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Taking turns: some aspects of behavioural lateralization in schooling fish

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Abstract

Lateralization of cognitive functions seems to be exceptionally widespread in nature and have been demonstrated to occur in multiple taxa. Previous studies using fish as models have suggested that social behaviours such as schooling may covary with behavioural lateralization at the population-level. Here, we assess the strength, degree and repeatability of behavioural lateralization in schooling fish. Two of the species studied (Aulorhynchus flavidus and Gasterosteus aculeatus) were found to express population-level symmetry in the direction of lateralization whereas one species (Ammodytes hexapterus) showed no indication of population-level lateralization. We also provide evidence that behavioral lateralization is repeatable over time. From our cross-species comparisons we conclude that population-level lateralization is not necessarily related to a gregarious life-style. Further studies should test repeatability over longer periods of time and the role of lateralization in schooling behavior.

Introduction

Important advance within the burgeoning field of behavioural ecology have revealed that fundamental traits previously regarded exclusively human are widespread in nature. A key human trait now considered to be ubiquitous among species as diverse as mammals, birds, fish, amphibians and reptiles is the division of cognitive function in either brain hemisphere, (for thorough reviews see e.g. Bradshaw 1988, Drea 1996, Bisazza et al. 1998b, Vallortigara et al. 1999). Laterality may be broadly defined as when responses derived from cerebral lateralization are manifested in behavioural side bias. Individuals in a population may exhibit the same
pattern of lateralization and express symmetry in the direction of bias. Conversely, profound variation in laterality may be expressed between individuals within a single population, with each individual expressing a tendency for either a left- or right- bias even though no symmetry in lateralization at the population level occurs (Rogers and Andrew 2002). Over the past two decades, teleost fish has emerged as important model organisms for studying many aspects of cerebral lateralization (Bisazza and Brown 2011). In fish, the phenomenon of laterality has been shown to have far-reaching implications for a diverse range of behaviours with ecological significance. For example, important studies have documented a positive relationship between the degree of lateralization and performance in schooling behavior (Bisazza and Dadda 2005), spatial orientation (Sovrano et al. 2005) and even escape performances (Dadda et al. 2010).

Belonging to a social group of animals may infer advantages in terms of food acquisition and predator avoidance (Pitcher et al. 1982, Magurran 1990). Needless to say, the cohesion of such units is dependent on synchronous maneuvers by the individual members. Accordingly, it has been suggested that bias for turning preference at the population level will occur to a higher degree in social species since it promotes the coordination among individuals (Rogers and Andrew 2002). Empirical evidence in support of this hypothesis comes from a cross-species comparison involving 16 species of fish from 13 different families. All species with a social behavior also expressed laterality at the population level whereas more solitary species tended to express laterality at the individual level (Bisazza et al. 2000).

In this study, we investigate the direction and strength of behavioral lateralization in three species of schooling fish, and test the repeatability of lateralized behavior in detour tests.

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Materials and methods

(a) Fish species

The species included in the study were Tubesnout (*Aulorhynchus flavidus*), Sandlance, (*Ammodytes hexapterus*) and three-spined stickleback (*Gasterosteus aculeatus*), all known to show marked schooling behaviour (Limbaugh 1962, Hart 1973, Ward et al. 2002). Beach seining around San Juan Islands, Washington USA was the primary method of collection, although an anadroumos population of sticklebacks was sampled by dip-netting in a nearby estuary. The fish were transported to Friday Harbor Laboratories and housed in seawater flow-through tanks at a temperature of 11.12±0.91°C and under natural light conditions. Tubesnouts (N=73), sandlances, (N=51) and sticklebacks, (N=36 + 48) were all acclimatized for at least three days before behavioural assays.

(b) Lateralization

We used a standard detour test to assess individual behavioural lateralization (Bisazza et al. 1998a). Fish were transferred into a double T-maze runway with a narrow channel with barriers at both ends (fig. 1). Prior to behavioural assays, we blocked one end of the runway were the fish were acclimatised during two minutes preceding the behavioural assay. When this time had elapsed, the fish were given access to the runway and coaxed with a dip-net until the subject were halfway into the runway. At this point, it was left to swim freely and without influence of the operator until it faced the opaque barrier where it made the decision to turn right or left. The detour behaviour to the left or the right when the fish faced the barrier was observed.
and resisted by a direct observer as well as by a video camera placed above the maze and facing down. Videos were recorded on “mov – mpeg4 format” using H264 Webcam deluxe ver3.68. The test apparatus were scaled to compensate for size-differences between species (sandlance and tubesnout, 263cm length x54cm width x15cm width channel with the barriers placed 15 cm away from runway ends, sticklebacks 122cm length x 40cm width x7cm width channel with the barriers placed 7 cm away from runway ends). Water depth in the maze was 10 cm for sandlances and tubesnouts, and 4 cm for sticklebacks. All trials were conducted within the natural light regime in order to match the circadian rhythm of the study species. For each individual, we conducted ten consecutive trials allowing for a relative lateralization index to be calculated. The index is ranging from -100 to 100, where the extreme values correspond to total left and right bias, respectively.

Figure 1. Schematic representation of the double T-maze apparatus. Fish could swim along the runway (alternately in opposite directions); left/right turning directions were recorded. OB: opaque barrier, DA: Decision area

(c) Repeatability of Lateralization

To determine whether lateralization is repeatable over time we tested 50 tubesnouts for lateralization following the procedures described in b). After the detour Lucas J, Branco P. & Hulthén K.
test each fish was tagged with a code of 3 colors visual implant elastomer (Northwest Marine Technology, Inc.) (VIE hereafter). In the following day the lateralization tests were repeated for the tagged fish.

(d) Data and statistical analysis

To make a comparison between fish and illustrate their lateralization, a relative lateralization index (\(L_r\) hereafter) (Bisazza et al. 1998) (formula1) was calculated:

\[
\frac{(\text{Turn to the right} - \text{Turn to the left})}{(\text{Turn to the right} + \text{Turn to the left})} \times 100
\]

Formula 1

In \(L_r\) index, fish were attributed a value that ranged between -100 (10 turns to the left) and 100 (10 turns to the right). To ascertain laterality, a one-sampled t-test was performed on the mean value of the distribution of \(L_r\) by testing it against a theoretical mean (0) expected for random decisions. If this discrepancy was found to be significant, the population was defined as lateralized.

To determine whether lateralization is repeatable over time we performed a Wilcoxon matched pair test to compared the values of the two lateralization tests and ascertained if an individual had maintained the same lateralization trend, or if it had changed its preference.

Results

1. Lateralization

Definition of lateralization in different coastal species:
**Tubesnouts**

73 tubesnouts were tested for lateralization with a detour test, and a bias towards right turns was discovered to exist at the population level \((t_{test} = 3.262, p = 0.0017; n = 73; \text{mean} = 12.88)\). Figure 2 shows that this population is moderately lateralized to the right, the mean is displaced to the right of 0, which would be the expected mean value of a random distribution.

![Frequency distribution of the relative lateralization index \((Lr)\) for tubesnouts](image)

Figure 2 – Frequency distribution of the relative lateralization index \((Lr)\) for tubesnouts. A value of -100 represents a fish with 10 left turns in 10 trials; 0 value corresponds to a fish with 5 turns to the left and 5 turns to the right; 100 value corresponds to a fish with 10 turns to the right in 10 trials.

**Sand lances**

51 sand lances were tested for lateralization with a detour test, and no statistical bias for asymmetrical left or right turns was discovered at the population level \((t_{test} = 1.139, p = 0.260; n = 51; \text{mean} = -5.49)\). In figure 3 is visible the non
laterality of this population, the distribution is similar to a normal distribution of random choice events.

Figure 3 – Frequency distribution of the relative lateralization index (Lr) for sandlances. A value of -100 represents a fish with 10 left turns in 10 trials; 0 value corresponds to a fish with 5 turns to the left and 5 turns to the right; 100 value corresponds to a fish with 10 turns to the right in 10 trials.

**Sticklebacks**

Two populations of sticklebacks were tested lateralization with a detour test; a marine population (n=36) and a anadromous population (n=48). The marine population shows a bias to left turns, at the population level (t test = 7.14, p = <0.0001; n = 36; mean = -32.78). The figure 4 shows that the distribution of the Lr is skewed to the left. The mean shows a high displacement to the left of 0, meaning that this is a highly lateralized population, different from a population ruled by random choice. The same general results were found for the anadromous population of Lucas J, Branco P. & Hulthén K.
sticklebacks. A bias towards left turns was discovered at the population level ($t_{\text{test}} = 7.32$, $p < 0.0001$; $n = 48$; mean = -30.83). The figure 5 shows the same trend as Figure 4 distribution of the $Lr$ is skewed to the left, showing a mean highly displaced from 0, meaning that this is a highly lateralized population, different from a population ruled by random choice.

![Figure 4](image.png)

**Figure 4** – Frequency distribution of the relative lateralization index ($Lr$) for the stickleback’s marine population. A value of -100 represents a fish with 10 left turns in 10 trials; 0 value corresponds to a fish with 5 turns to the left and 5 turns to the right; 100 value corresponds to a fish with 10 turns to the right in 10 trials.

2. **Repeatability**

The Wilcoxon Matched Pairs test shows that there are no statistical significant differences ($n = 50$, $Z = 1.094$, $p = 0.274$) between the score attained by each fish in the two trials.

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Figure 5 - Frequency distribution of the relative lateralization index ($L_r$) for the stickleback’s anadromous population. A value of -100 represents a fish with 10 left turns in 10 trials; 0 value corresponds to a fish with 5 turns to the left and 5 turns to the right; 100 value corresponds to a fish with 10 turns to the right in 10 trials.

**Discussion**

Between-species comparisons have shown that behavioral lateralization at the population-level seems to covariate with social behaviors, such as schooling (Rogers and Andrew 2002). Given that that behavioral lateralization in the same direction infers advantages in terms of school cohesion and alignment, we predicted population-level lateralization for the social and schooling species included in this study. First, tubesnouts tended to express a small bias towards left turns in our detour trials. Secondly, strong biases for left turns were found in both the marine and anadromous population of sticklebacks. It is interesting to note that bias seems to be shared by sticklebacks living in very different environments, (i.e. saline and brackish water). In addition, Aulorhynchidae (tubesnouts) and Gasterosteidae (sticklebacks)
are two closely related species (Nelson 1971). Hence, we conclude that the results for these two species are in line with previous observations of turning bias in the same direction for congeners of social species. (Bisazza et al. 2000). However, sandlances, another species known to aggregate into schools showed no indication of population level lateralization. Consequently, this result appear to be in contrast to earlier findings (Bisazza et al. 2000). However, although schooling behavior may serve as an effective defense against predation in a number of fish species (Neill and Cullen 1974), prey also parade alternative defense tactics. A key-strategy in the sand lance to reduce the risk of predation is to bury in the sediment when not foraging and during overwintering (Reay 1970, Pinto et al. 1984) We thus speculate that this complementary and more sedentary defense strategy may decrease the need for lateralization in the same directions as conspecifics. Lateralization of the brain is something that appears to be ubiquitous among animal groups (Vallortigara et al. 1999), but as this work shows, lateralization responses may or may not tend to be homogeneous in a population. When they are we have a lateralized population, when they are not we have lateralized individuals in an overall non lateralized population.

This work also proved that behavioral lateral bias when present is something repeatable over time. It is not something of random chance; it is part of the animal’s personality and as such the animal is coherent when choosing sides.

To conclude, our results suggest that behavioral lateralization is repeatable over time and might be, at the population level, a feature shared by closely related species. Lateralization appears not to be necessarily related to schooling behavior. Further work should focus on testing repeatability over longer periods of time, and on testing the direct influence of lateralization in school formation and behavior.
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Analysis of fast-start movements using accelerometry and video tracking in the Great Sculpin (*Myoxocephalus polyacanthocephalus*)

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Abstract

While the use of accelerometers in the aquatic environment becomes an increasingly used tool in remotely observing animals; however, the data obtained from deploying accelerometers still needs better understanding. Observations gathered by studies using accelerometers are largely limited to the identification of simple behaviours such as resting and swimming, yet fine-scale movements such as feeding and escape responses are mostly undetected. In this experiment, we aim to establish a link between acceleration traces and fast-start movements in the Great Sculpin (M. polyacanthocephalus) by the analysis of acceleration data from accelerometers and a high-speed video camera. Feeding events, escape events and spontaneous movements were triggered and observed using a 100Hz recording accelerometer (Little Leonardo Ltd, Japan) and a high-speed video camera for n = 7 great sculpin. Kinematic comparison between acceleration obtained from accelerometers and high-speed video camera were performed using vector transformation, yet prove to be difficult due to differences in reference frames and different sources of error. To establish a link between behaviour and acceleration, statistical analysis shows that the signature of spontaneous events can be described by the variation of the magnitude of acceleration which is significantly lower in spontaneous events compared to fast-start movements. Most of this information is lost (50%) if the accelerometer sampling rate is lower than 30Hz. Furthermore, two parameters (the value of $A_{\text{max}}$, the variation of acceleration in lateral and forward direction) allow us to differentiate between escape events and feeding events. These results are a valuable contribution to understanding acceleration data in the field and the issues associated with low sampling rates.
Introduction

Information on behaviour and locomotion of fish in their natural environment is fundamental for insights into their ecology and physiology. However, field observations in aquatic environments are challenging. Recently, the use of micro-accelerometer tags have provided an effective means of indirectly monitoring the behaviour and locomotion of fish in the field.

Observations obtained by previous studies using accelerometers have been limited to the identification of relatively simple behaviours encompassing a broad range of movements, such as resting and swimming. These were based on broad categorization of the acceleration signals, using the mean, maximum or minimum value of the acceleration intensity or frequency components of the signals (FFT and wavelet). Few studies have attempted to identify the acceleration signature of the behaviour involving fast-start locomotion, in spite of the ecological importance of the movement in terms of predator-prey interactions and activity. In addition, it is important to relate acceleration to kinematic movement of fish to find the missing link between acceleration signals and behaviour.

