How lipid content and temperature affect American shad (Alosa sapidissima) attempt rate and sprint swimming: implications for overcoming migration barriers

<table>
<thead>
<tr>
<th>Journal:</th>
<th>Canadian Journal of Fisheries and Aquatic Sciences</th>
</tr>
</thead>
<tbody>
<tr>
<td>Manuscript ID</td>
<td>cjfasm-2018-0406.R2</td>
</tr>
<tr>
<td>Manuscript Type:</td>
<td>Article</td>
</tr>
<tr>
<td>Date Submitted by the Author:</td>
<td>27-Feb-2019</td>
</tr>
<tr>
<td>Complete List of Authors:</td>
<td>Bayse, Shannon; Memorial University of Newfoundland Fisheries and Marine Institute, McCormick, Stephen; U.S. Geological Survey Castro-Santos, Theodore; USGS, S.O. Conte Anadromous Fish Research Center</td>
</tr>
<tr>
<td>Keyword:</td>
<td>American shad, Swim speed fatigue time, Fish passage, Survival analysis</td>
</tr>
<tr>
<td>Is the invited manuscript for consideration in a Special Issue?:</td>
<td>Not applicable (regular submission)</td>
</tr>
</tbody>
</table>
How lipid content and temperature affect American shad (Alosa sapidissima) attempt rate and sprint swimming: implications for overcoming migration barriers


U.S. Geological Survey, Leetown Science Center, S.O. Conte Anadromous Fish Research Laboratory, One Migratory Way, Turners Falls, MA 01376, USA.

*Corresponding author. Present address: Fisheries and Marine Institute, Memorial University of Newfoundland, 155 Ridge Road, St. John’s, NL A1C 5R3, Canada, Phone: 709-778-0386, Fax: 709-778-0661, Email: Shannon.Bayse@mi.mun.ca
Abstract

How seasonal effects, such as temperature increases and reduced lipid content affect the ability of anadromous fish to traverse high-velocity barriers and sprint swimming is poorly understood. Here we evaluate American shad (*Alosa sapidissima*) swimming performance in a flume against high flow velocities (2.5-3.7 m·s\(^{-1}\)) during the upstream migration period (April-May; temperatures 11.1-21.4\(^{\circ}\) C) to determine how their willingness to enter a velocity barrier (attempt rate) and swimming endurance changed during migration. American shad did not make attempts at low temperatures, and attempt rate gradually increased throughout the migration as temperatures warmed. American shad displayed two distinct non-sustained swimming modes (prolonged and sprint swimming) and endurance was different between sexes. Females swam at prolonged speeds more often at warmer temperatures and displayed a lower endurance at warm temperatures for long fish. Male swimming was primarily at sprint speeds and was affected by swimming speed, length, and lipid content. Our results indicate that American shad motivation and swimming endurance changes over the course of the migration as conditions change, potentially limiting their ability to pass barriers.
Introduction

Fishways exist to provide river connectivity and facilitate fish passage beyond barriers. In rivers with dams, fish passage via fishways is essential to provide fish access to spawning, nursery, and feeding grounds. However, fishways may also act as a velocity barrier that approaches, or exceeds, the behavioral and physiological limits of species they intend to pass (Haro et al. 2004). Many factors can affect fish passage efficiency through a fishway, including temperature, discharge, velocity, swimming endurance, among others (Castro-Santos 2004).

Typically, fishways are designed to pass specific species under current site conditions. However, passage efficiency at fishways is likely to change as temperature and flow regimes vary seasonally or are altered by climate change (Kemp et al. 2011) or anthropogenic thermal inputs (Webb 1996). Additionally, changes in precipitation rates are expected from climate change (Trenberth 2011), and shifts, either higher or lower, can affect fishway discharge, which can in turn influence passage efficiency (Ovidio and Philippart 2002). Rises in temperature and changes in flow may affect a fish’s endurance to traverse fishways, perhaps dramatically altering current passage efficiencies.

With few exceptions, fish are obligate poikilotherms that cannot produce enough metabolic heat to compensate rapid heat loss through the gills and epidermis (Fry 1968). This is converse to the regulated homeostatic state that fish can achieve with other environmental factors such as salinity, oxygen, and pH (Brett 1971). Temperature has a strong impact on all enzymatic reactions, and environmental temperature therefore has a profound effect on crucial fish physiological processes including growth, energetics, and swimming (Brett 1971).
The effect temperature has on fish swimming capacity is well documented for non-sprint swimming speeds. At sustained swimming speeds, speeds that can be maintained for longer than 200 min (Beamish 1978), fish rely on aerobic processes to power swimming. How temperature influences sustained swimming is directly related to oxygen consumption (Brett 1965), and generally, at sustained swimming speeds metabolic rate increases with higher temperatures until an optimum is reached, at which point temperature negatively affects metabolic rate (Fry 1947; Beamish 1978). Prolonged swimming speeds are thought to generally range from 20 s to 200 min (Beamish 1978) and rely on a combination of aerobic and anaerobic processes (Bilinski 1974), which can induce fatigue. Similar to metabolic rate during sustained swimming, prolonged swimming capacity increases with temperature to an optimum, and then negatively affects swimming performance (Beamish 1978).

How temperature affects sprint swimming is less clear. Sprint swimming is a high-rate, steady mode of swimming that leads to fatigue, typically in less than 20 s (Beamish 1978; Webb 1975). Sprint swimming relies almost exclusively on anaerobic metabolism, sustained by glycogen, adenosine triphosphate, and phosphocreatine stored in white muscle (Milligan 1996), and is thought to be largely independent of temperature (Brett 1971), although few studies have investigated this relationship.

Fish sprint swimming capacity has historically been studied in relatively short swimming chambers (Brett 1964; Videler 1993; Peake et al. 1997). Recent studies in large-scale flumes that allow fish to swim against greater flows, and with more volition, have found higher swim speeds, indicating that small swim chambers may limit detection of maximum sprint swimming speed.
ability (Haro et al. 2004; Peake and Farrell 2006; Castro-Santos et al. 2013). For example, Castro-Santos et al. (2013) observed brook trout (Salvelinus fontinalis) and brown trout (Salmo trutta) to swim at speeds > 25 body lengths (BL)·s\(^{-1}\), much higher than previous studies (< 12 BL·s\(^{-1}\); Bainbridge 1960; Peake et al. 1997). Additionally, previous studies in small fish chambers found no correlation between temperature and sprint swimming for performance or recovery (Brett 1964; Schreer et al. 2001), but very few studies have used large flumes and volitional swimming to investigate temperature or other environmental effects.

