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Review

Beyond U_{crit}: matching swimming performance tests to the physiological ecology of the animal, including a new fish 'drag strip'☆

J.A. Nelson[a,*], P.S. Gotwalt[a], S.P. Reidy[b], D.M. Webber[b]

[a]*Department of Biological Sciences, Towson University, Towson, MD 21252, USA*
[b]*Department of Biology, Dalhousie University, Halifax, NS, Canada B3H 4J1*

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Abstract

Locomotor performance of animals is of considerable interest from management, physiological, ecological and evolutionary perspectives. Yet, despite the extensive commercial exploitation of fishes and interest in the health of various fish stocks, the relationships between performance capacity, natural selection, ecology and physiology are poorly known for fishes. One reason may be the technical challenges faced when trying to measure various locomotor capacities in aquatic species, but we will argue that the slow pace of developing new species-appropriate swim tests is also hindering progress. A technique developed for anadromous salmonids (the U_{crit} procedure) has dominated the fish exercise physiology field and, while accounting for major advances in the field, has often been used arbitrarily. Here we propose criteria swimming tests should adhere to and report on several attempts to match swimming tests to the physiological ecology of the animal. Sprint performance measured with a laser diode/photocell timed 'drag strip' is a new method employing new technology and is reported on in some detail. A second new test involves accelerating water past the fish at a constant rate in a traditional swim tunnel/respirometer. These two performance tests were designed to better understand the biology of a bentho-pelagic marine fish, the Atlantic cod (*Gadus morhua*). Finally, we report on a modified incremental velocity test that was developed to better understand the biology of the blacknose dace (*Rhinichthys atratulus*), a Nearctic, lotic cyprinid.

Keywords: Fish; Exercise; Sprint; Burst swimming; Laser diode; Critical swimming

1. Introduction

Locomotor performance of feral animals is of considerable interest from management, physiological, environmental, ecological and evolutionary perspectives. For some animals, success in predator–prey interactions and dominance hierarchy encounters depend upon locomotor capacity (Webb, 1986; Garland et al., 1990). Similarly, the first response of motile animals to environmental perturbation is usually behavioral; successful movement to more suitable environments and therefore survival may depend upon locomotor capacity (e.g. Breitburg, 1992). Thus, locomotor performance is a potential fitness parameter and scientists have expended considerable effort over the past half-century trying to measure the relative ability of animals to move in several temporal contexts (see Beamish, 1978; Bennett and Huey, 1990; Garland and Carter, 1994; Garland and

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*Corresponding author. Tel.: +1-410-704-3945; fax: +1-410-704-2405.

E-mail address: jnelson@towson.edu (J.A. Nelson).

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Losos, 1994; Hammer, 1995; Kolok, 1999 for reviews).

The study of locomotor capacity of fishes, in particular, has a relatively long history (see reviews by: Beamish, 1978; Randall and Brauner, 1991; Hammer, 1995; Domenici and Blake, 1997; Kolok, 1999; Plaut, 2001); most of this work has focused on the mechanism of propulsion by fish and the use of exercise performance as a gauge of fish health, stress level or ability to deal with environmental change. Very little effort has gone into investigating differences in locomotor capacity among individual fish and whether these differences have ecological or evolutionary relevance (Kolok, 1999; Plaut, 2001). The majority of fish locomotion studies have employed a graded water velocity increment test first developed by Brett (1964), which was designed to evaluate the relative ability of salmonid fishes to ascend lotic waters to natal streams. A smaller number of studies have employed fixed-velocity tests, chasing regimes, filming of fish swimming behavior or other techniques (Beamish, 1978). The years following Brett's (1964) first description of the U_{crit} test saw the widespread and indiscriminant adoption of his procedure to a multitude of problems concerning swimming capacity in a variety of fish species (Hammer, 1995). Beamish (1978) extensively reviewed the state of fish locomotion research at this point in time, including an already large number of U_{crit} studies. Drawing largely upon his own work with centrarchids (Farlinger and Beamish, 1977), Beamish (1978) proposed guidelines for the magnitude and duration of velocity increments for subsequent U_{crit} studies. Brett himself (1967) had earlier proposed his own guidelines for U_{crit} studies. The guidelines proposed by Brett (1967) and Beamish (1978) have largely been adhered to by investigators in the 1980s and 1990s. One purpose of this presentation is to propose that graded velocity tests can have utility outside the parameters suggested by Brett (1967) and Beamish (1978). We developed a graded water velocity increment test for blacknose dace (*Rhinichthys atratulus*) with only 5-min time intervals that was repeatable over a period of 1 month and has revealed very interesting information about this species.

Taking into account the diversity of fishes and swimming styles, we propose the following criteria for gauging or establishing the utility of swimming tests: (1) the intra-individual variance of performance in the test over extended time periods (months to years) should be significantly smaller than inter-individual variance in performance among conspecifics (i.e. the test should be repeatable through time); (2) The locomotor performance required of the fish in the test should be within the range of performances experienced by the fish within the course of a lifetime and thus have possible relevance towards determining Darwinian fitness of fish in the field; (3) the results from the performance test should theoretically supply information relevant to the in situ biology of the animal, be it behavioral or physiological information. For the vast majority of published incremental velocity (U_{crit}) studies, it is either unknown or not reported whether the test conformed to these criteria (see Hammer, 1995, Kolok, 1999; and Plaut, 2001, for reviews). We also believe that new swimming tests, which are increasingly being developed by fish biologists (e.g. Jain et al., 1998; Cech et al., 1998; McDonald et al., 1998), should conform to these criteria to be of maximal utility.

A second purpose of this presentation is to introduce two new methods for measuring short-term exercise performance of fishes and to discuss briefly our attempts at determining whether they conform to the above-stated criteria. Studies of fish swimming performance have multifarious goals. However, if the goal of a study is to understand performance physiology or to use locomotor capacity as a potential fitness parameter, an isolated incremental velocity test will probably prove insufficient in most cases. As employed by most investigators, a critical swimming speed test causes the fish to use variant swimming modes at different times during the test. The onset and duration of these different swimming modes is quite variable among individuals of a species and the degree to which an individual uses anaerobic metabolism to power the swim can also vary substantially (Nelson, 1990; Hammer, 1995; Kolok and Farrell, 1994; Nelson et al., 1996). Thus, two conspecific fish may have identical U_{crit} values but may have used quite different physiologies and may have swum quite differently in arriving at U_{crit} (e.g. Nelson, 1990; Nelson et al., 1996). In other words, individual fish of a species show the variation in exercise physiology we have come to expect as routine from humans (e.g. Bouchard et al., 1989). Thus, if the goal of a study is to characterize the performance physiology of a spe-

cies or population, or to assign a performance level to an individual fish, additional performance tests will improve the veracity of the study. Thus, there is a need for more and diverse swimming tests for fish. At this point in time, there are very few published alternatives to the incremental velocity tests. It is also very poorly known how other types of swimming performance relate to U_{crit} performance in the same individual; this question has only been addressed in a few studies (Kolok, 1999; Reidy et al., 2000).

