

# Repeatability and correlation of swimming performances and size over varying time-scales in the guppy (*Poecilia reticulata*)

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## Summary

1. Although the repeatability of a trait is of interest for several reasons, few studies have critically examined the repeatability or correlation of metrics of swimming performance. We quantified repeatability of three swimming performances (burst speed during a c-start escape response, critical swimming speed [ $U_{crit}$ ], maximum speed [ $U_{max}$ ]) and size/shape measures over short (within-day), medium (days and weeks), and long (more than a year) time-scales in a small poeciliid fish, the Trinidadian guppy (*Poecilia reticulata*).

2. We found that individual differences in most swimming performance metrics were repeatable on various time-scales. Burst speed during c-start was repeatable within a day, as well as over days and weeks, but it was not repeatable over the span of a year. However, the medium term repeatability was only statistically significant when accounting for the variation of individuals within a day and correcting for attenuation.  $U_{crit}$ ,  $U_{max}$  and size were repeatable on medium time-scales, but only size and  $U_{max}$  were repeatable over the span of a year. Our index of shape was not statistically repeatable over any time-scales.

3. When examining the correlations of performances and size, only a few traits were related to each other.  $U_{crit}$  and  $U_{max}$  were significantly positively related to each other for trial 1 only, but not any of the others.  $U_{max}$  and the mean c-start burst speed were correlated on trial 2 only. No other traits were significantly related to each other, and no performance traits were related to size or shape.  $U_{crit}$  and  $U_{max}$  also did not differ in their overall mean values, suggesting that  $U_{max}$ , a more recently described assessment of fish swimming performance, may be a reliable alternative.

4. All traits, except our index of shape, shifted in their mean value over the span of a year.  $U_{crit}$  and c-start burst speed decreased significantly, consistent with previous findings on the effects of senescence on neuromuscular systems. However,  $U_{max}$  and size increased significantly over a year.

5. The measures of swimming performance used in this study are becoming widely used in ecological and evolutionary studies. Our results suggest that individual differences in these measures are relatively stable on various time-scales. We found no significant relation of these performance metrics with body size or a simple index of shape, and few relations among the performance measures, suggesting that they measure different aspects of locomotor physiology and/or motivation under the test conditions used. Finally, while  $U_{crit}$  and c-start burst speed decreased over the span of the year, consistent with theories of senescence,  $U_{max}$  significantly increased over a year, suggesting that further studies of this recently described performance metric are needed.

**Key-words:** c-start, critical swimming speed, individual variation, locomotion, performance, *Poecilia reticulata*, repeatability, swimming

## Introduction

The locomotor performance of organisms has many important ecological and evolutionary implications, with relevance

for finding food, finding mates, avoiding predators and dispersal (e.g., Irschick & Garland 2001, Perry *et al.* 2004, Oufiero & Garland 2007, Irschick *et al.* 2008, references therein). In fish, swimming performance has been categorized into three main modes: sustained, prolonged and burst (Beamish 1978). Within these three modes of swimming, prolonged and

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burst have been studied extensively, including aspects of their physiology, biomechanics and neurobiology. More recently, studies have been focusing on the ecological and evolutionary implications of variation in swimming performance (Nicoletto 1991, O'Steen *et al.* 2002, Ghalambor *et al.* 2004, Blake *et al.* 2005, Langerhans *et al.* 2005, Karino *et al.* 2006, Kruesi & Alcaraz 2007). Although many of these studies examine differences among populations, species or groups (McGuigan *et al.* 2003, Nelson *et al.* 2003, Odell *et al.* 2003, Chappell & Odell 2004, Langerhans *et al.* 2004), more are beginning to examine differences at the individual level (Kolok 1999). However, a key to studies at the individual level is assessing the amount of intraindividual variation, or repeatability, for the trait of interest.

The repeatability of locomotor performance has been well-documented in terrestrial vertebrates, with much attention in squamate reptiles (e.g., Sorci *et al.* 1995, Perry *et al.* 2004, and reviewed in Kolok 1999). However, the repeatability of swimming performances in teleost fish has received less attention and has only recently begun to be evaluated critically (Nelson & Claireaux 2005, Claireaux *et al.* 2007). The repeatability of a trait is important for several reasons (Bennett 1987, Boake 1989). First, and most simply, it indexes the reliability and reproducibility of the protocol by which a trait is measured (Losos *et al.* 2002). Second, significant repeatability sets the stage for investigations of the proximate (mechanistic) factors that contribute to variation in the trait (e.g. Bennett *et al.* 1989, Girard & Garland 2002; Harris & Steudel 2002, Rhodes *et al.* 2003, Oufiero & Garland 2007). Third, repeatability generally sets the upper limit of heritability for the trait (e.g., Brodie & Garland 1993, Boake 1989, but see Dohm 2002). Finally, repeatability also sets a general upper limit to the intensity of selection that can be applied to a trait, which is important both in laboratory experiments (Garland & Rose 2009) and in field studies that attempt to quantify selection in nature (e.g., Clobert *et al.* 2000; Irschick *et al.* 2008). In other

words, if a trait has low repeatability, then it is 'harder' for selection to act on it, that is, separate the favoured from disfavoured individuals.

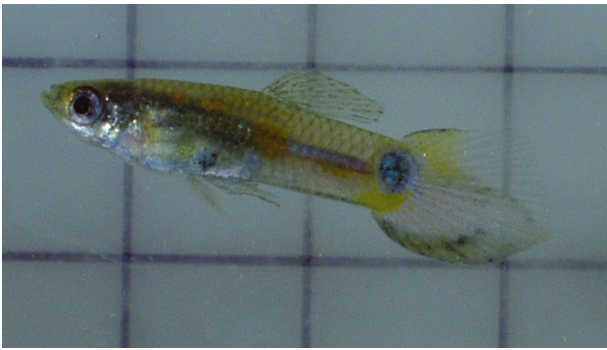
The repeatability of swimming performance in fish has been examined in several species, for several traits, and over varying time-scales (Table 1). Most of the research has focused on the critical swimming speed ( $U_{crit}$ ), an endurance measure that is often used as an indicator of health in fisheries science (Plaut 2001). More recent work has examined the repeatability of sprint speed in fish, similar to sprint-speed measures of squamate reptiles (Nelson *et al.* 2002). These studies have generally focused on larger-bodied, commercially important species. However, smaller species (mainly members of the Poeciliidae) are becoming model organisms in evolutionary and ecological research, with more studies examining swimming performances. Yet, the only studies of repeatability for poeciliid fish yet published are short-term and involve the c-start (Chappell & Odell 2004, Langerhans *et al.* 2004, Royle *et al.* 2006), which gauges burst-swimming capabilities (Domenici & Blake 1997).