Video analysis based on kinematic experiments on fast-start and spontaneous movement in fish in laboratory settings have demonstrated the relationship between acceleration and swimming motion. These observations indicate the potential for accelerometers to identify more detailed locomotion and behaviour. For example, it has been shown that fast-start locomotion in fish have two distinctive movements: C-starts and S-starts (Domenici, 1997, Hale 2002, Domenici et al. 2004, Wöhl and Schuster, 2007), which may be attributed to escape and feeding tasks. Additionally, these two movement patterns exhibit different acceleration signature. These
conclusions were based on the analysis of acceleration resulting from tracking fast-start movements recorded by high-speed video cameras. Accordingly, we predict that if acceleration is recorded at sufficient frequencies these fast-start movement patterns should be identifiable and can possibly be related to distinct kinematic events as defined in previous studies (e.g. Stage I, Stage II, S-start, C-start).

This acceleration signature could then be used in field studies to remotely monitor more complex fish behaviour than previously possible.

There are several objectives to this study:

1. Validate whether fast-start locomotion can be identified by accelerometer signals compared to spontaneous movement (such as swimming, turns etc.)
2. Validate whether detailed categorization of the fast-start locomotion is possible to identify escape and feeding tasks
3. If 1 (and/or 2) is possible, clarify how much recording frequency of the acceleration is needed to sample those fast-start movements.
4. Find the missing link between acceleration and fast-start movement in fish (e.g. what does a C-start look like in accelerometer-recorded acceleration?)

These research objectives were analyzed using a readily available, hardy model species; the Great sculpin *Myoxocephalus polyacanthocephalus*. 
Materials and Methods

Study Animal

Great sculpin (Myxocephalus polyacanthocephalus (Pallas, 1814)) were collected by beach seine in two locations on the southeast side of San Juan Island, Washington USA. Experiments were carried out at the Friday Harbor Laboratories. The fish were held in a 170cm diameter outdoor tank with flow-though seawater at 11±1°C with a water level of 1m. Fish were maintained in the tanks for at least one week prior to the experiments and tagging. The fish were not fed before the experiment to ensure responsiveness to prey during the predator-prey trials.

Accelerometer

An ORI-380D3GT microaccelerometer (Little Leonardo Ltd., Tokyo, Japan) was used to record tri-axial acceleration. The sampling rate was set to 100Hz (1 recording every 10ms) with a resolution of 12 bit and a 10h recording capacity. The tag is 12x45mm in dimension with a weight of 10g (2% or less of the overall body weight). The accelerometer recorded acceleration up to ± 4g.

Experimental Protocol

Fish were tagged with Petersen Disk tags several days before the experiments using MS222 as the anaesthetic agent. The Petersen Disks are made up of two plastic disks which are attached to each side of the fish at 0.5 cm below the first dorsal fin - assumed to be the least invasive position (Fig. 1A & B). The accelerometer was attached to the Petersen disk tag 30 min before the experimental trials via a male-female Velcro system. The male part of the Velcro is permanently attached to the
surface of one of the Petersen Disks, and the corresponding Velcro is attached to the accelerometer. The attachment of the accelerometer and direction of acceleration is shown in Fig. 1A.

![Diagram of fish with accelerometer and direction arrows](image)

**Figure 1A&B.** *M. polyacanthocephalus* tagged with a Petersen Disk Tag and accelerometer (Little Leonardo Ltd.)

For the experiments individual fish were transferred to an identical (170cm diameter) flow-through experimental outdoor tank (height 1m) filled with seawater to a depth of 50cm adjacent to the holding tank (same water temperature). The transferral and tagging time ranged between 2 and 3min. In addition to the accelerometer, a piece of reflective tape was attached to the caudal peduncle to allow high-speed video tracking. Due to short tagging times, none of the animals showed signs of stress post-tagging and settled quickly in the experimental tank. Fish were kept in the experimental tank 30min prior to the start of the experiment. Escape responses were triggered by manually thrusting a 140cm long pole (2.5 cm diameter) on the bottom of the tank 10cm from the end of the caudal fin when the animal was located at least 1BL away from the tank wall - a similar method has been used in escape response studies of other fish (Harper and Blake, 1990; Domenici 2004). Escape responses were elicited in 30-min intervals, allowing for a recovery time of 30 min after initial transport. During feeding experiments, 5 live sand lance (*Ammodytes spp.* - preferred prey of Great Sculpin) (less than 15cm in size) were introduced to the
experimental tank. Fish were observed while feeding ad libitum. Feeding and escape trials were carried out over several days for each animal. For each fish a minimum of 9 escapes were elicited. Depending on the responsiveness of the fish between 10 and 22 feeding events were observed. The observations for each fish are detailed in Table 1.

The escape and feeding responses of each fish were recorded with a high-speed camera (GiGE Vision, Fastec Imaging HiSpec 2G Mono) with a 25mm COSMICAR (Japan) lens at 500 frames s\(^{-1}\) as well as with the animal – borne accelerometer at 100 Hz. Additionally, swimming behaviour was constantly recorded using a 30Hz standard USB webcam, Microsoft LifeCam VX-1000. The distance between the tank bottom and the high-speed and USB camera was 260cm. Fast-starts were recorded using HiSpec Control Software. Behavioural observations were recorded with a H264 Webcam 3.83 software. The resolution of the HiSpec camera is 1280 × 1024 pixels at 500 frames s\(^{-1}\).

<table>
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<th>Fish</th>
<th>Length (FL) [cm]</th>
<th>Length (TL) [cm]</th>
<th>Weight in [g]</th>
<th># (E_f)</th>
<th># (E_e)</th>
<th># (E_s)</th>
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Analysis

Kinematics of Feeding: Video versus Accelerometer

Acceleration recorded from the high speed camera and the accelerometer was standardised to 100 Hz. Lanczo’s algorithm was used to smooth the acceleration trace over a moving window of five frames. To create a comparative acceleration trace between the video recorded acceleration and the tri-axial accelerometer, a low pass filter was used to extract the dynamic component of the $x$ and $y$ axes from the tri-axial acceleration trace. The $x$ and $y$ dynamic acceleration was then combined using the following equation to obtain the summed value of acceleration on 2 axes:

\[
\text{Comparison of the acceleration between video tracking and accelerometer}
\]

Acceleration obtained by the video tracking is defined in the coordinate fixed to the tank (hereafter referred to as video reference frame), while acceleration obtained by the accelerometer is defined in the coordinate fixed to the accelerometer (hereafter referred to as accelerometer reference frame). From the video reference frame, the accelerometer coordinate always changes when the accelerometer moves with the body of the fish, therefore it is necessary to transform the acceleration obtained by video tracking to the acceleration that would be sensed in the accelerometer reference frame, to be able to compare the two sources of acceleration data. To achieve that, following calculation was performed:

We assume that the acceleration coordinate is defined as the coordinate composed of two vectors, one of which is the vector ($Y_A$ axis) connecting the two markers.
attached to the both ends of the accelerometer in the longitudinal direction, the other
is the vector (X_A axis) perpendicular to the Y_A axis going through the centre of the two
markers. Assume M_t, M_{t+1}, and M_{t+2} to be the 2D points of the centre of the two
marker obtained by the video tracking at time t, t+1, and t+2 respectively in the video
coordinates. Acceleration obtained by the video tracking at time t should be defined
as the acceleration measured in terms of the change of the points M_t, M_{t+1}, and M_{t+2}
with the direction from M_t to M_{t+2}. On the other hand, acceleration obtained by the
accelerometer at time t is defined as the acceleration with the two directions of the X_A
axis and the Y_A axis at time t in the accelerometer reference frame. Therefore
acceleration A_{vt} obtained by the video tracking at time t is transformed to the
acceleration A_{xt} and A_{yt} in the accelerometer reference frame using the angle \alpha
between and the Y_A, following [2] and [3]

\[ A_{xt} = A_{vt} \sin \alpha \]  \[2\]
\[ A_{yt} = A_{vt} \cos \alpha \]  \[3\]
Finding a signature of acceleration corresponding to behavioural events

Selection and extraction of data

Acceleration data of events were extracted with the help of examining behavioural observations recorded by the video camera. At least 10 spontaneous 1s-interval events (such as random turns or swimming) were extracted per fish. Start of feeding and escape events in the acceleration record were chosen to be the first point of change in acceleration from rest when the stimulus was applied. Feeding events were extracted in a similar matter (since sculpin are sit-and-wait predators, start of feeding events may be categorized by change in acceleration from rest). The data extraction was performed using IGOR Pro 6 (Wave Metrics Inc., Lake Oswego, OR, USA). Acceleration data will be explored as 3-dimensional acceleration as well as the magnitude thereof (MA, not corrected for gravitational acceleration) to investigate statistical parameters that are descriptive for one and only one type of event. Parameters explored stem from the frequency domain (spectral and wavelet analysis), probability domain (probability density function, population parameters such as mean, maximum, variation in acceleration) and time domain (time change points that are biologically motivated such as the end of Stage I in fast-start movements). A technique of optimization over various parameters will be employed over highest variability within events as well as lowest variability amongst individuals. The precision and accuracy of resulting parameters will be tested on n=1 fish which is excluded from finding the parameter. Statistical analyses will be carried out using the program R and IGOR Pro 6.
Results

Kinematics of Feeding: Video versus Accelerometer

Acceleration was compared from the individual x, y and z axes of the accelerometer to that of the combined x and y of the video track (Fig. 2).

![Graph showing acceleration comparison](image)

**Figure 2.** Acceleration recorded at 100 Hz on the x, y and z axis of a tri-axial accelerometer and from video-tracking (acceleration derived from the x and y vectors of movement).

Differences are evident in the acceleration from the video track and that of the accelerometer, though the timing of minimum and maximum acceleration is comparable from the x-axis of the accelerometer and the video tracking signal (Fig. 2).
Figure 3. Transformed acceleration data from the x (a.) and y (b.) axes. RMSE x =0.65, y =1.26

Acceleration from the x (lateral) and y – axes (forward acceleration) of the high speed video was extracted and compared to that of the x and y trace from the tri-axial accelerometer. The location of the minima and maxima in the x-axis (lateral acceleration) is consistent, yet the y axis has few similarities with a much larger RMSE (Fig. 3).
Finding a signature of acceleration

The most desirable parameter for detection of behavioural events should have the following properties:

1. Low individual variation
2. Size-independent
3. Independent of absolute values (such as maximum acceleration in one axis)
4. Independent of comparison of escape vs. feeding cut off values

Properties of probability distributions of events posses most of these properties and were therefore investigated initially. Additionally, frequency properties of fast-start events were investigated using spectral analysis and wavelet analysis. However, both techniques suffer from low data density (at 100Hz an escape event that occurs over an average of 250ms will contain only 25 data points and therefore is suboptimal for spectral investigation) and were therefore dismissed.

It was impossible to find one powerful parameter that can be used to identify acceleration specific to spontaneous, escape and feeding events respectively. This lead to the development of a conceptual decision tree approach (Fig. 4) to identify behavioural movements in terms of testing a series of multiple parameters (Φ) that differentiate between spontaneous and fast-start movements and a family of parameters, Ω = [Ω₁, Ω₂,..., Ωᵢ] that allow to distinguish between feeding and escape events.
Figure 4. Conceptual approach to detecting behavioural events based on acceleration traces

**Detection of spontaneous movement**

The most powerful parameter (\( \Phi \)) that allows differentiating between spontaneous and fast-start movement is the variation (\( \sigma^2 \)) in combined acceleration, \( MA \), which is the magnitude of the acceleration vector in three dimensions [1].

[1]

The standard deviation of \( MA \), \( \sigma_{MA} \) is significantly lower in spontaneous activity than in fast-start events (Fig. 5, Table 2, \( n = 6 \)) based on a Wilcoxon Rank Sign test within fish. The summary statistics for each event can be found in Table 2.
Table 2. Summary Statistics of magnitude of acceleration for spontaneous (Eₘ), feeding (E₇) and escape events (Eₑ), p-values Wilcoxon Rank Sign test for $H_0: \mu_1 = \mu_2$

<table>
<thead>
<tr>
<th>Fish</th>
<th>Sample Size</th>
<th>Eₘ $\mu \pm SE$ [g]</th>
<th>E₇ $\mu \pm SE$ [g]</th>
<th>Eₑ $\mu \pm SE$ [g]</th>
<th>$H_0: \mu_1 = \mu_7$</th>
<th>$H_0: \mu_1 = \mu_e$</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>nₛ = 10, nₑ = 15, n₇ = 12</td>
<td>0.10±0.03</td>
<td>0.44±0.01</td>
<td>0.28±0.01</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>B</td>
<td>nₛ = 10, nₑ = 9, n₇ = 15</td>
<td>0.02±0.00</td>
<td>0.61±0.01</td>
<td>0.90±0.01</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>D</td>
<td>nₛ = 10, nₑ = 11, n₇ = 12</td>
<td>0.02±0.00</td>
<td>0.21±0.00</td>
<td>0.40±0.01</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>E</td>
<td>nₛ = 5, nₑ = 12, n₇ = 11</td>
<td>0.03 ±0.00</td>
<td>0.38±0.02</td>
<td>0.62±0.03</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>F</td>
<td>nₛ = 10, nₑ = 9, n₇ = 15</td>
<td>0.02±0.00</td>
<td>0.40±0.01</td>
<td>0.79±0.02</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>G</td>
<td>nₛ =11, nₑ = 9, n₇ = 10</td>
<td>0.02±0.00</td>
<td>0.62±0.02</td>
<td>0.83±0.05</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>
This parameter can now be used to detect fast-start movements in an acceleration trace \((MA)\) that spans several hours. We designed a 1-s window that calculates standard deviation of acceleration and, given a (conservative) cut-off parameter of \(\Phi = \sigma_{MA} = 0.3\) identifies fast-start movements. The window-estimate was tested using a random acceleration trace (from Fish C, Fig. 5). The parameter was able to pick up 100\% of the fast-start event without falsely detecting spontaneous movement (results were checked with behavioural observations from 30Hz camera).
Figure 5. Identification of fast-start movements based on $\Phi = \sigma_{\text{MA}}$, MA – magnitude of acceleration, SD - standard deviation, FS – fast start detections

Assessing the power of $\Phi$ given different sampling frequencies (subsampling)

The same acceleration data (from Fish C) was subsampled with a frequency ranging from 10 ~ 90 Hz at 10 Hz intervals, and then the fast-start movements were identified based on the same cut-off parameter of $\Phi$ as used for the data without subsampling. The detection rate of the fast-start movements decreased as the sampling frequency decreased (Fig. 6). Especially, the detection rate decreased to the rate less than 50% when the data was subsampled with the frequency less than 30 Hz.