The two new swim tests we report on here are attempts to resolve swimming performances of fish on the scale of seconds to minutes. Fast-start performance is usually considered the measure of performance with the most predictive value for predator–prey interactions (Domenici and Blake, 1997; Webb, 1986) and thus ecological/evolutionary relevance, yet has been studied relatively little in these contexts. Most studies on fast-starts and sprint locomotion were designed to discern mechanisms of propulsion by fish and employed hydrodynamic kinematics (Gero, 1952; Gray, 1953), high-speed filming, or high-speed filming coupled with digital image analysis to calculate swimming speed or acceleration (Domenici and Blake, 1997; Gamperl et al., 1991; Harper and Blake, 1990; Taylor and McPhail, 1985; Wardle, 1975; Webb, 1975, 1978, 1983). Recent technological advances have also allowed the use of piezoelectric accelerometers (Domenici and Blake, 1997; Harper and Blake, 1989, 1990) to accurately measure fish acceleration. Unfortunately, since we wanted to obtain repetitive measurements of performance on a large number of animals under conditions 'field-relevant' for Atlantic cod, none of the traditional techniques were optimal. Piezoelectric techniques require extensive animal handling. Therefore, to measure large numbers of animals with adequate recovery times is difficult. Likewise, although large numbers of fish can be filmed relatively quickly, analysis of films or videotapes to extract data can take inordinate amounts of time. High-speed filming also requires that the fish perform under fairly bright lights, a condition ecologically inappropriate for many fishes, including cod.

Huey et al. (1981) developed a computer driven, multi-beam photocell timing technique based upon an earlier dual photocell method introduced by Bennett (1980). Huey et al.'s (1981) method allowed acceleration and sprint velocity to be repeatably and accurately measured in large numbers of terrestrial animals relatively quickly (e.g. Hertz et al., 1983; Huey and Dunham, 1987; Bennett and Huey, 1990). The development of this computerized 'drag strip' contributed to the flourishing of knowledge concerning the physiological ecology and evolutionary biology of locomotion of small terrestrial vertebrates in the 1980s. We developed a system, similar to that described by Huey et al. (1981), but designed to measure sprint performance of aquatic organisms. This new method allows the investigator to obtain acceleration and swimming speed data from 'bursts' of locomotion on a large number of fishes relatively quickly. We report on the use of this method for measuring sprinting performance of Atlantic cod (*Gadus morhua*) over a 2-m distance. In addition, we report on the development of a constant acceleration test (CAT) for Atlantic cod utilizing a traditional swim tunnel/respirometer.

2. Methods and materials

2.1. New sprint performance method

2.1.1. Chamber construction

The fast start chamber was constructed from 1/4″ and 3/8″ opaque polyvinyl chloride 'flat stock' (Fig. 1). The dimensions of the actual raceway were 2.2 m length × 0.3 m width × 0.3 m height which separated a holding chamber and a receiving chamber each of equal dimension. We designed this chamber for use on 50 cm adult Atlantic cod; these dimensions should be scaled appropriately for fishes of different size. To allow passage of laser light, transparent windows were cut from Lexan® Plexiglas and secured to the raceway section of the chamber (Fig. 1).

Light-emitting laser diodes of 3 mW power output, 600–720 nm wavelength and 3 mm beam width were placed at 0, 0.3, 0.9, 1.5 and 2.1 m positions along the runway (Fig. 1). A 3-mm glass rod was attached to the front of the laser lens. This rod refracted the beam to project a vertical plane or 'curtain of light' across the raceway. The width, height and intensity of the beam could be modified by changing the diameter of the glass rod and the distance between the laser and the glass rod.

A group of six photodarlington detectors of detection wavelength 580–720 nm were obtained from a local electronics retailer and positioned vertically 2.5 cm apart directly across from each

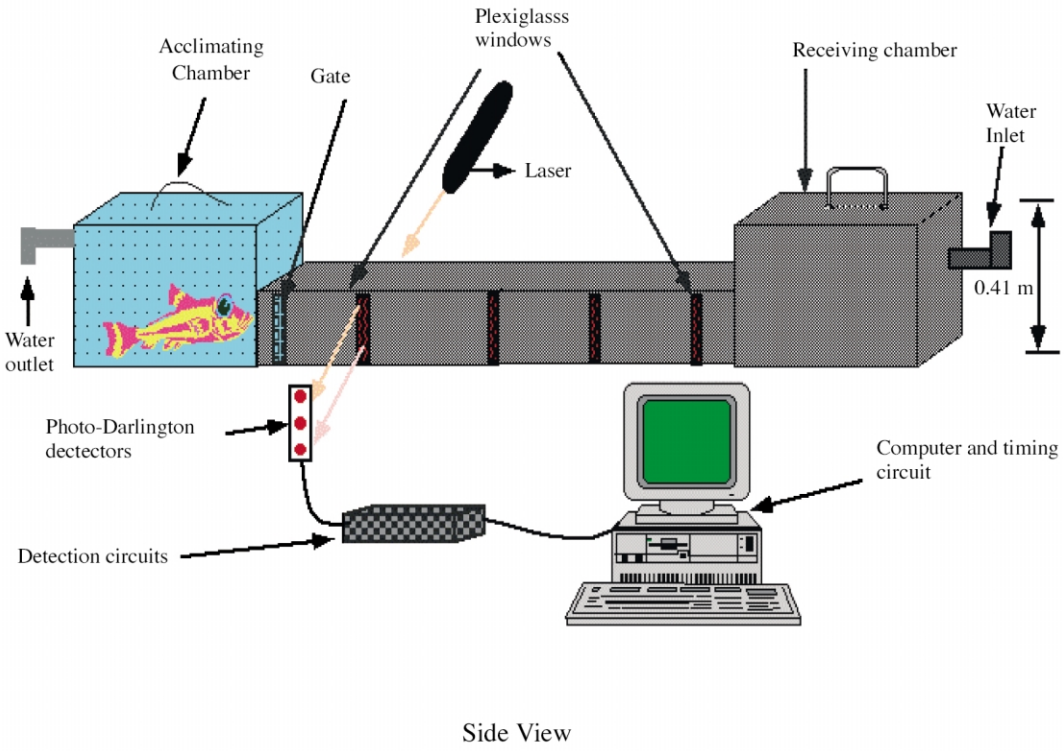

Fig. 1. Diagram of the fast-start chamber used to measure sprint performance in Atlantic cod (*Gadus morhua*): (a) top view (b) side view. All dimensions are in meters.

plexiglass window (total of 30 detectors). This separation distance assured that a beam would be broken with the first 2 cm of a fish that crossed it (for the size and shape of cod we used). Smaller fish would require a greater density of detectors. These detectors and lasers are produced commercially for various applications and are therefore readily available and inexpensive.