The repeatability of a performance measure will depend on the consistency of both psychological factors (e.g. motivation) and the physiological and morphological subordinate traits that govern physical performance abilities (Bennett 1987, Harris & Steudel 2002, Belke & Garland 2007). Obviously, the morphology of an organism can have strong effects on its performance capabilities (Arnold 1983, Garland & Losos 1994, Boily & Magnan 2002, Blake 2004, Billman & Pyron 2005). Additionally, different performance measures may trade-off with one another. For example, it has been proposed – and shown in some, but not all, cases – that endurance performance trades-off with sprinting performances, in part because of the different muscle fibre types associated with each performance (Garland & Losos 1994, Vanhooydonck *et al.* 2001). Few studies have examined this in fish; those that have often find no relation at the level of individual variation

**Table 1.** Repeatability values reported in the literature for a variety of fish and performance measures, sorted from shortest to longest interval between replicate trials for each type of performance

Species	<i>N</i>	Performance	Time period	Repeatability	Type	Source
<i>Poecilia reticulata</i>	86	C-start (speed)	2 min	0.805	P	Chappell & Odell 2004
<i>Xiphophorus helleri</i>	28	C-start (speed)	Minutes	0.23	I	Royle <i>et al.</i> 2006
<i>Gambusia affinis</i>	18	Burst speed	Within a day	0.89	I	Langerhans <i>et al.</i> 2004
<i>Gasterosteus aculeatus</i>	6	Burst speed	2 days	0.943	S	Garenc <i>et al.</i> 1999
<i>Dicentrarchus labrax</i>	32	Sprint	4 Weeks	0.43	S	Claireaux <i>et al.</i> 2007
<i>Gadus morhua</i>	23	Sprint	3 months	0.756	P	Reidy <i>et al.</i> 2000
<i>Dicentrarchus labrax</i>	52	Sprint	6 months	0.23 n.s.	S	Claireaux <i>et al.</i> 2007
<i>Micropterus salmoides</i>	7	$U_{crit}$	4 days	0.86 (11 °C)	S	Kolok 1992
	9			0.77 (22 °C)		
<i>Oncorhynchus mykiss</i>	8	$U_{crit}$	8 days	0.857	S	Gregory & Woods 1998
<i>Pimephales promelas</i>	10	$U_{crit}$	10–14 days	0.83	S	Kolok <i>et al.</i> 1998
<i>Rhinichthys atratulus</i>	14	$U_{crit}$	1 month	0.6	K	Nelson <i>et al.</i> 2003
<i>Gadus morhua</i>	12	$U_{crit}$	3 months	0.771	P	Reidy <i>et al.</i> 2000
<i>Dicentrarchus labrax</i>	52	$U_{crit}$	6 months	0.44	S	Claireaux <i>et al.</i> 2007

I, Intraclass correlation coefficient; P, Pearson product–moment correlation; S, Spearman rank correlation; K, Kendall's  $\tau$ . n.s. = not significant.



**Fig. 1.** Male Trinidadian guppy (*Poecilia reticulata*) originating from the Quare river drainage. Grid represents 1 cm scale.

(e.g., Chappell and Odell 2004, Claireaux *et al.* 2007, but see Reidy *et al.* 2000). Those that have shown a trade-off often demonstrate it at the population or species level (Langerhans 2006); however, as Bennett (1987) notes, 'If two factors are functionally related, they should be significantly correlated among individuals at the species levels' (presuming, of course, that the repeatability of the traits in question is high enough).

The purpose of the present study was to quantify the repeatability of several performance measures over varying time-scales in a small poeciliid fish, the Trinidadian guppy (*Poecilia reticulata*, Fig. 1). We examined the repeatability of burst speed during a c-start escape response (as measured as the maximum 2 ms velocity attained during an escape response) on a short (minutes), medium (days and weeks), and long time-scale (more than a year). We also examined the repeatability of critical swimming speed ( $U_{crit}$ ), as has been done in larger fish species, on a medium (weeks) and long (more than a year) time-scale. Finally, because guppies are generally too small to break photocell infrared beams used in sprint-performance measures, we used a measure of maximum or 'burst' speed in a flow tunnel, as described in Reidy *et al.* (2000) and more recently in Farrell (2008). We classified this measure as maximum speed ( $U_{max}$ ), and measured its repeatability on a medium (weeks) and long (more than a year) time-scale. Finally, we also tested for relations between the performance measures and with size traits.

Few studies have measured long-term repeatability of locomotor performance in vertebrates (Huey & Dunham 1987, van Berkum *et al.* 1989, Huey *et al.* 1990, Robson [2000] cited in Angilletta *et al.* 2002, Miles 2004). The longest measure in fish has been six months for critical swimming speed and sprint speed in European sea bass (Claireaux *et al.* 2007). Long-term repeatability has additional implications, including the effects of senescence on locomotor performance (Huey *et al.* 1990; Reznick *et al.* 2004; Bronikowski *et al.* 2006).

## Methods and materials

### ANIMAL HUSBANDRY

Male Trinidadian guppies originating from the Quare river drainage of Trinidad (*Poecilia reticulata*,  $N = 24$ , Fig. 1) were used to assess

the amount of individual variation associated with several swimming performance metrics in fish. The breeding stock was originally collected in 2002, and our sample was from approximately the 10th generation of captive breeding. For the present study, beginning at approximately 90 days of age, fish were individually maintained in 2½ gallon aquaria, with a 12L:12D light cycle. Water was pretreated (e.g., with UV sterilization and chemicals to maintain pH and rid water of chlorines and chloramines), and partial water changes were done approximately every two weeks. Fish were fed flake food, live brine shrimp, liver paste, and freeze-dried blood worms *ad libitum*. Prior to any of the performance measures, individuals were fasted for at least 24 h.