Figure 6. Detection rate of fast-start movements of the subsampled data based on $\Phi = \sigma_{\text{MA}}$
Detection of characteristic fast-start movements (escape vs. feeding)

Parameter: $\sigma_x - \sigma_y$

A parameter that was tested and resulted in significant differences in feeding and escape events is the variation in lateral acceleration compared to the variation in forward acceleration (Fig. 6)

**Figure 7.** Difference in variation of lateral and forward acceleration in n = 7 fish (A...G) for feeding and escape events.
Table 3. Summary statistics of lateral and forward acceleration in feeding (Ef) and escape events (Ee), p-values Wilcoxon Rank Sign test for $H_0: \mu = 0$, * indicates significance

<table>
<thead>
<tr>
<th>Fish</th>
<th>Sample Size</th>
<th>Ef $\mu_s \pm SE$ [g]</th>
<th>Ee $\mu_s \pm SE$ [g]</th>
<th>$H_0$: $\mu_{Ef} = 0$</th>
<th>$H_0$: $\mu_{Ee} = 0$</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>$n_e = 15$, $n_f = 12$</td>
<td>-0.03±0.01</td>
<td>0.1±0.01</td>
<td>0.38</td>
<td>0.01 *</td>
</tr>
<tr>
<td>B</td>
<td>$n_e = 9$, $n_f = 15$</td>
<td>-0.03±0.01</td>
<td>0.45±0.01</td>
<td>0.56</td>
<td>0.004 *</td>
</tr>
<tr>
<td>C</td>
<td>$n_e = 15$, $n_f = 22$</td>
<td>-0.00±0.00</td>
<td>0.14±0.00</td>
<td>0.57</td>
<td>&lt;0.001 *</td>
</tr>
<tr>
<td>D</td>
<td>$n_e = 11$, $n_f = 12$</td>
<td>0.04±0.01</td>
<td>0.20±0.01</td>
<td>0.30</td>
<td>&lt;0.001 *</td>
</tr>
<tr>
<td>E</td>
<td>$n_e = 12$, $n_f = 11$</td>
<td>0.04±0.01</td>
<td>0.42±0.01</td>
<td>0.41</td>
<td>&lt;0.001 *</td>
</tr>
<tr>
<td>F</td>
<td>$n_e = 9$, $n_f = 15$</td>
<td>-0.03±0.00</td>
<td>0.43±0.01</td>
<td>0.60</td>
<td>0.004 *</td>
</tr>
<tr>
<td>G</td>
<td>$n_e = 9$, $n_f = 10$</td>
<td>0.09±0.01</td>
<td>0.27±0.02</td>
<td>0.22</td>
<td>0.008 *</td>
</tr>
</tbody>
</table>

Summary statistics and as results from Wilcoxon Rank Sign test where the mean of the variation in acceleration is compared to zero are shown in Table 3. In all fish, variation in lateral acceleration does not differ significantly from variation in forward acceleration in feeding events, while in escape events, variation in lateral acceleration is always higher than variation in forward acceleration, and always significantly different from zero. Hence, $\Omega_1 = \sigma_x - \sigma_y$. 
Figure 7. Maximum acceleration in sway, surge, and heave axis. Asterisk (*) indicates a significant difference in sway and surge acceleration.
There is a difference between the maximum acceleration in the heave axis and the surge and sway axes in the feeding event (Fig. 7, Table 4.). Sway maximum acceleration seems to be significantly higher than surge $A_{\text{max}}$ in the escape event. No significant differences are found in the feeding events in $A_{\text{max}}$ for surge and sway. This is not the case for Fish C.

**Table 4.** Summary Statistics of Lateral and Forward maximum Acceleration in feeding ($E_f$) and escape events ($E_e$), p-values Wilcox Rank Sign test for $H_0: \mu_{\text{sway}} = \mu_{\text{surge}}$

<table>
<thead>
<tr>
<th>Fish</th>
<th>Sample Size</th>
<th>$H_0: \mu_{E_f\text{ sway}} = \mu_{E_f\text{ surge}}$</th>
<th>$H_0: \mu_{E_f\text{ sway}} = \mu_{E_f\text{ surge}}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>$n_e = 15$,  $n_f = 12$</td>
<td>0.93</td>
<td>0.06*</td>
</tr>
<tr>
<td>B</td>
<td>$n_e = 9$,  $n_f = 15$</td>
<td>0.15</td>
<td>&lt; 0.001*</td>
</tr>
<tr>
<td>C</td>
<td>$n_e = 15$,  $n_f = 22$</td>
<td>0.37</td>
<td>0.52</td>
</tr>
<tr>
<td>D</td>
<td>$n_e = 11$,  $n_f = 12$</td>
<td>0.71</td>
<td>0.003 *</td>
</tr>
<tr>
<td>E</td>
<td>$n_e = 12$,  $n_f = 11$</td>
<td>0.95</td>
<td>0.09 *</td>
</tr>
<tr>
<td>F</td>
<td>$n_e = 9$,  $n_f = 15$</td>
<td>0.16</td>
<td>0.002 *</td>
</tr>
<tr>
<td>G</td>
<td>$n_e = 9$,  $n_f = 10$</td>
<td>0.49</td>
<td>0.03 *</td>
</tr>
</tbody>
</table>

Hence, there is strong evidence to conclude that the this can be used as second signature parameter, i.e. $\Omega_2 = A_{\text{max}}$, forward vs. $A_{\text{max}}$, lateral
Discussion

Video vs. Acceleration

Two comparisons were made to assess the difference in acceleration defined by the accelerometer and the video tracking. Though there are some similarities in the timing of the maximum and minimum acceleration in the \( x \) axis, signal scaling and change over time is highly variable.

Differences found between the tri-axial accelerometer and the video tracking acceleration are likely influenced by the comparison of two-dimensional video recording to three dimensional accelerometer recording:

- The angle of the accelerometer causing multiple dimensions to be recorded on any given axis. For example the \( x \) axis has components of \( z \) and \( y \) acceleration.
- Drifts in the timing of the acceleration on the accelerometer resulting in difficulties comparing accelerometer data with video tracking

Though video recording is a useful tool for observing fine-scale movements of un-tagged animals (such as small changes in fin motion), where other accelerometer tagging methods fail, two dimensional recordings loose a component of acceleration which is important for aquatic animals. Tri-axial acceleration in comparison may be useful for recording fish motion in low light conditions, though is restricted by sampling rate and the amount of time animals can be monitored.

Transformation

The shape of the transformed acceleration from the accelerometer was very different from the shape of the acceleration from the video tracking, although the timings of the peaks of the accelerations were close (Fig 3). The difference may result
from the (different) measurement noise of both the accelerometer and video tracking.
In the video tracking, the acceleration was obtained by the double derivative of the
digitized position, hence a slight error of the position estimate, possibly coming from
image magnification, film speed, and digitization error by human, would make a large
difference in the acceleration even though appropriate smoothing technique was
performed (Harper and Blake 1989, Walker 1998). While the accelerometer, is
considered quite accurate compared to the video tracking (Harper and Blake 1989), it
still has a measurement error coming from its mechanism for discretizing the true
acceleration of the fish. However, most difference between the two measurement
apparatus of acceleration may be coming from the fact that accelerometer measured
gravity acceleration as well as movement acceleration at the same time, and the two
acceleration components cannot be accurately separated. If the movement of the fish
were steady, since the change of the gravity acceleration was considered much slower
than the change of movement acceleration, frequency based filtering method such as
low-pass filter (e.g. Tanaka et al. 2001) and running mean technique (e.g. Wilson et al.
2006) would accurately separate the gravity acceleration from the movement
acceleration. However, during unsteady movements, such as fast-start events, there is
no way accurately differentiating the movement acceleration from the gravity
acceleration because the fish might change its posture as quickly as the change of the
movement acceleration, i.e. there is no knowledge about how much posture (hence,
gravity acceleration) of the fish changed except for the accelerometer. If the posture
of the fish changed, the imaginary two dimensional plane created by the \( x \) and \( y \) axis
of the accelerometer is not parallel to the two dimensional plane of the video tracking,
which requires accurate posture information to transform the force measured in the
accelerometer axis to the force that would be measured in the axis of the video


tracking plane. We conclude therefore that all the error mentioned above accumulated and made the difference in the acceleration estimation. Hence, comparing video and acceleration to conclude the usefulness of either is very difficult given the intrinsic problems with the comparison.

**Identification of signatures**

Accelerometers are often used in settings where the sampling rate of the device depends on the technology and the size of the animal (high sampling frequencies generally mean larger battery and larger storage capability, both increasing the dimensions of the tag). They are attached to animals in the field, retrieved at some later point and analyzed. However, most of the time the researcher does not have a good idea of linking the acceleration trace to the ‘observed’ movement, and a lot of the analysis becomes guesswork. Simple parameters such as tail beat, ‘activity’ (i.e. when is the acceleration not zero) and the like are readily available, yet most of accelerometer data in the field still leaves too much to interpretation. Additionally body size constrained sampling frequencies might be too low to detect some more fine-scale movements. In this experiment we could detect one parameter that is characteristic to spontaneous movements. This parameter, $\Phi = \sigma_{MA}$, is the variation in acceleration and allows us to differentiate between fast-start movements and spontaneous movements such as feeding or escape response. The variation in the magnitude of acceleration which combines all three axes is much lower in spontaneous movements than fast-starts. This is kinematically sensible, since during escape and feeding responses great sculpin acceleration in all three dimensions while during spontaneous movements there is a change in acceleration in the lateral direction (tail beat during a e.g. a sharp turn or swimming), and a very slow (little)
change in the forward acceleration (increase of speed from rest), while there is little change in vertical acceleration. Consequently the variation of the combination of these axes is expected to be much lower than in fast-starts, where rapid changes in acceleration occur in all three dimensions. This parameter has been successfully tested and is very reliable with little false detections.

Additionally, we find that sampling acceleration at equal or less than 30Hz significantly reduces the detection of fast-start movements based on the parameter $\Phi$. Coincidentally, <30Hz is a standard sampling frequency used in many experiments (e.g. 16Hz in Kawabe et al. 2003, 32, 16 or 8Hz in Tsuda et al. 2006, or 5Hz in Murchi et al. 2011) and should be re-considered based on these findings. While one might argue that the focus of some of these studies is not so much the fine-scale movement but general activity, short burst acceleration such as spontaneous turns should be classified as ‘activity’ and are severely underestimated with a sampling frequency as low as 5Hz.

Not one single parameter may be used to differentiate between feeding and escape events. In general, feeding events are much longer and more variable than escape events (visible to the eye), yet both events exhibit variation in all three axes. We find two parameters that are characteristic to escape and feeding movements. The first parameter, $\Omega_1 = \sigma_x - \sigma_y$ shows that there is a significant difference in the variation of the forward and lateral acceleration in escape events (much higher variation in lateral acceleration), while the variation in forward and lateral acceleration is not different in feeding events. This means that there is a higher variation from mean acceleration within an escape event in the lateral direction than in the forward direction, while there is no difference in the variation from the mean
acceleration in the lateral direction compared to the forward direction within a feeding event.

The second parameter, \( \Omega_1 = A_{\text{max},x} - A_{\text{max},y} \) shows the relative difference in maximum acceleration of the lateral and forward direction. There is a significant difference in maximum acceleration for feeding and escape events. In escape events sway \( A_{\text{max}} \) is much higher than surge \( A_{\text{max}} \), this is not the case in feeding events. This means that during escape events the lateral maximum acceleration is higher than the forward acceleration, while in feeding events there is no difference in maximum acceleration for these two axes. This parameter is consistent with the parameter of different variation.

This difference may potentially result from the different movement of the fish for the respective tasks (i.e. feeding and escape). The fact that variation is larger in the lateral direction for escape means that on average the fish moves with larger variability from the mean in the lateral direction than in the forward direction. This may mean that the fish moves in the lateral direction with higher intensity compared to the mean (which is true given the higher values of \( A_{\text{max}} \) in the lateral direction for escape events, as described by the second parameter). While this is not possible to say, using only the standard deviation to describe the probability density function since it is not Gaussian, it is still reasonable for the prey during escape responses to show more variability in movement in the lateral direction than in the forward direction in order to establish unpredictability or to show high maneuverability for predators. Additionally, the C-bend and following unbend observed during escape responses requires more variation in acceleration in the lateral direction than in the forward direction; this is seldom the case in feeding responses. For predators, it is simple to reach and sustain a maximum speed when moving in the same direction continuously.
The same might apply to sculpin when feeding. During feeding events it may be easier to move to the same direction (forward, or in specific: towards the prey) to achieve maximum acceleration rather than turning (as during escape responses). However, sharp turning events do occur during feeding as well (depending on the position of the prey) and this may indicate the large variation in standard deviation observed in the analysis of the feeding events. This may also explain why Fish C, which has the highest variability in feeding events (and some of them seem somewhat unnatural when observed with the video camera), did not show this pattern. Our analysis did not indicate a significant difference between later and forward directional acceleration but this might just mean the fish didn’t move to the lateral direction with a large acceleration enough to change its directions.

Ideally, these parameters should be tested in terms of detection probability on a new set of animals or in the field. While this was not possible, the methodology developed in this study could be used in the future to develop tags that are more fitting for the size of the animal, the research question in mind and the sampling frequency required to answer these questions. Studies in the laboratory settings are necessary to understand what acceleration means in animal-terms and to better understand the vast amounts of data that are collected in the field with accelerometers.
**References**


Intraspecific variation in swimming- and escape performance in the labriform shiner perch, *Cymatogaster aggregata*

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Key words: fish swimming, oxygen consumption, respirometry, intraspecific variation, labriform, shiner perch, *Cymatogaster aggregata*

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Abstract

Intraspecific variation in swimming performance, morphology and escape responses was assessed in the labriform shiner perch, *Cymatogaster aggregata*. Shiner perch were videotaped while swimming in a respirometer during a critical swimming speed ($U_{\text{crit}}$) protocol. The degree of individual variation in both oxygen consumption rate ($\dot{M}O_2$) and kinematic performance was evaluated from this $U_{\text{crit}}$ challenge. Fast escape performance was also evaluated in the same group of fish. There was an approximately two-fold variation in metabolic rate of fish swimming at 0.5 body lengths per second (MR@0.5 bl s$^{-1}$) and active metabolic rate, aerobic scope for swimming and critical swimming speed. A significant correlation was found between active metabolic rate and aerobic scope and between MR@0.5 bl s$^{-1}$ and aerobic scope. Similarly there was a significant relationship between active metabolic rate and $U_{\text{crit}}$. Individual variation in swimming performance could not be explained by differences in morphology of the caudal or pectoral fins or variation in body shape or size. There were no significant relationships between stage of escape response completed and any metabolic variables indicating that anaerobic escape responses are independent of aerobic capacity of the fish.