2.1.2. Operational details

The light detection and computer timing circuitry for an individual detector of a bank is shown in Fig. 2 and a flow diagram describing the software protocol is illustrated in Fig. 3. In summary, when activated by light, the photodarlington detector signal is amplified and triggers a 2N2222 transistor which puts out a 5 V TTL signal to 1 of 8 inputs into an 8-input NAND Gate (7430). When all six detectors in a bank are saturated, the NAND gate output is low (<0.3 V). However, if one of the beams is broken, the corresponding input to the NAND gate goes low and forces the output of the NAND gate to go high (>0.3 V) (Fig. 2). Similar detectors, including 'on board' NAND gate circuitry, are now available as integrated circuits from Honeywell® Corporation. The computer and digital timer board (MCS6522 Peripheral Interface Adapter, Interactive Microware Inc. P.O. 771, State College, PA 16801, USA) continuously scan the outputs from NAND gates associated with each bank of detectors. Data from the original incarnation of this sprint chamber were collected by an interrupt driven timer software routine in assembler code on an Apple II computer, operating at 1.023 MHz (code will be supplied free of charge upon request). The software-timing cycle was capable of distinguishing events 10^{-5} s apart and would initiate upon breaking of the first light beam by a fish (Fig. 3). The response time of the detector circuitry was determined to be 10^{-6} s. Data from the described system can now be conveniently collected with commercial analog/digital systems such as Labview® or Powerlab®.

2.1.3. Test protocol

Twenty four hours prior to the initiation of a trial, a fish was lightly anaesthetized with MS-222

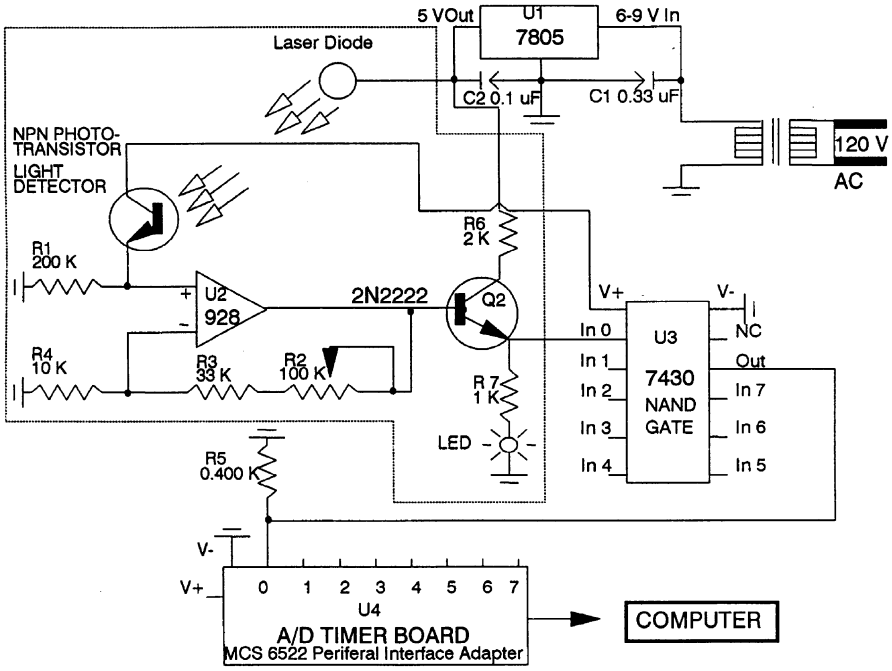

Fig. 2. Diagram of the electronic circuit used to indicate disruption of a laser beam. Note the section of the circuit replicated for each laser beam. Amperage for each circuit (excluding laser) ranged between 10 and 60 mA.

(50 mg/l Sigma®) and placed in the holding section of the chamber (Fig. 1). The fish was kept in this area by a gate that was used to separate the holding chamber from the raceway. Trials for Atlantic cod were conducted in 5 °C, 31‰ water at low, ambient red light so that the fish were in virtual darkness. The following morning, the gate was raised and the fish startled by grasping its caudal peduncle. Electrical, optical and auditory stimuli were also tried as ways to initiate a sprint by Atlantic cod, but tactile stimulation elicited the most intense and reproducible response. Following tactile stimulation, the fish burst down the raceway into the receiving chamber (Fig. 1) where devices for gently decelerating the fish could be located and/or another gate could be closed allowing the fish to rest in this chamber before subsequent treatments. Sprint swimming velocity and acceleration profiles were calculated by the computer software from the time elapsed between breakage of the first laser beam, breakage of subsequent laser beams and the distance between the laser banks.

Operation of the chamber was initially tested with a group of 7 wild Atlantic cod. The procedure was then used on a separate group of 23 Atlantic cod twice each, with 3 months elapsing between repetitive trials (Reidy et al., 2000); data from both groups of fish are presented here.

2.2. Constant acceleration test

This procedure has been previously described in Reidy et al. (2000) as the 'U_{burst}' test. Briefly, adult Atlantic cod were placed in a 96 l swim-tunnel/respirometer 24 h prior to the procedure. The following day, the water velocity was increased at a rate of 0.1667 cm s^{-2} (10 cm s^{-1} min^{-1}) until the fish was exhausted. The water velocity at which the fish exhausted was used as the measure of burst swimming performance (U_{burst}). The definition of exhaustion was when a 12 V electric field did not keep the fish off of the downstream retaining wall. The method was initially developed on a group of 8 Atlantic cod that were each tested twice 1 month apart and then 17 of the same 23 cod that were used for the sprint procedure (see above) were also tested with this protocol. Final U_{burst} velocities were corrected mathematically for the solid blocking effect according to Nelson et al. (1994).

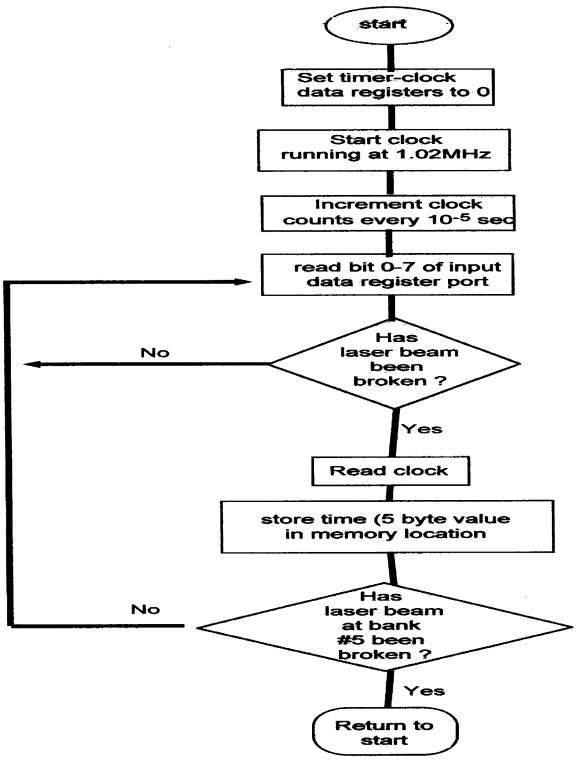

Fig. 3. Flow diagram of software protocol used to detect hardware laser beam breakage and timing between banks. Software was written in BASIC and Assembly Code and is available upon request.