### PERFORMANCE MEASURES

Burst speed was the first performance metric tested on all fish by eliciting an escape response often characterized by a c-start (Domenici & Blake 1997), and was measured similar to methods outlined in Chappell and Odell (2004). Burst speed was measured by placing individuals in a small arena (30 cm (L) × 30 cm (W) × 20.5 cm (H)) filled with approximately 4–5 cm of water to minimize vertical movement. A plastic cylinder (16.5 cm D × 20.5 cm H) was placed in the centre of the tank above a 10 cm grid. This plastic cylinder kept the fish in view of the high-speed video camera, and the grid allowed for distance calibration. Additionally, opaque plastic surrounded the plastic cylinder to reduce any outside visual distraction and stress levels of the fish being tested. A digital thermometer was placed inside the arena and temperature was recorded for each trial.

A mechanical stimulus (metal weights) weighing 51.92 g (approximately 4 cm D 1.5 cm H) was dropped from a height of 85 cm, outside of the fish's view, to elicit a c-start escape response. The stimulus was released after a 15-min acclimation, and responses were captured using a Red Lake Imaging high-speed video camera (Red Lake Motion Scope M1) at 500 frames per second. To obtain proper lighting, the arena was lit with two 120 W reflector bulbs placed approximately 85 cm above the testing arena. Because these lights tended to increase the temperature of the water, ice was added periodically outside of the testing arena, and the water was changed every couple of trials to try and maintain temperature at approximately 23 °C, similar to room and housing temperatures.

Three trials were recorded for each fish within approximately 30 min. If a fish did not respond, or responded poorly after the first stimulus, it remained in the tank for 2 min until the stimulus was released again; this procedure was repeated one additional time. If no successful trials were obtained from the fish, then the individual was removed from the study. If a fish responded well, it also remained in the arena for 2 min for an additional measurement; this procedure was repeated to obtain three successful measures of the individual's escape response (no more than 5 trials were conducted if 3 successful trials were not obtained initially).

The escape responses were converted to digital (.avi) files and the centre of mass of the fish was digitized from 10 ms prior to initiation of the c-start to 50 ms after the c-start began using custom Matlab software to obtain  $x,y$ -coordinates (Mathworks Inc., Natick, MA, USA). The velocity was obtained from a first order derivative of the displacement of the centre of mass from the  $x,y$ -coordinates. C-starts generally last only a few tenths of a second (Domenici and Blake 1997), so analysis up to 50 ms should capture the initial velocity during the c-start; a similar protocol has been used previously in guppies (Chappell and Odell 2004). The velocity data were also

smoothed using a 5-point moving average because there is often noise associated with estimating this parameter when they are calculated from the  $x, y$  coordinates (Walker 1998). We then found the maximum velocity over a 2 ms period for each trial and day and used this as our measure of burst speed during a c-start escape response. Additionally, we estimated the time to attain the maximum velocity for each trial to determine whether it was occurring during or after the c-start escape response. Previous studies have estimated the time to the end of stage 1 in guppies to be approximately 12 ms (Walker *et al.* 2005); therefore, we compared the time to maximum velocity to the time at the end of stage 1. The end of stage 1 was determined as the first decrease in velocity after the initiation of the c-start, even if it lasted for only one frame (2 ms). This allowed us to determine which stage of the c-start the maximum 2 ms velocity was reached.

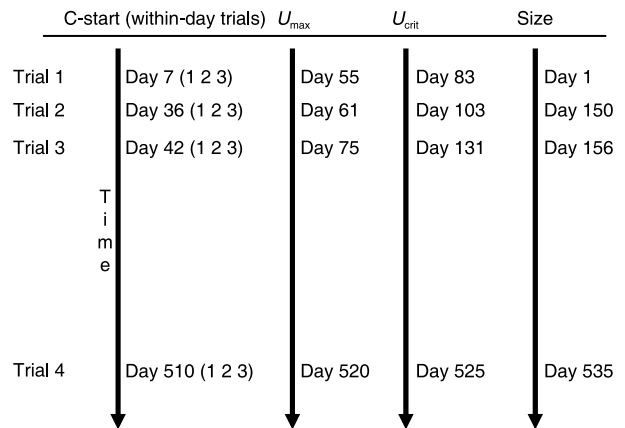
The second performance metric measured was maximum speed ( $U_{\max}$ ), similar to Reidy *et al.* (2000) and Farrell (2008). Fish were acclimated to a flow tunnel for 15 min at low velocity (approximately  $3 \text{ cm s}^{-1}$ ) to orient the fish to flow direction. The flow tunnel holds approximately 55 L of water and is 119.5 cm (L)  $\times$  15.3 cm (W)  $\times$  18.3 cm (H). The area where the fish was tested is 12 cm (L)  $\times$  15.3 cm (W)  $\times$  11.5 cm (H). This smaller area was obtained by placing grating throughout the working area of the flow tunnel, which also served to attain laminar flow. After the 15-min acclimation, flow was increased at approximately  $0.30 \text{ cm s}^{-2}$  until the fish could no longer maintain position in the flow and was pushed against the back grating or sides of the flow tunnel. The final speed for which the fish was able to maintain position was considered its maximum speed ( $U_{\max}$ ).

Finally, critical swimming speed ( $U_{\text{crit}}$ ) was measured following methods outlined in Brett (1964), Beamish (1978) and subsequent studies (Kolok 1999, Plaut 2001). Similar to  $U_{\max}$  measures, fish were acclimated to the flow tunnel for 15 min at a low velocity. After the 15-min acclimation period, flow was increased  $4.5 \text{ cm s}^{-1}/5 \text{ min}$ . Each fish was tested until it could no longer maintain position, and could not remove itself from the back grating after three taps on the side of the tunnel. Both  $U_{\text{crit}}$  and  $U_{\max}$  were measured at room temperature (approximately  $23 \text{ }^{\circ}\text{C}$ ).