Introduction

Locomotion in fishes, like in other animals, serves multiple purposes. Fish may swim to locate food, escape from predators, migrate, change environments or depths or engage in social interactions. Locomotion in fish can be divided into two forms; body-caudal fin (BCF) and median-paired fin (MPF) locomotion (Webb, 1998). Of the MPF swimmers, labriform locomotion is the most widespread. Labriform swimmers use their pectoral fins
for propulsion at low to medium swimming speeds but at a certain threshold speed a
transition in gait from only pectoral to a combination of pectoral and caudal fin
swimming occur. At high speeds caudal fin locomotion alone is utilized for rapid burst
swimming. This gait transition speed is termed $U_{p-c}$. The transition from MPF to BCF
has been believed to coincide with the switch from aerobic to anaerobic swimming
(Drucker and Jensen, 1996) but a recent study on the labriform striped surperch,
*Embiotoca lateralis*, found that gait transition occurred prior to the onset of anaerobic
swimming (Svendsen et al., 2010). The standard method of assessing swimming
performance is the critical swimming speed ($U_{crit}$) test, in which the fish is allowed to
swim against a water current of stepwise increasing speed until fatigue sets in and the fish
fall back onto the rear grid of the flume. Swimming at low to medium speeds (pectoral fin
swimming) uses red muscles that are fuelled by aerobic energy metabolism (oxidative
phosphorylation). As swimming speed approaches $U_{crit}$ a gradual transition to from
aerobic to anaerobic energy production occurs (Brett, 1964; Lee et al., 2003). There is a
switch to white anaerobic muscles which rely on anaerobic glycolysis for energy and thus
are only used for short bursts or for rapid acceleration. Survival in many fishes is
dependent on how they use the interplay of these systems to either capture prey or escape
from predators.

On a physiological level a trade-off may exist between the fishes ability to perform
very well at aerobically vs anaerobically. For example, individual fish that have a
relatively high aerobic scope, and hence possess a higher $U_{crit}$, may perform relatively
poorly in activities such as burst swimming and escape. Such a trade-off may exist since
a potential compromise may be found between the amount of red (aerobic) and white
(anaerobic) muscle present in a single fish. This has been emphasized by Kolok (1999) but very little attention has been given to intraspecific variation in swimming performance in relation to variation in aerobic metabolic performance. Additionally where such variations have been assessed, there has been a lack of proper corrections for the effect of body mass on oxygen consumption rate ($\dot{M}O_2$) (Reidy et al., 2000).

In addition to the confounding effects of body mass, many studies have shown aerobic metabolic performance in fish to be highly variable across species, populations, life-stages and under different environmental conditions (Post and Lee, 1996; Clarke and Johnston, 1999; Eliason et al., 2011). This variability leads to a diversity in species specific physiological responses with regards to activity, environmental conditions and performance. Intraspecific variation in swimming performance, as well as aerobic metabolic rate, is starting to receive more attention in fish. Kolok (1999) reviewed the literature for studies on intraspecific variation in prolonged swimming performance and concluded that individual variation in critical swimming speed is substantial and repeatable. The importance of individual variation becomes clear in the context of Darwinian fitness. Individual fish capable of swimming faster and/or longer (i.e. have greater stamina) may escape the attack of a predator better than a relatively slower conspecific. In terms of energy, fish with a greater aerobic capacity (greater absolute aerobic scope) will be less constrained by resource allocation making more energy available for routine activities.

In this study, $U_{pc}$ was located at 1.9 bl s\(^{-1}\) but an anaerobic component was not detected before 2.3 bl s\(^{-1}\). These swimming speeds represented 73 and 88% of $U_{crit}$. 
respectively, meaning that an anaerobic component of locomotion kicked in somewhere within this interval.

In the present study, we have assessed the extent of intraspecific variation in swimming performance, escape performance and external morphology of shiner perch, *Cymatogaster aggregata* Gibbons 1854, in relation to variation in aerobic metabolic performance.

**Materials and methods**

*Fish*

Shiner perch were collected by beach seining at Jackson Beach (48°31’N, 123°00’W), San Juan Island, Washington, USA and transferred to holding facilities at Friday Harbor Laboratories in August 2011. The fish were held in 60 x 22 x 115 cm flow through tanks at 12±1°C, supplied with a continuous flow of unfiltered seawater at a salinity of 34 ppt. 21 fish weighing between 22.5 and 27.3 g (mean 24.7±1.61 g) with a total length between 11.61 and 13.00 cm (mean 12.39±0.45 cm) were individually tagged using fluorescent visible implant elastomer (VIE) tags (Northwest Marine Technology, Shaw Island, Washington, USA). Tagging was performed on anesthetised fish (0.1 g l⁻¹ MS-222) and elastomers were injected immediately under the skin covering the left operculum using a 0.3 ml syringe. Fish were left to recover from tagging for at least 24 hours.
Respirometry was performed using computerised intermittent-closed respirometry (Steffensen et al., 1984). A swim tunnel with a volume of 5.3 l, containing a swimming section of 7.5 x 7.5 x 28 cm and a voltage controlled motor and propeller allowed for continuous recirculation of water at a given velocity past a swimming fish. Flow inside the respirometer was made rectilinear by a honeycomb plastic screen situated at the entrance of the swimming section. The motor was calibrated against water velocity by a handheld flow meter (Höntzsch GmbH, Waiblingen, Germany). The respirometer was immersed in an ambient tank of 41.5 l supplied with recirculating, fully aerated, water at 11.8± 0.1°C and a salinity of 34 ppt from a 60 l external reservoir. Oxygen saturation was assured by bubbling with atmospheric air and water oxygen tension ($P_{wO_2}$) was calculated as $P_{wO_2} = FO_2 (P_{BAR} – PH_2O)$, where $FO_2$ is the fraction of oxygen in the atmosphere (0.2095), $P_{BAR}$ is the barometric pressure, and $PH_2O$ is the water vapour pressure at given temperature and salinity. The flushing period replenished the respirometer with fully aerated water while at the same time removing metabolites. To obtain intermittent flow, the respirometer was equipped with an inlet pipe as well as an outlet chimney, allowing water to be exchanged inside the respirometer by means of a 5 l min$^{-1}$ Eheim 1046 pump (Eheim GmbH & Co., Deizisau, Germany).

Automated measurements of $\text{MO}_2$ were divided into periods of flush, wait and measurement. During the 3.5 min periods of flush, water inside the respirometer was exchanged with water from the ambient tank. This flush system was alternately turned on and off by a relay station, allowing fresh seawater to enter the respirometer. The 1.5 min wait period took into account a lag in system response assuring a linear decrease in $P_{wO_2}$.
over time, and was followed by the 5 min measurement period where changes in $P_{\text{w}}O_2$ due to fish respiration were recorded by an $O_2$-optode and monitored at 1 Hz by the AutoResp™ software (LoligoSystems, Tjele, DK). Prior to the experiment, the $O_2$-optode was calibrated against an anoxic solution of sodium sulphite dissolved in seawater and fully aerated water inside the respirometer. All variables, including duration of measurement, flush and wait periods, were typed into AutoResp™ software prior to the experiment. Oxygen consumption from microorganisms produced artificially high values of $\dot{M}O_2$, that was corrected for by measuring $\dot{M}O_2$ before the fish was introduced into the respirometer and again after the fish had been removed.

The respirometer was shielded from the surroundings during all measurements by means of black plastic drapings hanging from the ceiling, thereby minimising disturbance of the fish.

**Escape response**

An elliptical swimming arena (92 x 74 cm; 18 cm in height) marked with calibrated gridlines in the field of view was used to measure the escape response of individual fish. The arena was illuminated with two 500-watt and two 100-watt spotlights placed 1.66 m above the floor of the arena. Water level was maintained at 14 cm for all experiments and was changed every 2 hours to control for temperature (12.2-13.8°C). Escape responses were initiated by a stimulus made of a 4-oz (113.4 g) ball sinker fastened to a metal disc, which was dropped from 88 cm above the water surface using an electromagnet. The stimulus fell through an ABS pipe (7.5 cm diameter; 87 cm in length) positioned at one end of the arena, 36 cm from the centre, ending 1.0 cm above the water level to prevent
fish from seeing and reacting to the falling stimulus as done by Turesson et al. (Turesson et al., 2009). A mirror (7 x 5 cm) was placed 3 cm away from the end of the tube at 45° angle from the water surface so the camera would film when the stimulus made contact with the water, which was considered as initiation of stimulation.

A square piece of black plastic mesh (14 x 14 cm; mesh size 1 x 1 cm) was hung at the water surface with monofilament line, 6 cm away from the stimulus, acting as a refuge to ensure proper positioning of the fish prior to stimulation. This mesh provided some shading, which attracted the fish to remain underneath the refuge within 2.0 bl of the stimulus allowing the fish to escape in any direction and filming to occur through the mesh. Each escape response was initiated after the fish adjusted to the conditions of the arena for 30 minutes and was positioned perpendicular to the stimulus drop zone. The response was filmed at 250 frames s⁻¹ using a high-speed digital camera (FASTEC Imaging, Ranger, San Diego, CA, USA).

**Fin morphology**

To evaluate differences in fin morphology, pictures were taken with a handheld digital camera (Olympus µTough 8000) of the caudal fin and left pectoral fin of anaesthetised fish. The fins were fully distended manually and allowed to contract to a natural position before pictures were taken. A ruler was placed beside the fins as reference for subsequent digital analysis. The length of the leading edge of the pectoral fin, height of the caudal fin and surface area of both fins were measured in individual fish using ImageJ software v 1.44 (National Institutes of Health, USA). From these measurements, the aspect ratio (AR) of the pectoral fin was calculated using the equation
\[ AR = \frac{L^2}{s} \]

Where \( L \) is the length of the leading edge of the pectoral fin (mm) and \( s \) is the surface area of the pectoral fin (mm\(^2\)).

**Experimental protocol**

Shiner perch was transferred individually from the holding tanks into the swimming section of the respirometer. Transfer of the fish occurred within a water filled plastic bag, thereby avoiding aerial exposure of the fish. The fish was allowed to swim at 0.5 bl s\(^{-1}\) for 6-8 hours prior to experimental onset, allowing for estimates of routine metabolic rate (RMR) at this swimming speed. Following this, water velocity was increased in a stepwise fashion in intervals of 0.5 bl s\(^{-1}\). Three measurements of \( \dot{M}O_2 \) were recorded at each swimming speed (i.e. the fish were allowed to swim for 30 min at each speed). This stepwise increase in water velocity was performed until the fish fatigued and fell back onto the grid at the back of the swimming section for more than 10 s. At this point water velocity was returned to 0.5 bl s\(^{-1}\) and the fish was removed from the respirometer following completion of the ongoing measurement period. Three measurements of background respiration were recorded before another fish was introduced to the respirometer and allowed to swim at 0.5 bl s\(^{-1}\).

Following respirometer experiments, the fish were placed in a recovery tank for a minimum of 24 h prior to measuring escape responses as described above.
Data analysis

Linear regressions between $P_wO_2$ and time were calculated automatically by the AutoResp™ software for each period of measurement and slopes (k) derived from these regressions were used to calculate oxygen consumption by the fish according to the equation

$$\dot{MO}_2 = k V_{\text{resp}} \beta_wO_2 M^{-1}$$

where $\dot{MO}_2$ is the oxygen consumption rate (mg kg$^{-1}$ h$^{-1}$), k is the change in $P_wO_2$ over time (kPa h$^{-1}$), $V_{\text{resp}}$ is the volume of the respirometer minus volume of the fish (l), and $\beta_wO_2$ is the solubility coefficient of oxygen in water at given temperature and salinity (mg l$^{-1}$ kPa$^{-1}$) (Dejours, 1981). Presence of fish in the respirometer caused water velocity to increase. This solid blocking effect was corrected for by the AutoResp™ software as described by Steffensen et al. (Steffensen et al., 1984). The three measurements of $\dot{MO}_2$ at each swimming speed were averaged and a mean value of background respiration over the entire experimental period was subtracted to give the actual $\dot{MO}_2$ of the fish. Aerobic scope for swimming was calculated as AMR – $\dot{MO}_2$@0.5 bl s$^{-1}$. This method of presenting aerobic scope was used to avoid confounding effects of extrapolation back to SMR ($\dot{MO}_2$@0 bl s$^{-1}$).

Data of $\dot{MO}_2$ versus swimming speed (U) were fitted to the power function

$$\dot{MO}_2 = a + bU^c$$
where \( a \) is an estimate of SMR (\( \text{MO}_2@0 \text{ bl s}^{-1} \)), and \( b \) and \( c \) are parameter estimates derived from the fitting procedure using TableCurve\textsuperscript{TM}2D4 (Jandel Scientific Software, AISN Software Inc.) as suggested by Korsmeyer et al. (Korsmeyer et al., 2002).

Cost of transport (COT) was calculated as

\[
\text{COT} = \text{MO}_2 \ U^{-1}
\]

wherefrom the optimal swimming speed (\( U_{\text{opt}} \)) was obtained as the minimum COT value.

Critical swimming speed (\( U_{\text{crit}} \)) was calculated according to Brett (Brett, 1964) as

\[
U_{\text{crit}} = U_p + (t_p/t_i) \ U_i
\]

where \( U_p \) is the velocity at which the fish swam for the entire 30 min period (bl s\(^{-1}\)), \( t_p \) is the duration from obtaining maximum swimming speed to crash of the fish (min), \( t_i \) is the duration of the individual swimming periods at a given velocity (30 min) and \( U_i \) is the stepwise increase in swimming speed (0.5 bl s\(^{-1}\)).

