

Linking swimming performance, cardiac pumping ability and cardiac anatomy in rainbow trout

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Summary

We exploited the inherent individual diversity in swimming performance of rainbow trout *Oncorhynchus mykiss* to investigate the hypothesis that maximum cardiac performance is linked to active metabolic rate (AMR) and critical swimming speed (U_{crit}). Six hundred juveniles (body mass ~150 g) were screened using a swimming challenge of 1.2 m s^{-1} to identify 'poor swimmers' and 'good swimmers', i.e. the first and last 60 fish to fatigue, respectively. These 120 fish were individually tagged and then reared in common tanks for 9 months, where they grew at similar rates and achieved a similar body mass of approximately 1100 g. Critical swimming speed (U_{crit}) was then measured individually in tunnel respirometers, with simultaneous recordings of cardiac output *via* a ventral aortic flow probe. The group of individuals that were screened as poor swimmers remained so, with a significantly (27%) lower U_{crit} than good swimmers [$89 \pm 10 \text{ cm s}^{-1}$ vs $123 \pm 5 \text{ cm s}^{-1}$ (mean \pm S.E.M.), respectively, $N=6$], a 19% lower AMR ($147 \pm 12 \mu\text{mol min}^{-1} \text{ kg}^{-1}$ vs $181 \pm 11 \mu\text{mol min}^{-1} \text{ kg}^{-1}$, respectively), and a 30% lower maximum *in vivo* cardiac output ($47.3 \pm 4.7 \text{ ml min}^{-1} \text{ kg}^{-1}$ vs $68.0 \pm 5.2 \text{ ml min}^{-1} \text{ kg}^{-1}$, respectively). When cardiac performance was compared with an *in situ* heart

preparation, hearts from poor swimmers had a significantly (26%) lower maximum cardiac output ($45.9 \pm 1.9 \text{ ml min}^{-1} \text{ kg}^{-1}$ vs $56.4 \pm 2.3 \text{ ml min}^{-1} \text{ kg}^{-1}$, respectively) and a 32% lower maximum cardiac power output at a high afterload ($3.96 \pm 0.58 \text{ mW g}^{-1}$ vs $5.79 \pm 1.97 \text{ mW g}^{-1}$, respectively). Cardiac morphology was visualised *in vivo* by Doppler echography on anaesthetised individual fish and revealed that poor swimmers had a significantly more rounded ventricle (reduced ventricle length to height ratio) compared with good swimmers, which in turn was correlated with fish condition factor. These results provide clear evidence that maximum cardiac performance is linked to AMR and U_{crit} and indicate that a simple screening test can distinguish between rainbow trout with lower active metabolic rate, U_{crit} , maximal cardiac pumping capacity and a more rounded ventricular morphology. These distinguishing traits may have been retained for 9 months despite a common growing environment and growth.

Key words: swimming, metabolism, cardiovascular performance, heart morphology, domestication, rainbow trout, *Oncorhynchus mykiss*.

Introduction

It is generally held that maximum aerobic performance, as measured by critical swimming speed (U_{crit}) tests, utilizes the maximum pumping capacity of the heart in salmonids such as rainbow trout (*Oncorhynchus mykiss*). Experimental support for this contention has come in four main forms. Foremost are the measurements of cardiac output (\dot{Q}) during U_{crit} tests, which show that a plateau is reached for \dot{Q} just prior to U_{crit} (see, for example, Kiceniuk and Jones, 1977; Kolok and Farrell, 1994; Thorarensen et al., 1996a,b; Gallaughner et al., 2001). Second, *in vitro* measurements of maximum cardiac pumping are often

comparable with the maxima measured *in vivo* during U_{crit} swim tests (Farrell, 2002). Third, oxygen uptake (\dot{M}_{O_2}), like \dot{Q} , can also show a plateau just before fish reach U_{crit} (Lee et al., 2003), suggesting that in addition to \dot{Q} , arterial oxygen transport may also have reached a maximum. Fourth, a blood doping and blood removal study with rainbow trout showed that the optimum hematocrit (Hct) for U_{crit} and maximum \dot{M}_{O_2} was only marginally higher than routine Hct (Gallaughner et al., 1995), again lending support to the idea that arterial oxygen transport reaches a maximum during swimming at U_{crit} .