For all swimming performance metrics we assume that motivation of the individuals was maximal or near-maximal. Additionally, we did not observe any fish that seemed to refuse to swim for any of the trials.

#### SIZE AND SHAPE

We also examined repeatability of an index of size and shape, and correlated them with the performance measures. During testing, fish were anaesthetized using MS-222, and body mass (to the nearest 0.001 g), total length (TL: from tip of snout to end of the caudal fin, to the nearest 0.01 mm), standard length (SL: from the most anterior portion to the insertion [base] of the caudal fin, to the nearest 0.01 mm), and body depth (BD: at the insertion of the dorsal fin, to the nearest 0.01 mm) were measured. We used TL as our index of size since it had the lowest coefficient of variation (mass: coefficient of variation [CV] = 13.16%, SL: CV = 6.92%, TL: CV = 3.61%, BD: CV = 6.91%). For shape we used body depth/total length. These measurements were recorded a total of three times over a span of months and days, using digital calipers under a dissecting scope. After all performance measures had been taken, the fish were euthanized with a lethal dose of MS-222 and again measured. Results from a principal components analysis of the measures were similar and so are not reported.



**Fig. 2.** Timeline for locomotor and size measures. Because the interval between measurements varied, we simplified the measures by referring to each measurement day as a trial; however, we also report the actual day each trait was measured with size being measured on Day 1. Additionally, there are three measures of within-trial repeatability for c-start only.

#### EXPERIMENTAL DESIGN

We assessed the repeatability of these performance measures several ways and over varying time-scales (Fig. 2). Because the time between measures varied for each performance metric we refer to each day as a trial, Figure 2 outlines the timing between each trial for the three performance metrics and size measures with the day given that each trait was measured. We measured size first and used this as Day 1. For burst speed during c-start, we examined short, medium and long-term repeatability. Short-term repeatability was measured similar to previous studies, where the time between trials was in minutes, making it a within-day comparison (Fig. 2). Medium-term repeatability was assessed over a month (trial 2: day 36), and again over a week (trial 3: day 42). Finally, long-term repeatability was measured by testing the fish 16 months later (trial 4: day 510). Thus, we have a total of four trials, each with 1–3 c-start escape responses in each. For  $U_{\max}$ , we assessed only medium- and long-term repeatability over a period of days and weeks (trial 1: day 55, trial 2: day 61, trial 3: day 75) and over a year (trial 4: day 520). Similarly,  $U_{\text{crit}}$  was assessed over a period of weeks (trial 1: day 83, trial 2: day 103, trial 3: day 131) and over a year (trial 4: day 525). Finally, the repeatability of size and shape was assessed over a period of months (trial 1: day 1, trial 2: day 150), days (trial 3: day 156), and 11 months later (trial 4: day 535). The time-scale was chosen based on the time it took to measure each swimming performance metric three times: each performance metric had the first three trials measured before beginning a new performance measure. Due to the difference in time to measure all individuals for the trait, this produced the various time-scales of days, weeks or months.

#### STATISTICAL ANALYSIS

Repeatability of the various trials (see Fig. 2) was analysed in several ways. Because we obtained approximately 12 c-start trials per fish, over short, medium and long time periods, the repeatability was determined depending on which time frame we were assessing. For short-term repeatability of c-start burst speed, we used the intraclass correlation coefficient ( $I_{cc}$ ) (Lessells & Boag 1987). This test was used because we had up to three trials per fish, and the mean value of the

within-day trials were not expected to change. The  $I_{cc}$  has been used in other studies examining within-day repeatability of escape response burst speed (Langerhans *et al.* 2004, Royle *et al.* 2006). For the medium- and long-term repeatability, we used Pearson's product-moment correlation coefficient. This test was used because the means might change over this longer time period (which would artificially reduce the magnitude of the  $I_{cc}$ ), and because we were interested in the relationship between each specific set of days (i.e. trials over a week versus over a month or over a year).

Studies often use the maximum velocity of the fish on a given day as its value of c-start burst speed (Chappell & Odell 2004, Langerhans *et al.* 2004); however, at least one study has demonstrated in amphibians that the mean velocity on a given day might also be important (Austin & Shaffer 1992). Therefore, we tested for repeatability of the single highest velocity obtained on a given day among the four trials (i.e. the highest 2 ms burst speed velocity attained among the three within day c-start trials). We also averaged the highest velocity for each of the three within-day measures and compared them among trials, and also included a comparison of the means while correcting for attenuation (Adolph & Hardin 2007). For the long-term repeatability of c-start burst speed, we compared trial 3 to trial 4 by examining the single highest maximum for the respective day, as well as the mean for the day and the mean corrected for attenuation. Repeatability for  $U_{crit}$ ,  $U_{max}$ , size and shape is not as complicated due to the lack of repeated measures within a day. Therefore, we used Pearson's correlation coefficient to test for repeatability among the four trials. For size, we examined the repeatability of total length, for shape we examined the repeatability of body depth/total length. Similar to c-start velocity, when testing for long-term repeatability in  $U_{max}$ ,  $U_{crit}$  and size/shape we again compared trial 3 to trial 4 using Pearson's correlation coefficient to be consistent with our measure of repeatability. We also looked for differences in mean values over the span of a year using a paired *t*-test for all traits (including all size measures) to compare the mean value for trial 3 to the mean value of trial 4.

Finally, we examined relations between the performance metrics and between performance metrics and size/shape using Pearson correlations for values taken at a given trial. For our measure of size, we again used total length of the fish because it had the least amount of variance compared to the other size measures. We also correlated the performance traits to our index of shape, body depth/total length. For all of these analyses, we judged statistical significance at  $P < 0.01$  (2-tailed) to reduce problems of multiple comparisons.

The temperature during  $U_{max}$  and  $U_{crit}$  was maintained at room temperature and did not vary substantially. However; the temperature did vary for c-start measurements because of the lights used to illuminate the arena. Therefore, to test if temperature was a predictor of velocity during c-start, we regressed velocity against the temperature for the trial.