Temperature can play a large role in the ability or willingness of important species to pass fishways. The role of temperature in limiting fish passage is important to understand from a management perspective to improve current passage, as well as to be prepared for potential changes as temperatures increase via climate change. Few studies have directly examined how temperature affects the willingness and ability of a fish to pass a velocity barrier (a zone where fish must swim at unsustainable speeds to pass upstream; Castro-Santos et al. 2013). Castro-Santos (2004) quantified how several covariates, including temperature, affect the attempt rate of white sucker (Catostomus commersoni) and walleye (Sander vitreus) against a velocity barrier of water velocities at 1.5-4.5 m·s\(^{-1}\) through an open-channel flume. This study found that increasing temperatures increased the attempt rate for both species. Haro et al. (2004) included temperature when investigating the sprint swimming performance of several fish species. These authors found a positive correlation between temperature and maximum distance traversed while sprint swimming for blueback herring (Alosa aestivalis) and walleye, and a negative correlation for American shad (Alosa sapidissima).
American shad present an interesting species to investigate how temperature can affect the attempt rate and swimming endurance of a diadromous fish, and the subsequent implications for fish passage. American shad are a numerous anadromous species on the east coast of North America, and are a commercially and ecologically important species throughout their range (Greene et al. 2009). The species is distributed from Florida to Newfoundland, and individuals across that range may experience wide seasonal and geographical variations in temperature. For example, American shad in the Connecticut River, USA, experience a large change in temperature over the course of their spawning migration, from approximately < 5°C at the beginning stages in April to > 25°C in late July at the end stages (Castro-Santos and Letcher 2010), and have been shown to be sensitive to rising temperatures for both swimming endurance (Haro et al. 2004) and passage delays (Castro-Santos and Letcher 2010).

Coupled with the seasonal increase in temperature is a gradual reduction in lipid content. American shad do not feed during the fresh water portion of their migration, which can be greater than 228 km in the Connecticut River. In fresh water, lipid stores are gradually reduced below 0.5% of their total somatic content (Bayse et al. 2018), and total energetic depletion can range from 35 to 60% over the course of the migration (Leonard and McCormick 1999). The level of lipid reserves may influence both motivation and swimming endurance, and likely changes over the course of the migration as temperatures rise and lipid resources become reduced.

Seasonally, increased temperatures and reduced lipid content could potentially decrease American shad passage ability. Reduced passage ability likely would increase passage
delay length, further exposing American shad to deleterious temperatures, and further using up energetic resources; all of which could amplify current passage difficulties. We investigated the effect that seasonal temperature increases – temperature was not manipulated and increased naturally – and loss of lipid content have on motivating American shad to pass fishways, and how these factors affect American shad sprint swimming endurance over the course of their spawning migration.

Materials and methods

Flume

Swimming performance was measured in a flume at the S.O. Conte Anadromous Fish Research Laboratory (U.S. Geological Survey) in Turners Falls, Massachusetts (Fig. 1). The flume was similar to the one used in previously described studies (Haro et al. 2004; Castro-Santos et al. 2013; Duguay et al. 2018) and was 35 m long, 0.64 m wide, 0.64 m deep, and set with a 1% slope. It was constructed from transparent acrylic sheeting supported within aluminum and steel framing and a downstream staging area (53 m² x 1 m deep). During trials, fish volitionally entered the flume from the low-velocity staging area.

Flow to the flume was gravity supplied and adjusted by an intake valve connected to a 76.2 cm supply pipe from the adjacent hydroelectric power canal. Flow entered the flume under an adjustable sluice gate and continued down the flume and entered the staging area with a clockwise recirculatory pattern providing a low velocity zone. Flow exited the staging area through a semicircular wall (10.4 m²) of perforated steel plate (50% open) into a channel
equipped with an adjustable-height weir, which was used to regulate the water surface level in the staging area (Fig. 1).

Water depth and velocity within the flume were regulated by a combination of valve opening, sluice gate height, and staging area depth. Flow rates were monitored with a GF Signet 2552 Magmeter (Schaffhausen, Switzerland) within the supply pipe. Water surface levels were manually measured every 1.8 m up the length of the flume for each trial. Flume water temperatures were measured at the start of each treatment by a Taylor 9841 Extended Range Thermometer (Oak Brook, IL).

**Instrumentation**

Fish movements within the flume were monitored using a passive integrated transponder (PIT) telemetry system. The PIT system consisted of 16 antennas spaced every 1.8 m for the first 23.4 m, and every 3.6 m for the remaining 11.6 m. Each antenna was tuned to detect tags over a 0.5 m range and interfaced to a separate half-duplex PIT reader (TIRIS Series 2000 system, Texas Instruments, Dallas, Texas, USA). Readers were configured to charge and read tags at 10 Hz and interfaced through a multi-port serial-to-USB converter (Edgeport/16; Digi International, Inc., Minnetonka, Minnesota, USA) to a personal computer that records PIT identification number, antenna number, and time to the nearest 0.01 s. Further details of the PIT system can be found in Castro-Santos et al. (2013).

**Fish collection**
Migrating adult American shad were collected from the Connecticut River at the fish lift at Holyoke Dam, Holyoke, MA (rm 139—the first river barrier) between 28 April and 30 May 2016. Collections were timed to be representative of the entire upstream migration season and cover the extent of the temperature regime that fish encounter while still migrating upstream. Fish collection was subject to availability, and began at the start of passage at Holyoke Dam until the migration began to decline.

Collected American shad were transported to the flume via a fish transport truck (1,000 L) supplied with recirculating, oxygenated, Connecticut River water. Upon arrival, fish were measured (fork length), sex was determined, lipid content measured (see lipid content section), externally PIT tagged, and released into the flume staging area. Tags were half-duplex Oregon RFID 12 mm half-duplex PIT tags attached to fish hooks, and were placed in the cartilage at the base of the dorsal fin (Castro-Santos et al. 1996). Drag incurred by the tags was considered negligible due to their extremely small size (0.17 g; 12 mm) relative to an adult American shad (~3 kg; 430 mm). Additionally, Castro-Santos (2002) observed no difference in swimming performance for American shad with or without tags. The gate to the flume was closed to prevent entry and the fish were kept overnight in the staging area at a discharge of 0.08 m$^3$.s$^{-1}$.