2.3. U_{crit} procedure modified for a small, lotic cyprinid

Blacknose dace (*Rhinichthys atratulus*) from three watersheds and five separate locations within Maryland, USA were collected with a Smith-Root Inc®. Model 15-D backpack electroshocker. Approximately 20 fish from each site (40–60 mm total length, TL) were returned to the laboratory and restricted to an area of their holding tanks where the current speed was between 1 and 3 cm/s. Animals were fed daily with live adult brine shrimp (*Artemia* sp.), but were fasted 24 h prior to a swimming trial. Fish to be swum, were anaesthetized with MS-222, water concentration of 50 mg/l, until they reached phase I of anaesthesia (loss of equilibrium; Iwama et al., 1989). Anaesthetized fish were transferred to a laminar-flow swim flume (Nelson, 1989) and acclimated to a 5 cm/s current at 24($\pm$1 °C) for 1 h. Fish were then exposed to increasing velocity increments of 5 cm/s at 5 min intervals until exhausted. Exhaustion was defined as the point at which a fish no longer responded to gentle prodding with a rubber eraser. Critical swimming velocity, U_{crit}, was calculated according to Brett (1964). The formula used was:

$$^{5}_{5}U_{crit} = U_i + (T_i / T_{ii} \times U_{ii})$$

where U_{crit} = critical swimming speed (cm/s), U_i = highest velocity maintained for a full 5 min interval, T_i = time of fatigue at last current velocity (min), T_{ii} = interval length (5 min), and U_{ii} = velocity increment (5 cm/s). A subset of these fish were re-swum approximately 1 month from the date of initial swimming.

Current speeds of the holding tanks and swim flume were measured with a freshly calibrated Marsh-McBirney Inc.® Model 2000 flow meter. In each case, a three-dimensional grid was established (108 stations in the 180×55×35 cm holding tanks, 27 stations in the 32×10×10 cm swimming section of the swim flume) and current readings made at each station. To establish the calibration for the swim flume, current readings were repeated for each station at 5 V increments of the variable transformer supplying power to the motor.

2.4. General

All performance tests were conducted without investigator knowledge of that particular individual's performance in any previous test.

3. Results and discussion

3.1. New sprint performance method

The results from our trials with Atlantic cod show that the chamber described here can effectively measure sprinting performance in an aquatic medium and should be of use to investigators interested in the ecological and evolutionary ramifications of aquatic performance. We support this contention with evidence that the method is significantly repeatable over a period of several months and a finding that the intra-individual variance of swimming speed in repetitive trials is smaller than inter-individual variance.

3.1.1. Swimming speed

Swimming speed increased in a linear fashion when plotted as a function of elapsed time. Thus,

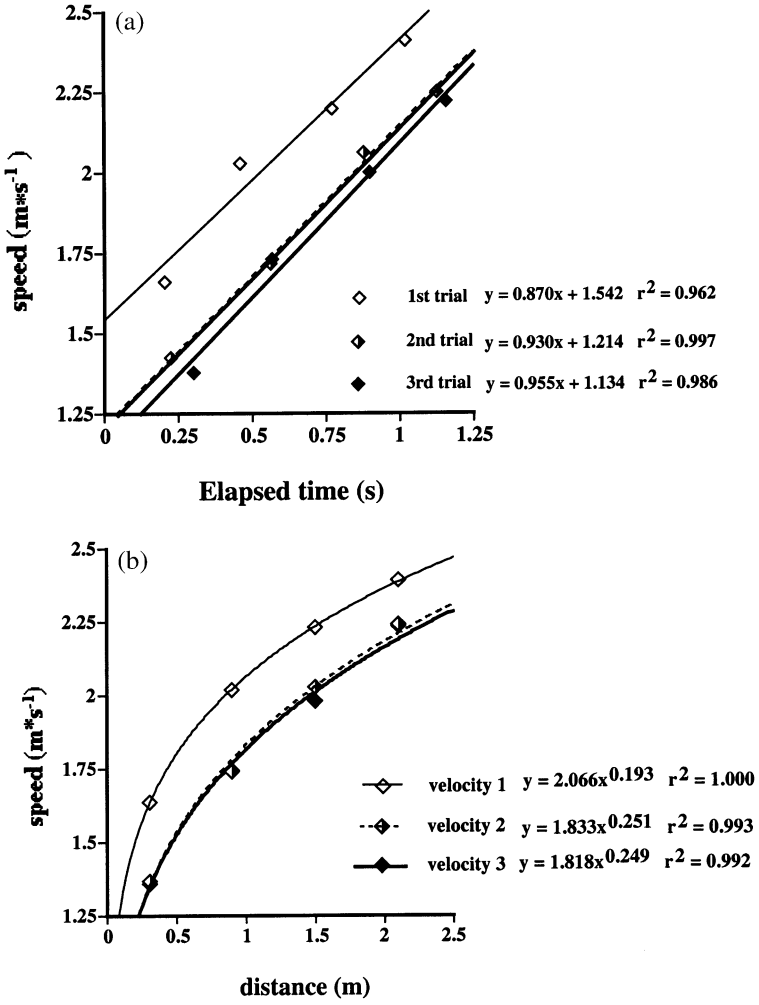

Fig. 4. Swimming speed of an individual Atlantic cod as it burst through the 2.2 m runway after tactile stimulation. Figures show three consecutive trials of the same animal run in a single day. (a) Swimming speed as a function of elapsed time; the equation and correlation coefficient of the least squares linear regression describing each line are included. (b) The same trials depicted in Fig. 4a with swimming speed plotted as a function of distance traversed; the equation and correlation coefficient of the best-fit power function are included.

the relationship of time versus swimming speed was fit with least-squares linear regressions, the lines and equations of which are presented in Fig. 4a for a single animal swum repeatedly, thrice in the same day. When swimming speed is plotted as a function of distance traveled, the relationship was best described by a power function; Fig. 4b shows the same three trials as Fig. 4a, but with speed plotted as a function of distance. Fig. 4 illustrates that much of the variability in repetitive runs occurs with initiation of the fast start; the three runs were virtually indistinguishable after the fish passed the second detector array (first data point). This result can be seen numerically by comparing the slopes of the regression lines and the power function exponent (Fig. 4).

Comparison of the velocity profiles for six additional cod (Fig. 5) demonstrates substantial inter-individual variance in sprint performance measured with our apparatus. This graph (Fig. 5) presents the best of three performance trials, run in a single day for each of the six fish. Four of the six fish had similar ‘best swims’ after the first detector array. In contrast, Fish #2 accelerated better than any other fish through the first two detector arrays but then basically decelerated