Relationships between oxygen consumption variables were compared using linear regression.

Sequential video images of each escape response of individual fish were uploaded to a computer running WINanalyze (v1.5) 3D Software. The centre of mass and the center of the head were digitized frame by frame from a dorsal view. The software produced coordinates, calibrated to the grids, per frame that were used to calculate maximum velocity (m s\(^{-1}\)), turning radius (cm), turning rate (degrees s\(^{-1}\)), and time to respond to
stimulus (ms). During each escape response the completion of stages 1 through 3 were qualitatively described and the maximum velocities during each stage were estimated for each fish.

Results

Oxygen Consumption Variables

All oxygen consumption data is summarized in Table 1. All significant relationships between oxygen consumption variables are shown in Figures 1-4. As seen in Table 1, there was large variation in absolute aerobic scope and active metabolic rate between individuals. These two variables showed a strong positive relationship (Fig. 1).

Table 1. Oxygen consumption rate and swimming performance measures.

<table>
<thead>
<tr>
<th></th>
<th>Mean</th>
<th>Minimum</th>
<th>Maximum</th>
<th>Factorial variation</th>
<th>CV (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>MR@0.5</td>
<td>84.5±3.43</td>
<td>46.2</td>
<td>108.1</td>
<td>2.34</td>
<td>18.6</td>
</tr>
<tr>
<td>AMR</td>
<td>676.1±18.11</td>
<td>463.8</td>
<td>802.1</td>
<td>1.73</td>
<td>12.3</td>
</tr>
<tr>
<td>ASS</td>
<td>591.6±19.92</td>
<td>370.6</td>
<td>755.8</td>
<td>2.04</td>
<td>15.4</td>
</tr>
<tr>
<td>FAS</td>
<td>8.5±0.63</td>
<td>5.0</td>
<td>17.3</td>
<td>3.46</td>
<td>34.0</td>
</tr>
<tr>
<td>U_p-c</td>
<td>3.9±0.14</td>
<td>2.5</td>
<td>5.0</td>
<td>2.00</td>
<td>16.0</td>
</tr>
<tr>
<td>U_crit</td>
<td>4.6±0.08</td>
<td>3.6</td>
<td>5.0</td>
<td>1.39</td>
<td>8.1</td>
</tr>
<tr>
<td>U_opt</td>
<td>2.3±0.13</td>
<td>1.5</td>
<td>3.5</td>
<td>2.33</td>
<td>25.8</td>
</tr>
</tbody>
</table>

MR@0.5, metabolic rate at 0.5 bl s\(^{-1}\); AMR, active metabolic rate; ASS, aerobic scope for swimming; FAS, factorial aerobic scope; U\_p-c, swimming speed at gait transition; U\_crit, critical swimming speed; U\_opt, optimal swimming speed; CV, coefficient of variation. Means are presented ± s.e. Factorial variation is maximum divided by minimum.
Metabolic rate at 0.5 bl s\(^{-1}\) varied less between fish but there was still a significant relationship with aerobic scope (Fig. 2). Individuals that had a higher metabolic rate at 0.5 bl s\(^{-1}\) had a lower aerobic scope for swimming (Fig. 2). Also correlated with aerobic scope was \(U_{\text{crit}}\). In general fish with a larger scope for activity were found to have a higher critical swim speed (Fig. 3). In addition, \(U_{\text{crit}}\) was also found to have a positive relationship with active metabolic rate (Fig. 4). Other variables calculated from oxygen consumption do not show significant relationships with each other and generally show less individual variation (Table 1).

![Graph showing relationship between aerobic scope and active metabolic rate.](image)

Figure 1. Relationship between aerobic scope and active metabolic rate for individual fish. \(N = 21, p < 0.001, r^2 = 0.977\).
Figure 2. Relationship between aerobic scope and metabolic rate at 0.5 bl s\(^{-1}\) for individual fish. N = 21, p = 0.005, \(r^2 = 0.4\).

Figure 3. Relationship between aerobic scope and \(U_{\text{crit}}\) for individual fish. N = 21, p < 0.001, \(r^2 = 0.6\).
Figure 4. Relationship between $U_{\text{crit}}$ and active metabolic rate for individual fish. N = 21, $p < 0.001$, $r^2 = 0.56$.

Escape Responses

Less than 10% of fish failed to respond to the stimulus, 43% completed a stage 1 response, 24% responded with a stage 1 and 2 response and the final 24% completed stages 1, 2, and 3. There were no significant correlations between escape responses and the fishes aerobic scope for activity (Fig. 5).
Figure 5. Measures of aerobic variables at different stages of the escape response.
**Body and Fin Morphology**

Tables 4 and 5 summarize the general morphological characteristics of both body and fin size. No significant relationships were found between oxygen consumption variables and size or between fin characteristics and oxygen consumption variables.

Table 4. General morphological parameters.

<table>
<thead>
<tr>
<th></th>
<th>Mean</th>
<th>Minimum</th>
<th>Maximum</th>
<th>CV (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>M (g)</td>
<td>24.7±0.33</td>
<td>22.5</td>
<td>27.3</td>
<td>6.1</td>
</tr>
<tr>
<td>TL (cm)</td>
<td>12.43±0.10</td>
<td>11.61</td>
<td>13.00</td>
<td>3.5</td>
</tr>
<tr>
<td>Depth (cm)</td>
<td>3.55±0.02</td>
<td>3.38</td>
<td>3.74</td>
<td>2.8</td>
</tr>
<tr>
<td>Width (cm)</td>
<td>1.46±0.01</td>
<td>1.34</td>
<td>1.60</td>
<td>4.5</td>
</tr>
<tr>
<td>Fineness ratio</td>
<td>3.50±0.03</td>
<td>3.15</td>
<td>3.76</td>
<td>4.3</td>
</tr>
</tbody>
</table>

M, body mass; TL, total length; CV, coefficient of variation.
Means are presented ± s.e.

Table 5. Pectoral and caudal fin characteristics.

<table>
<thead>
<tr>
<th></th>
<th>Mean</th>
<th>Minimum</th>
<th>Maximum</th>
<th>CV (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pectoral fin</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Length (mm)</td>
<td>24.1±0.25</td>
<td>21.7</td>
<td>27.3</td>
<td>4.8</td>
</tr>
<tr>
<td>Area (mm$^2$)</td>
<td>282.8±6.63</td>
<td>217.2</td>
<td>344.5</td>
<td>10.8</td>
</tr>
<tr>
<td>AR</td>
<td>4.1±0.07</td>
<td>3.6</td>
<td>4.7</td>
<td>8.0</td>
</tr>
</tbody>
</table>

Caudal fin

<table>
<thead>
<tr>
<th></th>
<th>Mean</th>
<th>Minimum</th>
<th>Maximum</th>
<th>CV (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Height (mm)</td>
<td>28.7±1.09</td>
<td>15.9</td>
<td>39.5</td>
<td>17.4</td>
</tr>
<tr>
<td>Area (mm$^2$)</td>
<td>310.6±10.99</td>
<td>204.5</td>
<td>428.1</td>
<td>16.2</td>
</tr>
</tbody>
</table>

AR, aspect ratio; CV, coefficient of variation.
Pectoral fin length refers to the leading edge of the fin. Means are presented ± s.e.
Discussion

Individual variation is a field that has been well studied in humans but only little is known about intraspecific variation in other vertebrates (Burton et al. 2011). One of the major objectives of this study was to investigate the degree of individual variation in swimming and escape performance in a labriform fish, the shiner perch. With regard to swimming and metabolic performance we showed an approximately two-fold variation in almost all of the parameters tested (Table 1). Variation also existed when the fish performed anaerobically during the escape response trials. This observed variation seems to be unsubstantiated by differences in morphology since none of the morphological parameters investigated (fineness ratio, pectoral fin length, area and aspect ratio, as well as caudal fin height and area) could explain the variation in swimming performance. Such lack of correlation between external morphology and swimming performance is also reported by Reidy et al. (2000) where total fin surface area of Atlantic cod (Gadus morhua) showed no relations with neither aerobic, nor anaerobic, swimming performance.

Aerobic scope for swimming of the shiner perch was found to correlate very tightly with active metabolic rate, and less so with metabolic rate at 0.5 bl s\(^{-1}\). This means that it was the metabolic ceiling (i.e. active metabolic rate) that was responsible for most of the two-fold variation in aerobic scope observed.

The positive correlation between aerobic scope for swimming and \( U_{crit} \) observed here in the shiner perch also agree with the findings of Reidy et al. (2000). These authors found a similar correlation between aerobic scope and \( U_{crit} \) in the Atlantic cod supporting the notion that metabolism is fuelled mainly aerobically throughout the swimming trial.
Further support for this comes from a study on striped surfperch, *Embiotoca lateralis* (a labriform swimmer similar to the shiner perch from the present study), where no indication of any anaerobic component of swimming was present below swimming speeds between 73 and 88% $U_{crit}$ (Svendsen et al., 2010).

In addition to the positive correlation between $U_{crit}$ and aerobic scope for swimming, the study by Reidy et al. (2000) also found a significant negative correlation between $U_{crit}$ and burst performance. Since burst swimming is mainly anaerobic, this finding suggests a trade-off between aerobic and anaerobic swimming performance within individual fish. In our study on shiner perch we also tested for potential interactions between aerobic and anaerobic swimming performance by evaluating the number of stages completed during a fast escape response. We did not find any interactions between such anaerobic escape performance and either $U_{crit}$, active metabolic rate or aerobic scope for swimming. This lack of correlation could indicate that the two components of the locomotory machinery, the red aerobic and the white anaerobic muscle, does not trade off within the individual shiner perch. It is possible that the fish with a higher aerobic capacity (aerobic scope for swimming) were able to obtain a higher $U_{crit}$ because they possessed more red muscle than their conspecifics but, since red muscle comprise less than a few percent of total muscle mass (Thorsen and Westneat, 2005), such increase in red muscle mass does not necessarily lead to a noticeable reduction in the amount of white muscle and thereby anaerobic locomotor performance.

In conclusion, a high degree of intraspecific variation was found within the group of shiner perch, with both aerobic metabolic performance and swimming performance varying approximately two-fold. Aerobic scope for swimming correlated with $U_{crit}$ but no
relations were observed between aerobic and anaerobic swimming performance. The variation in aerobic scope for swimming was explained mainly by variation in active metabolic rate. None of the morphological parameters correlated with any measure of swimming performance.

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References


The presence of a refuge affects escape responses in the Staghorn Sculpin

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Abstract

The influence of a refuge on escape responses was observed on Staghorn sculpins (*Leptocottus armatus*; Girard, 1854). The refuge was offered in sequences to compose four treatments to test if fish showed different behavioral responses. The locomotor performance to mechanical stimulation was observed by the use of high speed video tracking. There was no significant difference in velocity and acceleration between each treatment, except when responses were sorted in short and long latencies. To eliminate the possibility that the stimulus did not induce an escape response, angular velocities in startled fish were compared with the turning rate in routine swimming.

This study showed that the presence of a refuge had an impact on the fish trajectories in the treatment where fish were orientated towards the refuge. As it was only in the treatment mentioned above a difference in trajectory was found after stage 2, a final trajectory at point F was used. In treatments where fish had the refuge behind them or where the stimulus hit the water, analysis of trajectories at point F showed a tendency that fish changed direction after a fast start towards the refuge. This may show that mauthner neuron induced responses were only towards a refuge if it was visually mediated in advantageous positions.
Introduction

The kinematics of escape responses in animals is widely described and recently reviewed (Domenici et al., 2011a; b). In general, animal escape responses are away from the stimulus and are normally highly variable, but confined to an angle of 90-180 degrees away from the threat. Responses can differ by factors like the presence of a refuge and obstacles in the surroundings (Eaton et al., 1981; Eaton and Emberley, 1991; Cooper, 1997; Kramer and Bonenfant, 1997; Domenici et al., 2011a). In most fishes, escape responses consist of a Mauthner neuron initiated C-start, where one of the two Mauthner neurons fires an impulse to the muscles on one of the sides, resulting the fish going in to a C-shape (Webb, 1976; Eaton et al., 1981). The C-shape is the beginning of one of two stages in an escape response (Domenici and Blake, 1997).

Many animals inhabit refuges and they may either be a part of their natural habitat or small complex areas. This is also the case for fishes of which many species use stones, seaweed or other natural occurring hiding places. The reason for this is that it gives an increased protection from predators at the expense of lost feeding opportunities (Dill, 1990; Hixon and Beets, 1993; Krause et al., 1998). Responses are described by escape trajectories that are the angle between the fish and the fish at a set point. This can either be at a fixed time interval, or by using a movement behavior, or simply as the end trajectory after the stimulation (Eaton and
Emberley, 1991; Domenici et al., 2008). Previous work has shown that crabs and lizards nearly always flee to their refuge even if it means an escape towards the threat (Woodbury, 1986; Cooper, 1997), and in some cases a small fraction of a population escapes towards the threat even though there is no refuge. These towards responses with no refuge present might be beneficial for the population in terms of unpredictability (Comer, 2009).

The study of seeing if a refugee can make an animal differ from its normal kinematic behavior has so far mostly been done on mammals, reptiles and birds (Cooper, 1997; Kramer and Bonenfant, 1997; Rueda et al., 2008). Experiments which test the relationships between a fish and a refuge have focused on the reaction time and velocity of the fish at different distances to its refuge (Dill, 1990), but it has been done within a confined angle of escape to the refuge. The purpose of this study is to see if staghorn sculpins show different trends in a sequence of treatments, where the refuge is at four different positions. It is hypothesized that fish will seek out the refuge when startled.
Materials and Methods

Study species

Staghorn sculpins (*Leptocottus armatus*; Girard, 1854) were collected during August 2011 using beach seining off San Juan Island, Washington, U.S.A. Individuals were maintained in an aquarium of 60x120x90 cm that received a constant flow of seawater (34 ppm) at ambient water temperature (12-13 °C). Fish were held in ambient light and exposed to a natural 24-hour cycle without feeding. 67 sculpins (length 14.2 ± 0.8 cm, weight 41.4 ± 6.8 g) were tested individually to determine escape response with the presence of a refuge; fish were tested only once. 10 to 15 fish were transferred to an acclimation tank the day before the experiments were performed. The acclimation tank was constructed exactly like the experimental tank, except more shelters were offered. Fresh seawater was supplied consistently to the acclimation tank.

