxygen content as function of time (Steffensen *et al.* 1984; Svendsen *et al*. 2016):

$$MO_2 = V_{resp} M_{fish} O_2 \qquad \textbf{\#1}$$

Where O_2 is the slope of the change in oxygen saturation as function of time, V_{resp} is the relative volume of the respirometer subtracted by the volume of the fish (M_{fish}), assuming the density of the water equals the density of the fish (Green and Carrit 1967).

Microbial respiration was determined prior and after each measurement trial, with an extended measurement phase (30 min; $r^2 \geq 0.8$) to ensure linear decline of the oxygen content over time and was subtracted the oxygen consumption.

Experimental protocol

To ensure enough space for turning within the working chamber in the bidirectional flow treatment the dimensions of the respirometer were relatively large; As a result, the mean respirometer:organism volume-ratio was, on average, 398, thereby exceeding the recommendations (<150) for swimming respirometers according to Svendsen *et al*. (2016). Therefore, to ensure linear decline (with an $r^2 \geq 0.8$) of oxygen as function of time, oxygen uptake rates were determined from a series of continuously, intermittent experimental cycles, consisting of a 900 s measurement phase, followed by a 240 flush- and 60 s wait-phase.

Steady swimming protocol

MO_2 (**eq. 2**) at varying unidirectional water flow velocities was established following a modified standard critical swimming speed protocol (U_{crit}) (Brett 1964). Prior to each swimming trial, individual fish (n = 7) were introduced to the respirometer 3-4 h before measurement at a steady speed of 0.5 BL s^{-1}, allowing the fish to habituate and stabilize MO_2 (Chabot *et al.* 2016; Roche *et al*. 2014). Water flow velocity (U) was incrementally increased by 0.5 BL s^{-1} following every third experimental cycle. Measurements were stopped following U = 3.0 BL s^{-1}, and all individuals were transferred to their holding tanks. No fish were observed to fatigue at any of the flow velocities.

A three-parameter, hydrodynamic-based power function was fitted to MO_2 as function of swimming speed (Roche *et al.* 2014) for the steady flow swimming protocol. The relations are described by the following equation:

$$MO_{2Ucrit} = a + b*U^c \quad \#\,\mathbf{2}$$

Where a is equal to the theoretical oxygen consumption at standard metabolic rate at zero speed ($MO_{2,\,@SMR}$), b is the linear coefficient and U is the water flow velocity increasing with its exponent c.

Unsteady swimming protocol

A Computer-generated sine-wave function (**eq. 3**) simulated moderate wave periods (5 s) similar to the San Juan Islands, Washington inshore coasts (Finlayson 2006; Roche et al 2014) (Figure 1). The relations are described by the following equation:

$$U(t) = \alpha \cdot [2/\pi] \text{ \# } \mathbf{3}$$

Where U is the flow velocity as function of time (t), andαequals the amplitude of the sinusoidal wave (2.5 BL s^{-1}) (Figure 1: solid, red line and dashed, blue line).

(1) Applying the sinusoidal wave function properties to the flow regimes, the water direction was reversed every half of a wavelength period (2.5 s) (hereafter referred to as bidirectional flow regime) (Figure 1: dashed red line).
(2) A one-way, uniform wave function (hereby referred to as unidirectional flow regime) equalled a one-half of the wavelength of the sinusoidal wave period (2.5 s), *e.i.* corresponding to the amplitude (α) and velocity (Figure 1: dashed purple line).

Prior to each swimming trial, test subjects (n = 11) were introduced to the respirometer 3-4 h before measurement at a steady speed of 0.5 BL s^{-1}, allowing the fish to habituate and stabilize MO_2 (Chabot *et al*. 2016; Roche *et al.* 2014). For each individual, the unidirectional or bidirectional flow regime were randomly introduced: Data acquisition were collected through three experimental cycles, before returning to the initial steady flow (0.5 BL s^{-1}), allowing them to settle down and restore baseline MO_2 (steady state), before being introduced to the alternative flow regime for an additional three experimental cycles.