## Results

### SIZE AND SHAPE

Mean values for all size and shape measurements and all trials are summarized in Table S1 in the Supporting Information. Total length (Fig. 3a, Table 2) was repeatable across all trials (trial 1 vs. 2:  $r = 0.7111$ ,  $P < 0.001$ ; trial 2 vs. 3:  $r = 0.8452$ ,  $P < 0.001$ ; trial 3 vs. 4:  $r = 0.4600$ ,  $P = 0.031$ ). In contrast, our index of shape, body depth/total length was not repeatable among any of the trials (trial 1 vs. 2:  $r = 0.1179$ ,  $P = 0.592$ ;

trial 2 vs. 3:  $r = 0.3632$ ,  $P = 0.081$ ; trial 3 vs. 4:  $r = 0.2111$ ,  $P = 0.346$ , Table 2). All size measures increased significantly over the span of a year (Table 3); however, body depth/total length did not change over a year.

### BURST SPEED DURING C-START

Burst speed during a c-start escape response was not significantly related to temperature for any trial. Therefore, we did not include temperature in any further analyses. The average time it took fish to reach maximum velocity was  $18.297 \pm 3.223$  ms, and in 86.18% of the trials maximum velocity was achieved at the end of stage 1; in the remaining trials maximum velocity was achieved during stage 2. Within-day repeatability for velocity was not statistically significant for trial 1 (intraclass correlation coefficient [ $I_{cc}$ ] = 0.1395,  $P = 0.1622$ ), but was for trials 2 ( $I_{cc} = 0.2482$ ,  $P = 0.0272$ ) and trial 3 ( $I_{cc} = 0.3803$ ,  $P = 0.0027$ ). When the fish were tested again more than a year later, repeatability was marginally non-significant ( $I_{cc} = 0.2713$ ,  $P = 0.0604$ ).

Mean burst speed was significantly repeatable between trials 2 and 3, and after correction for attenuation was also repeatable between trials 1 and 2 (Table 2, see also Fig. 3b). After a year, the mean and maximum velocity attained during c-start decreased significantly (Table 3, Fig. 3b), and neither was repeatable, even after correcting the mean velocity for attenuation (Table 2).

### MAXIMUM SPEED ( $U_{max}$ ) AND CRITICAL SWIMMING SPEED ( $U_{crit}$ )

The maximum speed the fish maintained in the flow tunnel ( $U_{max}$ , Fig. 3c) was significantly repeatable over a time period of weeks (Table 2, trials 2 and 3) and more than 14 months (trials 3 and 4). Surprisingly,  $U_{max}$  increased significantly over the span of 14 months (Fig. 3c, Table 3,  $t = -4.491$ ,  $P < 0.001$ ).

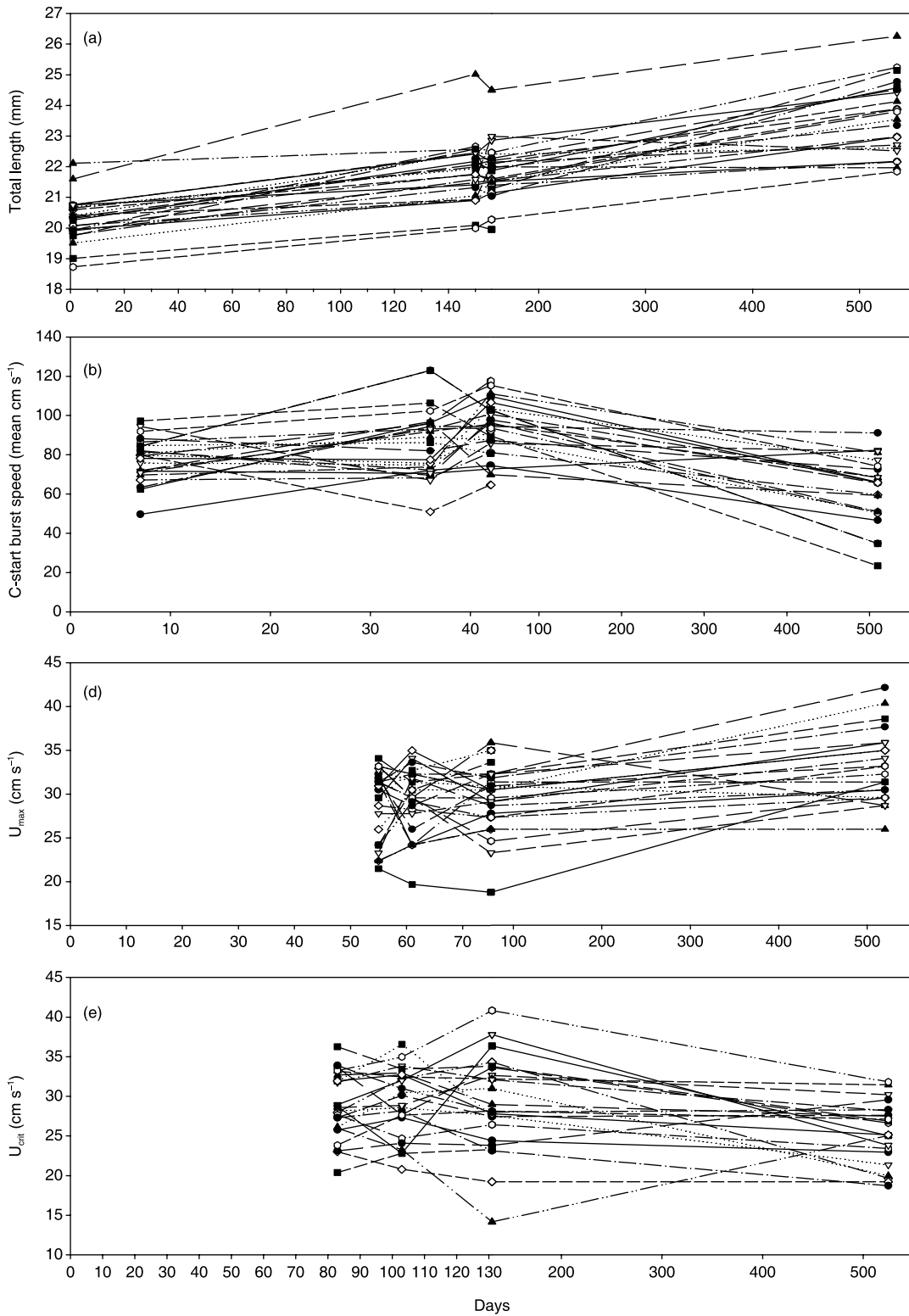
Critical swimming speed ( $U_{crit}$ ) was repeatable over weeks, but not over a year (Table 2). Similar to c-start burst speed,  $U_{crit}$  declined significantly over the span of a year (Fig. 3d, Table 3).