While these data are compelling, they are not conclusive evidence and may not even be applicable to other fish species. Indeed, Soofiani and Priede (1985) and later Reidy et al. (1995) both showed that \dot{M}_{O_2} in Atlantic cod *Gadus morhua* was greater post-exercise rather than during exercise. In fact, the suggestion was made that metabolic scope for Atlantic cod evolved to accommodate post-prandial and post-exercise peaks in oxygen demand rather than those during locomotor activity (Soofiani and Priede, 1985). However, while feeding greatly increases \dot{M}_{O_2} in all fish including salmonids (Jobling, 1981; Brett, 1983; Legrow and Beamish, 1986), active metabolic rate (AMR) in salmonids is typically 2–3 times higher than the post-exercise \dot{M}_{O_2} measured in Atlantic cod (Nelson et al., 1996). Furthermore, while feeding increases \dot{M}_{O_2} by 50–100% in salmonids, AMR during post-prandial swimming is no higher than in unfed fish (Thorarensen, 1994; Alsop and Wood, 1997). In fact, because U_{crit} is compromised post-prandially, it seems likely that there is no excess capacity for salmonids to pump blood to the intestine and liver to maximise digestion, as well as to skeletal muscles to maximise locomotion (Farrell et al., 2001). Indeed, gut blood flow decreases dramatically during exercise (Thorarensen et al., 1993). Thus, while the weight of evidence supports the idea that cardiac pumping is maximal during swimming at U_{crit} , some room for doubt still remains, especially given the recent finding that exercising monitor lizards have a higher AMR post-prandially compared with in the unfed state (Bennett and Hicks, 2001) and that in Atlantic cod the post-prandial increase in \dot{M}_{O_2} for a fixed meal size increased with swimming activity (Blaikie and Kerr, 1996).

A difficulty with interventional experiments is the degree of inherent individual diversity that exists in physiological performance traits, which can often be greater than the change elicited by the experimental intervention. In the present study we exploited inherent individual diversity and reasoned that if cardiac performance is indeed closely linked with swimming performance, then poor swimmers should have poorer cardiac performance than good swimmers. Therefore, we screened a large group of hatchery-raised rainbow trout to identify good and poor swimmers within the population. These fish were then individually tagged and allowed to grow together for a further 9 months, at which point their cardiac performance was measured both *in vivo* and *in vitro* to compare good and poor swimmers. In addition, and because cardiac abnormalities are frequently reported for cultured fish (Poppe and Taksdal, 2000; Brocklebank and Raverty, 2002; Poppe et al., 2002, 2003; Gamperl and Farrell, 2004), we compared simple cardiac morphometrics to determine if differences in cardiac morphology were associated with poor cardiac performance and swimming.

Materials and methods

Fish holding and screening

Fish rearing and experiments were conducted at the Station Expérimental Mixte IFREMER-INRA (Sizun, France). Rainbow trout *Oncorhynchus mykiss* Walbaum eggs, from a spring spawning strain, were fertilised on April 16th 2002 and

Table 1. Length, mass and condition factor of adult rainbow trout at the time of screening into good and poor swimmers, and the same fish 9 months later

	Good swimmers	Poor swimmers
Initial conditions (January 2003)		
Mass (g)	142±3	148±3
Length (cm)	21.8±0.7	21.7±0.7
Condition factor CF	1.37±0.02	1.44±0.02*
Final conditions (September 2003)		
Mass (g)	1110±37	1101±39
Length (cm)	42.5±0.5	41.8±0.5
Condition factor CF	1.44±0.02	1.51±0.03*

Values are mean ± S.E.M. (N=60).
*Significant difference between groups (Student's *t*-test; *P*<0.05).

emergent fry started feeding on May 29th 2002. In July, fish were moved to outdoor rearing tanks supplied with aerated ambient temperature freshwater, diverted from a nearby spring. On January 10th 2003 (water temperature=7.0°C), six batches of 100 fish were successively transferred to a 3 m diameter circular tank, in which plastic meshing delimited a swimming ring (inner circumference=8.5 m; width=0.8 m), and left undisturbed for 15 min. By manipulating the valve that controlled the water supply to the tank, water velocity was progressively raised (within 15 min) from 0.2 to 1.2 m s⁻¹, as measured using a flowmeter (Marsh-McBirney 200, Frederick, MD, USA). Fish swam against this current until they fatigued and fell back against a mesh screen just upstream of the water inflow. The first ten fish to fatigue (termed poor swimmers) among the 100 fish were removed from the tank, anaesthetised, their length and body mass measured (Table 1), and a Passive Integrated Transponder (PIT-tag) inserted into the peritoneal cavity. The glass PIT tags (8 mm long and 2.12 mm diameter) were purchased from ORDICAM DSC (Ordicam, Rambouillet, France) and were read using a Planete 128 DSC reader (Ordicam) at 134.2 kHz. The water current was maintained and fatigued fish removed until the last 10 fish (termed good swimmers) reached exhaustion. Good swimmers were also fitted with a PIT tag. Poor swimmers fatigued within 10–15 min, while good swimmers avoided the back grid for 45–60 min. This procedure was repeated for each batch of 100 fish. The resulting 120 fish were then mixed in two outdoor holding tanks (12 m³) until September 2003, when biometrics of all the fish were remeasured (Table 1) and the experiments performed. No mortality occurred during the 9-month growing period, during which fish were fed twice a day *ad libitum* with commercial feed (BioMar, Brande, Denmark). The rearing temperature followed the normal seasonal changes in the spring water and ranged between 7°C in winter and 18°C in summer.