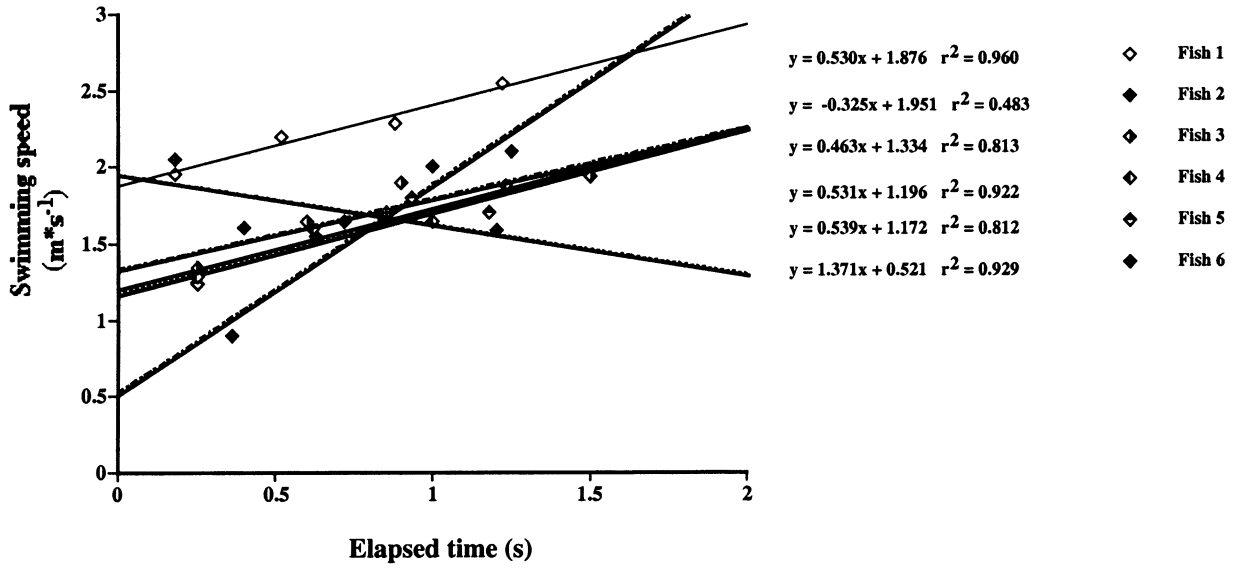

Fig. 5. Swimming speed of 6 additional Atlantic cod as they burst through the 2.2 m runway after tactile stimulation. The line for each fish represents the best of three trials, all performed in a single day, for each individual. Swimming speed is plotted as a function of elapsed time; the equation and correlation coefficient of the least squares linear regression describing each line are included.

through the remainder of the chamber while Fish #6 had the slowest start of any fish, but had the greatest rate of acceleration (approximately 2 $m\,s^{-2}$) throughout the remainder of the chamber (Fig. 5).

The fish depicted in Fig. 4 was intermediate in performance between 'fish 6' and the three similar-performing fish (1, 4 and 5). These results can also be seen numerically by examining the equations; the slope of the line is the acceleration

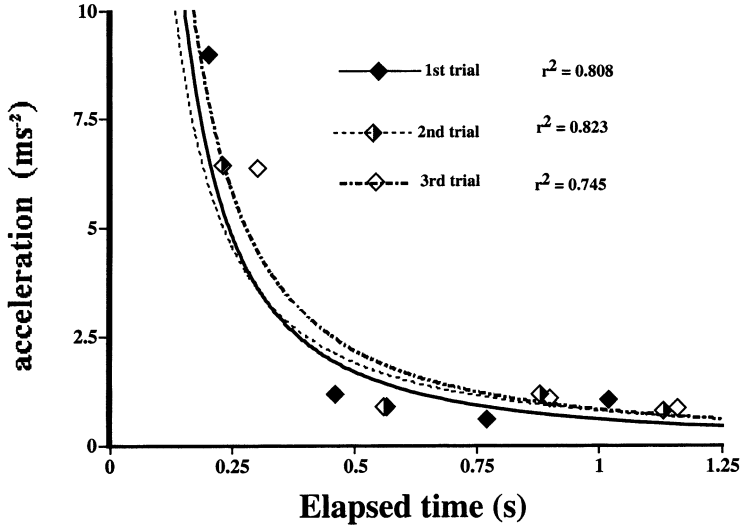

Fig. 6. Acceleration of Atlantic cod as they burst through the 2.2 m runway after tactile stimulation. The equation and correlation coefficient of the 'best-fit' power function for each curve are included. Acceleration curves are for the same three consecutive trials depicted in Fig. 4.

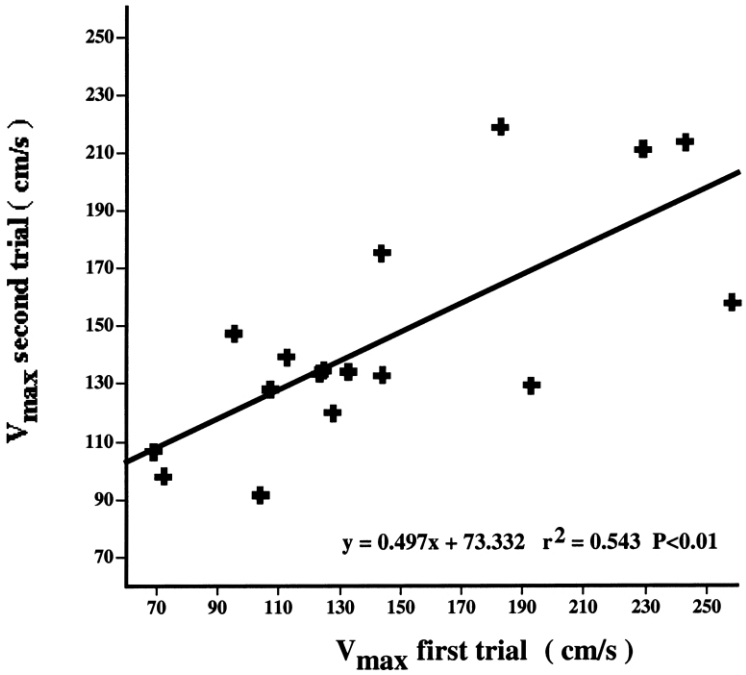

Fig. 7. Maximum swimming speed recorded in two separate sprint trials performed approximately 3 months apart, for each of 17 Atlantic cod. The equation of the least squares linear regression (solid line) and the square of the Spearman rank correlation coefficient are included.

through the last three detector arrays and the *y*-intercept can be considered a rough measure of the animal's starting ability (velocity at 0 time).

3.1.2. Acceleration

Fig. 6 plots the acceleration data corresponding to the three trials depicted in Fig. 4. Again, this was the same animal swum three times in a single day.

These data show that the maximal rate of acceleration for this cod undoubtedly occurred before our first detector array (0.3 m) and, that although the fish continued to positively accelerate throughout the swim chamber, the magnitude declined to a steady level after 0.3 m (Fig. 6). These data are in accord with accelerometer data collected on rainbow trout (*Salmo gairdneri*) and northern pike (*Esox lucius*) by Harper and Blake (1990). These authors found maximum acceleration for all types of fast-starts to occur within the first 0.15 s of swim initiation. To get a realistic number for maximal acceleration by Atlantic cod of this size, one would need to have the second photodetector array positioned much closer than 0.3 m from the starting position. For this reason, acceleration data are not analyzed further, although the inter-individual heterogeneity in acceleration data also exceeded intra-individual acceleration variance (not shown).

3.1.3. Long-term repeatability

The sprint performance technique described above was included in an ongoing study on the locomotor performance of Atlantic cod (Reidy et al., 2000). As one of many measurements in Reidy et al. (2000), 17 Atlantic cod had their sprint performance tested twice, with the trials falling approximately 3 months apart. Here we reiterate the maximal swimming velocity reached by the 17 cod for each of the two trials because it illustrates an important point (Fig. 7).