### RELATIONS BETWEEN PERFORMANCE METRICS AND MORPHOLOGY

Relations of performance metrics and morphology are summarized in Table 4. Critical swimming speed and  $U_{max}$  were consistently positively related, and significantly so at the first trial. Mean c-start burst speed significantly positively correlated with  $U_{max}$  at trial 2, but this appears to be an anomalous result considering the lack of correlation at other trials. None of the performance measures were significantly related to body size or shape.

## Discussion

Our results demonstrate that individual differences in three swimming performance metrics, which characterize two



**Fig. 3.** Plots of individual values for total length (a) mean c-start burst speed (b), maximum speed ( $U_{max}$ ) (c) and critical swimming speed ( $U_{crit}$ ) (d). Each point represents an individual's value for that day. Lines connect an individual's value among the 4 days.

different modes of swimming in teleost fish, as well as body size, are repeatable on various time-scales, including long-term repeatability more than a year later for at least one performance metric ( $U_{max}$ ) and size (TL). This relative stability of individual

differences is important for two general ecological/evolutionary reasons. First, if individual differences are not consistent over time, then natural or sexual selection cannot 'target' particular phenotypes with any degree of accuracy. Second, repeatability

**Table 2.** Repeatabilities of performance measures comparing trials 1 with 2, 2 with 3, and 3 with 4. Pearson correlation coefficient reported, except for C-start Burst Speed (mean), where we also report  $r$  values corrected for attenuation (Adolph & Hardin 2007; see 'Statistical Analysis' in Methods and materials). Bold indicates statistically significant correlations based on 2-tailed tests

	Trials 1 vs. 2	Trials 2 vs. 3	Trial 3 vs. 4
$U_{\max}$	$r = 0.3529$ $P = 0.091$	<b><math>r = 0.5410</math></b> $P = 0.006$	<b><math>r = 0.4252</math></b> $P = 0.048$
$U_{\text{crit}}$	<b><math>r = 0.7365</math></b> $P < 0.001$	<b><math>r = 0.5782</math></b> $P = 0.003$	$r = 0.3427$ $P = 0.118$
C-start burst speed (maximum)	$r = 0.1396$ $P = 0.515$	$r = 0.1636$ $P = 0.445$	$r = 0.0778$ $P = 0.744$
C-start burst speed (mean)	$r = 0.1841$ $P = 0.389$	<b><math>r = 0.4301</math></b> $P = 0.036$	$r = 0.1565$ $P = 0.510$
C-start burst speed (mean, corrected for attenuation)	<b><math>r_{\text{corr}} = 0.4563</math></b> $P = 0.0328$	<b><math>r_{\text{corr}} = 0.7574</math></b> $P < 0.0001$	$r_{\text{corr}} = 0.2677$ $P = 0.4002$
Total length	<b><math>r = 0.7111</math></b> $P < 0.001$	<b><math>r = 0.8452</math></b> $P < 0.001$	<b><math>r = 0.4600</math></b> $P = 0.031$
Body depth/TL	$r = 0.1179$ $P = 0.592$	$r = 0.3632$ $P = 0.081$	$r = 0.2111$ $P = 0.346$

generally sets an upper limit to heritability (but see Dohm 2002), and hence the rate of evolutionary (genetic) response to selection of a given intensity.

We found that most traits change in an expected fashion over a time span of a year or more, as fish progress through middle-to-old age. For instance, it is predicted that performance metrics will decrease during later ages due to the effects of senescence on the neuromusculature system (Huey *et al.* 1990, Reznick *et al.* 2004, Bronikowski *et al.* 2006). As expected, we found that both  $U_{\text{crit}}$  and burst speed during a c-start escape response decreased significantly after a year (Table 3, Fig. 3). However,  $U_{\max}$  surprisingly increased significantly (Table 3). Not surprisingly, body size increased significantly (Table 3), although the increase was less than 5%, which is typical for mature male poeciliids (Bisazza 1993).

The escape response of a fish, as characterized by the c-start, has been well-studied from many disciplines (Domenici & Blake 1997, Domenici 2002); however, only a few studies have examined its repeatability. We examined the velocity attained

during a c-start escape response and found short-term repeatabilities (Table 2, and see Results) within the range of those reported previously (Garenc *et al.* 1999, Chappell & Odell 2004, Langerhans *et al.* 2004, Royle *et al.* 2006). We did not find significant long-term repeatability of c-start performance, and no previous study has reported comparable values.

Our results for c-start mean burst speed suggest that using the mean value of several measures separated by short time intervals, and correcting for attenuation (Adolph & Hardin 2007), increases repeatability (Table 2, Fig. 3b). Few other studies have examined medium- and long-term repeatability of speed during a c-start escape response. For example, the longest study on burst speed in fish found significant repeatability over 2 days, on six individual *Gasterosteus aculeatus* (Garenc *et al.* 1999). Although many studies of terrestrial vertebrates have found repeatability of maximum speeds (e.g., Huey & Dunham 1987, Djawdan & Garland 1988, Huey *et al.* 1990, Brodie & Garland 1993, van Berkum *et al.* 1989, Garland & Losos 1994, Chappell *et al.* 2007, but see Losos *et al.* 2002), our results suggest that in future studies it may be beneficial to obtain multiple trials for an individual fish and use the mean velocity during a c-start escape response instead of the single maximum velocity (Table 2).