**Lipid content**

Lipid content was measured for each American shad with a Fat Meter (Distell Model 692 Fish Fat Meter, Distell Inc., West Lothian, Scotland, U.K.). The meter is a handheld device that uses microwave technology to determine lipid content. The meter actually measures water content in fish tissue and relates this to lipid content via the inverse relationship of lipid and
water content (Craig et al. 1978). Measurements followed the methods described in Bayse et al. (2018), which involved two readings directly under the dorsal fin, targeting white muscle just above the midline. Both replicate measurements were taken on the left side and totaled less than 10 s. The meter used the manufacturer setting “herring-1,” and the readings were adjusted with the regression formula provided by Bayse et al. (2018) to be used in conjunction with American shad.

**Trials**

The experiment alternated between a moderate (2.5 m·s⁻¹) and high-velocity (3.5 m·s⁻¹) treatment for each trial, alternating which velocity went first each day to prevent order effects. High velocities were chosen to focus results on factors that affected sprint swimming. Previous research has shown that American shad typically swim volitionally at sprint swimming speeds against flow velocities > 2.2 m·s⁻¹ (Castro-Santos 2005). To capture a broad range of sprint speeds, we tested flow velocities from 2.5-3.7 m·s⁻¹. Each trial began at approximately 8:00 the morning following collection, and each treatment lasted approximately 3 h, typically with two treatments a day.

**Statistical analysis**

**Attempt rate**

Individual attempts were defined as entry past the initial gate and into the flume. These were counted, compared among treatments, and the associated attempt rate was quantified using time-to-event analysis (or survival analysis) (Allison 1995; Castro-Santos 2004). Attempt
rate was measured as the “hazard,” or proportion of the available fish staging attempts at any
given time (Hosmer and Lemeshow 1999). Time was included from the start of each trial until
the first attempt, and the intervals elapsed between attempts. Fish that did not initiate
attempts were included in the analysis as censored observations, with the trial duration
substituted for event time. This allowed all available fish to contribute to the rate calculation,
whether an attempt was staged or not. Additionally, for fish that staged attempts, the time
elapsed between their last attempt time and the end of the trial was included as a censored
observation.

How temperature, lipid content, velocity, fish length, and sex affected attempt rate was
modeled with a Cox proportional hazard regression (coxme function in the coxme package
(Therneau 2015) with R Development Core Team (2009)) (Allison 1995; Castro-Santos and Perry
2012). Individual fish and trial were included as random effects on the intercept (Goerig and
Castro-Santos 2017). A positive coefficient value indicates that a positive change in the
covariate increases attempt rate (see Castro-Santos et al. 2013 for more details).

**Swimming endurance**

Swimming endurance was defined as the relationship between swim speed ($U_s$) and
fatigue time and was quantified using survival analysis (Castro-Santos 2005). For each
swimming speed, swim speed-fatigue times were calculated for the attempt in which maximum
distance of ascent was achieved. Any ascent that reached the upper end of the flume
(considered antenna 15 for this study; 27.5 m from entrance) was included as a censored
observation. Regression models were fit with the survreg function from the survival package in
Moving-point regression models were used to investigate mode shifts (Brett 1964; Castro-Santos et al. 2013). This was modelled following the moving-point regression methods of Castro-Santos et al. (2013), using the model:

\[
\ln(T) = \beta_0 + \beta_1 C_{ps} + \beta_2 U_s + \beta_3 C_{ps} U_s + \epsilon
\]

where fatigue time \( T \) is determined by the intercept term \( \beta_0 + \beta_1 C_{ps} \) and a slope term \( \beta_2 U_s + \beta_3 C_{ps} U_s \). The \( \beta \) terms are regression coefficients and \( C_{ps} \) is a binary categorical variable that is zero for observations less than an incrementing hypothetical swim speed threshold \( U_{ps} \) where fish change modes, and one for observations greater than this value. Hence, two intercept values \( \beta_0 \) and \( \beta_0 + \beta_1 \) and two slope values \( \beta_2 \) and \( \beta_2 + \beta_3 \) were calculated, one for each side of the mode shift (if two modes). Separate regression models were considered for each value of \( U_{ps} \), and the best model was selected based on the minimum Akaike information criterion (AIC) (Burnham and Anderson 2002). Each fitted model had a unique AIC value, allowing the examination of individual model variability, and the error term \( \epsilon \) had a Weibull distribution (see Castro-Santos et al. 2013 for more details).

Temperature changes followed the natural increase in temperature during spring, and it was not possible to manipulate temperature due to the need for very large water volumes in the flume. As such, the temperature-related effects we describe are also closely linked to seasonal changes over time. Originally, ordinal date (day of the year 1-365) was investigated as an independent variable to represent the change of time over the course of the migration for both attempt rate and swimming endurance models. However, ordinal date was correlated with temperature, having a Pearson's product moment correlation coefficient (Pearson 1948).
of 0.99 (where 0 indicates no correlation and -1 and 1 indicate complete correlation).

Additionally, the variance inflation factor (VIF) was > 10, and rendered the slope of fit models illogically, which is common when multicollinearity is present. The VIF quantifies the severity of multicollinearity where a value > 5 indicates a problematic amount of collinearity (James et al. 2014). Additionally, models that included ordinal day and did not include temperature had poor model fits. Best fit models that included ordinal day and excluded temperature retained several variables that were not significant ($p > 0.05$) and were illogical, which is a sign of multicollinearity. Models that included temperature, and excluded ordinal day, were logical with several significant variables included. Thus, we excluded ordinal date from the analysis and retained temperature with the understanding that temperature was a “temperature-related” effect that also had a seasonal component.