Fig. 7 shows that this method is significantly repeatable ($P<0.01$ Spearman-rank order $r_s=0.756$) over a period of 3 months in a population of wild fish held in the laboratory. By correcting for differences in body size, this relationship became even more robust (not shown). The slope of the regression line relating second trials to first trials is only 0.5; this was largely dictated by the four best performing fish having fairly large reduc-

tions in performance the second time (Fig. 7). Data points at the extreme of a linear regression have a disproportionate effect on the location of the 'best fit' line (Draper and Smith, 1981). Since nine of the fish had a faster second trial, seven fish had a faster first trial, and one fish had identical trials, we feel safe in concluding that there was no learning effect nor did the fish's health deteriorate over the 3-month period between trials.

3.1.4. Method advantages

The major advantage of this technique is that it allows the investigator to obtain acceleration and swimming speed data on a large number of fish under natural light levels fairly quickly. The rate at which animals can be processed can be increased by making the chamber bi-directional or by reducing acclimation time. Although filming fast-starts of fish is no more time-intensive than our method, high-speed cinematography must occur at light levels that are appropriate only for neustonic fishes. Extracting acceleration and swimming speed data from films can also take hours per fish; the apparatus described above produces swimming speed and acceleration data that can be stored in a computer file, saved to a spreadsheet, or printed immediately. Films also have a number of technical problems described thoroughly by Harper and Blake (1989) and reviewed by Domenici and Blake (1997).

Accelerometers, when properly deployed, are the optimum way to obtain an accurate measurement of a fish's ability to fast-start (Harper and Blake, 1990), however, their use is precluded for small fishes. The extensive animal handling and surgery required for accelerometer implantation also renders their use impractical for evolutionary or ecological studies requiring large sample sizes. The use of accelerometers also requires labor-intensive calibrations, and to obtain the ultimate degree of accuracy these instruments are capable of, one must also film the fish to correct for tangential accelerations (Harper and Blake, 1990).

3.1.5. Method disadvantages

The major disadvantage of the chamber described here is that the performance measurements are relative. Errors induced by wall effects (Webb, 1993) and non-linear swimming paths of the fish compromise the ability of this chamber to measure absolute values of swimming performance. Furthermore, fish with more pointed snouts will break the portion of the beam impinging upon a detector with greater variance than those with blunter snouts. Thus, deviations of measured speeds and accelerations from actual values will be specific to each species and size class of animal. For example, the 0.3 m horizontal distance between the first two detector banks in our prototype was insufficient to resolve the maximum acceleration capability of Atlantic cod with confidence. This type of error can be limited by reducing the vertical and horizontal distance between photodetectors and more narrowly defining the starting position of the fish (Fig. 1). It is also possible with more complex circuitry to monitor each phototransistor in a bank and thereby quantify and correct for any error due to a vertical swimming component but this will incur further costs and analysis complexity. Likewise, lateral deviations from linearity can be corrected for by also filming the trials. We believe that for studies requiring only relative measures of short-duration swimming performance between individuals, a laser detection 'sprint chamber' like the prototype described here will prove optimal.

3.2. Constant acceleration test

Although interesting information was obtained from this test (Reidy et al., 2000), for the preliminary group of eight fish we used, the test did not conform to the criterion of repeatability over time (Fig. 8).

The relationship between performance in a second trial and initial performance was insignificant ($F=1.6$; $P=0.26$), this was largely due to two of the fish having large ($\sim$20%) improvements in performance in the second trial. This was apparent by removing these two fish from the data set, which produced a significant least squares regression between the first and second trial ($F=11.34$ $P=0.028$) despite the sample size of only six fish (Fig. 8). Although the CAT cannot be considered repeatable at the moment, we feel that the question needs to be investigated further before rejecting the method as useful. Even considering the two fish with large improvements in performance between repetitive trials, inter-individual variance in performance exceeded maximal intra-individual variance in performance for this test (Fig. 8). The ecological relevance of this test is discussed in Reidy et al. (2000).

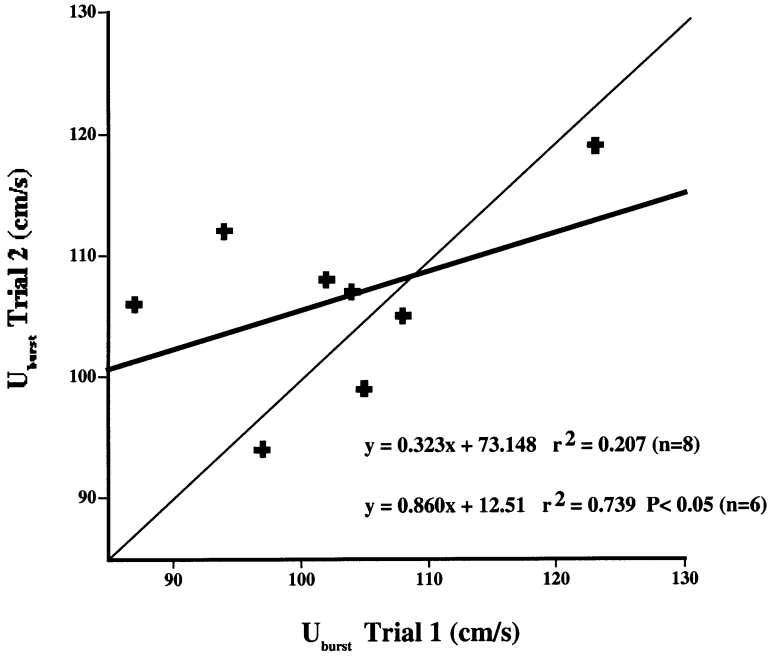

Fig. 8. Swimming speed at fatigue for the 8 Atlantic cod used to initially develop the constant acceleration protocol (U_{burst} of Reidy et al., 2000) recorded in each of two separate trials performed approximately 1 month apart. The equation of the least squares linear regression (dark line) for all eight fish and the same equation with the two worst performing fish removed (both of which had substantial improvement on a second trial) and respective correlation coefficients are included. The lighter line is the line of perfect identity.

3.3. U_{crit} procedure modified for a small, lotic cyprinid

The modified U_{crit} procedure we employed to gauge performance of blacknose dace was very repeatable (Fig. 9). The line relating second performance to first was highly significant by both least squares ($F=62.5$, $P<0.0001$) and non-parametric techniques (Spearman rank order $r=0.771$; $P=0.001$). There was also substantial inter-individual variance in performance among dace that was not attributable to the size of the fish. The size of the dace in this study was intentionally limited, but among the dace used, there was absolutely no relationship between length and swimming performance. Even the regression of the logarithm of TL plotted against the logarithm of critical swimming speed was insignificant (Spearman rank order $r=0.22$; $P=0.19$; $n=40$).