Estimated oxygen uptake rates

We estimated MO_2 ($MO_{2,\text{ Estimated}}$) (Figure 1: Solid green line) as a reference-profile in relation to the nonlinear oxygen consumption properties (Roche *et al*. 2014), associated with the effects of maintaining spatial position in sinusoidal varying water flow velocities (Roche *et al*. 2014). This was achieved by integrating the MO_2 (**eq. 2**) obtained in the steady,

unidirectional swimming protocol at varying flow velocities into into the sinusoidal function (**eq. 3**). The relations are summarized by the following equation:

$$MO_{2,\,Estimated} = a + b * (\alpha \cdot [2/\pi])^{c} \qquad \#\ \mathbf{4}$$

According to the sinusoidal water flow direction (Figure 1. solid, red line), negative sign of flow velocities indicate reversed flow direction, hence, average water velocity (1.59 body length s^{-1} [BL s^{-1}]) was calculated as the average of the positive-half of the sine-wave function ($\alpha \cdot [2/\pi]$, α = 2.5 BL s^{-1} [maximum water velocity]) (corresponding to 70% of U_{crit} for *C. aggregata*; (Roche *et al*. 2014)).

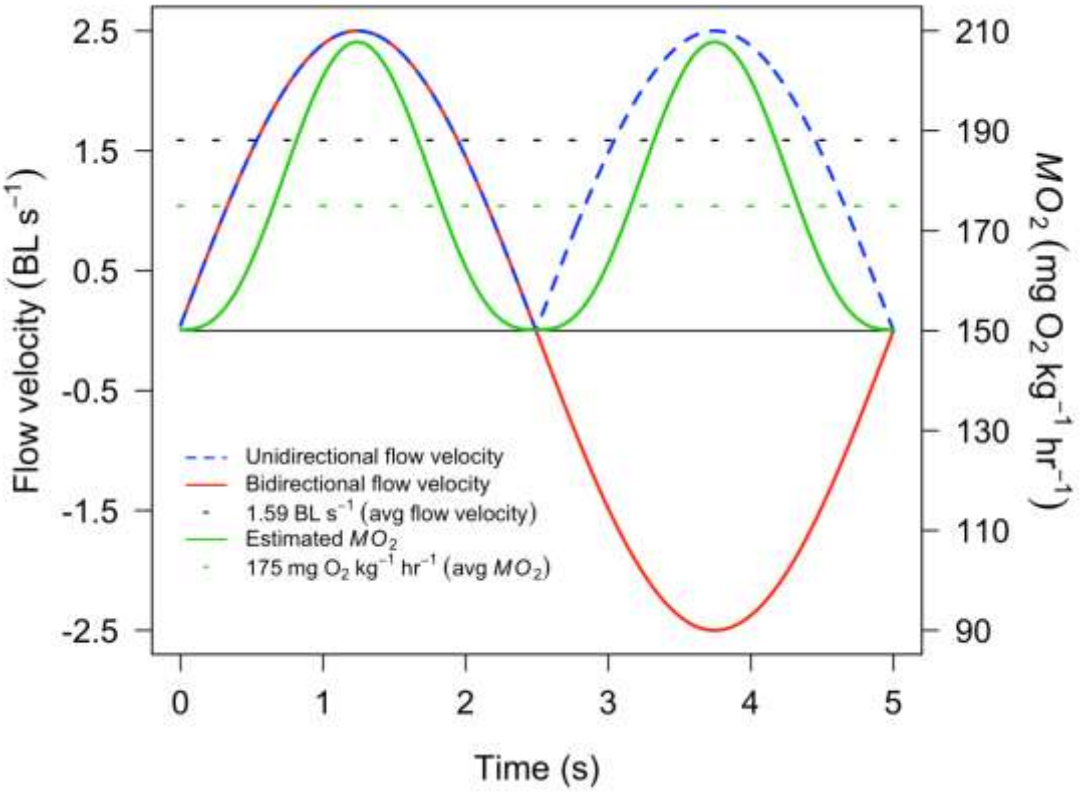

Figure 2: Computer generated wave functions for uni- and bidirectional flow regimes: Conceptualization of the bidirectional (solid red line) and unidirectional (dashed blue line) wave flow (double wavelength period = 5 s; α= 2.5 BL s^{-1}), both treatments had an average flow velocity of 1.59 BL s^{-1} (dashed black line). $MO_{2,\,Estimated}$ (solid green line) and mean $MO_{2,\,Estimated}$ (dashed green line) is based on the integration of MO_2 (**eq. 2**) into a sinusoidal function (**eq. 3**), hereby predicting the dynamic, nonlinear oxygen uptake rates associated with the varying velocities for the opposing flow regimes (**eq. 4**).