Experimental set-up

The experimental tank was 106 cm in diameter, with white plastic covering bottom and wall. All of the experimental set-up was surrounded by black plastic except for the top, where camera, lamps and stimulus were located. To test sculpin behavior in response to predators, a dropping stimulus was created to model predation. The stimulus was a 50 ml plastic centrifuge tube (weight 205g) which was filled with water and screws. The stimulus was set to be 136 cm in height attached to stands and was
controlled by an electric magnetic. The hitting point of stimulus on the water surface was less than 10 cm from the tank wall. A PVC shelter (cut in half lengthwise of 10.5 cm in diameter and 12 cm in length) was offered as a refuge, which proved to be an effective shelter in pre-tests. The position of the stimulus was randomized between one of four positions, which were symmetrically located at the above stands, and thereby avoiding substantial fish preference to objects above the tank. The refuge was offered in sequences to compose four treatments at “0°”, “90°”, “180°”, “270°” angle between stimulus dropping point, fish and refuge. A fifth treatment was performed as a control where no refuge was offered.

**Fig. 1.** The five treatments: In treatment A no refuge was offered. In treatment B there was in a 180° angle between stimulus, fish and refuge. In treatment C there was a 90° angle between stimulus, fish and refuge. In treatment D there was a 270° angle between stimulus, fish and refuge and in treatment E the angle between stimulus, fish and refuge was 0°. 1) Stimulus, 2) fish, 3) refuge

A Fastec Imaging Troubleshooter high-speed video camera (250 frames per second) at 480x420 pixels mounted at a height of 210 cm was used to record fish behavior. The camera recorded fish behavior over a time-loop of four seconds, and recordings were saved on a computer as the stimulus was released. Two 150W halogen lamps were used to provide
illumination. Experiments were conducted during the day from 08:00 AM to 08:00 PM. Before each testing, each individual was transferred from the acclimation tank to the experimental tank, with a reflective tape mark at 35% of total lengths from the snout tip, which was proved to be center mass for sculpins (Paglianti and Domenici, 2006). Fish were allowed to acclimate in the refuge at its position in the upcoming treatment for 25 minutes. Five minutes before stimulation, the shelter and the fish were moved to the center of the tank. Prior to stimulation, the refuge was removed from above and relocated to be at one of four locations in the tank, or taken out of the tank, corresponding to different treatments, and the stimulus was released within 30 seconds. The orientation of fish was determined as the angle between the line that joins the fish snout with the center mass of the fish and the line that joins the center mass of fish with the stimulus. The initial orientation was set to be 90° before the stimulus was released, mimicking predation from the side. The refuge was always 10 cm away from the tank wall, implying that the stimulus in treatment “A” did not hit the refuge. Fresh seawater was supplied consistently, except after the shelter was removed, preventing any flow effect on fish behavior.
**Fig. 2.** Experimental set-up. A: Top view of the tank with fish and refuge in a “0 degree” treatment, where stimulus hits the water behind the refuge. B: Side view of tank with fish and refuge in at “0 degree” treatment, where stimulus drops behind the refuge. 1) Camera, 2) lamp, 3) stands with four possible positions of stimulus, 4) curtain, 5) stimulus, 6) tank, 7) computer, 8) refuge, 9) fish.

**Data analyses**

In cases where fish responded to stimulation, recordings were analyzed in WINanalyze, an automated tracking program. Prior to each analysis, the program was calibrated using the diameter of the experimental tank as a known distance. As the refuge was placed 10 cm from the tank wall, recordings were analyzed from the moment the stimulus hit the water to the fish snout reached distance of 10 cm from the wall. The final position, point F, was defined as the frame where the fish had moved from the start location to a distance that corresponded to the distance between the tip of the snout and the refuge at the initial orientation. Response latencies were defined as the time from the stimulus hit the water to the first movement.
of the fish. Each frame was analyzed and fish were tracked at the center of mass (CM) and at the tip of the head. Escape trajectory was defined as the difference in angle between the lines that connects the tip of the head with CM at compared with the initial orientation. The trajectory was measured from the start of stage 1 to the end of stage 2 as defined in by Domenici and Blake (Domenici and Blake, 1997). The trajectory at the final orientation, point F, was also found so final positions could be compared between treatments.

![Fig. 3](image.png)

**Fig. 3.** The dotted circle represents the final position, point F. A) The initial distance of the fish snout to the refuge (grey line) and CM to refuge (black line). B) The final position for the fish, point F, and the corresponding angle from the start CM to the final CM that gives the final escape trajectory.

For treatment A where no refuge was present, an average of the distances between refuge and CM of the fish (27.24±2.18 cm) from the other trials was used. The initial distance from fish to refuge, d, was calculated as

\[ d = \sqrt{\Delta x^2 + \Delta y^2} \]

Where d is the initial distance from fish to refuge in millimeters, \( \Delta x \) is
change in $x$-coordinates [mm] and $\Delta y$ is change in $y$-coordinates [mm]. The maximum velocity [$\text{ms}^{-1}$] and maximum acceleration [$\text{ms}^{-2}$] was found for stage 1 and 2 and calculated using a continuous five point polynomial curve fit on raw data from video analysis (Lanczos, 1956). The same was done for the turning rate (fig. 4). To compare the fast starts of the treatments with routine swimming, 10 random fish were recorded in the tank. Angular velocities [$^\circ \text{s}^{-1}$] from spontaneous turns were measured and compared to angular velocities from fast starts. Angular velocity was measured as turning of the head between the start point and the end point of stage 1.

Fig. 4 An example of smoothed data from a fast start with time [ms] on the x-axis and turning rate [$^\circ \text{ms}^{-1}$] on the y-axis. The dotted curve represents raw data from video analysis. The solid curve represents smoothed data, using a continuous five point polynomial fit (Lanczos, 1956). Stage 1 and stage 2 was defined as described by Domenici and Blake (Domenici and Blake, 1997)
Results

In this study a total of 46 individuals responded to stimulation. A $\chi^2$ test was used to test if fish had a significantly different responsiveness to a certain treatment. In all cases no difference was found ($p > 0.05$). The angular velocity of spontaneous turns was compared to angular velocity of fast starts, and significant difference was found ($p < 0.0001$; Mann-Whitney non parametric test)

![Frequency distribution of angular velocities](image)

**Fig. 5** Frequency distribution of angular velocities [$^\circ$ s$^{-1}$]. Solid bars represent fast starts. Open bars represent turns in spontaneous swimming as done by Meager (*Meager et al.*, 2006)

Maximum velocity and maximum acceleration for escape responses in the five treatments was calculated as described in *data analyses*. An ANOVA multiple sample test was used to compare velocities and acceleration. No difference was found at the 5% level of significance in either max velocity or max acceleration.
Fig. 6. 1: The maximum velocities (y-axis) in the five different treatments (x-axis). Velocities were not significantly different between treatments (p > 0.05). 2: The maximum accelerations (y-axis) in the five different treatments. Accelerations were found not to be significantly different (p > 0.05).

As there were found no differences in velocities or accelerations between treatments, data was sorted in pools of short latencies (response time less than 70 ms) and long latencies (response time more than 70 ms), and a Mann-Whitney non-parametric test was used to compare average max velocities and average max accelerations from short latencies with average max velocities and average max accelerations from long latencies. The comparison of accelerations showed no difference at the 5% level of significance. The short latency max velocity was found to be significantly higher than the long latency max velocity (p < 0.01).
Fig. 7. 1: The maximum velocities from short and long latencies. A comparison showed that velocities from short latency responses were significantly different from the velocities from long latency responses (p < 0.01). 2: Max acceleration from short and long latency responses. Comparison showed that accelerations were not significantly different (p > 0.05).

As described in fig. 3, trajectories were found at the end of stage 2 and at point F. Circular plots were used to visualize fish behavior in the different treatments. Average angular vectors at the end of stage 2 and at point F were compared. At the end of stage 2, only trajectories from treatments A & C and B & C were significantly different (p < 0.05 and p < 0.01). At point F, angular vectors from treatment A & B, A & C and B & C were found to be significantly different (P < 0.05; p < 0.05 and p < 0.001). As it was only in treatment B and C more than 10 fish responded to stimulation, a Mardia-Watson-Wheeler test was performed to compare only said treatments. Other treatments were compared with Watson-Williams F test. Comparison of trajectories at the end of stage 2 and at the final orientation within the same treatments showed no significant difference (p > 0.05, Watson-Williams F test).
Table 1. Comparison of the average angular vector between treatments A to E. 1: Comparison of angular vectors after stage 2. 2: Comparison of angular vectors at point F. * A Mardia-Watson-Wheeler test was used to compare treatments B and C. All other treatments were compared with Watson-Williams F test.

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Fig. 8. The Escape trajectories plotted in circular graphs. Angular standard deviation is marked with error bars on each plot. 

1: The five treatments as shown in fig. 1. 

2: The escape trajectories at the end of stage 2. 

3: The trajectories at point F. All angular vectors were compared. See table 1 for p-values. Each line corresponds to 10° and each circle on the plots corresponds to one fish.
Discussion

The strategy for avoiding predators in teleosts is stimulation of muscles by the Mauthner neurons (Eaton et al., 1981), where response comes within 50ms from stimulations. As seen in various studies, a percentage of tested animals do not respond to the stimulation (Paglianti and Domenici, 2006). Furthermore some fish respond towards the threat as seen in Domenici and Blake (Domenici and Blake, 1993). In this study only three fish had a trajectory towards stimulus at the end stage 2. It is argued by Comer and Domenici that towards responses in some cases can be advantageous for animals, because it increases the unpredictability of the escape (Comer, 2009; Domenici et al., 2011a). In species where refuges are a natural part of the habitat, it seems reasonable for the animal to hide when predators strike. The effect of escape responses with the presence of a refuge has been tested in lizards and crabs. Said studies concluded that these animals prefer to go to their refuges, even if it means towards response (Woodbury, 1986; Cooper, 1997).

In this study Staghorn Sculpins were offered a piece of PVC tube as refuge. As shown in fig. 6 and fig. 7, max velocities and max accelerations were not significantly different between treatments. Walker found that the probability of a successful avoidance of predator strike is strongly correlated to latencies, as the fast start gives more time to accelerate (Walker et al., 2005). Experiments done by Dill shows that distance to
cover only has an influence on flight initiation distance (Dill, 1990). A tendency on flight speed correlated to distance is seen but not found to be significant (Dill, 1990). Even though the distance from fish to refuge in this study varied a little, no effect was seen in the results as described above. It is therefore argued that the variable distance can be ignored.

Experiments showed that fish in treatment B and C indeed sought out the refuge when startled (fig. 8 and table 1). Because the refuge in said treatments was either in front of the fish or away from the stimulus, the hide was in advantageous positions, and therefore there was no conflicting information in these treatments. In table 1, p-values from comparisons in groups show that only treatment C is different from the control treatment at the end of stage 2. This shows that because fish collect information on surrounding obstacles before the stimulation, they still have the ability to correct trajectories within stage 1 and stage 2, in spite the fast Mauthner neuron initiation (Eaton and Emberley, 1991). At the end of stage 2, treatments A and B were found not to be significantly different (table 1). The angular vector in treatment A was 138° and in treatment B 147°. As found in other of species, preferred escape angles are between 90° and 180° (Domenici and Blake, 1993; Domenici and Batty, 1997). The reason why angular vectors in treatments A and B are not significantly different could be because the refuge in treatment B is within the preferred angles of escape. Also treatments D and E were not significantly different from
treatment A at the end of stage 2. In these treatments most responses were away from the stimulus with an escape vector not different from the escape vector in treatment A. Even though treatment D and E at the end of stage 2 and at point F are not different, the angular deviation shows that some fish turn around and swim towards the refuge. This supports arguments in (Eaton and Emberley, 1991) that fishes are aware of their surroundings. The importance of refuges for animals exposed to predation has been shown in (Hixon and Beets, 1993) where the abundance of prey is positively correlated to the number of refuges as fish don’t compete for hides when predators are close. In this study, results show that fish change their trajectory if a refuge is in an advantageous position, and it is therefore concluded that the presence of a refuge affects escape responses for the Staghorn sculpin.

Acknowledgment

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We will not acknowledge the group of retarded sculpins which didn’t respond to any kind of stimulation.

References


Surf’s up! The energetic costs of labriform swimming in unsteady flows

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ABSTRACT

Swimming represents the primary means through which fishes interact with their environment, and factors influencing swimming performance can profoundly affect their distribution and survivorship. Traditional measures of swimming performance are estimated under laboratory conditions using steady water flow. However, these experiments potentially underestimate the actual cost of swimming under unsteady water flows that characterize natural systems. Using a swimming respirometer and video recordings, we swam *Cymatogaster aggregata* using a standard $U_{\text{crit}}$ swimming trial under one of three flow conditions with the same mean water velocity at each speed increment: steady flow (control), low amplitude water velocity fluctuations ($A=0.5\text{BLs}^{-1}$) and high amplitude water velocity fluctuations ($A=1\text{BLs}^{-1}$). We found that unsteady flows increase the metabolic cost of swimming, but only when high flows push a swimming fish beyond the threshold for exclusively aerobic metabolism (beyond $U_{\text{burst}}$). Furthermore, unsteady flows at the highest amplitude treatment decreased the maximum mean velocity ($U_{\text{crit}}$) and mean gait transition velocity ($U_{\text{pc}}$) achieved by individuals compared to the control and low amplitude unsteady flows. However, lower costs of swimming than predicted in low amplitude flows below $U_{\text{burst}}$ suggest that fish are able to take advantage of the cyclical wave patterns and economize energy expenditure. Mean pectoral fin beat frequency did not differ significantly among treatments. This is the first study exploring the costs of swimming under unsteady flow in a marine labriform swimmer.

