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Effects of intraspecific variation in reproductive traits, pectoral fin use and burst swimming on metabolic rates and swimming performance in the Trinidadian guppy (*Poecilia reticulata* Peters)

Jon C. Svendsen[1,2,*], Amanda I. Banet[3,4], Rune H. B. Christensen[5], John F. Steffensen[2] and Kim Aarestrup[1]

[1]Technical University of Denmark, National Institute of Aquatic Resources, Freshwater Fisheries, Vejlsøvej 39, DK-8600 Silkeborg, Denmark;
[2]University of Copenhagen, Marine Biological Laboratory, Biological Institute, Strandpromenaden 5, DK-3000 Helsingør, Denmark;
[3]Department of Biology, University of California, Riverside, CA 92521, USA;
[4]University of British Columbia, Vancouver, BC, V6T 1Z4, Canada;
[5]Technical University of Denmark, Department of Applied Mathematics and Computer Science, Richard Petersens Plads, Building 324, Room 220, DK-2800 Lyngby, Denmark;

ABSTRACT. There is considerable intraspecific variation in metabolic rates and locomotor performance in aquatic ectothermic vertebrates, however, the mechanistic basis remains poorly understood. Using pregnant Trinidadian guppies (*Poecilia reticulata* Peters), a live-bearing teleost, we examined the effects of reproductive traits, pectoral fin use, and burst-assisted swimming on the swimming metabolic rate, standard metabolic rate ($MO_{2\text{std}}$) and prolonged swimming performance (U_{crit}). Reproductive traits included reproductive allocation and pregnancy stage; the former defined as the mass of the reproductive tissues divided by the total body mass. Results showed that the metabolic rate increased curvilinearly with swimming speed. The slope of the relationship was used as an index of swimming cost. There was no evidence that reproductive traits correlated with the swimming cost, $MO_{2\text{std}}$, or U_{crit}. In contrast, data revealed strong effects of pectoral fin use on swimming cost and U_{crit}. *Poecilia reticulata* employed body-caudal fin (BCF) swimming at all tested swimming speeds, however, fish with a high simultaneous use of the pectoral fins exhibited increased swimming cost and decreased U_{crit}. These data indicated that combining BCF swimming and pectoral fin movement over a wide speed range, presumably to support swimming stability and control, is an inefficient swimming behaviour. Finally, transition to burst-assisted swimming was associated with an increase in aerobic metabolic rate. Our study highlights factors other than swimming speed that affect swimming cost and suggests that intraspecific diversity in biomechanical performance, such as pectoral fin use, is an important source of variation in both locomotor cost and maximal performance.

Keywords: basal metabolic rate, energetics, gait transition, gravidity, life history, respiratory physiology

* Corresponding author: jos@aqua.dtu.dk

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Jon C. Svendsen[1,2,*], Amanda I. Banet[3,4], Rune H. B. Christensen[5], John F. Steffensen[2] and Kim Aarestrup[1]

[1]Technical University of Denmark, National Institute of Aquatic Resources, Freshwater Fisheries, Vejlsøvej 39, DK-8600 Silkeborg, Denmark, [2]University of Copenhagen, Marine Biological Laboratory, Biological Institute, Strandpromenaden 5, DK-3000 Helsingør, Denmark, [3]Department of Biology, University of California, Riverside, CA 92521, USA, [4]University of British Columbia, Vancouver, BC, V6T 1Z4, Canada, [5]Technical University of Denmark, Department of Applied Mathematics and Computer Science, Richard Petersens Plads, Building 324, Room 220, DK-2800 Lyngby, Denmark.

*Author for correspondence (jos@aqua.dtu.dk)

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SUMMARY

There is considerable intraspecific variation in metabolic rates and locomotor performance in aquatic ectothermic vertebrates, however, the mechanistic basis remains poorly understood. Using pregnant Trinidadian guppies (*Poecilia reticulata* Peters), a live-bearing teleost, we examined the effects of reproductive traits, pectoral fin use, and burst-assisted swimming on the swimming metabolic rate, standard metabolic rate ($\boldsymbol{M}\mathbf{O}_{\mathbf{2std}}$) and prolonged swimming performance ($\boldsymbol{U}_{\mathbf{crit}}$). Reproductive traits included reproductive allocation and pregnancy stage; the former defined as the mass of the reproductive tissues divided by the total body mass. Results showed that the metabolic rate increased curvilinearly with swimming speed. The slope of the relationship was used as an index of swimming cost. There was no evidence that reproductive traits correlated with the swimming cost, $\boldsymbol{M}\mathbf{O}_{\mathbf{2std}}$, or $\boldsymbol{U}_{\mathbf{crit}}$. In contrast, data revealed strong effects of pectoral fin use on swimming cost and $\boldsymbol{U}_{\mathbf{crit}}$. *Poecilia reticulata* employed body-caudal fin (BCF) swimming at all tested swimming speeds, however, fish with a high simultaneous use of the pectoral fins exhibited increased swimming cost and decreased $\boldsymbol{U}_{\mathbf{crit}}$. These data indicated that combining BCF swimming and pectoral fin movement over a wide speed range, presumably to support swimming stability and control, is an inefficient swimming behaviour. Finally, transition to burst-assisted swimming was associated with an increase in aerobic metabolic rate. Our study highlights factors other than swimming speed that affect swimming cost and suggests that intraspecific diversity in biomechanical performance, such as pectoral fin use, is an important source of variation in both locomotor cost and maximal performance.

Key words: basal metabolic rate, energetics, gait transition, gravidity, life history, respiratory physiology.

INTRODUCTION

Intraspecific variation in metabolic rate and locomotor performance remain poorly understood in many taxa. Variation in physiological traits may be important, however, because it can be functionally significant and reflect behavioural or physiological trade-offs, where the costs or benefits of any phenotype are variable and may depend on internal and external factors (Williams, 2008; Biro and Stamps, 2010; Burton et al., 2011). For example, intraspecific variation in metabolic rate and locomotor performance may be associated with disruptive selection regimes leading to variation in foraging strategy and predator avoidance (Marras et al., 2010). Moreover, studies of physiological diversity may reveal the physiological basis of intraspecific variation in life history traits (Speakman, 2005; Arnott et al., 2006; Williams, 2012). Finally, phenotypic diversity may be indicative of genetic diversity and the degree to which a population can adjust to environmental change (Hayes and Jenkins, 1997; Bolnick et al., 2003; Sears et al., 2009).

Reproductive status may be a source of intraspecific variation in metabolic rate and locomotor performance. Elevated metabolic rate in relation to gravidity or pregnancy has been reported in many animals, including eastern garter snake (*Thamnophis sirtalis* Linnaeus) (Birchard et al., 1984), mountain spiny lizard (*Sceloporus jarrovi* Cope) (DeMarco, 1993), yellowtail rockfish (*Sebastes flavidus* Ayres) (Hopkins et al., 1995), Korean rockfish (*Sebastes schlegeli* Hilgendorf) (Boehlert et al., 1991), sailfin molly (*Poecilia latipinna* Lesueur) (Timmerman and Chapman, 2003), striped surfperch (*Embiotoca lateralis* Agassiz) (Webb and Brett, 1972) and European eelpout (*Zoarces viviparus* Linnaeus) (Skov et al., 2010). Several studies have demonstrated diminished locomotor performance caused by gravidity or pregnancy. Examples include the northern death adder (*Acanthophis praelongus* Ramsay) (Webb, 2004), side-blotched lizard (*Uta stansburiana* Baird and Girard) (Miles et al., 2000), short-horn sculpin (*Myoxocephalus scorpius* Linnaeus) (James and Johnston, 1998) and mosquitofish (*Gambusia affinis* Baird and Girard) (Plaut, 2002; Belk and Tuckfield, 2010). Using the Trinidadian guppy (*Poecilia reticulata* Peters), Ghalambor and colleagues provided evidence that pregnancy may constrain fast-start swimming performance employed to evade predators (Ghalambor et al., 2004). It has been suggested that diminished swimming performance in live-bearing pregnant fish may be attributed to metabolic constraints caused by the embryos (Plaut, 2002), however, to our knowledge such relationships have not been examined.

The impact of pregnancy on female performance could have important ecological and evolutionary consequences. For example, pregnant bighorn sheep (*Ovis canadensis* Shaw) spend less time in optimal foraging areas, where the predation risk is highest, than females that have recently given birth (Berger, 1991). Such differences in behaviour may reduce the predation risk associated with diminished locomotor performance at the cost of resource acquisition. From an evolutionary point of view, cost of reproduction represents one of the most prominent elements in life history evolution (Stearns, 1989). Using free-ranging lizards, Miles and colleagues demonstrated that a decrement in performance is associated with current reproductive investment and represents a cost of reproduction expressed as diminished locomotor performance and lowered survivorship to next clutch (Miles et al. 2000).