Laterality

Swim tunnel turning behavior

To investigate the degree of lateralization in the respirometer, we monitored full-length bidirectional flow-treatments (n = 9) using a Panasonic MiniDV camcorder (10 FPS, 640 x 420). The turning chosen to either the right- or left side was recorded for five consecutive time periods (each 1 min) (to a total of 120 turns). Time periods were selected at least five minutes following the learning period, which we defined as time the test subject used to perform ten unimpeded, consecutive turns in the respirometer, without being compromised by the respirometer walls. This was conducted, as the embedded adjustment to the respirometer conditions comprised increased respiratory oxygen demands, not being equivalent to perceived natural conditions. Following the trial, fish were placed into individual recovery tanks.

To investigate lateralization and turning preference of *C. aggregata* each test subject (n = 9) was transferred from the recovery tank to the center of a double T-maze runway (127 x 60 x 25 cm, length x width x height; center runway: 76 x 17 cm, length x width) (Figure 3), to undergo a detour test at least 4 hours following MO_2 measurements.

Following habituation (10 m) in the center of the test arena, the detour to the left - or right for each test subject was observed from a total of ten trials: For each trial the relative lateralization (L_R) was indexed from -100 to +100 based on the priority of turning right or left, respectively, quantifying the direction and degree of lateralization (Bisazza *et al*. 1998; Dadda *et al*. 2010)

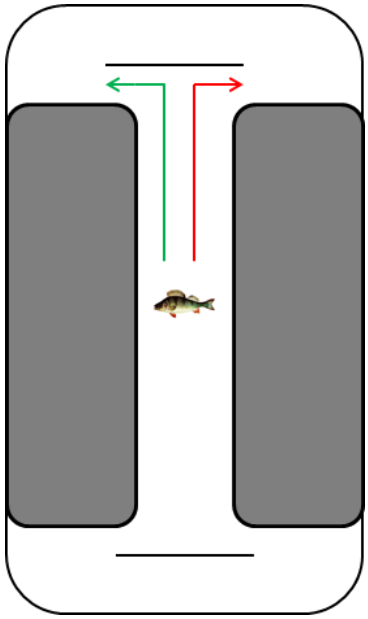

Figure 3: Double T-maze for detouring test setup. The preference of left- or right turning for individual test subject was recorded using a relative lateralization index, according to Bisazza et al. (2010). See text for details.

Data analysis

An analysis of variance (ANOVA), along with a Tukey's post-hoc test was used to compare MO_2 for bidirectional, unidirectional and mean $MO_{2,\ Estimated}$ from our power-function (Fig. 2).