The c-start escape response of fish has been proposed to be important in predator-prey interactions, and recent studies have begun to highlight the importance of various aspects of this response. Walker *et al.* (2005) examined several aspects of c-start escape response in relation to successful escapes during staged encounters between guppies (*P. reticulata*) and a natural fish predator (*Crenicichla alta*), and recorded the trials with a high-speed video camera. Successful escapes were associated with long approach distances, low predator strike velocities, increased initial flight angles, increased accelerations, and increased stage 1 turning velocities (Walker *et al.* 2005). Because we only measured velocity during a c-start escape response, more studies are warranted on the repeatability of other aspects of this performance.

Our results for repeatability of critical swimming speed ( $U_{\text{crit}}$ ) are consistent with previous studies, which have reported repeatabilities of 0.44–0.86 (Table 1), and are similar to endurance repeatabilities in squamate reptiles (Kolok

**Table 3.** Mean values ( $\pm$  standard deviations) for trials 3 and 4 in all fish. All traits, except the ratio of body depth to total length, changed significantly over the span of a year, as indicated by a paired  $t$ -test

	$N$	Trial 3	Trial 4	$T$	d.f.	2-Tailed $P$
$U_{\max}$ (cm s <sup>-1</sup> )	22	29.13 $\pm$ 3.65	33.13 $\pm$ 4.12	-4.491	21	< 0.001
$U_{\text{crit}}$ (cm s <sup>-1</sup> )	22	29.26 $\pm$ 4.02	25.43 $\pm$ 3.93	2.966	21	= 0.007
C-start maximum burst speed (cm s <sup>-1</sup> )	20	95.65 $\pm$ 22.89	66.73 $\pm$ 16.18	4.793	19	< 0.001
C-start mean burst speed (cm s <sup>-1</sup> )	20	93.76 $\pm$ 12.85	63.52 $\pm$ 16.93	6.904	19	< 0.001
Mass (g)	22	0.105 $\pm$ 0.015	0.136 $\pm$ 0.021	-7.433	21	< 0.001
Standard length (mm)	22	16.26 $\pm$ 0.83	17.43 $\pm$ 1.19	-5.049	21	< 0.001
Total length (mm)	22	21.94 $\pm$ 0.86	23.61 $\pm$ 1.20	-7.12	21	< 0.001
Body depth (mm)	22	4.10 $\pm$ 0.28	4.31 $\pm$ 0.33	-3.146	21	= 0.005
Body depth/Total length	22	0.187 $\pm$ 0.009	0.183 $\pm$ 0.012	1.439	21	= 0.165

**Table 4.** Pearson correlations for the four swimming performance traits, total length as a measure of body size, and body depth/total length as an index of shape.  $r_1 =$  trial 1, etc. Significant correlations ( $P < 0.01$ ) based on 2-tailed tests and not corrected for multiple comparisons are marked in bold and with asterisk (\*)

	$U_{\text{crit}}$	C-start burst speed (maximum)	C-start burst speed (mean)	Total length	Body depth/TL
$U_{\text{max}}$	$r_1 = \mathbf{0.550^*}$	$r_1 = -0.243$	$r_1 = -0.505$	$r_1 = 0.378$	$r_1 = 0.098$
	$r_2 = 0.380$	$r_2 = 0.261$	$r_2 = \mathbf{0.616^*}$	$r_2 = -0.080$	$r_2 = -0.063$
	$r_3 = 0.221$	$r_3 = -0.131$	$r_3 = 0.048$	$r_3 = 0.145$	$r_3 = 0.151$
	$r_4 = 0.525$	$r_4 = -0.060$	$r_4 = -0.258$	$r_4 = 0.277$	$r_4 = 0.074$
$U_{\text{crit}}$		$r_1 = 0.010$	$r_1 = -0.143$	$r_1 = -0.117$	$r_1 = 0.141$
		$r_2 = 0.208$	$r_2 = 0.253$	$r_2 = 0.173$	$r_2 = 0.147$
		$r_3 = 0.173$	$r_3 = 0.067$	$r_3 = 0.209$	$r_3 = 0.138$
		$r_4 = 0.193$	$r_4 = -0.069$	$r_4 = 0.361$	$r_4 = 0.197$
C-start burst speed (maximum)			$r_1 = \mathbf{0.779^*}$	$r_1 = -0.398$	$r_1 = 0.256$
			$r_2 = 0.457$	$r_2 = 0.068$	$r_2 = 0.095$
			$r_3 = 0.410$	$r_3 = -0.204$	$r_3 = -0.414$
			$r_4 = 0.439$	$r_4 = 0.328$	$r_4 = 0.434$
C-start burst speed (mean)				$r_1 = -0.474$	$r_1 = 0.128$
				$r_2 = -0.036$	$r_2 = -0.110$
				$r_3 = -0.346$	$r_3 = -0.375$
				$r_4 = -0.299$	$r_4 = 0.031$
Total length					$r_1 = 0.191$
					$r_2 = 0.142$
					$r_3 = 0.310$
					$r_4 = -0.138$

\* $P < 0.01$ .

1999, Perry *et al.* 2004). Although no studies have yet demonstrated the direct fitness benefits of an increased in  $U_{\text{crit}}$  or endurance in fish, some studies have begun to explore its relationship with environmental characteristics, such as water velocity (McGuigan *et al.* 2003, Nelson *et al.* 2003).

Maximum swimming speed ( $U_{\text{max}}$ ) is a recently described performance trait and has not been evaluated for adaptive significance or repeatability. We found its repeatability to be similar to that of  $U_{\text{crit}}$ , at least on a medium time-scale (Table 2).  $U_{\text{crit}}$  and  $U_{\text{max}}$  tended to be positively correlated, and significantly so for at least the first trial (Table 4). Surprisingly, their mean values were not significantly different for the first three trials (trial 1:  $t = 0.788$ ,  $P = 0.439$ ; trial 2:  $t = 0.301$ ,  $P = 0.766$ , trial 3:  $t = 0.478$ ,  $P = 0.637$ , and see Table S1). However, there was a difference in their means for the fourth trial ( $t = 9.204$ ,  $P < 0.001$ ), with fish having a significantly higher  $U_{\text{max}}$ . These results suggest that the measure of  $U_{\text{max}}$  may be an equally reliable trait to measure in fish performance, but see also Farrell (2008). Because the ramping velocity is much faster in  $U_{\text{max}}$ , the trials are much shorter (approximately 95 s in our study) than  $U_{\text{crit}}$  trials (approximately 22 min in our study) (Reidy *et al.* 2000, Farrell 2008). However, caution must be taken because our results demonstrate a significant increase in  $U_{\text{max}}$ , but a decrease in  $U_{\text{crit}}$ , causing a significant difference in their means on a long time-scale. Therefore, more definitive tests of  $U_{\text{max}}$  are needed to determine its relationship to  $U_{\text{crit}}$  (and see Farrell 2008).