Recent studies on the metabolic rates of swimming fish have included measurements of gait transitions occurring as a function of swimming speed (Korsmeyer et al., 2002; Jones et al., 2007; Cannas et al., 2006; Svendsen et al., 2010). A gait is “a pattern of locomotion characteristic of a limited range of speeds described by quantities of which one or more change discontinuously at transitions to other gaits” (Alexander, 1989). However, as far as is known, no previous studies have investigated how intraspecific variation in fin use within a single gait affects swimming cost and maximal performance. Moreover, while previous studies have examined the metabolic rates associated with the transition from rigid-body, median or paired-fin (MPF) swimming to undulatory, body-caudal fin (BCF) swimming (Korsmeyer et al., 2002; Cannas et al., 2006; Svendsen et al., 2010), the energetics of the gait transition from steady BCF swimming to unsteady BCF swimming (i.e. burst-assisted) remain poorly understood (Farrell, 2007).

The objective of this study was to examine whether diversity in reproductive traits and swimming behaviour correlate with intraspecific variation in metabolic rates and maximal locomotor performance. Reproductive traits included reproductive allocation and pregnancy stage; the former defined as the mass of reproductive tissues divided by the total body mass. Swimming behaviour included use of the pectoral fins and gait transition from steady BCF swimming to unsteady BCF swimming (i.e. burst-assisted). To this end, we used *P. reticulata* Peters 1859 captured in Trinidad for swimming trials at increasing speeds.

Poecilia reticulata is a live-bearing species producing one litter every 3 - 4 weeks (Reznick and Yang, 1993). Reproductive allocation in female *P. reticulata* tends to vary with season (Reznick, 1989), resource availability (Reznick and Yang, 1993) and predation regime (Reznick and Endler, 1982). In terms of locomotion, *P. reticulata* is an acanthomorph fish (Chen et al., 2003) and as such, the pectoral fins are located relatively high on the body, at an approximately mid-dorsal position and relatively close to the centre of mass of the fish (Drucker et al., 2006). Compared to less derived fishes, the pectoral fins of acanthomorph fishes are generally associated with a wider range of motion and a correspondingly greater propulsor diversity (Drucker et al., 2006). Moreover, *P. reticulata* is a BCF swimmer that may switch to burst-assisted swimming (Pohlman et al., 2001). Several studies have used Trinidadian *P. reticulata* to investigate factors causing intraspecific variation in relation to evolutionary ecology (Magurran, 2005), and *P. reticulata* is a key organism for empirical tests of theoretical life history models (Reznick et al., 1990; Reznick et al., 1996; Ghalambor et al., 2003). We used individual female *P. reticulata*, varying in reproductive traits, to document swimming metabolic rates, standard metabolic rate, swimming behaviour and prolonged swimming performance. Measurements of excess post-exercise oxygen consumption (Lee et al., 2003b) were included because individual variation in swimming performance might be related to processes associated with anaerobic rather than aerobic power production.

We predicted that reproductive allocation and/or pregnancy stage would correlate positively with metabolic swimming cost and negatively with prolonged swimming performance. Further, we predicted that standard metabolic rate would correlate positively with reproductive allocation and/or pregnancy stage. In terms of fin use, we predicted that fish extending their pectoral fins would experience increased drag and increased swimming cost, as hypothesised by previous studies (Webb, 1998; Weihs, 2002; Green and Hale, 2012). Inefficient fin use at increasing speeds could translate into decreased swimming performance. For example, if extending the pectoral fins causes a consistent increase in the swimming cost, a fish with extensive pectoral fin use at increasing speeds could exhibit inferior swimming performance, because the fish would reach the maximum metabolic rate at a relatively slow swimming speed. Finally, as a consequence of gait transition to burst-assisted swimming, aerobic metabolic rate (i.e. oxygen consumption rate during exercise) should either plateau; or the rate of increase, as a function of swimming speed, should decline because burst-assisted swimming is partly covered by anaerobic metabolism (Farrell, 2007).

MATERIALS AND METHODS

Experimental animals

A total of 18 female *P. reticulata* (body mass: 0.296 ± 0.009 g; total length: 3.0 ± 0.0 cm (mean ± S.E.)) was captured using butterfly nets in the Naranjo River in Trinidad. The river is a low predation tributary to the Aripo River. The mean current velocity at the collection site was 12.7 ± 1.2 cm s^{-1}. In the laboratory, fish were kept in five identical holding tanks (30 l each) using filtered water originating from the Arima Valley. Each tank housed 4 – 5 individuals including one male. One air stone in each tank secured normoxic conditions. Each tank was cleaned and water was replaced every third day. Prior to experimentation, fish were acclimated to the laboratory for 2 - 3 weeks. Fish were fed daily on commercial flake food to satiation. Mean water temperature in the holding tanks was 25.8°C (range: 24.6 – 26.9°C). All fish holding procedures were identical for the five tanks.

P. reticulata is a lecithotrophic species. Lecithotrophic means that there is no placenta-like connection between the mother and young (Reznick and Yang, 1993), such that yolk stored in the egg is assumed to be the only source of embryo nutrition. Recent work within the Poeciliidae has indicated, however, that some mother-to-embryo nutrient transfer may occur in species thought to be lecithotrophic (Marsh-Matthews et al., 2005; Marsh-Matthews et al., 2010). To our knowledge, no attempt has been made to quantify any post-fertilization provisioning in *P. reticulata*, and it is not known to what degree there is a limited transfer of oxygen or small molecules.

Respirometry

A 0.170 l Bläzka-type swimming respirometer (Model SW10000; Loligo Systems Aps, Tjele, Denmark) was used to measure oxygen consumption rate (MO_2; mg O_2 kg^{-1} h^{-1}) as a function of swimming speed (*U*). The respirometer was submerged in an ambient tank (0.9 × 0.35 × 0.39 m) supplying water for the respirometer. Water temperature was maintained at 26.0°C (range: 25.9-26.1°C) using two cooling peltier elements (IceProbe; Cool Works Inc., San Rafael, CA, USA) and a submersible heater (50 W; AkvaStabil; Haderslev, Denmark). An air stone in the ambient tank maintained oxygen levels > 95% air saturation.

The inner dimensions of the cylindrical observation section in the respirometer were 26 × 100 mm (diameter × length). An impeller, placed downstream of the observation section, was driven by an external electric motor that generated the re-circulating flow. Deflectors situated upstream of the observation section collimated the flow. To promote rectilinear flow and a uniform velocity profile in the observation section, water was passed through an upstream honeycomb (3 mm cell diameter) producing a micro turbulent flow. A grid (2 × 2 mm) in the downstream direction bounded the observation section. Water speeds in the observation section were measured using a Laser Doppler Anemometer consisting of a 4W Ar-ion laser, a Fiberprobe and BSA data processors (Dantec Dynamics, Skovlunde, Denmark). The measurements were used to correlate water speed with voltage output from the external motor controller. Additional details have been published previously (Poulsen et al., 2012)

Polystyrene sheets covered the majority of the ambient tank to minimize any outside stimuli affecting the fish during the experiment. A small opening was used for behavioural observations. Fish were encouraged to swim in the most upstream part of the observation section using a darkening hide.

Oxygen partial pressure in the respirometer was measured using fibre optic sensor technology (PreSens, Regensburg, Germany). Intermittent-flow respirometry was applied in accordance with previous studies (Steffensen, 1989). The respirometer was fitted with an inlet port and a standpipe outlet, through which the volume of water in the respirometer could be replaced with a computer-actuated pump. The software AutoResp (Loligo Systems Aps, Tjele, Denmark) was used to control the flush (240 s), wait (120 s) and measurement (360 s) phases. These settings provided one MO_2 measurement per 12 min. Preliminary trials demonstrated that the R^2 associated with each MO_2 measurement was always > 0.95, similar to previous studies (Claireaux et al., 2006; Svendsen et al., 2012). The oxygen content never fell below 18.4 kPa. Standard equations were used to calculate MO_2 (Svendsen et al., 2010). Water in the ambient tank was recirculated through a loop consisting of a separate mechanical filter (Pick-up 2006; Eheim, Deizisau, Germany) and a UV-sterilizer (UV-10000; Tetra Pond, Melle, Germany). Between experimental runs, the entire setup was cleaned using a chlorine solution, flushed repeatedly, and refilled with water from the same source as used for the fish holding.

Fish for experiments were starved for 24 h prior to respirometry to ensure a post-absorptive state. Fish mass (to nearest 0.001 g), length, depth and width (all to nearest 0.5 mm) were determined for pre-experimental calculation and correction of the solid blocking effects, ranging from 2.2 to 4.2%. Calculations of solid blocking effects followed previous studies (Bell and Terhune, 1970).