RESULTS

Intermittent-flow respirometry

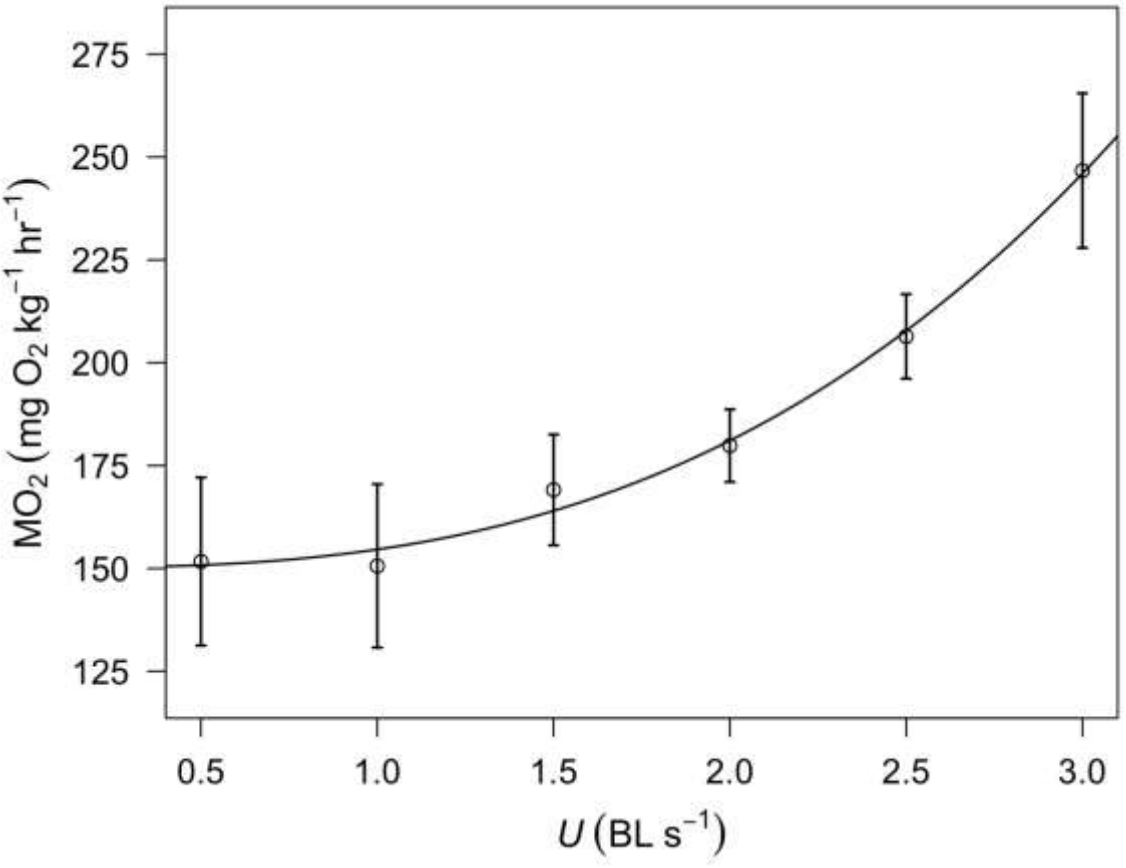

Figure 4: Oxygen consumption rate (MO_2: mg O_2 kg^{-1} hr^{-1}) in relation to swimming speed for *Cymatogaster aggregata* (n = 7) in laminar flow (mean ± S.D.), fitted to a power function: MO_2 = 150.16±3.38 + 4.47 ± $1.74U^{2.79\pm0.34}$. r^2 = 0.99. Swimming speeds were selected below gait-transitioning ($U_{p\text{-}c}$) to ensure fish were demonstrating aerobic swimming (Roche et al. 2014).

A three-parameter hydrodynamic based power function was fitted to the oxygen uptake (MO_2) as function of the swimming speed (U), according to the following equation (**eq. 5**) (Fig. 4) (Roche et al. 2014):

$$MO_2 = 150.16\pm3.38 + 4.47\pm1.74U^{2.79\pm0.34} \qquad \textbf{\# 5}$$

MO_2 increased consistently with swimming speed (U) through the entire water velocity range (0.5 - 3 BL s^{-1}), hence, on average, $MO_{2,\ Estimated}$ was determined to be 175.4 $mgO_2\ kg^{-1}\ hr^{-1}$, calculated as the integrated area under the curve (Figure 1: solid green line) (mean $MO_{2,\ Estimated}$) of the sinusoidal function (cf. **eq. 3**), corresponding to an average water velocity of 1.59 BL s^{-1})