**Results**

Over the course of the migration, temperatures ranged from 11.1-21.4°C. Five fish collections were made and ranged between 30 and 43 fish per collection, 191 in total. The first two collections (28 April and 8 May 2016) had either zero attempts (28 April collection) or very few attempts (8 May collection) and these fish were tested an additional day, either 48 (28 April collection) or 24 h (8 May collection) later to ensure a long enough time had passed to generate attempts. Similar results were observed for repeated tests: both trials for the 28 April collection had zero attempts, and 5 and 19 attempts for the 09 May and 10 May trials, respectively, for the 8 May collection (Table 1).
Female American shad totaled 52.1% of fish collected and males were 47.9%. Fish ranged in fork length 35.2-50.9 cm with females (mean ± standard deviation; 45.2 ± 2.4 cm) being significantly longer than males 40.2 ± 2.6 cm (ANOVA, $df = 1$, $F = 191.4$, $p < 0.001$) (Table 2), which is typical for migrating American shad (Leonard and McCormick 1999). Lipid content measurements ranged from 0.6-9.4% with males (mean ± standard deviation; 6.3 ± 2.1 %) having significantly higher percent lipid content than females 4.1 ± 2.1 % (ANOVA, $df = 1$, $F = 40.7$, $p < 0.001$; Table 2).

The Fat Meter was unavailable for the last trial date, May 31, 2016. Therefore, all model results (model results below) contain two separate model data sets, data set one – all fish and all trials without lipid content measurements, and data set two – all fish and all trials except the trial and fish tested on May 31, 2016, and include lipid content measurements. A stipulation of analyses using AIC is that models from different data sets cannot be compared.

**Attempt rate**

A total of 197 attempts were observed (Table 1). Several attempts ($n = 54$) had a missed reading at the first antenna due to equipment malfunction. To include these attempts in the swimming endurance analysis, we extrapolated the time value at the first antenna with a linear model for each attempt. The time values at the next three antennas were used if they had a linear trend and extrapolated with the predict function (stats package) in R. Attempts with zero slope or irregular relationships between time and distance were not extrapolated, and these attempts were removed ($n=13$) from swimming endurance analysis. The remaining extrapolations were reasonable: each model had a coefficient of determination ($R^2$) ≥ 0.99.
American shad made more attempts as temperatures increased (Fig. 2). Attempts more than doubled as temperatures rose above 14.0°C, with a total of 28 attempts < 14.0°C and 67 attempts between 14.0 and 15.0°C (139.3% increase; Table 1). Attempts continued to increase as temperatures rose above 15.0°C to 21.4°C up to 102 attempts, a 52.2% increase (Table 1).

Attempt rate (attempts-individual⁻¹-day⁻¹) increased from 0.6 attempts-individual⁻¹-day⁻¹ at temperatures < 14.0°C to 3.8 attempts-individual⁻¹-day⁻¹ (533.3% increase) at temperatures between 14.0 and 15.0°C (Fig. 2). Attempt rate continued to increase as temperatures rose up to 21.4°C, with 6.9 attempts-individual⁻¹-h⁻¹, which is an 81.6% increase from the attempt rate between 14.0 and 15.0°C.

Flow velocities ranged from 2.5-2.9 m·s⁻¹ for the moderate velocity condition and 3.3-3.7 m·s⁻¹ for the high velocity condition (Table 1). American shad made more attempts against higher flow velocities (117 to 80 high to low), however the attempt rate was 0.1·individual⁻¹·day⁻¹ for both conditions.

The best fit attempt rate model for data set one (including all data but without considering lipid content) included an interaction between the temperature and velocity parameters (Table 3). This interaction term had a negative coefficient value indicating that as one parameter increases, and the other decreases, attempt rate changes. To visualize this relationship, the model was plotted with the survfit function from the survival package. This function is unable to plot models with random effects; therefore, random effects were removed to enable plotting; note that trends between the two models were consistent and aid in demonstrating model interpretation (Table 3; Fig. 3). At low temperatures (10 and 14°C; Fig.
301 3a,b), attempt rate was higher at high flow velocity (3.5 m·s$^{-1}$) versus moderate (2.5 m·s$^{-1}$). At
302 18° C, attempt rate was about the same between flow velocities (Fig. 3c). At high temperatures
303 (22° C), attempt rate was higher for moderate flow velocity (Fig. 3d), opposite of the lowest
304 temperatures. Conversely, within a given flow treatment the relative effect of temperature
305 remained consistent, with greater rates associated with higher temperatures (Fig. 4).
306
307 The best fit model for data set two (included lipid content) similarly contained
308 temperature and velocity parameters as the main factors effecting attempt rate, but the
309 interaction between temperature and velocity was no longer retained, and an interaction
310 between lipid content and length was included (Table 3). This interaction term has a positive
311 slope, indicating that as lipid content is higher, longer fish have a higher attempt rate, whereas
312 shorter fish have higher attempt rates at low lipid content levels. The effect of lipid content and
313 length even though significant ($p < 0.05$) was slight, with a slope of 0.004.
314
315 **Swimming endurance**
316
317 Due to differences in length and body shape between female and male American shad,
318 swimming endurance was investigated separately for each sex. Female American shad swam at
319 speeds from 6.2 to 12.8 BL·s$^{-1}$, and a moving-point regression on the relationship between
320 swim speed-fatigue time showed a significant breakpoint (a change in slope of the swim speed-
321 fatigue time relationship) between two modes at 8.0 BL·s$^{-1}$ (Fig. 5; Table 4). Therefore,
322 swimming speeds ≤ 8.0 BL·s$^{-1}$ were considered mode one or prolonged swimming, and speeds >
323 8.0 BL·s$^{-1}$ were considered mode 2 or sprint swimming. Males swam at speeds from 6.9 to 15.1
BL·s$^{-1}$ (Fig. 6; Table 5), and a moving-point regression indicated a significant breakpoint between two modes at 9.5 BL·s$^{-1}$.

To further examine the existence and effect of a break between non-sustained swimming speeds (prolonged and sprint), the best fit model was investigated for each sex including both speeds, and if the break was significant, each speed was modelled separately. For data set one, the best fit model with both swimming speeds for female American shad included significant parameters: break, swim speed, length, and an interaction between break and swim speed (Table 4). Length had a negative coefficient indicating that smaller fish have greater endurance at a given relative swim speed. The interaction between break and swim speed had a positive coefficient, meaning that the slope of the swim speed-fatigue time relationship was steeper for prolonged swimming speeds than for sprint swimming speeds. (Fig. 5).