Each *P. reticulata* was introduced to the working section and given at least 8 h (overnight) to acclimate while swimming at 2 L_T s^{-1} (total body lengths per second). Preliminary trials demonstrated that 2 L_T s^{-1} was the minimum swimming speed that secured positive rheotaxis (i.e. upstream orientation of the anterior body part). After the acclimation period, fish maintained a low MO_2, even when exposed to a few stepwise increases in the swimming speed. Occasionally, the acclimation period was extended to meet this criterion. Subsequently, each individual fish was exposed to progressive increments in the swimming speed of 0.5 L_T s^{-1} every 12 min until fatigue. MO_2 was measured at each swimming speed. Preliminary trials demonstrated that the critical (maximum) swimming speed (U_{crit}) was 9-17 L_T s^{-1}. The speed increment (0.5 L_T s^{-1}) was chosen to ensure an adequate number of MO_2 measurements (>12) at increasing speeds in individual fish. This type of data was required because we aimed at describing the relationship between U and MO_2 using an equation representing each individual fish. Maximum MO_2 (MO_{2max}) was estimated as the highest MO_2 measured during the swimming protocol (McKenzie et al., 2003).

Immediately after fatigue, the swimming speed was returned to 2 L_T s^{-1} (acclimation speed), following Lee and colleagues (Lee et al., 2003b). Using this swimming speed, MO_2 was measured for 1 h to quantify any excess post-exercise oxygen consumption (EPOC) (Lee et al., 2003b). Levels of background respiration were estimated from blank runs and used to correct the MO_2 measurements following past studies (Jones et al., 2007).

Swimming behaviours and critical swimming speed (U_{crit})

Behavioural data were collected during the swimming trials, similar to previous studies (Swanson et al., 1998). During the measurement phase (6 min) of the respirometric loop (12 min), time spent swimming with extended pectoral fins, caudal undulation, and using burst-assisted swimming were recorded. Use of each behaviour was recorded over a 1 min time interval (i.e. 3 min in total). These data were collected during each 12 min interval, starting at the acclimation speed (2 L_T s^{-1}) and ending at fatigue. End point values were the percentages of time allocated to these swimming behaviours at each swimming speed (Korsmeyer et al., 2002; Webb and Fairchild, 2001). The values were used to calculate the average fin and gait use (% of time) during the complete swimming trial for the individual fish. Finally, the gait transition speed (U_{STmax} (Peake, 2008)) from steady to unsteady swimming (i.e. burst-assisted) was recorded as the highest swimming speed without unsteady swimming.

The equation provided by Brett was used to calculate U_{crit} (Brett, 1964). Oufiero and Garland demonstrated that the U_{crit} protocol yields critical swimming speeds that are repeatable for individual *P. reticulata*, indicating that they represent actual measures of organism performance (Oufiero and Garland, 2009).

Reproductive traits

Immediately after the swimming trial, fish were euthanized using an over-doze of MS-222 and preserved in 6% formaldehyde. Wet and dry reproductive allocation (RA) was quantified using methods similar to Reznick (Reznick, 1983). Briefly, embryos and associated reproductive tissues were separated from female somatic tissue. Stage of embryonic development (i.e. pregnancy stage) was determined morphologically following standard procedures (Haynes, 1995). Development ranged from stage 0 (an egg with yolking ova) to stage 50 (fully developed embryos, ready to be born). Wet weights of the reproductive and somatic tissues were measured using a Mettler AE163 analytical balance (Mettler-Toledo, Columbus, OH, USA) and recorded to the nearest 0.00001 g. Subsequently, the tissues were air dried for 24 h at 600°C and weighed again following the same procedure. RA was calculated as the mass of reproductive tissues divided by the total body mass.

Data analysis

As indicated, we aimed at describing the relationship between U and MO_2 using an equation representing each individual fish. Previous studies have used power, exponential and polynomial models to describe the relationship between U and MO_2 (Korsmeyer et al., 2002; Arnott et al., 2006; Tudorache et al., 2011). Webb recommended that a certain model should not be assumed, but rather a model should be used that best describes the available data (Webb, 1993). Accordingly, we examined various models before determining the most appropriate model. Using the model for individual fish, MO_2 was extrapolated to zero swimming speed to estimate standard metabolic rate ($MO_{2\mathrm{std}}$), following previous studies (Brett, 1964; Arnott et al., 2006). The model was also used to estimate metabolic swimming cost in individual fish, expressed as the slope of the relationship between U and MO_2.

To test the predictions of this study, reproductive traits and pectoral fin use (considered the independent variables) were correlated with swimming cost and $MO_{2\mathrm{std}}$, both derived from the identified model, as well as U_{crit} (considered the dependent variables). Reproductive traits and pectoral fin use were not manipulated experimentally. Instead, the analyses relied on *post-hoc* intraspecific variation resulting from differences among individuals. To test our predictions, linear least square regression was used to examine if the reproductive traits correlated positively with $MO_{2\mathrm{std}}$ and swimming cost and negatively with U_{crit}. In terms of pectoral fin use, we tested if this variable correlated positively with swimming cost and negatively with U_{crit}. To assess such relationships further, we also tested for a negative correlation between U_{crit} and swimming cost.

The final objective of this study was to test the prediction that gait transition from steady BCF to unsteady BCF swimming would cause MO_2 to either plateau; or the rate of increase, as a function of swimming speed, would decline. To examine this prediction, we compared MO_2 before and after transition to burst-assisted swimming at increasing speeds using a sign test.

Because the five fish holding tanks were identical, maintained in an identical fashion and kept in the same room, we have no reason to believe that the different tanks affected the fish differently. Therefore, tank origin was not included in any analyses.

Estimates of $MO_{2\mathrm{std}}$ and $MO_{2\mathrm{max}}$ were used to estimate the metabolic scope (MS). The MS was defined as the difference between $MO_{2\mathrm{std}}$ and $MO_{2\mathrm{max}}$, following past studies (Farrell and Richards, 2009). The speed where fish transitioned from steady to unsteady BCF swimming (U_{STmax}), and the simultaneous $M\mathrm{O}_2$ measurements, were used to partition the MS into the proportion attributed to steady swimming and the proportion attributed to unsteady swimming.

To detect EPOC, we compared the individual pre-exercise $M\mathrm{O}_2$ with the first post-exercise $M\mathrm{O}_2$ using a paired t-test after examining the assumptions of normal distribution of data and homogeneity of variance. Both data sets were collected while the fish was swimming at 2 L_{T} s^{-1} (acclimation speed). If post-exercise $M\mathrm{O}_2$ was significantly higher than pre-exercise $M\mathrm{O}_2$, it was considered evidence of EPOC and anaerobic power production, following previous studies (Svendsen et al., 2010).

The free statistical software R (R Development Core Team, 2011) was used for statistical analyses. The R-package nlme (Pinheiro et al., 2011) was used to fit models. Results were considered significant if $\alpha = 0.05$. All values are reported as means ± s.e.m. unless noted otherwise.

RESULTS

The behavioural data showed that *P. reticulata* employed the caudal fin for swimming (i.e. BCF swimming) at all speeds (data not shown). In contrast, use of the pectoral fins and burst-assisted swimming varied with the swimming speed (Fig. 1). As swimming speed increased, the use of the pectoral fins decreased, however, there was no distinct threshold speed at which fish discontinued using the pectoral fins (Fig. 1). In fact, two individuals used the pectoral fins at all swimming speeds (Fig. 1).

Most fish (15 out of 18) employed burst-assisted swimming at the highest swimming speeds (Fig. 1). Burst-assisted swimming was less variable than use of the pectoral fins. All fish that started using burst-assisted swimming continued doing so throughout the remaining swimming trial (Fig. 1). The average gait transition speed (U_{STmax}) from steady swimming to unsteady swimming (i.e. burst-assisted swimming) was 40.85 ± 1.79 cm s^{-1}, equivalent to 13.48 ± 0.59 L_{T} s^{-1}. This measure included the maximum recorded steady swimming speed of three individuals that did not perform burst-assisted swimming (Fig. 1). The mean U_{crit} was 44.99 ± 1.84 cm s^{-1}, equivalent to 14.89 ± 0.66 L_{T} s^{-1}. There was no significant relationship between fish total length (cm) and U_{crit} (cm s^{-1}) ($P > 0.1$; $R^2 < 0.16$).

In terms of metabolic rates, $M\mathrm{O}_2$ as a function of U in individual fish was best described by the exponential function:

$$M\mathrm{O}_2 = a \exp(U\, b) \qquad (1)$$

where a is the $M\mathrm{O}_2$ at zero speed (i.e. $U = 0$), and b is the rate of increase in the $M\mathrm{O}_2$ as a function of U. The equation has been used to describe relationships between $M\mathrm{O}_2$ and U in a number of studies (Brett, 1964; McKenzie et al., 2003; Arnott et al., 2006). Equation (1) provided an estimate of the $M\mathrm{O}_{2\mathrm{std}}$ (i.e. a) and swimming cost (i.e. b) measured at increasing U in individual fish.