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INTRODUCTION

The energetic costs of locomotion can form a large and variable component of the daily energy budgets of mobile organisms (Boisclair and Sirois, 1993). Environmental factors that influence locomotor performance can therefore have profound effects on individual fitness (Arnold, 1983; Irschick and Garland, 2001). In fishes, the energetic costs of swimming have traditionally been estimated by measuring oxygen consumption in steady-flow respirometers over a range of swimming speeds (e.g. Steffensen et al., 1984). While steady-flow respirometry has provided invaluable insights into the swimming physiology of fishes, it may fall short of describing the true costs of swimming in nature, where water flows are often unsteady and can vary dramatically over short time-scales (Liao, 2007; Webb et al., 2010).

Water flow in the form of waves and currents is an important physical property of aquatic systems and has major effects on community structure and species distributions (Bellwood et al., 2002; Webb et al., 2010). Many fish species living in habitats routinely exposed to high intensity water motion have developed a range of morphological, physiological and behavioural adaptations to exploit these environments (e.g. Johansen et al., 2008; Langerhans, 2008). However, an increasing number of studies suggest that global changes in climate and water flow regimes are increasing both the frequency and intensity of climatic events such as storms, floods and wave surges (Bronstert, 2003; Harley et al., 2006; Seymour, 2011; Wang and Swail, 2001). The ability of fishes to adapt to changes in their hydrodynamic environment will therefore depend on whether and how increases in unsteady water flow affect the cost of locomotion. Recent work in freshwater systems has examined the performance and energetic costs of fish swimming in turbulent...
flows (Enders et al., 2003; Enders et al., 2005; Taguchi and Liao, 2011; Tritico and Cotel, 2010), yet similar studies in marine organisms are nonexistent. This oversight is surprising given the ubiquity and importance of unsteady, wave-driven water motion in coastal habitats around the globe (Fulton and Bellwood, 2005; Gourlay and Colleter, 2005).

Here, we examined whether the metabolic costs of fish swimming in unsteady water flow conditions is higher than (1) the costs of swimming in steady flows (standard $U_{crit}$ trial) and (2) the costs of swimming predicted by theoretical models based on sinusoidal, wave-like flows with fixed amplitudes. We hypothesized that fish swimming in unsteady flows incur greater energetic costs than fish swimming in steady flows because the relationship between swimming speed ($U$) and oxygen consumption ($MO_2$) is non-linear (Brett, 1964). We also hypothesized that theoretical models based on the standard curve would underestimate the cost of locomotion in unsteady flows as fish may incur additional costs from maintaining their position and stability during changes in surrounding water velocity.

**Study system**

Members of the surfperch family (*Embiotocidae*) are near-shore swimmers with a wide distribution along the Pacific coast of North America, from Baja California to southern Alaska (Eschmeyer et al., 1983). Surfperches are labriform swimmers; they use their pectoral fins to generate thrust via positive and negative lift forces at each fin-beat cycle in a movement analogous to the wing-beat cycle of birds in flight (Webb, 1973; Webb, 1975). In labriform fishes, routine locomotion at low to intermediate speeds is
powered by pectoral girdle muscles, which consist mostly of red-oxidative (aerobic) muscle fibres (Drucker and Jensen, 1996b; Westneat and Walker, 1997). At higher swimming speeds, these fishes exhibit a distinctive switch from pectoral only to caudal-assisted swimming, a gait transition known as $U_{p,c}$ (Cannas et al., 2006; Drucker and Jensen, 1996b). Recruitment of the caudal fin for fast steady swimming speeds appears to be achieved aerobically (Svendsen et al., 2010). However, unsteady fast swimming speeds (e.g., fast-starts, burst swimming) are powered by the segmented myotomal musculature along the body axis, consisting primarily of white-glycolytic (anaerobic) muscle fibres (Beamish, 1978; Kendall et al., 2007). This transition to anaerobic metabolic pathways at high swimming speeds can generate high mechanical power outputs, but comes with an elevated cost of transport and incurs an oxygen debt (Kendall et al., 2007; Svendsen et al., 2010). Consequently, these speeds can only be sustained for a short time and result in elevated rates of oxygen consumption post-exercise (excess post-exercise oxygen consumption; EPOC) in order to repay the oxygen debt (Beamish, 1978; Brett, 1964; Lee et al., 2003). Although beach surf zones are physically dynamic environments that routinely experience high amounts of wave exposure, previous studies on surfperches have only investigated the kinematics and physiology of swimming in steady state laminar flow conditions (Cannas et al., 2006; Drucker and Jensen, 1996a; Drucker and Jensen, 1996b; Mussi et al., 2002; Svendsen et al., 2010). Given this, we swam shiner surfperch (Cymatogaster aggregata Gibbons) in a flow-through respirometer at progressively higher speeds using traditional steady state flow and compared measures of oxygen consumption and swimming performance to those recorded in unsteady, undulating flow at set amplitudes. Our flow treatments mimicked a
unilateral wave scenario (i.e., sinusoidal variations in water flow velocity in a single direction, around a constant mean velocity).

**MATERIALS AND METHODS**

We collected 20 adult *C. aggregata* (total length \( L_T = 14.84 \pm 0.11 \text{cm} \); mass = \( 46.3 \pm 1.4 \text{g} \); means ± s.e.) in August 2011 using a beach seine net at Fourth of July Beach and Jackson’s Beach on San Juan Island, Washington, USA. Fish were immediately transported to the Friday Harbor Laboratories, University of Washington, and kept in flow-through tanks at an ambient light regime. Tanks were continuously supplied with filtered seawater (salinity 34 ppm) at a mean temperature of 12 °C (range 11 to 13 °C). Fish were acclimated for a minimum of 3 days and fasted for 24 h before experimental trials to ensure that satiation was standardized across individuals (Johansen et al., 2010; Niimi and Beamish, 1974).

**Respirometry**

We measured oxygen consumption (\( M_{O_2} \): mg O\(_2\)kg\(^{-1}\)h\(^{-1}\)) for 20 solitary fish in a 8.31 l clear Plexiglas swimming respirometer with a working section of 9.0×26.0×10.0 cm (width × length × depth). Oxygen levels in the respirometer were recorded using a fiber optic oxygen meter (Presense Fibox 3) monitored with Oxyview v.5.31 (Presens). To reduce bacterial growth and respiration in the system, we rinsed the respirometer thoroughly in freshwater after every 6\(^{th}\) trial. This procedure ensured that background respiration rates (measured at the beginning and end of each trial) remained below 20% of the oxygen consumption of fish.

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We calibrated the flow in the working section of the respirometer from 0 to 80 ± 0.5 cm s\(^{-1}\) (mean ± SE) using a digital TAD W30 flow-meter (Höntzsch, Germany). Solid blocking effects of the fish in the working section were corrected by the respirometry software (AutoResp, Loligo Systems) following Bell & Terhune (1970). Fish were placed in the respirometer and left to acclimate for a minimum of six hours at a swimming speed of 0.5 body lengths per second (BLs\(^{-1}\)) until their oxygen consumption reached a steady state. We measured oxygen consumption at 0.5 BLs\(^{-1}\) by averaging the three MO\(^2\) measurements immediately prior to the onset of the first trial (1 BLs\(^{-1}\)). These points fall within 10% of the average three lowest MO\(^2\) measurements at 0.5 BLs\(^{-1}\) in over 50% of the fish tested.

We measured oxygen consumption as a function of swimming speed (U) following a standard critical swimming speed (U\(_{\text{crit}}\)) protocol for intermittent flow respirometry (Plaut, 2001; Steffensen, 1989; Steffensen et al., 1984). U\(_{\text{crit}}\) trials were initiated at 1.0 BLs\(^{-1}\) and swimming speed was increased by increments of 0.5 BLs\(^{-1}\) every 30 min. A swimming trial ended when the fish could no longer swim against the flow and was swept downstream onto a retaining grid for a minimum of 3s. We took three consecutive recordings of MO\(_2\) at every swimming speed; each determination consisted of a 225s flush, 75s wait and 300s measurement period, for a total of 10 minutes. We constructed a standard curve using six test subjects (treatment A=0; \(L_T=14.92 \pm 0.24\) cm; mass = 44.7 ± 2.2g), in which a constant velocity was maintained at each swimming speed increment (e.g. Johansen et al., 2010). We repeated the same step-wise procedure for the remaining 14 fish, but varied the swimming speed using low amplitude (A= 0.5BLs\(^{-1}\)) fluctuations (n=7, \(L_T=14.89 \pm 0.18\) cm; mass = 48.5 ± 3.46g)
and high amplitude ($A=1.0 \text{ BLs}^{-1}$) fluctuations ($n=7$, $L_t= 14.64 \pm 0.22\text{ cm}$; mass = $45.2 \pm 1.2\text{ g}$) around the mean at each increment of $0.5 \text{ BLs}^{-1}$ (e.g., $1.0 \pm 0.5 \text{ BLs}^{-1}$ and $1.0 \pm 1.0 \text{ BLs}^{-1}$). These sinusoidal variations in water velocity were created by programming the propeller’s rotational speed in the respirometer (TracerDAQ Pro™ Software). Water velocities followed a sinusoidal function with a period of 5s, which is representative of moderate wave periods in the Puget Sound and San Juan Islands (Finlayson, 2006).

We measured EPOC after each $U_{\text{crit}}$ trial by integrating the area under the curve of the relationship between $MO^2$ and time at $U_{\text{crit}}$ until the time when the fish’s oxygen consumption reached a steady state (Lee et al., 2003). Since most fish did not reach an oxygen consumption rate as low as that measured prior to the onset of the trial (see Svendsen et al., 2010), we determined the end of EPOC as the first 10 min time interval in which $MO^2$ increased by 5% after having stabilized. We added EPOC to each fish’s $MO^2$ measurements proportionally to the count of burst swimming events at each distinct swimming speed during the $U_{\text{crit}}$ trial. We attributed EPOC to burst swimming behaviours only, excluding pectoral and caudal fin swimming. In the confamilial, co-occurring striped surfperch *Embiotoca lateralis* Agassiz, Svendsen et al. (2010) showed that the pectoral-caudal gait transition ($U_{p-c}$) is not a threshold for anaerobic swimming; instead burst activity is the single most important determinant of EPOC.

*Video analysis: swimming performance and fin beats*

We recorded the swimming behaviour of test subjects during each trial with a video camera (Canon Vixic HV30) positioned above the respirometer’s working section. A mirror was placed at $45^\circ$ adjacent to the working section in order to record the top and
side view of the fish in a single frame. $U_{p-c}$ was determined when a fish changed from strictly pectoral to pectoral-and-caudal swimming for more than 5s (Johansen and Jones, 2011). For each speed increment of 0.5 BLs$^{-1}$, one observer (MT) examined the video footage using ODlog (Macropod Software) and recorded the frequency and amount of time each fish spent (1) swimming only with its pectoral fins (P), (2) swimming with a combination of its pectoral and caudal fins (P+C), or (3) bursting and coasting with caudal fin only (BC). One count was made for each pectoral fin beat (i.e. one full rotation of the pelvic fin) during both P and P+C swimming. One BC event was defined as a period of time that included caudal fin beats (typically 1, 2 or 3 beats) and the subsequent forward glide motion.

Statistical analysis

We calculated a fish’s critical swimming speed ($U_{crit}$) and gait transition speed ($U_{p-c}$) following Brett (1964):

$$U_{crit} \text{ and } U_{p-c} = U + U_i \times \left( \frac{t}{t_i} \right)$$

(1)

where $U$ is the penultimate swimming speed before the fish fatigued and stopped swimming ($U_{crit}$) or before the fish changed gait from P to P+C swimming ($U_{p-c}$); $U_i$ is the swimming speed at which the fish was unable to continue swimming or changed swimming gait (i.e., swimming speed at increment $i$); $t$ is the length of time the fish swam at the final swimming speed where fatigue or gait change occurred; $t_i$ is the amount of time fish were swam at each speed interval in the trial (30 min). We tested for differences in fish swimming performance ($U_{p-c}$, $U_{burst}$, $U_{crit}$) among treatments using one-way ANOVAs followed by a Tukey HSD post-hoc test.

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The analysis of physiological response curves requires taking into account the temporal autocorrelation of data points (Peek et al., 2002). In our case, measurements of oxygen consumption for a given $U_{\text{crit}}$ trial were not independent since we carried out repeated measurements on the same fish. Therefore, we used a linear mixed effect model to test for differences in the relationships between swimming speed ($U$) and oxygen consumption ($MO_2$) across flow treatments (Bolker et al., 2009; Peek et al., 2002). Relationships are based solely on the aerobic component of swimming trials ($MO_2$ measurements at speeds below $U_{\text{burst}}$). The data were log10 transformed to meet the assumptions of linearity, normality and homogeneity of variance. All analyses were conducted in R v2.11.1 (R Development Core Team, 2010).

RESULTS

Respirometry

Oxygen consumption rate ($MO_2$) was best described as a power function for fish in all three flow treatments (Figure 1).

Treatment A=0 BLs$^{-1}$ (steady flow treatment; standard curve):

$$MO_2 = 2.64 \times \text{Speed}^{3.86} + 135.14$$

Treatment A=0.5 BLs$^{-1}$ (unsteady flow treatment with amplitude 0.5 BLs$^{-1}$ around $U_{\text{mean}}$):

$$MO_2 = 3.66 \times \text{Speed}^{3.45} + 127.52$$

Treatment A=1.0 BLs$^{-1}$ (unsteady flow treatment with amplitude 1.0 BLs$^{-1}$ around $U_{\text{mean}}$):

$$MO_2 = 8.67 \times \text{Speed}^{3.12} + 144.89$$
The relationship between MO$_2$ and U$_{\text{mean}}$ for the unsteady flow treatment (A=0.5 BL/s$^{-1}$) was not significantly different from the standard curve steady flow (A=0) treatment ($t=-0.975$, $p>0.30$) as determined by the linear mixed effect model (Figure 2). In contrast, the relationship between MO$_2$ and U$_{\text{mean}}$ for the 1.0 BL/s$^{-1}$ amplitude flow treatment significantly differed from the standard curve ($t=2.57$, $p=0.02$). The mean MO$_2$ differed significantly among treatment groups at swimming speeds of 2.5 BL/s ($F_{2,17}=9.45$, $p<0.001$), 3.0 BL/s ($F_{2,17}=9.66$, $p<0.001$) and 3.5 BL/s ($F_{2,16}=5.92$, $p=0.012$). This was not the case at 4.0 BL/s ($F_{2,11}=0.45$, $p=0.65$). Post-hoc tests revealed that fish in the high amplitude treatment consumed significantly more oxygen than fish in the control group at 2.5 BL/s and 3.0 BL/s (all $p$s<0.05), but not at 3.5 BL/s ($p=0.20$).