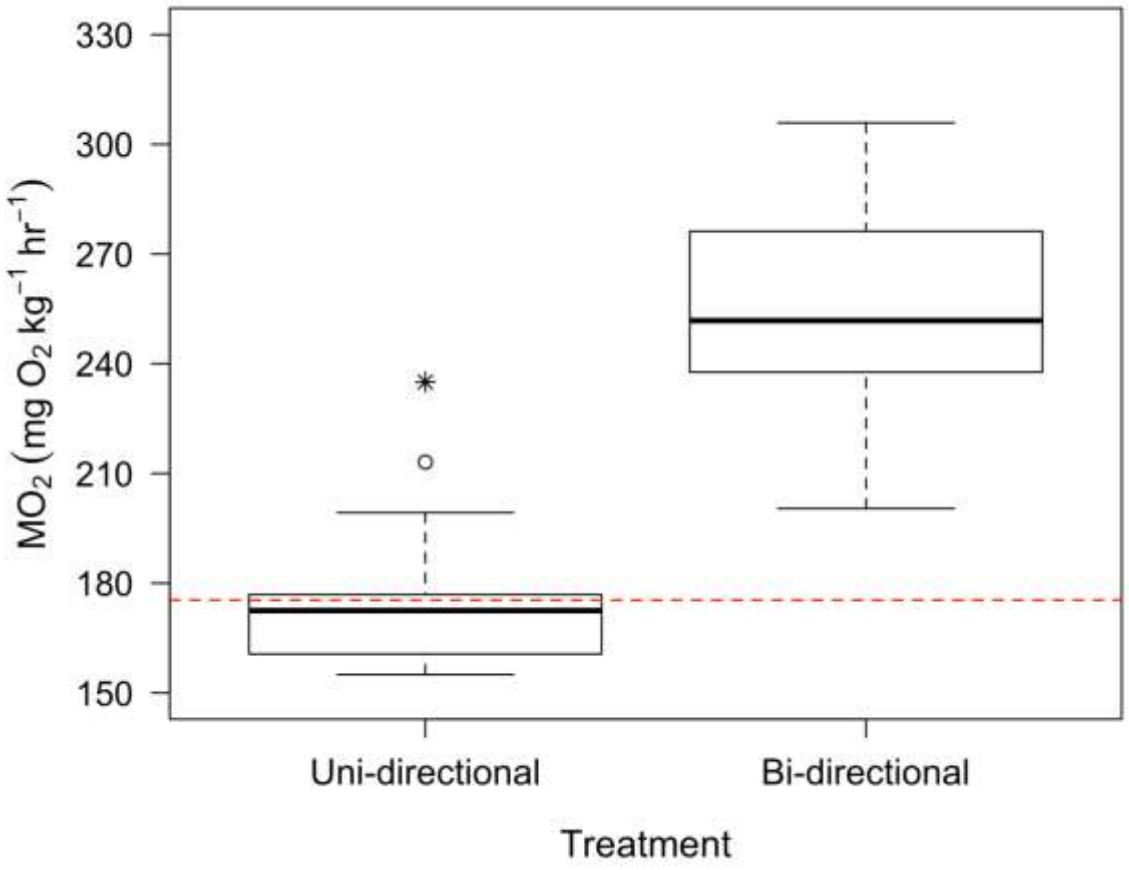

Figure 5: Oxygen consumption rate (MO_2: mg O_2 kg^{-1} hr^{-1}) for unidirectional and bidirectional dynamic flow treatments (n = 11). Boxes represent the first- and third quartiles; bold lines indicating median values. Whiskers denote variability outside of the quartiles and open circles represent outliers. Asterisk indicates $p < 0.001$. The red, dashed line indicates the mean $MO_{2,\ Estimated}$ (175.4 $mgO_2\ kg^{-1}\ hr^{-1}$), derived from the nonlinear relationship between MO_2 and sustained swimming in dynamic flow velocities (**eq. 4**).