Maximum likelihood estimation was done on the logarithm of the $M\mathrm{O}_2$. The log $M\mathrm{O}_2$ was described by the following linear random coefficient model for the i'th observation on the j'th fish:

$$\mathrm{Log}\ M\mathrm{O}_{2\mathrm{ij}} = a'_{\mathrm{j}} + U_{\mathrm{i}}\, b_{\mathrm{j}} + e_{\mathrm{ij}}, \quad \mathrm{i} = 1, \ldots, n_{\mathrm{j}}, \quad \mathrm{j} = 1, \ldots, 18. \qquad (2)$$

The fish specific intercepts a_j' and slopes b_j were assumed to follow normal distributions with means μ_a and μ_b and variances σ_a^2 and σ_b^2. Moreover, a correlation between a_j' and b_j, ρ, was allowed. An AR1 (autoregressive of order 1) autocorrelation was allowed in the residuals using the equation:

$$e_\mathrm{ij} = \varphi\ e_\mathrm{i-1,j} + \varepsilon_\mathrm{ij} \qquad (3)$$

where φ is the autoregressive parameter. The ε_ij are assumed to be independently and identically normally distributed with mean zero and variance σ_ε^2. The MO_2 at zero U is a_j = exp(a_j') for the j'th fish, while b_j is the rate of increase in the MO_2, as a function of swimming speed, for the j'th fish. Thus, a_j and b_j represent $MO_{2\mathrm{std}}$ and swimming cost, respectively, for the j'th fish.

Measurements of MO_2 in relation to U in individual fish are plotted in Fig. 2. Data indicated that the rate of increase in MO_2, as a function of U, was lower at speeds when burst-assisted swimming was not employed (steady swimming) than at speeds when burst-assisted swimming was employed (unsteady swimming) (Fig. 2). Consequently, the parameters in equation (1) were estimated using observations with steady swimming only (Figs 1, 2).

The mean $MO_{2\mathrm{std}}$ was exp(μ_a) = exp(5.76) = 318.05 mg O_2 kg^{-1} h^{-1}. The 95% confidence interval was 294.01 – 344.05 mg O_2 kg^{-1} h^{-1}. The average rate of increase in the MO_2 as a function of U was 0.0262 (Fig. 2). Estimates of the parameters for equations (1-3) are provided in Table 1. The average $MO_{2\mathrm{max}}$ was 1270.69 ± 40.50 mg O_2 kg^{-1} h^{-1}. Body mass correlated weakly with $MO_{2\mathrm{std}}$ and $MO_{2\mathrm{max}}$ in a positive and negative fashion, respectively, but none of the relationships were significant (P>0.05).

After completing the swimming trial, fish were dissected and $\mathrm{RA_j}$ (i.e. fish specific RA) and fish specific pregnancy stage were quantified as described above. Measurements showed that both wet and dry RA varied between individuals (Table 2). Likewise, the pregnancy stages varied between individuals (Table 2). Wet and dry $\mathrm{RA_j}$ and fish specific pregnancy stage were related to $\hat{b}_\mathrm{j}$ (i.e. estimated fish specific steady swimming cost), $\hat{a}_\mathrm{j}$ (i.e. estimated fish specific $MO_{2\mathrm{std}}$) and $U_\mathrm{crit,j}$ (i.e. fish specific U_crit). The tests revealed no significant relationships (all P>0.1). These findings indicated that steady swimming cost, $MO_{2\mathrm{std}}$ and U_crit did not correlate with the reproductive traits.

The same tests were carried out using average pectoral fin use instead of the reproductive traits. These tests revealed that steady swimming cost (i.e. $\hat{b}_\mathrm{j}$) correlated positively with the average pectoral fin use (P<0.001; R^2=0.56; Fig. 3). Hence, *P. reticulata* spending more time with extended pectoral fins had increased steady swimming costs (Fig. 3). There was no correlation between $\hat{a}_\mathrm{j}$ and the average pectoral fin use, indicating that $MO_{2\mathrm{std}}$ and average pectoral fin use were unrelated (P=0.42).

There was a negative correlation between the average pectoral fin use and $U_\mathrm{crit,j}$ (P<0.0001; R^2=0.70; Fig. 4). Hence, *P. reticulata* spending more time with extended pectoral fins had a low U_crit (Fig. 4). There was no correlation between average pectoral fin use and $M\mathrm{O}_{2\mathrm{max,j}}$ (fish specific $MO_{2\mathrm{max}}$), or between $U_\mathrm{crit,j}$ and $M\mathrm{O}_{2\mathrm{max,j}}$ (both P>0.25), indicating that $MO_{2\mathrm{max}}$ did not influence the average pectoral fin use or U_crit.

The average pectoral fin use by individual fish was calculated using three different methods: 1) the average pectoral fin use throughout the complete swimming trial (i.e. from acclimation speed to U_crit); 2) the average pectoral fin use up to the initiation of burst-assisted swimming; and 3) the average pectoral fin use up to 9.5 L_T s^{-1} (equivalent to

28.8 cm s^{-1}). This swimming speed represented the highest swimming speed that all fish managed to maintain for a complete respirometric loop (Figs 1, 2). The average pectoral fin use data presented in Figs 3 and 4 were based on methods 2) and 1), respectively. The relationships shown in Figs 3 and 4 were present and significant (all $P<0.02$; $R^2>0.31$) regardless of the method employed to calculate the average pectoral fin use for the individual fish. These findings indicated that the relationships between average pectoral fin use and steady swimming cost (Fig. 3) and U_{crit} (Fig. 4) were not artefacts caused by the variable swimming performance of the fish.

Fish condition index was calculated following previous studies (Marras et al., 2011) and correlated with average pectoral fin use. Employing methods 1) and 2) to calculate average pectoral fin use, there was no significant correlation between fish condition index and average pectoral fin use (both $P>0.11$). When method 3) was employed, fish condition index correlated negatively with the average pectoral fin use (P=0.01; $R^2=0.34$). Because of the inconsistent relationships, a possible effect of condition index on pectoral fin use was not considered any further.

Finally, $\hat{b}_{\mathrm{j}}$ correlated negatively with $U_{\mathrm{crit,j}}$ ($P=0.002$; $R^2=0.46$) (Fig. 5). Hence, *P. reticulata* with a low U_{crit} had increased steady swimming costs in comparison with fish with a high U_{crit} (Fig. 5). Collectively, Figs 3, 4 and 5 indicated that elevated pectoral fin use increased steady swimming costs, which translated into a low U_{crit}. It appeared that increased steady swimming costs meant that *P. reticulata* with elevated pectoral fin use reached the maximum metabolic rate at a relatively low speed and therefore had a low U_{crit}. The findings suggested that inefficient fin use at increasing swimming speeds is coupled with a low U_{crit}.

Metabolic rate data collected when unsteady swimming occurred were insufficient to estimate the actual rate of increase in the $M\mathrm{O}_2$ as a function of U, specific for this swimming gait (Fig. 2). It was clear, however, that the vast majority of the $M\mathrm{O}_2$ data points during unsteady swimming were higher than what would be expected based on extrapolation of the values representing steady swimming (Fig. 2). To examine these observations statistically, a sign test was used to investigate if observations involving unsteady swimming (Fig. 2) were distributed around the prediction of the exponential equation (1) with an equal probability against the two-sided alternative. Differences between predicted values, using equation (1), and the actual observations involving unsteady swimming were aggregated for each fish and the mean difference was used as the end point value. These calculations showed that for all 15 fish performing unsteady swimming, the mean difference was positive (i.e. higher mean $M\mathrm{O}_2$ than expected). Testing the data using the sign test revealed a highly significant result ($P<0.001$), showing that the metabolic rate increased after transition to burst-assisted swimming.

The $M\mathrm{O}_{2\mathrm{max}}$ was 1270.69 ± 40.50 mg O_2 kg^{-1} h^{-1}. This value was recorded as the highest $M\mathrm{O}_2$ measured during the swimming protocol (McKenzie et al., 2003). In four fish, the maximum metabolic rate was not associated with the highest swimming speed, but with the second highest swimming speed (Fig. 2). Thus, the mean $M\mathrm{O}_2$ recorded during the highest swimming speed (1258.76 ± 39.73 mg O_2 kg^{-1} h^{-1}) was slightly lower (1%) than $M\mathrm{O}_{2\mathrm{max}}$.

The metabolic scope (MS) was calculated as $M\mathrm{O}_{2\mathrm{max}} - M\mathrm{O}_{2\mathrm{std}}$ following past studies (Farrell and Richards, 2009) and was on average 952.64 mg O_2 kg^{-1} h^{-1}. Depending on the fish, $M\mathrm{O}_{2\mathrm{max}}$ occurred during steady or unsteady swimming (Fig. 2). The highest $M\mathrm{O}_2$ recorded during steady swimming was on average 1015.61 mg O_2 kg^{-1} h^{-1}. This measure included $M\mathrm{O}_{2\mathrm{max}}$ of three individuals that did not perform any burst-assisted swimming (Figs 1, 2). The $M\mathrm{O}_2$ increased by 255.08 mg O_2 kg^{-1} h^{-1} during the part of the swimming protocol that involved unsteady swimming (Fig. 2). In proportions of the MS, steady swimming accounted for 73.2%, whereas unsteady swimming accounted for 26.8%. These findings showed that unsteady swimming contributed significantly to MS.