Among females, the best fit model for prolonged swimming speeds in data set one included temperature, length, swim speed, and an interaction between temperature and length. Swim speed had a negative coefficient meaning that fatigue time is greater at lower swim speeds. The interaction term had a negative coefficient and is illustrated in Fig. 5. At high temperatures ($20^\circ$ C), fatigue time was lower for long fish (+1 standard deviation (SD) of mean length) (solid red line; Fig. 5), versus cold temperatures ($12^\circ$ C) (solid blue line; Fig. 5). For short fish (-1 SD of mean length), the opposite relationship was shown. At high temperatures, fatigue time was higher for short fish (dashed red line; Fig. 5), and lower for long fish at cold temperatures (dashed blue line; Fig. 5).
The best model for females at sprint swimming speeds and data set one included swim speed and length, both with a negative coefficient, indicating greater endurance at lower swim speeds and for shorter fish. The sprint swim speed-fatigue time relationship to fish length was illustrated in Fig. 5 for short fish (dashed black line) versus long fish (solid black line).

Only 10 observations were made at a prolonged swimming speed for male American shad. This indicated that tested flow velocities were appropriate to concentrate male swimming at sprint speeds. Consequently, we did not further model prolonged swimming. The best fit model for sprint swimming for data set one included swim speed and length, both with negative coefficients (Table 5). Figure 6 shows the relationship between swim speed and fatigue time for sprint swimming with short fish (dashed black line) having a higher swim speed-fatigue time relationship versus long fish (solid black line).

For data set two, prolonged swimming attempts for female American shad dropped to only 6 observations that also had a lipid content measurement, and data set two was not further analyzed. Sprint swimming was investigated for both female and male attempts. For females, only swim speed was included in the best fit model, with a negative slope indicating swim speed and fatigue time decreased as swimming speed increased. The male model included swim speed and length with almost the same slope as without lipid content (data set one); however, lipid was included in this model, having a positive slope that indicates that endurance increases with higher lipid content (Table 5).

Discussion
Few studies have investigated temperature or lipid content’s impact on American shad swimming endurance, and to our knowledge no studies have addressed how both factors influence swimming endurance for any fish. Leonard et al. (1999) swam American shad in a respirometer at two temperatures, and a higher standard metabolic rate and swim speed (sustained swimming speeds) were observed in the high temperature group. Castro-Santos (2002; 2005) and Haro et al. (2004) tested sprint swimming in American shad in a flume similar to the one in this study, and observed a negative correlation with temperature, distance traversed, and passage performance. Male American shad actually traversed greater distances at higher temperatures on their first attempt whereas females travelled shorter distances (Castro-Santos 2002).

Several studies have addressed how adult salmon (Salmonidae) migrations will be affected by changing temperatures and energy content (Rand and Hinch 1998; Young et al. 2006; Hanson et al. 2008; Lennox et al. 2018). These studies considered performance over the entire migration focusing on migration speed, successful passage, and bioenergetic models; they differed from our design, which focused on sprint swimming performance in a controlled flume. Generally, these studies found that fish size, swimming activity, and temperature greatly influenced energy demand, spawning success, mortality, and rates of iteroparity. When compared to this study, similar results were found in relation to fish size, with smaller animals performing better at warmer temperatures (temperature-size rule; Kingsolver and Huey 2008).

Increased temperature was shown to increase energetic costs, but energetic cost was more sensitive to fish size and swimming speed (Lennox et al. 2018). For migration speed, Hanson et al. (2008) observed a negative correlation with high energetic status in the ocean to migration
speed, and migration speed was positively correlated to temperature once in the river.

Additionally, Young et al. (2006) found that low energetic content led to higher mortality for early-run sockeye salmon (*Oncorhynchus nerka*). Similar conditions of increased temperatures and lower lipid content could similarly affect American shad migrations, increasing mortality, and reducing spawning success and iteroparity as suggested in a simulation model for Connecticut River American shad (Castro-Santos and Letcher 2010).

Lipid content and length were different for each sex, which potentially affected motivation and endurance. In the Connecticut River, large fish expend 2-21% more energy (mostly lipid) during migration, when compared to smaller fish (Leonard and McCormick 1999), and lipid and length together affected attempt rate in this study. Sex was not included in any attempt rate final model, but perhaps length slightly masked a sex effect because females were longer. Additionally, migration to upriver spawning areas were considered to be 50-100% more energetically expensive for females (Leonard and McCormick 1999), and increased temperatures, or longer periods of being in high temperatures, will further increase the metabolic rate of American shad. Each of these factors could prove more challenging to large females that have the combination of decreased relative swimming capacity and increased depletion of lipid reserves in higher temperatures. During prolonged swimming attempts, higher temperatures decreased the swimming endurance of females for longer fish, indicating that temperature negatively affects their performance. Unfortunately, not enough prolonged swimming attempts had an associated lipid content measurement, as a low lipid content of these fish may have played a role in their decreased capacity. If an increased amount of work is
required to pass barriers (additional failed attempts) due to reduced endurance during prolonged swimming, a further reduction in available lipid is likely to ensue.

Our results are informative to management for determining how temperature changes affect American shad passage at fishways and other zones of high-velocity flow, which is an increasing concern with the realities of climate change effects on temperature and water flows (Silva et al. 2017). Other anthropogenic increases in temperature, such as those caused by powerplant discharge, surface runoff, reservoir heating, and others further exacerbate these effects, and may be both more acute and greater in magnitude than climate-driven changes (Webb 1996; Mustard et al. 1999). Both direct and indirect anthropogenic effects, as well as natural climate cycling can be expected to affect both motivation and ability of American shad to pass barriers.