The reason for the increase in  $U_{\text{max}}$  between trials 3 and 4 is unknown, but may be related to the simultaneous increase in body size (Table 3, Fig. 3c, Table S1). Yet,  $U_{\text{max}}$  is not significantly related to size for any of the trials (Table 4). Additionally,

$U_{\text{crit}}$  decreased over a year, and it is positively correlated with  $U_{\text{max}}$  on the fourth trial ( $r = 0.525$ , 2-tailed  $P = 0.012$ ). The increase in  $U_{\text{max}}$  was also not a function of training affects, as the fish were not intentionally exercised for a year. Gibert *et al.* (2001) found that the walking speed of fruit flies increased with age when reared at cold temperatures, but decreased when flies were reared at warm temperatures. However, all fish in our study were reared under similar conditions, so temperature acclimation cannot explain the increase in  $U_{\text{max}}$ . In our study, fish were mature prior to trial 1, so the increase in  $U_{\text{max}}$  is not likely a function of developmental effects on the neuromusculature system. However, there is an example of post-maturational age-related increase in stamina in natural populations of *Sceloporus merriami* (Huey *et al.* 1990). In any case, further studies are needed to determine why this performance metric increased with age.

A negative relationship may exist between burst speed and endurance because they are affected by many of the same subordinate traits, such as muscle fibre type composition (Webb 1984, Weihs 1989, Garland & Losos 1994, Vanhooydonck *et al.* 2001, Bonine *et al.* 2005). This relationship has been investigated at the individual level in one previous study of guppies. Chappell and Odell (2004) examined the relationship between burst speed during an escape response and maximum oxygen consumption (a likely component of endurance capacity) in male and female Trinidadian guppies, and for both sexes found no evidence of a trade-off. Claireaux *et al.* (2007) also examined this relationship at the individual level in the European sea bass (*Dicentrarchus labrax*) by measuring  $U_{\text{crit}}$  and sprint speed in a group of marked fish, over a period of weeks and months. They found no relationship between

$U_{crit}$  and sprint speed in any of their analyses (e.g. the entire cohort, survivors over 6 months, and non-survivors). They suggest that while these two modes of swimming, burst and prolonged, rely on overlapping morphological and physiological systems, they are still distinct for each mode of swimming. For instance, prolonged is powered by aerobic respiration and may be limited by fuel or delivery of oxygen to the muscle, whereas burst is powered by anaerobic means and may be limited by neuromusculature factors. Therefore, they conclude, that 'This disconnection may account for the fact that  $U_{crit}$  and sprint speeds respond differently to environmental constraints, and it introduces the possibility of independent selection trajectories for the two performance metrics (Claireaux *et al.* 2007).' Our results (Table 4) also indicate no relationship between burst (escape response) and prolonged ( $U_{crit}$ ) swimming, whether examining the mean c-start burst speed or the maximum. More studies are needed to tease apart this relationship between sprint and endurance performance in fish; both at the interspecific and intraspecific levels, corrections for 'measurement error' may reveal new and interesting results (see Adolph & Hardin 2007; Ives *et al.* 2007).

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## Supporting Information

Additional Supporting Information may be found in the online version of this article:

### Table S1. Descriptive statistics for all traits and trials

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**Table S1.** Descriptive statistics for all traits measured for all trials. Sample sizes (N), means, and standard deviations reported.

<b>Trait</b>	<b>N</b>	<b>Mean</b>	<b>Standard Deviation</b>
<b>Maximum speed (<math>U_{max}</math>)</b>			
Trial 1	24	29.41	3.797
Trail 2	24	29.41	3.653
Trail 3	24	29.56	3.785
Trial 4	22	33.13	4.121
<b>Critical swimming speed (<math>U_{crit}</math>)</b>			
Trial 1	24	28.81	4.063
Trail 2	24	29.13	4.327
Trail 3	24	28.94	6.002
Trial 4	22	25.43	3.930
<b>C-start burst speed (maximum)</b>			
Trial 1	24	83.32	10.228
Trail 2	24	96.03	11.248
Trail 3	24	95.96	22.564
Trial 4	20	66.73	16.181
<b>C-start burst speed (mean)</b>			
Trial 1	24	77.62	10.944
Trail 2	24	84.27	15.851
Trail 3	24	92.94	14.313
Trial 4	20	63.52	16.931
<b>Mass</b>			
Trial 1	23	0.0805	0.01059
Trail 2	24	0.1048	0.01683
Trail 3	24	0.1037	0.01627
Trial 4	22	0.1361	0.02051
<b>Standard length (SL)</b>			
Trial 1	23	14.89	1.030
Trail 2	24	16.14	0.880
Trail 3	24	16.17	0.876
Trial 4	22	17.43	1.195
<b>Total length (TL)</b>			
Trial 1	23	20.28	0.733
Trail 2	24	21.82	1.014
Trail 3	24	21.86	0.906
Trial 4	22	23.61	1.198
<b>Body Depth (BD)</b>			
Trial 1	23	3.796	0.2621
Trail 2	24	4.218	0.3217
Trail 3	24	4.093	0.2973
Trial 4	22	4.314	0.3309
<b>Body depth/total length</b>			
Trial 1	23	0.1872	0.0099
Trail 2	24	0.1932	0.0104
Trail 3	24	0.1871	0.0088
Trial 4	22	0.1828	0.0120