Immediately after fatigue, the swimming speed was reduced to the acclimation speed (2 L_T s^{-1}), following Lee and colleagues (Lee et al., 2003b). Starting at the MO_2 recorded during the highest swimming speed (1258.76 ± 39.73 mg O_2 kg^{-1} h^{-1}), post-exercise MO_2 declined rapidly and approached the pre-exercise MO_2 (Fig. 6). The first measure of post-exercise MO_2 was significantly higher than the pre-exercise MO_2 ($P<0.001$), providing evidence of EPOC and anaerobic power production in *P. reticulata* (Fig. 6). The rapidly declining MO_2, and the fact that we had no MO_2 data between 0 h and 0.2 h (Fig. 6), precluded an accurate estimation of EPOC (mg O_2 kg^{-1}). Post-exercise MO_2 declined until 0.69 h and approached the pre-exercise MO_2. The last two measurements of post-exercise MO_2 at 0.89 h and 1.08 h remained slightly elevated relative to the pre-exercise MO_2 (Fig. 6). The majority of the post-exercise decline in the MO_2 occurred within 0.3 h after the swimming speed was returned to the acclimation speed (Fig. 6).

DISCUSSION

Contrary to predictions, we found no evidence of correlations between reproductive traits and steady swimming cost, standard metabolic rate (MO_{2std}) and prolonged swimming performance (U_{crit}). In contrast, pectoral fin use correlated positively with swimming cost and negatively with U_{crit}. We suggest that the use of pectoral fins indicated a mechanism to maintain swimming stability, rather than generate forward thrust. Further, we propose that elevated use of pectoral fins indicated an elevated need to support swimming stability resulting in increased swimming cost and therefore decreased U_{crit}. Finally, we found that the aerobic metabolic rate increased after transition to burst-assisted swimming suggesting that unsteady swimming constituted 26.8% of the metabolic scope (MS).

Although pregnancy may influence metabolic rates and swimming performance in live-bearing fish, this study found no evidence of reproductive allocation (RA) or pregnancy stage correlating with MO_{2std}, steady swimming cost or U_{crit} in wild *P. reticulata* from a low predation river. A number of studies have reported elevated metabolic rate (Webb and Brett, 1972; Boehlert et al., 1991; Hopkins et al., 1995; Timmerman and Chapman, 2003; Skov et al., 2010) and diminished swimming performance (Plaut, 2002; Ghalambor et al., 2004; Belk and Tuckfield, 2010) in pregnant live-bearing fish. The studies differ from the present study in a number of ways. Firstly, two previous studies tested pregnancy effects on fast-start swimming performance (Ghalambor et al., 2004; Belk and Tuckfield, 2010) rather than U_{crit}. It is possible that fast-start swimming performance is more sensitive to pregnancy than U_{crit}. Secondly, previous studies followed individual fish over the course of the gestation period for repeated measurements (Webb and Brett, 1972; Plaut, 2002; Timmerman and Chapman, 2003) or made comparisons between gestating females and reproductively inactive females or males (Boehlert et al., 1991; Hopkins et al., 1995; Skov et al., 2010). We were unable to make repeated measurements on individual fish because of the destructive nature of measuring RA, and our samples included no reproductively inactive fish (all wet RA ≥ 4.93%). Finally, our study examined low predation *P. reticulata* only. It is well known, however that high predation *P. reticulata* (e.g. from Aripo River) have considerably higher RA than low predation *P. reticulata* (Reznick and Endler, 1982). Further studies should test for pregnancy effects on metabolic rates and U_{crit} in both high and low predation fish, while also controlling for age and genetic background (Ghalambor et al., 2004; Belk and Tuckfield, 2010).

Previous studies have covered the energetics of gait transitions from 1) exclusive pectoral fin propulsion to combined pectoral and caudal fin propulsion (Korsmeyer et al., 2002; Cannas et al., 2006; Jones et al., 2007; Kendall et al., 2007; Svendsen et al., 2010); 2) steady swimming to unsteady swimming (Svendsen et al., 2010); 3) dorsal and anal fin propulsion to caudal fin propulsion (Korsmeyer et al., 2002); and, 4) free stream swimming to Karman gaiting (Liao et al., 2007; Taguchi and Liao, 2011). By contrast, little attention has been devoted to the energetic effects of fin use variation within a single gait. The present study found that within the steady BCF swimming gait, swimming cost correlated strongly with pectoral fin use. Fish that ceased using the pectoral fins at low speeds reduced swimming cost in comparison with fish that ceased using the pectoral fins at a higher speed or not at all. According to previous studies, BCF swimmers extending their paired fins should experience increased swimming costs (Webb, 1998; Webb, 2002) because of the additional drag (Videler and Wardle, 1991; Weihs, 2002; Green and Hale, 2012), however, this hypothesis has rarely been tested. Although furling of the pectoral fins at relatively low speeds is common (Drucker and Lauder, 2003), some BCF swimmers employ both caudal and pectoral fins at relatively high swimming speeds. For example, in the field, brook trout (*Salvelinus fontinalis* Mitchill) combine the use of the caudal and pectoral fins at a wide range of swimming speeds (McLaughlin and Noakes, 1998). Notably, *S. fontinalis* using their pectoral fins swim with a higher caudal fin beat frequency at a given swimming speed than those not using their pectoral fins (McLaughlin and Noakes, 1998). Because there is a positive relationship between caudal fin beat frequency and MO_2 (Ohlberger et al., 2007), these findings indicate that *S. fontinalis* using the pectoral fins experienced increased swimming cost. The observations on *S. fontinalis* are consistent with the present study demonstrating a positive relationship between pectoral fin use and steady swimming cost in *P. reticulata*. Our data suggest that combining the caudal and pectoral fins over a wide speed range is an inefficient BCF swimming behaviour.

What proximate mechanism could underpin the observed intraspecific variation in pectoral fin use? A likely mechanism involves variable needs to support swimming stability and control. In BCF swimmers, pectoral fins are not used for forward thrust generation, but play an important role as trimming and/or powered correction systems to maintain swimming stability (Webb, 2002). The former involves positioning the fins to dampen or correct perturbations, whereas the latter involves active movements of the fins independent of the body to correct perturbations (Webb, 2002). Stability and control can be a major problem in swimming (Videler and Wardle, 1991; Webb, 1998; Webb, 2002). For example, there are six possible recoil motions for a rigid body resulting from propulsor movements, three of them translational and three rotational (Hove et al., 2001). BCF swimming generates large side forces that cause the anterior parts of the body to recoil (yaw and/or sideslip) (Hove et al., 2001; Weihs, 2002; Lauder, 2006), which may represent a major stability problem in BCF swimming (Webb, 1988; Weihs, 2002). The yaw movements generated by the caudal fin are usually countered by movements of the pectoral or pelvic fins (Lauder, 2006). Such needs for stability control by balancing forces have led recent studies to emphasize the importance of multiple fins employed by swimming fish (Hove et al., 2001; Drucker et al., 2006; Lauder and Tytell, 2006, Tytell et al., 2008; Blake et al., 2009). The use of paired fins to maintain stability and control is most pronounced at lower speeds. At higher swimming speeds, various stability problems persist, but control is shifted more towards the body-caudal fin. In the present study, individual *P. reticulata* employed the pectoral fins at a variable speed range, and increased pectoral fin use was associated with increased steady swimming costs and a low U_{crit}. Although the exact function of extending the pectoral fins remains unknown, it is likely that the variation in pectoral fin use reflected, at least partly, different needs to balance forces and support swimming stability and control. According to this hypothesis, *P. reticulata* that continued using the pectoral fins at high swimming speeds did so to support swimming stability. As such, the extent of pectoral fin use at increasing speeds could be interpreted as an index of swimming stability in individual fish, with extensive pectoral fin use indicating a swimmer with stability problems.

What is the metabolic cost of stability control in swimming fishes? The metabolic cost of stability control is not known (Lauder, 2006), but the cost likely represents a significant part of the total swimming cost (Webb, 2002). In the present study, the pectoral fins were presumably not employed to generate forward thrust, but to support stability control. Using the pectoral fins as a trimming and/or powered correction system to stabilize BCF swimming should increase swimming costs (Webb, 1998; Weihs, 2002) because of the increased lateral surface and hence additional drag (Videler and Wardle, 1991; Weihs, 2002; Green and Hale, 2012). Correspondingly, we suggest that the positive relationship between pectoral fin use and steady swimming cost reflected, at least partially, cost of stability control incurred when using the pectoral fins as a trimming and/or powered correction system. In the same vein, the negative relationship between pectoral fin use and U_{crit} could be explained by the fact that pectoral fin use correlated positively with swimming cost. Fish that made extensive use of the pectoral fins exhibited a significant increase in the swimming cost, which may have resulted in inferior swimming performance, because the fish reached the maximum metabolic rate at a relatively slow swimming speed.