American shad commonly experience migration delays, which can be up to a week or more at individual fishways (Sullivan 2004; Castro-Santos and Letcher 2010), and can be affected, at least partially, by temperature. Fish were collected for this study at a dam 139 km from the river mouth in a fish lift. These fish were motivated to migrate from the ocean and upriver, but were unwilling to enter the experimental flume (velocity barrier) until temperatures reached 14°C, after which attempt rates increased several fold with individual fish making multiple attempts. These results imply that, at temperatures below 14°C, Connecticut River American shad are clearly motivated to migrate to spawning grounds, but are behaviorally and perhaps physiologically limited, from passing high flow regions. A constructed velocity barrier, such as a dam, could be directly limiting early season migration range, which in
turn reduces spawning success of some individuals, and decreases the overall population fecundity of a spawning season. However, as temperatures increase with climate change, perhaps warmer temperatures earlier in the year will promote a higher passage motivation at this stage. It should be noted, however, that we were unable to measure the effect of high temperature (>22 °C) on motivation and endurance because it did not occur during the upstream migration, but may in the future. Based on previous research on prolonged swimming in other species, it seems likely that high temperature (above an optimal) will decrease swimming capacity at prolonged swimming speeds, either by directly limiting delivery of metabolic substrates, or by reduced aerobic scope, which would increase recovery times required between attempts (Brett and Glass 1973). This could directly affect female American shad that preferentially swam at prolonged speeds at high temperatures. Thus, any benefits of elevated temperature early in migration may result in a trade-off if temperatures are also elevated at the end of the migratory period.

However prolonged swim speed fatigue events for male American shad were low in number, and were excluded from analysis. The study design was to target flow velocities that would only produce sprint swimming, but that was not met for female American shad, which had a very distinct separation between swimming modes. This approach was partially successful for males, with 10 fatigue events at prolonged swimming speeds. Therefore, our results for female American shad were split, lowering the number of attempts at sprint speed, and leaving an unknown effect of temperature and lipid content for male American shad at prolonged swimming speeds.
Although the two non-sustained swimming modes described in this study included speeds greater than typically associated with prolonged speeds, it is interesting to note that the break in the swim speed-fatigue time relationship happened at 20 seconds, which is identical to what Brett (1964) described as the threshold between prolonged and sprint swimming. In this way our terminology differs somewhat from the Sprint1 and Sprint2 modes described by Castro-Santos et al (2013), who described a break at speeds greater than 19 BL s$^{-1}$ and endurance times of $<10$ s. Recognizing this, Castro-Santos et al. (2013) did not refer to their observations as prolonged speeds. In the present study, however, the mode switch at 8.0 BL s$^{-1}$ is more consistent with the literature, and so the lower speeds likely correspond with what is traditionally considered prolonged speeds. This is important, because prolonged swimming is generally believed to comprise both aerobic and anaerobic musculature and metabolic processes (Brett 1964; Jayne and Lauder 1994). Thus, the observed temperature effects at prolonged swimming are consistent with the expectation that aerobic metabolism is more strongly affected by temperature than anaerobic metabolism, and that sprinting ability is relatively temperature-independent.

In this study, we normalized swim speeds to BL s$^{-1}$. This is standard practice for numerous and well-established biomechanical and metabolic reasons (Alexander 2005). However, the approach implicitly assumes an isometric relationship of speed, which is also known to be inaccurate; over a large range of body sizes this imposes error caused by as-yet poorly-understood allometries in swimming ability. Because of this we found a significant effect of body length, with smaller fish having greater endurance at relative swim speeds than larger fish. These effects, however, were less than the overall greater absolute performance of larger
fish. For example, a 45 cm male American shad would fatigue at 10 seconds when swimming at 11.3 BL s\(^{-1}\) (5.1 ms\(^{-1}\)) and a 40 cm male would have the same endurance when swimming at 12.4 BL s\(^{-1}\) (5.0 m s\(^{-1}\)), thus the improved endurance at relative swim speeds is not sufficient to offset the overall speed advantages afforded by greater length. It does, however, reduce the magnitude of the benefit of added size, and may in part account for why some researchers have failed to detect significant length effects on swimming performance when measured in SI units (Peak and Farrell 2006). This study was not, however, designed to explicitly quantify length effects, and more work is needed to improve our understanding on this important driver of swimming performance.

These results raise significant concerns regarding models of swimming performance currently used in fish passage engineering manuals and guidelines (Beach 1984; Bell 1991; Larinier 2002), all of which assume that temperature has a strong limiting effect on sprinting ability. These data suggest that a more nuanced view is required, and further emphasizes the need for empirical data on sprinting ability for migratory fishes in general, and temperature effects in particular.

We have shown that attempt rate was directly affected by temperature and lipid content, and that swimming endurance at prolonged swimming speeds was affected by temperature for female American shad. As temperatures increase with climate change and other anthropogenic effects, a mix of impacts can be expected for American shad. An improvement in early season passage could be a benefit from higher temperatures (and not yet extinguished lipid resources), ultimately perhaps selecting for earlier migrations. Limits to
swimming motivation and/or endurance, however, could prove detrimental to American shad’s ability to pass barriers, preventing access to important spawning grounds, which could decrease spawning success, decrease spawning at upriver sites, and potentially reduce the rate of iteroparity.

Acknowledgements

We express our thanks and appreciation to the many individuals that assisted with this study including: K. Sprankle (U.S. Fish and Wildlife Service; USFWS); E. Goerig (Harvard University); S. Leach and B. LaFlamme (Normandeau Associates); R. Murray (Holyoke Gas & Electric); J. Noreika, S. Walk, S. Parker, A. Regish, A. Skrzynska, and L. Guo (U.S. Geological Survey; USGS).

This work was funded by USGS/USFWS Science Support Partnership (SSP) Program (grant number SSP-15-R5-02). Any use of trade, product, or firm names is for descriptive purposes only and does not imply endorsement by the U.S. Government. All experiments were carried out under U.S. Geological Survey Institutional Animal Care and Use Committee Guidelines under protocol No.C09076.

References


Beach, M.H. 1984. Fish pass design—criteria for the design and approval of fish passes and other structures to facilitate the passage of migratory fish in rivers. Ministry of Agriculture, Fisheries, and Food; Directorate of Fisheries Research; Fisheries Research Technical Report 7–8. Lowestoft.


**Figure captions**

**Fig. 1.** Experimental flume and staging area (Aquatic Biomechanics and Kinematics Station, or ABiKiS) used for sprinting performance studies. Figure modified from Duguay et al. (2018) and approximately to scale.

**Fig. 2.** American shad attempt rate (attempts·individual$^{-1}$·day$^{-1}$) for each day a trial occurred (bars), and temperature at the beginning of each trial (line).

**Fig. 3.** Percent of American shad staging attempts as a function of time (data set one), estimated from a Cox model, where $U_f$ is constant and temperature increases (a-d). The solid line represents the mean curve, shaded areas are the 95% confidence intervals, and the dashed line indicates the time at which 50% of the fish had attempted to swim up the flume.