In vivo swimming studies

In vivo studies were performed on six poor swimmers of

mean (\pm S.E.M.) mass 1204 ± 105 g and fork length 419 ± 11 mm, and six good swimmers with a mean mass 1030 ± 62 g and fork length 419 ± 10 mm. The water temperature was $16 \pm 0.4^\circ\text{C}$ during these experiments. Fish were anaesthetised in 0.1 mg l^{-1} MS-222 buffered with 0.1 mg l^{-1} NaHCO_3 before being transferred to an operating table, where their gills were irrigated with aerated water containing diluted anaesthetic (0.05 mg l^{-1} MS-222 and NaHCO_3). The ventral aorta was exposed *via* an incision in the cleithrum, a Transonic flow probe (Transonic Systems, Ithaca, NY, USA) placed around it and the incision closed with silk sutures. The dorsal aorta (DA) was then cannulated using the technique described by Soivio et al. (1975). Following surgery, fish were transferred to individual opaque PVC chambers, where they recovered for 48 h in a continuous flow of normoxic water. The DA cannula was flushed daily with heparinised (10 IU l^{-1}) Cortland's saline (Wolf, 1963). Following this recovery period, trout were carefully transferred, without air-exposure, into a water-filled plastic bag and then into a swimming respirometer, where they recovered for at least 4 h while swimming gently against a water velocity of 30 cm s^{-1} .

The U_{crit} swimming tests were performed using two Brett-type swim-tunnel respirometers, designed to exercise individual fish in a non-turbulent water flow with a uniform velocity profile (Steffensen et al., 1984). One respirometer constructed of PVC has been described in detail previously (McKenzie et al., 2001). The second was of a similar design and size, but constructed in stainless steel, with a total water volume of 48 l and a swim chamber with a square cross-sectional area of 290 cm^2 . Water flow was generated by a thermo plastic composite propeller downstream of the swim chamber, attached to a variable speed, low inertia, brushless servo-motor (Ultact II, Phase Motion Control S.R.L., Milan, Italy), calibrated to deliver water velocities in cm s^{-1} and swimming speeds in body lengths s^{-1} ($BL \text{ s}^{-1}$). The respirometer was thermostatted by immersion in a large outer stainless steel tank that received a flow of aerated water. In both respirometers, swimming speeds were corrected for the solid blocking effect of the fish, as described by Bell and Terhune (1970).

Each trout was exposed to progressive water velocity increments of 10 cm s^{-1} every 30 min, until fatigue. Fish were considered to be fatigued when they were unable to remove themselves from the posterior screen of the swimming chamber despite gentle encouragement with a sudden increase in water velocity. Measurements of oxygen uptake (\dot{M}_{O_2}) were collected at each swimming speed, as described in McKenzie et al. (2001). These measurements were used to derive: (i) the notional metabolic rate of the immobile fish (IMR); (ii) the maximum metabolic rate of activity (AMR) during swimming (this occurred at speeds approaching U_{crit}); and (iii) net aerobic scope relative to IMR (McKenzie et al., 2003a). Critical swimming speed was calculated in both absolute (cm s^{-1}) and relative ($BL \text{ s}^{-1}$) terms, as described by Brett (1964). Three fish from each experimental group swam in each of the two respirometers. Prior to actual experiments, preliminary U_{crit}

tests were run on four, non-instrumented trout (two good swimmers, two poor swimmers) in both the PVC and steel respirometers. There were no systematic differences in either \dot{M}_{O_2} or swimming performance linked to a particular respirometer (mean \pm S.E.M.): AMR was $181 \pm 39 \mu\text{mol kg}^{-1} \text{ min}^{-1}$ in the PVC tunnel and $195 \pm 37 \mu\text{mol kg}^{-1} \text{ min}^{-1}$ in the steel tunnel, while U_{crit} was $2.45 \pm 0.28 BL \text{ s}^{-1}$ in the PVC tunnel and $2.40 \pm 0.21 BL \text{ s}^{-1}$ in the steel tunnel.