Many fish species transition from steady to unsteady swimming at increasing swimming speeds. Few studies, however, have quantified the metabolic rate associated with burst-assisted swimming. Metabolic rate studies concerned with burst-assisted swimming at high speeds remain challenged by the facts that the gait can be maintained for only a short period of time (Farrell, 2007) and involves anaerobic metabolism (Burgetz et al., 1998; Lee et al., 2003b; Svendsen et al., 2010) that may complicate the measurements (Farrell, 2007; Ellerby, 2010). Anaerobic metabolism is evidenced by the activation of white muscles and the occurrence of glycolysis followed by EPOC (Burgetz et al., 1998; Lee et al., 2003b; Farrell, 2007; Svendsen et al., 2010). Farrell reviewed past studies and discussed the paradox that the relationship between swimming speed and aerobic $M\mathrm{O}_2$ often is exponential and not sigmoidal as predicted by the anaerobic influence on the total metabolic cost (Farrell, 2007). The exponential relationship may be explained by a number of factors including white muscles working in a partially aerobic fashion, and pooling fish that vary considerably in their individual U_{crit} values, as this would tend to obscure any individual plateaus in $M\mathrm{O}_2$ (Farrell, 2007). The present study examined the relationship between swimming speed and $M\mathrm{O}_2$ up to U_{crit} in individual fish and found no evidence of a sigmoidal relationship. Instead, $M\mathrm{O}_2$ continued to increase during burst-assisted swimming, and the data points during unsteady swimming were higher than what would be expected based on extrapolation of the values representing steady swimming. Correspondingly, unsteady swimming constituted 26.8% of the MS. The absence of $M\mathrm{O}_2$ plateauing during unsteady swimming at increasing speeds suggests that anaerobic metabolism played a limited role in fuelling the swimming, even close to U_{crit}. This inference is consistent with past studies suggesting limited anaerobic capacity (Kieffer, 2000) and dependence on anaerobic power production during swimming (Goolish, 1991) in small fish (< 10 cm in body length). On the other hand, we did find evidence of EPOC, indicating that anaerobic power production occurred during the swimming trial. The majority of the post-exercise metabolic decline occurred within 0.3 h. Previous studies measuring metabolic recovery after U_{crit} tests have reported recovery times from approximately 0.2 to 4 h (Brett, 1964; Bushnell et al., 1994; Reidy et al., 1995; Lee et al., 2003a; Lee et al., 2003b). Scaling relationships between body size and the partitioning of aerobic and anaerobic power production in swimming fish remain an important future avenue of empirical research.

In many terrestrial animals, gait transitions reduce metabolic cost of locomotion (Griffin et al., 2004; Rubenson et al., 2004; Nudds et al., 2011), but the proximate mechanism driving the transition may not be metabolic *per se*, but rather related to mechanical factors, such as musculoskeletal force (Farley and Taylor, 1991) and bone strain (Biewener and Taylor, 1986). Compared to terrestrial locomotion, gait transitions in aquatic locomotion remain poorly understood. The present study analysed metabolic consequences of the gait transition from steady to unsteady BCF swimming and found that the metabolic rate increased after the transition. These data raises the question why an animal would perform a transition to a gait that is less energy efficient. In labriform swimmers, it has been suggested that the pectoral-caudal gait transition is driven by a need to supply additional mechanical power rather than to minimize metabolic swimming costs (Alexander, 1989; Korsmeyer et al., 2002; Cannas et al., 2006; Jones et al., 2007; Kendall et al., 2007). Only small amounts of muscle can be packed around the paired fins while larger amounts can be accommodated about the axial skeleton driving caudal propulsion (Webb, 1998). The transition from steady to unsteady BCF swimming could have a similar mechanistic basis, because additional mechanical power from white muscle fibres may be available after transition to burst-assisted swimming. However, this remains speculation, and additional studies of muscle recruitment patterns at increasing speeds combined with aerobic and anaerobic metabolic rates of disparate muscle types are required to evaluate the hypothesis.

The U_{crit} protocol provides a measure of physiological endurance capacity, initially used in fisheries sciences (Brett, 1964; Beamish, 1978). Recently, studies have started to examine U_{crit} in ecological and evolutionary contexts (Claireaux et al. 2007; Oufiero et al. 2011; Dalziel and Schulte, 2012; Dalziel et al., 2012a; Dalziel et al., 2012b). Using individual fish, the present study demonstrated a relationship between pectoral fin use, steady swimming cost and U_{crit}. Fish with a low U_{crit} spent more time with extended pectoral fins and exhibited increased steady swimming cost. We found no evidence that $M\mathrm{O}_{2\mathrm{max}}$ correlated with U_{crit}. These findings indicate that U_{crit} does not only reflect the physiological endurance capacity of individual fish, but indeed also the biomechanical performance.

This study shows that fish with a low U_{crit} may spend more energy on swimming, in comparison with fish with a high U_{crit}, because the former fish have higher swimming costs during steady swimming. This may be particularly relevant in species living in lotic habitats. For example, Nelson and colleagues reported a positive relationship between home-stream current velocity and U_{crit} in blacknose dace (*Rhinichthys atratulus* Hermann) (Nelson et al., 2003). Our finding that U_{crit} correlates negatively with steady swimming costs indicates that not only will a high U_{crit} allow fish to traverse fast flowing riffles without fatigue, as suggested by Nelson et al. (2003), a high U_{crit} also implies lower steady swimming cost at current velocities that represent sub-maximal swimming speeds. Thus, because of superior biomechanical performance, a high U_{crit} may allow fish to inhabit relatively high stream current velocities and yet have relatively low swimming costs. As such, the observed intraspecific variation in pectoral fin use, swimming cost and U_{crit} could have implications for habitat use in individual fish. For example, Ellerby and Gerry showed that habitat use varies with individual differences in energy economy, steady-state swimming and maneuverability in bluegill sunfish (*Lepomis macrochirus* Rafinesque) (Ellerby and Gerry, 2011). Although *P. reticulata* often occupy distinct pools in rivers, it remains to be tested if intraspecific variation in pectoral fin use, swimming cost and U_{crit} influence habitat use within pools and to what degree such relationships (or lack of) affect daily energy expenditures (Careau and Garland, 2012).

In summary, this study found that elevated pectoral fin use is associated with increased swimming cost and decreased U_{crit}. It is unclear why some *P. reticulata* continued using the pectoral fins while others quickly ceased using them at increasing swimming speeds. We propose that use of the pectoral fins is related to stability and control rather than generation of forward thrust. Extending the pectoral fins may help maintain swimming stability, but it comes with increased swimming cost, which in turn is associated with reduced U_{crit}. The causes and consequences of variation in pectoral fin use remain, however, poorly understood and further study is warranted. Finally, we found that the aerobic metabolic rate increased after transition to burst-assisted swimming, and unsteady swimming constituted 26.8% of the MS.

LIST OF SYMBOLS AND ABBREVIATIONS

a	oxygen consumption rate at zero swimming speed
a'_{j}	fish specific log oxygen consumption rate at zero swimming speed
$\hat{a}_{\mathrm{j}}$	estimated fish specific oxygen consumption rate at zero swimming speed
a_{j}	fish specific oxygen consumption rate at zero swimming speed
AR1	autoregressive process of order 1
b	rate of increase in the oxygen consumption rate as a function of swimming speed. The parameter is an index of swimming cost
b_{j}	fish specific rate of increase in the oxygen consumption rate as a function of swimming speed
$\hat{b}_{\mathrm{j}}$	estimated fish specific rate of increase in the oxygen consumption rate as a function of swimming speed
e_{ij}	autocorrelated residuals
EPOC	excess post-exercise oxygen consumption
$\varepsilon_{\mathrm{ij}}$	uncorrelated residuals assumed to be independently and identically normally distributed
L_{T}	total body length
$M\mathrm{O}_2$	metabolic rate
$M\mathrm{O}_{2\mathrm{ij}}$	metabolic rate for the i'th observation on the j'th fish
$M\mathrm{O}_{2\mathrm{max}}$	maximum metabolic rate
$M\mathrm{O}_{2\mathrm{max,j}}$	fish specific maximum metabolic rate
$M\mathrm{O}_{2\mathrm{std}}$	standard metabolic rate
MS	metabolic scope
μ_{a}	mean fish specific log oxygen consumption rate at zero swimming speed
μ_{b}	mean fish specific rate of increase in the oxygen consumption rate as a function of swimming speed
φ	autoregressive (AR1) parameter
ρ	correlation between the fish specific log oxygen consumption rate at zero swimming speed (a'_{j}) and the fish specific rate of increase in the oxygen consumption rate as a function of swimming speed (b_{j})
σ_{a}^2	variance of the fish specific log oxygen consumption rate at zero swimming speed
σ_{b}^2	variance of the fish specific rate of increase in the oxygen consumption rate as a function of swimming speed
σ_{ε}^2	variance of the uncorrelated residuals ($\varepsilon_{\mathrm{ij}}$)
RA	reproductive allocation
RA_{j}	fish specific reproductive allocation
U	swimming speed

U_{crit}	critical swimming speed
$U_{\mathrm{crit,j}}$	fish specific critical swimming speed
U_{STmax}	gait transition speed from steady to unsteady swimming

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Figure Legends

Figure 1. Use of pectoral fins and burst-assisted swimming (% of time) in 18 individual *P. reticulata* as a function of swimming speed (U; cm s^{-1}). Pectoral fin use is indicated using a blue line, whereas burst-assisted swimming is indicated using a red line (unsteady swimming). The caudal fin was employed at all swimming speeds (i.e. body-caudal fin swimming). Each fish is represented by an average of 28 measurements of pectoral fin use and burst-assisted swimming.