3.4. General

Although the U_{crit} method developed by Brett (1964) has produced a wealth of information on both the performance and metabolism of swimming fish, we think that the time has come for the development of new swimming tests which are more targeted at individual species and swimming modes. The new sprint performance method described here may prove useful to investigators interested in the ecological and evolutionary implications of variance in aquatic locomotor performance. Most of the work in this arena has employed reptilian models (see Bennett and Huey, 1990; Garland and Carter, 1994; Garland and Losos, 1994 for reviews). The few fish studies that have expressed interest in variance of performance have primarily used critical swimming speed as the measure of locomotor capacity (reviewed by Kolok, 1999 and Plaut, 2001). Because a final U_{crit} value is a complex product of multiple swimming modes and changing metabolic support, we predict diminishing utility for this test in studies designed to discern biological causality or draw ecological or evolutionary inference (Nelson et al., 1994, 1996). Indeed, a factor analysis of fin areas and aspect ratios of the cod used in the Reidy et al. (2000) study turned up significant relationships with both the new sprint performance method and the constant acceleration method but none with

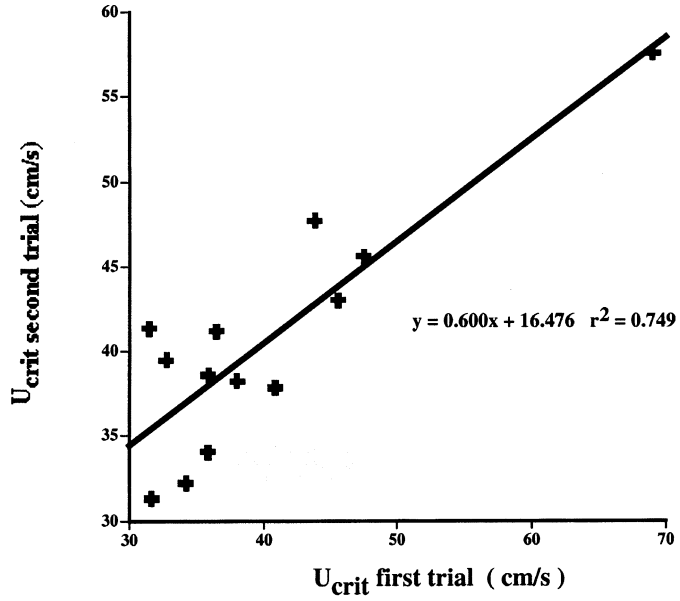

Fig. 9. Critical swimming speed $^{5}_{5}U_{crit}$ recorded in each of two separate trials run approximately 1 month apart, for each of 14 blacknose dace. The equation of the least squares linear regression (solid line) and the square of the correlation coefficient are included.

the U_{crit} procedure. The U_{crit} procedure also suffers from not mimicking the natural swimming of many fish species; this test was designed to simulate conditions for salmonids ascending lotic freshwaters of decreasing order and should not be indiscriminately applied to all fishes and questions. What is needed for the future are swimming tests designed for individual species and questions that conform to the criteria elaborated here.

Fast start and sprint performances of fish are biologically relevant to factors that can directly relate to success for many species (Webb, 1986). Presumably, the dearth of studies on ecological/evolutionary relevance of fast starts and sprints in fishes comes, in part, from the lack of convenient methods for studying these performances in large numbers of fish. The chamber described here allows investigation of sprint swimming performance under any light levels without a huge investment of investigator time and money. We used this prototype sprint chamber to show that inter-individual variance in performance was greater than intra-individual variance of repetitive trials. The maximum sprint velocity of 17 Atlantic cod measured with this method was significantly repeatable over a period of 3 months (Reidy et al., 2000). This suggests that the method can be used to explore mechanisms of differences in sprint performance and their ecological/evolutionary relevance. This method has been subsequently used to follow sprint performance of individual cod through cycles of starvation and feeding (Martinez et al., 2002). Interestingly, the relative ranking of sprint performances of cod was maintained throughout a feeding/starvation regime whereas those of various muscle metabolic capacities were not. With this degree of interesting information coming from a prototype chamber utilizing a species not generally known for its swimming prowess, we are confident that this method will be of general use for studies of other aquatic animals.

Although the CAT (U_{burst}) has not been rigorously shown to be repeatable, it has already helped to increase our understanding of cod biology. The significant, negative relationship with U_{crit} (Reidy et al., 2000) hints that there are physiological or morphological tradeoffs between the types of swimming used in these two tests. In addition, factor analysis of cod fin areas produced a factor, loaded heavily for pelvic fin areas, that correlated strongly and significantly with the CAT (U_{burst}) test ($r=0.84$; $F=23.6$; $P<0.001$). Since cod use a 'flap and glide' swimming style throughout most of the CAT test, this result is logical. Cod depress their pelvic fins during the 'glide' phase of this swimming style to limit backward movement; fish

with relatively large pelvic fins are apparently able to do this better, leading to a better final performance value in this test.

The U_{crit} procedure has been modified in practically every manner possible (Beamish, 1978; Hammer, 1995); rarely have these modifications been subject to the most fundamental measure of scientific veracity, that of reproducibility. Fortunately, when the reproducibility of U_{crit} procedures has been tested, they usually are (Randall et al., 1987; reviewed in Kolok, 1999). Here we add a ${}^{5}_{5}U_{crit}$ procedure with blacknose dace to the list of significantly repeatable incremental velocity tests. In addition to the significant repeatability of this method, there was substantial inter-individual variation, not attributable to size, which will make this test useful for ecological and evolutionary studies on dace. The large differences in locomotor performance among populations of blacknose dace was strongly correlated with differences in current flow at the site of their capture and will be the subject of a separate communication. Thus, although the ${}^{5}_{5}U_{crit}$ we used was outside the guidelines suggested by Brett (1967) and Beamish (1978), because the test is significantly repeatable, mimics conditions encountered by dace in their environment and is yielding important new information about this species, we claim that it is a valid, new test for this species.

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References

Beamish, F.W.H., 1978. Swimming capacity. In: Hoar, W.S., Randall, D.J. (Eds.), Fish Physiology, vol. 7. Academic Press, New York, pp. 101–187.

Bennett, A.F., 1980. The thermal dependence of lizard behaviour. Anim. Behav. 28, 752–762.

Bennett, A.F., Huey, R.B., 1990. Studying the evolution of physiological performance. In: Futyma, D.J., Antonovics, J. (Eds.), Oxford Surveys of Evolutionary Biology, vol. 7. Oxford Univ. Press, Oxford, pp. 251–283.

Bouchard, C., Tremblay, A., Nadeau, A., et al., 1989. Genetic effect in resting and exercise metabolic rates. Metabolism 38, 364–370.

Breitburg, D.L., 1992. Episodic hypoxia in Chesapeake Bay: interacting effects of recruitment, behavior, and physical disturbance. Ecol. Monogr. 62, 525–546.

Brett, J.R., 1964. The respiratory metabolism and swimming performance of young sockeye salmon. J. Fish Res. Bd. Canada 21, 1183–1226.

Brett, J.R., 1967. Swimming performance of sockeye salmon (Oncorhyncus nerka) in relation to fatigue time and temperature. J. Fish Res. Bd. Canada 24, 1731–1741.