**Fig. 4.** Percent of American shad staging attempts as a function of time (data set one), estimated from a Cox model, where temperature is constant and $U_f$ increases (a-c). The solid line represents the mean curve, shaded areas are the 95% confidence intervals, and the dashed line indicates the time at which 50% of the fish had attempted to swim up the flume.

**Fig. 5.** Swim speed-fatigue time relationship of female American shad (data set one) BL = body length. Vertical dashed line indicates the location of a break point between mode 1 (left) and mode 2 (right) swimming speeds. Triangles indicate fatigue and circles indicate censored observations. Modelled relationships are demonstrated for mode 1: long fish (+1 standard deviation (SD) fork length) at cold temperatures ($12^\circ$ C) (solid blue line), long fish at hot temperatures ($20^\circ$ C) (solid red line), short fish (-1 SD fork length) at cold temperatures (dashed blue line), and short fish at hot temperatures (dashed red line). Models for mode 2 include short fish (dashed black line) and long fish (solid black line).

**Fig. 6.** Swim speed-fatigue time relationship of male American shad at mode 2 swimming speeds (data set one). Triangles indicate fatigue observations. Modelled relationships are demonstrated as: short fish (-1 SD) (dashed black line) and long fish (+1 SD) (solid black line).
Table 1. Trial date, subtrial, mean flow velocity ($U_f$), temperature at start of trial, and sample sizes for sprinting performance experiments. Sample sizes include number of fish available (Number Available), number of fish that made an attempt (Number Attempting), and total attempts made (Total Attempts).

<table>
<thead>
<tr>
<th>Trial date</th>
<th>Subtrial</th>
<th>$U_f$</th>
<th>Temperature</th>
<th>Number Available</th>
<th>Number Attempting</th>
<th>Total Attempts</th>
</tr>
</thead>
<tbody>
<tr>
<td>29 Apr 2016</td>
<td>1</td>
<td>2.9</td>
<td>11.1</td>
<td>30</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>29 Apr 2016</td>
<td>2</td>
<td>3.7</td>
<td>11.3</td>
<td>30</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>02 May 2016</td>
<td>1</td>
<td>3.6</td>
<td>11.1</td>
<td>30</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>02 May 2016</td>
<td>2</td>
<td>2.8</td>
<td>11.2</td>
<td>30</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>09 May 2016</td>
<td>1</td>
<td>3.5</td>
<td>11.2</td>
<td>42</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>09 May 2016</td>
<td>2</td>
<td>2.6</td>
<td>11.5</td>
<td>42</td>
<td>3</td>
<td>3</td>
</tr>
<tr>
<td>09 May 2016</td>
<td>3</td>
<td>3.5</td>
<td>11.6</td>
<td>42</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>10 May 2016</td>
<td>1</td>
<td>2.6</td>
<td>12.0</td>
<td>42</td>
<td>5</td>
<td>5</td>
</tr>
<tr>
<td>10 May 2016</td>
<td>2</td>
<td>3.5</td>
<td>12.2</td>
<td>42</td>
<td>13</td>
<td>14</td>
</tr>
<tr>
<td>16 May 2016</td>
<td>1</td>
<td>2.6</td>
<td>13.9</td>
<td>43</td>
<td>3</td>
<td>4</td>
</tr>
<tr>
<td>16 May 2016</td>
<td>2</td>
<td>3.4</td>
<td>14.3</td>
<td>43</td>
<td>25</td>
<td>42</td>
</tr>
<tr>
<td>20 May 2016</td>
<td>1</td>
<td>3.4</td>
<td>14.6</td>
<td>35</td>
<td>9</td>
<td>11</td>
</tr>
<tr>
<td>20 May 2016</td>
<td>2</td>
<td>2.5</td>
<td>15.0</td>
<td>35</td>
<td>13</td>
<td>14</td>
</tr>
<tr>
<td>31 May 2016</td>
<td>1</td>
<td>3.3</td>
<td>20.6</td>
<td>41</td>
<td>20</td>
<td>25</td>
</tr>
<tr>
<td>31 May 2016</td>
<td>2</td>
<td>2.6</td>
<td>20.9</td>
<td>41</td>
<td>30</td>
<td>54</td>
</tr>
<tr>
<td>31 May 2016</td>
<td>3</td>
<td>3.7</td>
<td>21.4</td>
<td>41</td>
<td>20</td>
<td>23</td>
</tr>
</tbody>
</table>
Table 2. Fork length and lipid content of American shad used in swimming studies. Fork length includes all collected fish, female *n*=100 and male *n*=92, and lipid content includes all fish excluding 30 May 2016 collection, female *n*=72 and male *n*=79. SD is standard deviation.

<table>
<thead>
<tr>
<th>Measurement</th>
<th>Female</th>
<th>Male</th>
<th><em>F</em>-value</th>
<th><em>p</em>-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean length ± SD (cm)</td>
<td>45.2 ± 2.4</td>
<td>40.2 ± 2.6</td>
<td>191.4</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>Mean lipid content ± SD (%)</td>
<td>4.1 ± 2.1</td>
<td>6.3 ± 2.1</td>
<td>40.7</td>
<td>&lt; 0.001</td>
</tr>
</tbody>
</table>
### Table 3. Output of Cox's proportional hazards model of time between attempts for American shad stratified by attempt number