Figure 2. Oxygen consumption rate (MO_2; mg O_2 kg^{-1} h^{-1}) as a function of swimming speed (U; cm s^{-1}) in 18 individual *P. reticulata*. Grey symbols represent MO_2 when no burst-assisted swimming occurred (steady swimming), whereas red symbols represent MO_2 when burst-assisted swimming occurred (unsteady swimming) (see Fig. 1). Each datum represents a 12 min period. Equation (1) was fitted to the data without burst-assisted swimming (black lines) (see Table 1). The slope of each black line represents the rate of increase in MO_2 as a function of U and is considered an index of steady swimming cost in individual fish.

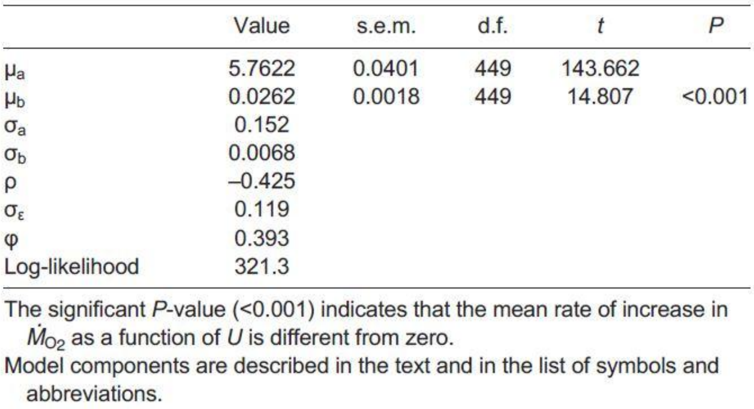
Figure 3. Steady swimming cost as a function of the average pectoral fin use (% of time) in 18 individual *P. reticulata*. Steady swimming cost was measured as the rate of increase in the oxygen consumption rate as a function of swimming speed (see Fig. 2; Eqn 1; Table 1). Both steady swimming cost and average pectoral fin use were based on measurements up to the swimming speed where burst-assisted swimming occurred (see Fig. 1).

Figure 4. Critical swimming speed (U_{crit}; cm s^{-1}) as a function of average pectoral fin use (% of time) in 18 individual *P. reticulata*. Average pectoral fin use was based on measurements up to U_{crit} (i.e. including burst-assisted swimming) (see Fig. 1).

Figure 5. Steady swimming cost as a function of critical swimming speed (U_{crit}; cm s^{-1}) in 18 individual *P. reticulata*. Steady swimming cost was measured as the rate of increase in the oxygen consumption rate as a function of swimming speed (see Fig. 2; Eqn 1; Table 1). Steady swimming cost was based on measurements up to the swimming speed where burst-assisted swimming occurred (see Fig. 1).

Figure 6. Oxygen consumption rate (MO_2; mg O_2 kg^{-1} h^{-1}) at the highest recorded swimming speed (closed circle), and post-exercise MO_2 during the subsequent recovery phase (open circles). The swimming speed was adjusted to 2 L_T s^{-1} (total body lengths per second) during the recovery phase. Pre-exercise MO_2 at 2 L_T s^{-1} is indicated using a solid line (95% CL; dashed lines).

Table 1

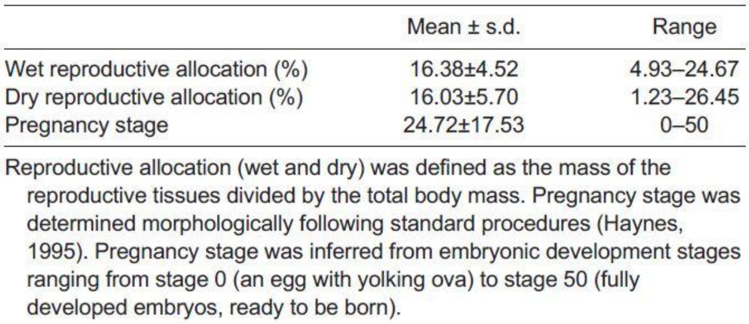


	Value	s.e.m.	d.f.	*t*	*P*
μ_a	5.7622	0.0401	449	143.662	
μ_b	0.0262	0.0018	449	14.807	<0.001
σ_a	0.152				
σ_b	0.0068				
ρ	–0.425				
σ_ε	0.119				
φ	0.393				
Log-likelihood	321.3				

The significant *P*-value (<0.001) indicates that the mean rate of increase in $\dot{M}_{O_2}$ as a function of *U* is different from zero.

Model components are described in the text and in the list of symbols and abbreviations.

Table 2



	Mean ± s.d.	Range
Wet reproductive allocation (%)	16.38±4.52	4.93–24.67
Dry reproductive allocation (%)	16.03±5.70	1.23–26.45
Pregnancy stage	24.72±17.53	0–50

Reproductive allocation (wet and dry) was defined as the mass of the reproductive tissues divided by the total body mass. Pregnancy stage was determined morphologically following standard procedures (Haynes, 1995). Pregnancy stage was inferred from embryonic development stages ranging from stage 0 (an egg with yolking ova) to stage 50 (fully developed embryos, ready to be born).

Figure 1

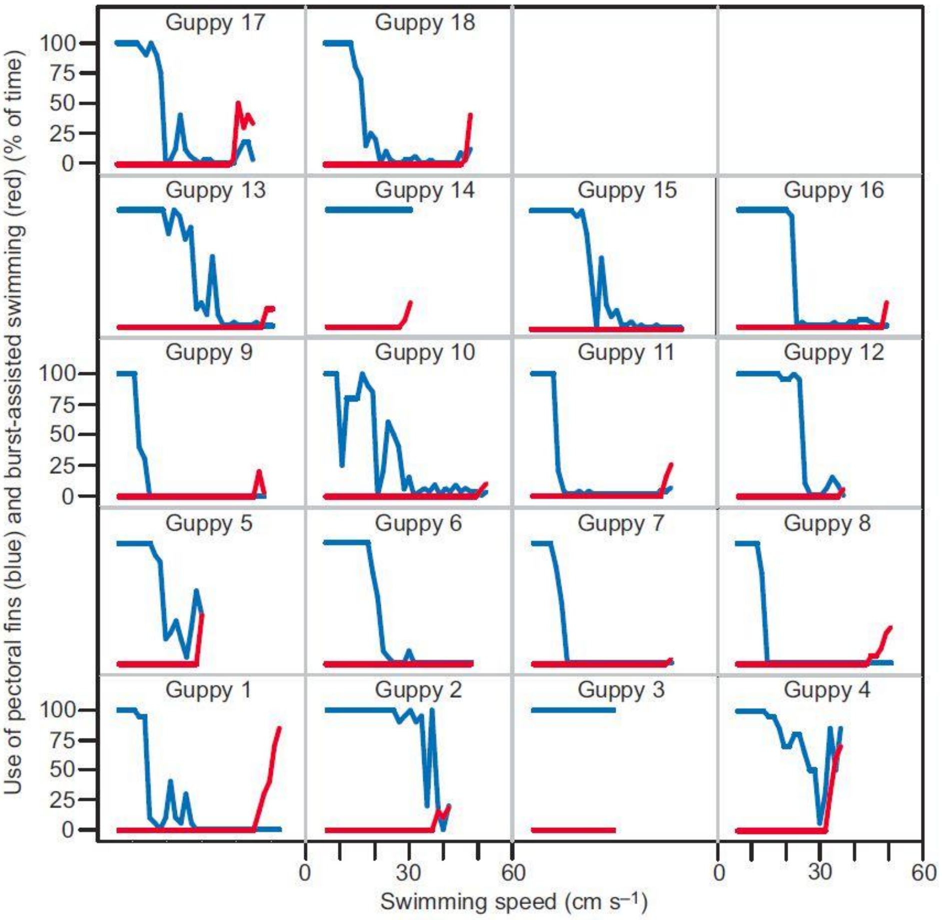

Guppy 17
Guppy 18
Guppy 13
Guppy 14
Guppy 15
Guppy 16
Guppy 9
Guppy 10
Guppy 11
Guppy 12
Guppy 5
Guppy 6
Guppy 7
Guppy 8
Guppy 1
Guppy 2
Guppy 3
Guppy 4
100
75
50
25
0
Use of pectoral fins (blue) and burst-assisted swimming (red) (% of time)
0
30
60
Swimming speed (cm s–1)