There was a significant difference in MO_2, on average, between the unidirectional (173.6 mg O_2 kg^{-1} hr^{-1})- and bidirectional (255.8 mg O_2 kg^{-1} hr^{-1}) flow treatments (ANOVA; $F_{(2,18)} = 29.02$, $p < 0.001$) (Fig. 5). The bidirectional flow treatment demonstrates a 47% increase in mean MO_2 compared to the unidirectional treatment. No statistical significance (Tukey's HSD, $p > 0.5$) was revealed between mean $MO_{2,\ Estimated}$ (175.4 mg O_2 kg^{-1} hr^{-1}) (cf.

eq. 4) and MO_2 of the unidirectional flow treatment (173.6 mg O_2 kg^{-1} hr^{-1}). MO_2, on average, of bidirectional treatments (255.8 mg O_2 kg^{-1} hr^{-1}) was significantly different from mean MO_2 of the unidirectional treatments (Tukey's HSD; $p = 0.008$ and $p < 0.001$).

Quantifying laterality

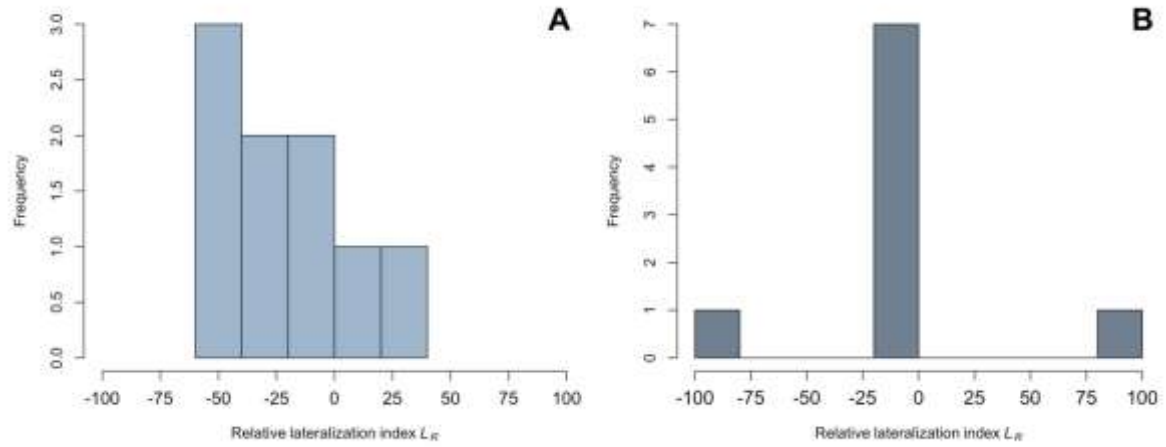

Figure 6: Relative lateralization index of each fish (n = 9) for both the detour-test (A) and in the swim tunnel (B).

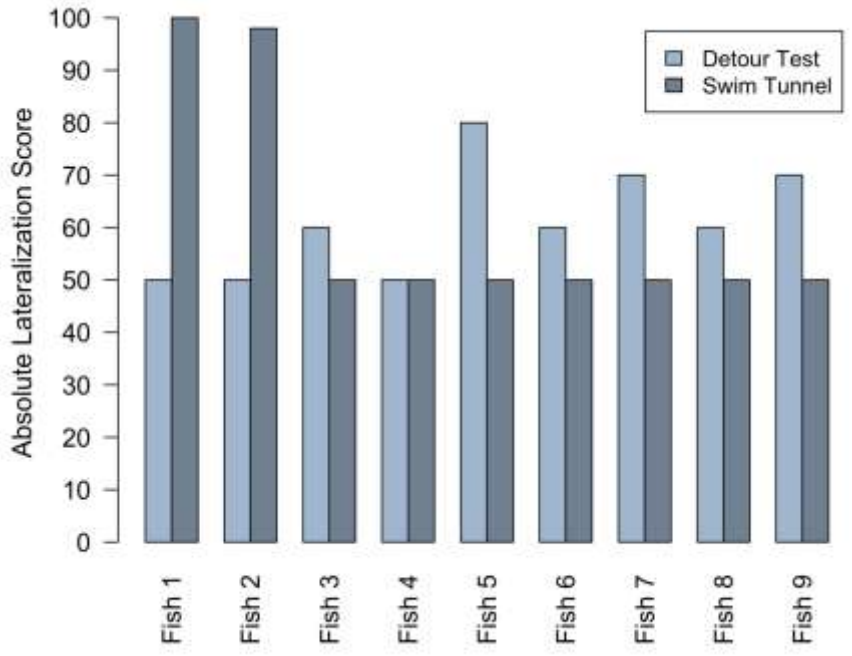

Figure 7: Absolute lateralization score of each fish for the detour-test and in the swim tunnel (n = 9).