*Swimming performance*

Gait transition speed from P only to P+C propulsion (U$_{p-c}$) occurred at significantly different mean speeds among treatments (1-way ANOVA, $F_{2,17}=3.8158$, $p=0.005$, Figure 3). Fish in large amplitude treatments (1BL/s$^{-1}$) reached U$_{p-c}$ at significantly lower mean speeds than fish in both low (A=0.5BL/s$^{-1}$) amplitude ($p=0.006$) and steady (A=0BL/s$^{-1}$) flow ($p=0.027$) treatments. However, there was no difference in the mean gait transition speeds between fish in low amplitude and steady flow treatments ($p=0.847$). Transition speeds from pectoral-caudle swimming (P+C) to a burst and coast gait (U$_{\text{burst}}$) were not significantly different among treatments ($F_{2,17}=1.968$, $p=0.17$). The mean maximum swimming speed (U$_{\text{crit}}$) reached by fish was significantly different among treatments ($F_{2,17}=3.871$, $p=0.041$). Fish in the high amplitude treatment (A=1.0 BL/s$^{-1}$) reached U$_{\text{crit}}$ at significantly lower speeds than fish in the low amplitude treatment.
(p=0.043); fish in the steady flow treatment did not differ significantly in their maximum mean speed from either high (p=0.129) or low (p=0.883) amplitude treatments.

Fin beats

Pectoral fin beat frequency had a significant effect (LMM, F = 489.100, P < 0.001) and explained 60% of the variance in MO$_2$ when controlling for P+C and BC events (Figure 4, Table 1). P+C beat frequency was significant (LMM, F = 112.887, P < 0.001) and explained 26% of the variance in MO$_2$ when controlling for P and BC events. The frequency of burst and coast events was significant (LMM, F = 13.752, P < 0.001), but only explained 4% of model variance when controlling for all other predictors.

Mean pectoral fin beat frequency was greatest in the A=1.0 BLs$^{-1}$ treatment, followed by the A=0.5 BLs$^{-1}$ amplitude flow treatment and control treatment. However, the difference among flow treatments was not significant during pectoral only (LMM, F = 2.272, P = .131) and P+C fin swimming (LMM, F = 2.393, P = 0.119) even when controlling for swim speed (LMM, F = .581, P = 0.560).

DISCUSSION

We showed that *C. aggregata* incur greater energetic costs when swimming in high, unsteady wave-like flows (A=1.0 BLs$^{-1}$) than in steady flows with the same mean velocity (A=0 BLs$^{-1}$). However, our results suggest that *C. aggregata* are only affected by unsteady flows that vary in velocity beyond a certain threshold: fish swimming in unsteady flows with lower variations in velocity (A=0.5 BLs$^{-1}$) did not experience energetic costs superior to that of fish in steady flows (A=0 BLs$^{-1}$). Furthermore, swimming performance measures (U$_{p-c}$, U$_{crit}$) were lower for fish swimming at 1.0 BLs$^{-1}$

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amplitude versus the control (\(U_{\text{p.c}}\)) and the 0.5 BLs\(^{-1}\) amplitude treatment. Considering that P+C and BC swimming are predictive of \(MO_2\) consumption, fish swimming in high unsteady flows experience peak velocities that push them to recruit their caudal fin at lower mean velocities than in steady flows. We showed that some portion of the variance in aerobic oxygen consumption (26% and 4%) is likely caused by the recruitment of the caudal tail muscles. Therefore, we would expect that the increase in \(MO_2\) consumption at a given speed of high amplitude water flow would be the result of a greater frequency of fin beats and/or a greater proportion of caudal fin beats. While P or P+C fin beats were highest in the 1.0 BL/s amplitude group, followed by the 0.5 BL/s amplitude and control group, the difference among treatments was not significant. A larger sample size may have been able to detect a significant change in fin beats among treatments.

*Metabolic costs of swimming in unsteady flows*

Traditionally, the field of fish swimming has described the kinematics, physiology, morphology and behaviour of fish movement. Increasingly, scientists have been able to apply this knowledge in practical ways such as developing more effective fishways for impounded rivers (e.g. Peake et al., 1997) and relating swimming performance measures to ecologically meaningful traits (Plaut, 2001). One limitation of this approach is that traditional performance measures taken under controlled laboratory conditions do not always accurately reflect the swimming abilities of fishes in natural conditions, where temperature and water flow velocity and turbulence are never constant. Freshwater researchers have attempted to replicate more natural conditions in swimming performance experiments, which may be more ecologically relevant and applicable to management objectives (Enders et al., 2003; Enders et al., 2005; Liao, 2007; Liao et al., M.K. Taylor, D.G. Roche, S.A. Binning
2003; Taguchi and Liao, 2011). However, similar experiments are lacking in marine systems. Studies of unsteady water flow on fishes have focused on body-caudle swimmers whereas the different swimming morphologies and kinematics of other locomotor modes may have a profound influence on the costs of unsteady swimming (Fulton, 2010; Webb and Cotel, 2010).

Of the few studies that have looked at the metabolic costs of fishes swimming in unsteady flows, the results are unclear. Enders et al. (2003) found a 1.3- to 1.6-fold increase in the metabolic cost of swimming in juvenile Atlantic salmon (Salmo salar) as turbulence increased. Under a more controlled type of turbulent condition (Von Karmen wake), Liao et al. (2003) demonstrated that rainbow trout (Oncorhynchus mykiss) are able to exploit the regular pattern of vortices and reduce the muscular activity needed to swim at a given speed. More recently, Taguchi and Liao (2011) suggest that turbulent flow can reduce the energetic costs of swimming in rainbow trout as long as certain energy-saving behaviours are employed. Our study represents the first attempt at measuring the metabolic costs of swimming in unsteady water flow for a marine, labriform fish. We found that unsteady flows increase the metabolic cost of swimming, but only at levels of flow that require burst swimming behaviour powered by the anaerobic body-caudal swimming muscles. In C. aggregatea, this change in energetic metabolic pathway was clearly visible in a change from P+C assisted population to BC propulsion powered solely by the caudle fin. However, all mean swimming speeds tested, low amplitude fluctuations (A=0.5 BLs\(^{-1}\)) did not increase the energy required to swim relative to steady flow. Rather, low amplitude fluctuations seem to require less energy for swimming at 3BLs\(^{-1}\) and 3.5BLs\(^{-1}\) than steady flow swimming or the predicted costs based on the
sinusoidal function, although these trends were not significant. If water flow and wave patterns occur at regular intervals, it is possible that fish can anticipate the motion of the water and use the periods of low flow to economise energy and ride the wave. This behaviour may be similar to the energy-efficient wave and bow-riding behaviour observed in some cetaceans (Fish and Hui, 1991; Williams et al., 1992), and warrants further investigation.

_Unsteady flows and swimming performance_

Measures of swimming performance in the laboratory have been criticized by ecologists, who struggle to extrapolate the results of such tests to ecologically meaningful performance measures in natural systems (Peake, 2004; Plaut, 2001). However, as many fish species lack anti-predator defences other than effective escape and avoidance behaviours, swimming capability likely has a major impact on Darwinian fitness (Plaut, 2001). Although swimming capabilities measured under laboratory conditions may not accurately reflect an individuals’ ability to outswim a predator or migrate upriver in the wild, these measures provide useful baselines that ecologists can then use to test against treatments that mimic more natural conditions. In our study, we used metabolic rates, swimming performance measures and fin beat frequencies in standard steady flow trials as a control for our two fluctuating speed treatments, which mimicked the unsteady water flow conditions a fish is likely to experience in rough or stormy weather days. The fact that only some of these performance measures differed among treatments provides interesting insights into the swimming behaviours of these fishes. For instance, the caudle fin was recruited earlier in the high amplitude treatment due to the destabilizing effect that maximum flows had on a fish swimming. However, once these fish were able to
steady themselves with their caudle fins, they were able to continue swimming using aerobic energy pathways as long as the other two treatment groups, as indicated by the similar $U_{\text{burst}}$ speeds in all treatments. The ability of labriform-swimming fishes to recruit their caudle fins for stability while still swimming aerobically with their pectoral fins may provide a substantial advantage over strictly body-caudle swimming fishes, which must use their caudle fins for thrust and stability during both aerobic and anaerobic swimming phases. Using a labriform swimmer in this study provided us with a unique means of partitioning aerobic from anaerobic energy metabolism given that pectoral fin beats correlate with oxygen consumption and gait transition speed ($U_{\text{burst}}$) can visually be used as an index of anaerobically-powered activity (Johansen et al., 2010; Svendsen et al., 2010; Tudorache et al., 2009). Interestingly, fish in the high amplitude group did not reach $U_{\text{crit}}$ earlier than the control group, although they did fail at lower speeds than the low amplitude treatment fish. This discrepancy may be due to a lack of power to be able to detect significant differences among these treatment groups. However, a significant difference between high and low amplitude treatments suggests that fish do experience a higher cost of swimming when they are pushed beyond their aerobic capacities. Although these swimming performance measures do not represent all of the complexities and challenges of swimming in the wild, they can provide interesting insights into the effect of a single parameter that mimics natural conditions when compared with a highly controlled swimming trial that allows a fish to reach the theoretical maxima for a given measure.

**Conclusion**

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Understanding the effects that waves and current have on the swimming capacity of fishes is important for both practical and theoretical reasons. Rivers are being increasingly degraded, and flow regimes altered as a result of numerous anthropogenic activities, compounded by climate change (Kingsford, 2011). Similarly, coastal marine systems are routinely exposed to intense bouts of water motion due to tidal action, wind-driven waves and weather patterns that are predicted to become more frequent and intense (Harley et al., 2006; Seymour, 2011). Whether or not fishes have the ability to adapt to these changes in their hydrodynamic environments depends on their swimming performance and physiology; traits which can be used to make predictions about the cost of locomotion in altered environments. Although controlled laboratory swimming trials under steady flow conditions have taught us much about the physical and physiological limits of swimming in fishes, this study provides an important advancement in our understanding of the costs of locomotion in unsteady flow conditions. Future studies should build on this research by exploring the influence of other important wave parameters such as periodicity on a variety of fishes with a range of swimming modes.

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trials. J.F. Steffensen, P. Domenici and J.L. Johansen helped with the set-up and design of the experimental protocol. Fish collections were carried out under the University of Washington animal care protocol number 4238-04. Partial support for this project was provided by the Adopt-a-Student Fund (MKT, DGR) and the Wainwright Fellowship Fund (SAB) from UW, the ANU Vice Chancellor’s Special Needs Research Grant (DGR, SAB), and the Fisheries Society of the British Isles (MKT).
REFERENCES


TABLES AND FIGURES

Table 1. Parameter estimates for Linear Mixed Model predicting Log(MO$_2$) based on pectoral only fin beat frequency, pectoral+caudal fin beat frequency and burst event frequency.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Estimate (SE)</th>
<th>df</th>
<th>t</th>
<th>95% Confidence Lower</th>
<th>95% Confidence Upper</th>
<th>Sig</th>
</tr>
</thead>
<tbody>
<tr>
<td>Intercept</td>
<td>1.82520</td>
<td>111.565</td>
<td>68.004</td>
<td>1.77201</td>
<td>1.87838</td>
<td>0.00</td>
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<tr>
<td>Pectoral</td>
<td>0.00073</td>
<td>333.963</td>
<td>22.116</td>
<td>0.00066</td>
<td>0.00079</td>
<td>0.00</td>
</tr>
<tr>
<td>P+C</td>
<td>0.00138</td>
<td>339.468</td>
<td>10.625</td>
<td>0.01127</td>
<td>0.00164</td>
<td>0.00</td>
</tr>
<tr>
<td>Burst</td>
<td>0.00051</td>
<td>328.760</td>
<td>3.708</td>
<td>0.00024</td>
<td>0.00078</td>
<td>0.00</td>
</tr>
</tbody>
</table>
Figure 1. Mean oxygen consumption (MO$_2$ in mg O$_2$ kg$^{-1}$h$^{-1}$) as a function of mean swimming speed (U$_{\text{mean}}$) for *C. aggregata* in one of three treatments: constant flow velocity (standard curve) in red (n=6), unsteady flow with an amplitude of 0.5 BLs$^{-1}$ around U$_{\text{mean}}$ (n=7), and unsteady flow with an amplitude of 1.0 BLs$^{-1}$ around U$_{\text{mean}}$ (n=7). Relationships are based on the aerobic component of swimming trials and include MO$_2$ measurements at speeds below U$_{\text{burst}}$ (filled symbols). Empty symbols are MO$_2$ measurements that include EPOC to provide the metabolic swimming cost that included both the aerobic and anaerobic swimming components. The three coloured lines are Eqn () (blue), Eqn () (green) and Eqn () (red), illustrating the relationship between exercise MO$_2$ and swimming speed. Error bars are standard errors. The grey lines are theoretical

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predictions of MO₂ vs. speed based on the standard curve for the 0.5 BLs⁻¹ amplitude treatment (solid) and the 1.0 BLs⁻¹ amplitude treatment (dashed).
Figure 2. Same as Figure 1 – data was log10 transformed to meet the assumptions of the linear mixed effect model.
**Figure 3:** Mean water velocity achieved by fish for three different swimming performance measures ($U_{pc}$, $U_{burst}$ and $U_{crit}$) in three different water flow treatments (control no fluctuation= standard, low amplitude fluctuation= 0.5BL, high amplitude fluctuation= 1.0BL). Asterisks indicate significant differences between the high fluctuation treatment and both low amplitude and control groups (**, p<0.01) and between high and low fluctuation treatments (*, P<0.05).
Figure 4: Scatterplot of the relationship between pectoral fin beat frequency and MO$_2$ grouped within three different flow treatments (control= 0BL$^{-1}$, low amplitude fluctuation= 0.5BL$^{-1}$, high amplitude fluctuation= 1.0BL$^{-1}$). MO$_2$ was log10 transformed for the model, but was back-transformed for this figure.