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Coefficient</th>
<th>SE</th>
<th>z value</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>All attempts (n=197)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Temperature</td>
<td>0.937</td>
<td>0.230</td>
<td>4.080</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>Velocity</td>
<td>2.531</td>
<td>0.861</td>
<td>2.940</td>
<td>0.003</td>
</tr>
<tr>
<td>Temperature*Velocity</td>
<td>-0.148</td>
<td>0.047</td>
<td>-3.150</td>
<td>0.002</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Random effects</th>
<th>Variable</th>
<th>SD</th>
<th>Variance</th>
</tr>
</thead>
<tbody>
<tr>
<td>Individual</td>
<td>Intercept</td>
<td>0.810</td>
<td>0.657</td>
</tr>
<tr>
<td>Trial</td>
<td>Intercept</td>
<td>1.412</td>
<td>1.995</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Coefficient</th>
<th>SE</th>
<th>z value</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Attempts with lipid measurement (n=95)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Temperature</td>
<td>1.227</td>
<td>0.342</td>
<td>3.580</td>
<td>0.061</td>
</tr>
<tr>
<td>Velocity</td>
<td>0.519</td>
<td>0.277</td>
<td>1.870</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>Lipid</td>
<td>-1.799</td>
<td>0.830</td>
<td>-2.170</td>
<td>0.030</td>
</tr>
<tr>
<td>Length</td>
<td>-0.026</td>
<td>0.012</td>
<td>-2.270</td>
<td>0.023</td>
</tr>
<tr>
<td>Lipid*Length</td>
<td>0.004</td>
<td>0.002</td>
<td>2.070</td>
<td>0.038</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Random effects</th>
<th>Variable</th>
<th>SD</th>
<th>Variance</th>
</tr>
</thead>
<tbody>
<tr>
<td>Individual</td>
<td>Intercept</td>
<td>1.074</td>
<td>1.154</td>
</tr>
<tr>
<td>Trial</td>
<td>Intercept</td>
<td>1.112</td>
<td>1.237</td>
</tr>
</tbody>
</table>
Table 4. Relationship between swim speed ($U_s \text{ m·s}^{-1}$) and fatigue time ($\ln(T)$) for female American shad.

<table>
<thead>
<tr>
<th></th>
<th>Coefficient</th>
<th>SE</th>
<th>z value</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Female: Mode 1 and Mode 2 (n=59)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Intercept</td>
<td>11.016</td>
<td>1.371</td>
<td>8.034</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>Break</td>
<td>-3.228</td>
<td>0.989</td>
<td>-3.264</td>
<td>0.001</td>
</tr>
<tr>
<td>$U_s</td>
<td>-0.716</td>
<td>0.128</td>
<td>-5.586</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>Length</td>
<td>-0.005</td>
<td>0.002</td>
<td>-3.272</td>
<td>0.001</td>
</tr>
<tr>
<td>Break:$U_s$</td>
<td>0.409</td>
<td>0.130</td>
<td>3.149</td>
<td>&lt; 0.002</td>
</tr>
</tbody>
</table>

Female: Mode 1 (n=20)

<table>
<thead>
<tr>
<th></th>
<th>Coefficient</th>
<th>SE</th>
<th>z value</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Intercept</td>
<td>-18.326</td>
<td>5.871</td>
<td>-3.122</td>
<td>0.002</td>
</tr>
<tr>
<td>Temperature</td>
<td>1.361</td>
<td>0.271</td>
<td>5.027</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>Length</td>
<td>0.056</td>
<td>0.012</td>
<td>4.628</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>$U_s</td>
<td>-0.544</td>
<td>0.133</td>
<td>-4.095</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>Temperature:Length</td>
<td>-0.003</td>
<td>0.001</td>
<td>-4.897</td>
<td>&lt; 0.001</td>
</tr>
</tbody>
</table>

Female: Mode 2 (n=39)

<table>
<thead>
<tr>
<th></th>
<th>Coefficient</th>
<th>SE</th>
<th>z value</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Intercept</td>
<td>7.915</td>
<td>0.992</td>
<td>7.979</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>$U_s</td>
<td>-0.308</td>
<td>0.034</td>
<td>-8.967</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>Length</td>
<td>-0.005</td>
<td>0.002</td>
<td>-2.842</td>
<td>0.004</td>
</tr>
</tbody>
</table>

Female: Mode 2 with lipid (n=22)

<table>
<thead>
<tr>
<th></th>
<th>Coefficient</th>
<th>SE</th>
<th>z value</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Intercept</td>
<td>4.656</td>
<td>0.534</td>
<td>8.712</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>$U_s</td>
<td>-0.220</td>
<td>0.054</td>
<td>-4.087</td>
<td>&lt; 0.001</td>
</tr>
</tbody>
</table>

Table 5. Relationship between swim speed ($U_s \text{ m·s}^{-1}$) and fatigue time ($\ln(T)$) for male American shad.

<table>
<thead>
<tr>
<th></th>
<th>Coefficient</th>
<th>SE</th>
<th>z value</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Male: Mode 2 (n=39)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Intercept</td>
<td>7.797</td>
<td>0.653</td>
<td>11.943</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>$U_s</td>
<td>-0.264</td>
<td>0.025</td>
<td>-10.768</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>Length</td>
<td>-0.006</td>
<td>0.001</td>
<td>-5.057</td>
<td>&lt; 0.001</td>
</tr>
</tbody>
</table>

Male: Mode 2 with lipid (n=29)

<table>
<thead>
<tr>
<th></th>
<th>Coefficient</th>
<th>SE</th>
<th>z value</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Intercept</td>
<td>7.462</td>
<td>0.741</td>
<td>10.075</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>$U_s</td>
<td>-0.269</td>
<td>0.028</td>
<td>-9.532</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>Length</td>
<td>-0.006</td>
<td>0.001</td>
<td>-4.455</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>Lipid</td>
<td>0.038</td>
<td>0.014</td>
<td>2.754</td>
<td>0.006</td>
</tr>
</tbody>
</table>
Fig. 1
Fig. 2

The figure shows the attempt rate (attempts-individual\(^{-1}\)-day\(^{-1}\)) and temperature over time from 4/29 to 5/31. The attempt rate increases significantly on 5/16, with a further increase on 5/20. The temperature rises steadily from 4/29 to 5/31.
Fig. 3

Temperature 10°C

Temperature 14°C

Temperature 18°C

Temperature 22°C

Percent Attempting

Time (h)

U, m s⁻¹ 2.5 3.5

0.00 0.25 0.50 0.75 1.00

0.00 0.25 0.50 0.75 1.00

0.00 0.25 0.50 0.75 1.00

0.00 0.25 0.50 0.75 1.00

Time (h)
Fig. 4
Fig. 6

![Graph showing the relationship between length (cm) and fatigue time (s) vs swim speed (BL/s).]

- **Length (cm)**: 450, 425, 400, 375
- **Fatigue Time (s)**: 20, 10, 5
- **Swim Speed (BL/s)**: 10, 11, 12, 13, 14, 15

The graph illustrates a negative correlation between length and swim speed, indicating that longer fish tend to have higher fatigue times at lower swim speeds.