Shiner perch took an average of 286 ± 199 s (mean ± S.D.) to adjust to the bidirectional flow. There was no overall relative lateralization of fish in either the detour-test

or the swim tunnel. Fish displayed no absolute lateralization in both the detour-test (61.1 ± 10.5) or the swim tunnel (60.9 ± 21.6) (n = 9) (Fig. 7). There was no significant difference in the absolute lateralization score for the detour-test and in the swim tunnel (t-test; t = 0.027, *df* = 11.6, p > 0.5) (Fig. 5). Of the fish tested only two of the nine demonstrated laterality in the swim tunnel, while only one test subject of the nine demonstrated laterality during the detour-test (Fig. 3). There was no correlation between lateralization and learning time or MO_2.

DISCUSSION

Unsteady water dynamics are ubiquitous throughout the natural world and assuming that fish, and other marine organisms, swim in steady flows highly underestimates the true costs of swimming. Shiner perch had an increased oxygen uptake rate by 47% (82.2 mg O_2 kg^{-1} hr^{-1}) when swimming in the bi-directional flow, while swimming in unsteady, unidirectional flow showed no overall change in oxygen uptake compared with estimated oxygen uptake rates from steady swimming measurements. The excess energetic costs from the bi-directional flow, relative to the unidirectional flow treatment, is the cost required for fish to turn as water directions change. Fish that station-hold must turn regularly to resist displacement, maintain their position, and/or maximise food encounter rate; therefore fish must make trade-offs between energy consumption (foraging) and energy expenditure (swimming and turning).

Station-holding is a common behaviour seen in fish dependent on currents for foraging, mating, and/or site attached fish (Webb 1989). While station-holding can be beneficial (e.g., increased prey encounter rates) if the velocity of the current is too high, fish may face a tradeoff between maintaining position and increased energetic requirements associated with turning. If fish in ecosystems experiencing frequent surge, are unable to contend with the energy required to maintain their position, they must seek out refuges or areas of lower flow, at the cost of decreased foraging (Webb 1989), or else risk being displaced to potentially deleterious habitats (e.g., onto shore or into pelagic zones) (Carlson and Lauder 2010).

Most open shorelines rarely experience prolonged periods of steady flows; therefore, to understand the energetic requirements of fish (and other organisms) it is necessary to simulate natural conditions. Failing to account for unsteady flows, such as wave surge, leads to underestimating the cost of swimming for fish in these ecosystems (Roche et al. 2014). These additional costs associated with unsteady environments are often overlooked due to the difficulty in determining their true value (Liao 2007). While Roche et al. (2014) found a 25.3% increase in MO_2 for fish in unsteady water flow compared to steady, we show that swimming and turning in a bidirectional surge was 47% more costly than when swimming in a uni-directional unsteady flow. Furthermore, this cost was 53% greater than the costs predicted by integrating the cost of swimming in a steady flow along the generated wave.

Overall this species did not show any relative or absolute lateralization in both the swim tunnel and the detour test. This is consistent with previous findings that have been

shown for this species to be non-lateralized (Dadda *et al*. 2010). Two fish showed lateralization in the swim tunnel and not the detour test, which could be due to individual variation, or indicative of lateralization for a certain behavior, which could be beneficial as fish that are lateralized for certain behaviors have been shown to be good at multitasking (Vallortigara and Rogers 2005). However, there was no correlation between MO_2 and laterality.

Further studies should begin to incorporate other factors (*i.e.* environmental conditions) in conjunction with variable hydrodynamic conditions, for example temperature, which is a key driver known to affect fish performance (Johansen and Jones 2011). Hydrodynamic conditions are unavoidable for fish in most ecosystems, while these conditions can be beneficial, fish may be faced with constant energetic trade-offs to maximize fitness.

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