Cech, J., Swanson, C., Young, P.S., 1998. Swimming behavior of splittail in multi-vector flow regimes: applications for fish screens. In: Mackinley, D., Howard, K., Cech Jr., J. (Eds.), Fish Performance Studies. American Fisheries Society, Bethesda, pp. 111–114.

Domenici, P., Blake, R.W., 1997. The kinematics and performance of fish fast-start swimming. J. Exp. Biol. 200, 1165–1178.

Draper, N., Smith, H., 1981. Applied Regression Analysis. second ed. Wiley, New York, NY.

Farlinger, S., Beamish, F.W.H., 1977. Effects of time and velocity increments on the critical swimming speed of largemouth bass (*Micropterus salmoides*). Trans. Am. Fish. Soc. 106, 436–439.

Gamperl, A.K., Schnurr, D.L., Stevens, E.D., 1991. Effect of a sprint-training protocol on acceleration performance in rainbow trout (*Salmo gairdneri*). Can. J. Zool. 69, 578–582.

Garland, T.J., Carter, P.A., 1994. Evolutionary physiology. Annu. Rev. Physiol. 56, 579–621.

Garland, T.J., Losos, J.B., 1994. Ecological morphology of locomotor performance in squamate reptiles. In: Wainwright, P.C., Reilly, S.M. (Eds.), Ecological Morphology: Integrative Organismal Biology. University of Chicago Press, Chicago, pp. 240–302.

Garland, T.J., Hankins, R.E., Huey, R.B., 1990. Locomotor capacity and social dominance in male lizards. Funct. Ecol. 4, 243–250.

Gero, D.R., 1952. The hydrodynamic aspects of fish propulsion. American Museum Novit. 1601, 1–32.

Gray, J., 1953. The locomotion of fishes. In: Marshall, S.M., Orr, P. (Eds.), Essays in Marine Biology. Elmhirst Memorial Lectures. Oliver and Boyd, Edinburgh, pp. 1–16.

Hammer, C., 1995. Fatigue and exercise tests with fish. Comp. Biochem. Physiol. 112A, 1–20.

Harper, D.G., Blake, R.W., 1989. On the error involved in high-speed film when used to evaluate maximum accelerations of fish. Can. J. Zool. 67, 1929–1936.

Harper, D.G., Blake, R.W., 1990. Fast-start performance of rainbow trout *Salmo gairdneri* and northern pike *Esox lucius*. J. Exp. Biol. 150, 321–342.

Hertz, P.E., Huey, R.B., Nevo, E., 1983. Homage to Santa Anita: thermal sensitivity of sprint speed in agamid lizards. Evolution 37, 1075–1084.

Huey, R.B., Dunham, A.E., 1987. Repeatability of locomotor performance in natural populations of the lizard Sceloporus merriami. Evolution 41, 1116–1120.

Huey, R.B., Schneider, W., Erie, G.L., Stevenson, R.D., 1981. A field-portable racetrack for measuring acceleration and velocity of small cursorial animals. Experientia 37, 1356–1357.

Iwama, G.K., McGeer, J.C., Pawluk, M.P., 1989. The effects of five fish anaesthetics on acid–base balance, hematocrit, blood gases, cortisol and adrenaline in rainbow trout. Can. J. Zool. 67, 2065–2073.

Jain, K.E., Birtwell, I.K., Farrell, A.P., 1998. Repeat swimming performance of mature sockeye salmon following a brief recovery period: a proposed measure of fish health and water quality. Can. J. Zool. 76, 1488–1496.

Kolok, A.S., Farrell, A.P., 1994. Individual variation in the swimming performance and cardiac performance of northern squawfish, Ptychocheillus oregonensis. Physiol. Zool. 67, 706–722.

Kolok, A.S., 1999. Inter-individual variation in the prolonged locomotor performance of ectothermic vertebrates: a comparison of fish and herpetofaunal methodologies and a brief review of the recent fish literature. Can. J. Fish Aquat. Sci. 56, 700–710.

Martinez, M., Guderley, H., Nelson, J.A., Webber, D., Dutil, J.D., 2002. Once a fast cod, always a fast cod: maintenance of performance hierarchies despite changing food availability in cod (Gadus morhua). Physiol. Biochem. Zool. 75, 90–100.

McDonald, D.G., McFarlane, W.J., Milligan, C.L., 1998. Anaerobic capacity and swim performance of juvenile salmonids. Can. J. Fish Aquat. Sci. 55, 1198–1207.

Nelson, J.A., 1989. Critical swimming speeds of yellow perch Perca flavescens: comparison of populations from a naturally acidic lake and a circumneutral lake in acid and neutral water. J. Exp. Biol. 145, 239–254.

Nelson, J.A., 1990. Muscle metabolite response to exercise and recovery in yellow perch (Perca flavescens): comparison of populations from naturally acidic and neutral waters. Physiol. Zool. 63, 886–908.

Nelson, J.A., Tang, Y., Boutilier, R.G., 1994. Differences in exercise physiology between two Atlantic cod (*Gadus morhua*) populations from different environments. Physiol. Zool. 67, 330–354.

Nelson, J.A., Tang, Y., Boutilier, R.G., 1996. The effects of salinity change on the exercise performance of two Atlantic cod (Gadus morhua) populations inhabiting different environments. J. Exp. Biol. 199, 1295–1309.

Plaut, I., 2001. Critical swimming speed: its ecological relevance. Comp. Biochem. Physiol. (A) 131, 41–50.

Randall, D.J., Brauner, C., 1991. Effects of environmental factors on exercise in fish. J. Exp. Biol. 160, 113–126.

Randall, D.J., Mense, D., Boutilier, R.G., 1987. The effects of burst swimming on aerobic swimming in chinook salmon (Oncorhynchus tshawytscha). Mar. Behav. Physiol. 13, 77–88.

Reidy, S.P., Kerr, S.R., Nelson, J.A., 2000. Aerobic and anaerobic swimming performance of individual Atlantic cod. J. Exp. Biol. 203, 347–357.

Taylor, E.B., McPhail, J.D., 1985. Burst swimming and size related predation of newly emerged coho salmon Oncorhynchus kisutch. Trans. Am. Fish. Soc. 114, 456–551.

Wardle, C.S., 1975. Limit of fish swimming speed. Nature (London) 255, 725–727.

Webb, P.W., 1986. Locomotion and predator–prey relationships. In: Feder, M.E., Lauder, G.V. (Eds.), Predator–Prey Relationships. University of Chicago Press, Chicago, pp. 24–41.

Webb, P.W., 1975. Acceleration performance of rainbow trout Salmo gairdneri and green sunfish Lepomis cyanellus. J. Exp. Biol. 63, 451–465.

Webb, P.W., 1978. Fast-start performance and body form in seven species of teleost fish. J. Exp. Biol. 74, 115–226.

Webb, P.W., 1983. Speed, acceleration, and maneuverability of two teleost fishes. J. Exp. Biol. 102, 211–222.

Webb, P.W., 1993. The effect of solid and porous channel walls on steady swimming of steelhead trout Oncorhynchus mykiss. J. Exp. Biol. 178, 97–108.