Figure 2

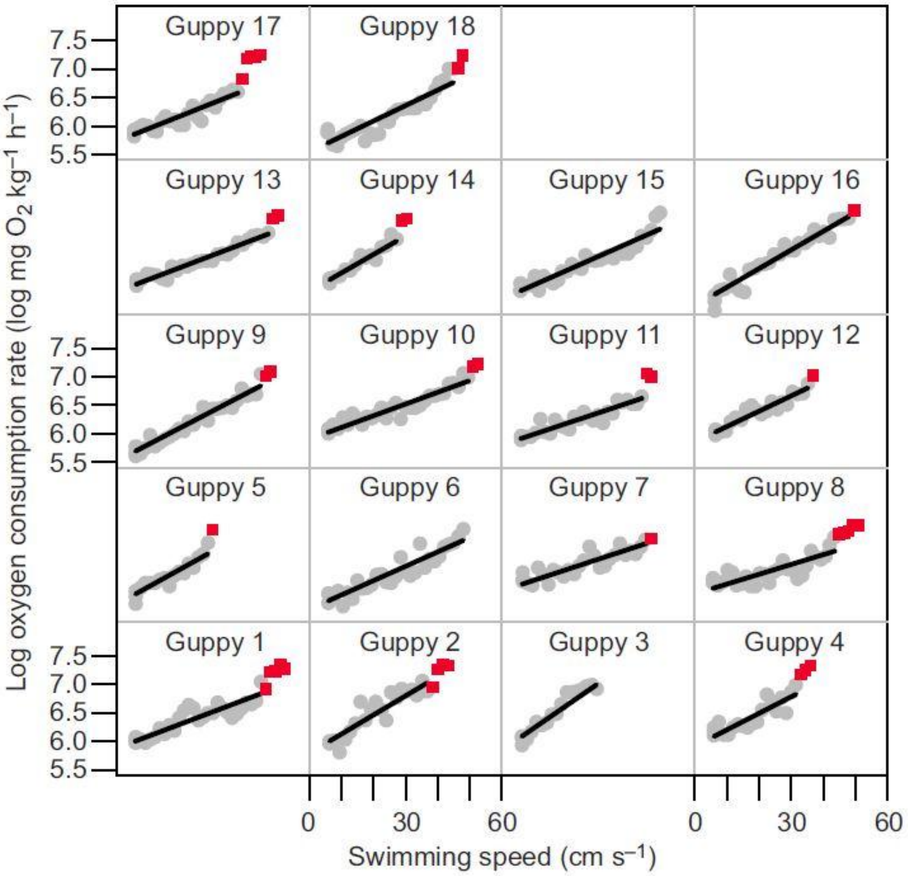

Guppy 17
Guppy 18
Guppy 13
Guppy 14
Guppy 15
Guppy 16
Guppy 9
Guppy 10
Guppy 11
Guppy 12
Guppy 5
Guppy 6
Guppy 7
Guppy 8
Guppy 1
Guppy 2
Guppy 3
Guppy 4
7.5
7.0
6.5
6.0
5.5
Log oxygen consumption rate (log mg O_2 kg^{-1} h^{-1})
0
30
60
Swimming speed (cm s^{-1})

Figure 3

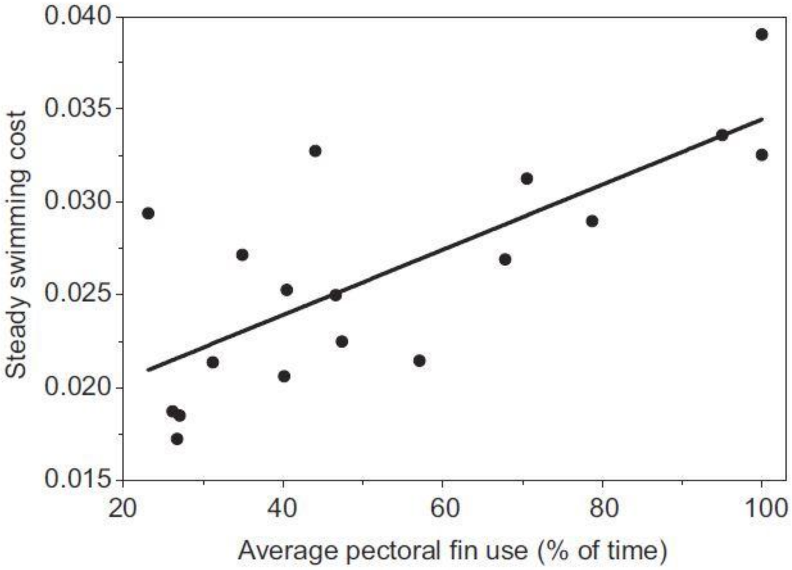
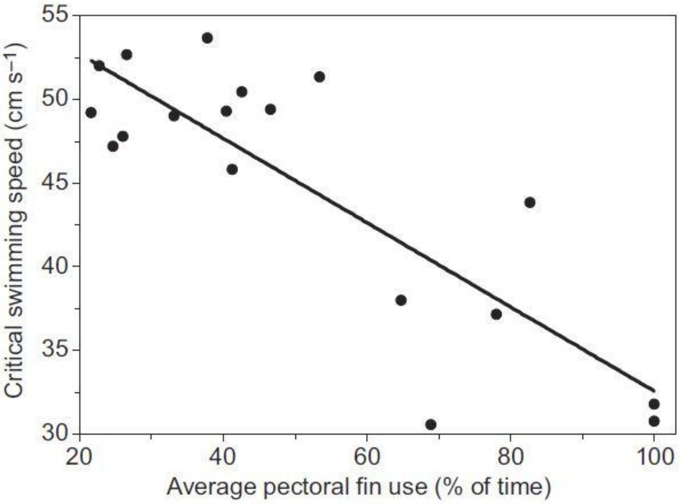

0.040
0.035
0.030
0.025
0.020
0.015
Steady swimming cost
20
40
60
80
100
Average pectoral fin use (% of time)

Figure 4


Critical swimming speed (cm s⁻¹)
55
50
45
40
35
30
20
40
60
80
100
Average pectoral fin use (% of time)

852 **Figure 5**

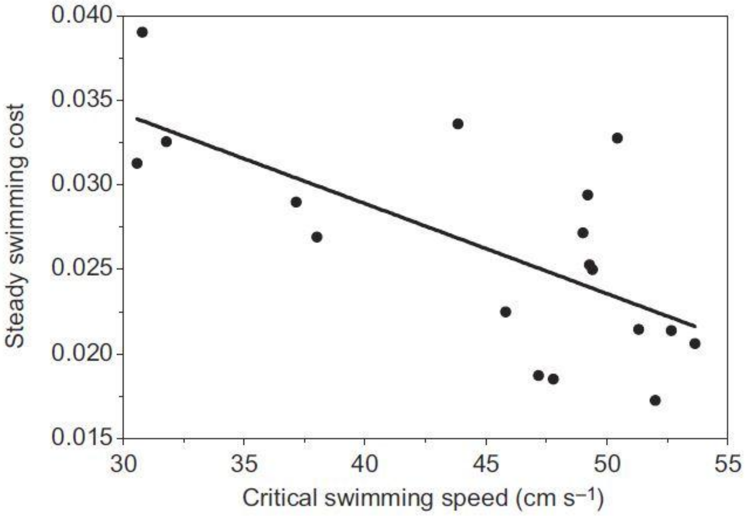


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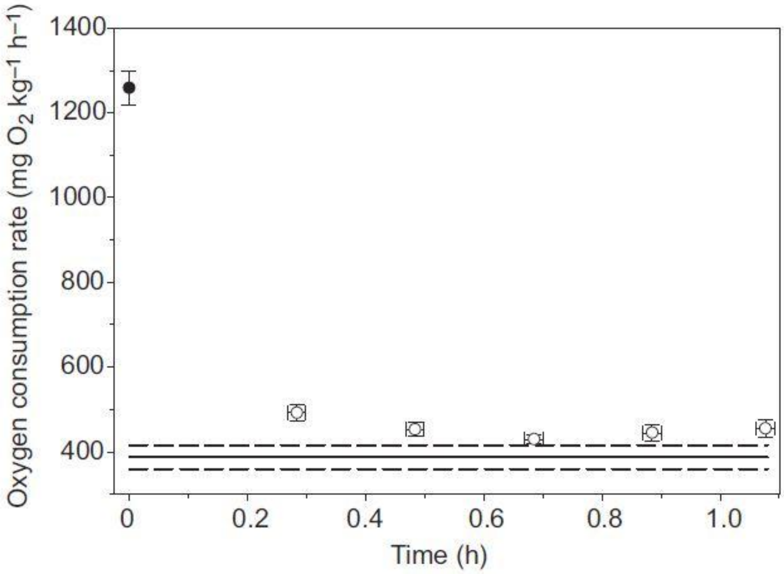
Figure 6


Oxygen consumption rate (mg O_2 kg^{-1} h^{-1})
1400
1200
1000
800
600
400
0
0.2
0.4
0.6
0.8
1.0
Time (h)