Measurements of cardiac output (\dot{Q}) were made at each swimming speed. Data from the flow probe were acquired and displayed real-time on a PC with LabVIEW software (Axelsson et al., 2002). Measurements of dorsal aortic blood pressure (P_{DA}) were also made at each speed by connecting the saline-filled DA cannula to a physiological pressure transducer (Statham P23XL, Statham Instruments, Oxnard, CA, USA), with the amplified (Gould Universal amplifier, Gould Instruments, Valley View, OH, USA) signal then acquired and displayed on the PC with LabVIEW software (Axelsson et al., 2002). Heart rate (f_{H}) was calculated automatically from the flow probe signal, and used to derive cardiac stroke volume (V_{s}) (Gallaughner et al., 2001). Total systemic vascular resistance (R_{sys}) during swimming was calculated from the measurements of \dot{Q} and P_{DA} (Gallaughner et al., 2001). Maximum values for \dot{Q} , f_{H} and V_{s} were identified from the cardiovascular measurements made at each swimming speed, as was the minimum value for R_{sys} . These extreme values always occurred at speeds near U_{crit} .

Arterial blood samples ($100 \mu\text{l}$) were collected from the DA cannula (and replaced with an equal volume of saline) at swimming speeds of 40 cm s^{-1} and 80 cm s^{-1} , as well as just prior to fatigue (i.e. at U_{crit}). Arterial blood total O_2 content (Ca_{O_2}) was measured using the method of Tucker (1967), as described in McKenzie et al. (2003b). The measurements of Ca_{O_2} and maximum \dot{Q} (see above) were then used to calculate maximum rates of arterial blood O_2 transport (\dot{T}_{O_2}), as described by Gallaughner et al. (2001).

In vitro perfused heart studies

The *in vitro* studies were performed on 15 fish (good swimmers: body mass = 1148 ± 63 g, ventricular mass = 0.87 ± 0.07 g; poor swimmers: body mass = 1106 ± 59 g, ventricular mass = 0.92 ± 0.08 g). The *in situ* heart preparation used to assess maximum cardiac performance has been described in detail by Farrell et al. (1986) and included the modifications outlined by Farrell et al. (1988). Briefly, fish were anaesthetised, transferred to an operating sling where their gills were irrigated with aerated buffered anaesthetic at 4°C , and injected with 0.6 ml of heparinised (100 IU ml^{-1}) saline *via* the caudal vessels. A stainless steel input cannula was secured into the sinus venosus through a hepatic vein and perfusion begun immediately with oxygenated saline containing a tonic level of adrenaline (5 nmol l^{-1} adrenaline). Silk threads were used to occlude any remaining hepatic veins and the ducti Cuvier. A stainless steel output cannula was

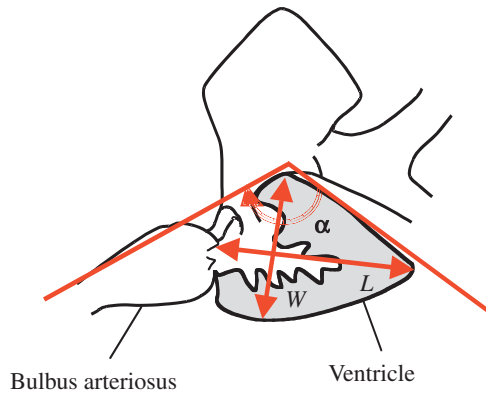


Fig. 1. A schematic diagram illustrating the measurements made during the echo-Doppler examination of the hearts. Ventricle length (L), ventricle width (W) and the angle (α) subtended between the ventral aorta and the ventral surface of the ventricular wall.

advanced into the ventral aorta until the tip was in the bulbus arteriosus and tied firmly in place. These procedures, which were completed in 15–20 min, isolated the heart in terms of saline input and output, while leaving the pericardium intact. The preparation was then immersed in a saline-filled, temperature-controlled organ bath at 16°C, where the input and output cannulae were attached to constant pressure heads. The heart was perfused with an oxygenated physiological saline (Farrell et al., 1988) and filling (input) pressure of the heart was adjusted to give a routine \dot{Q} of 25 ml min⁻¹ kg⁻¹ body mass. Mean output pressure was set at ~5 kPa to simulate routine *in vivo* mean ventral aortic pressures (Stevens and Randall, 1967). The heart maintained this control level of performance for a period of 15–20 min before the assessment protocol began.

The maximum pumping ability of the heart was assessed first by measuring maximum \dot{Q} when filling pressure was increased (i.e. a Starling response) and then by increasing output pressure to 8 and 9 kPa to elicit an increase in cardiac power output while the heart continued to pump maximally. When rainbow trout swim, or when they are given an intra-arterial adrenaline injection, both \dot{Q} and diastolic ventral aortic pressure increase, but mean pressure rarely exceeds 8 kPa (Kiceniuk and Jones, 1977; Gamperl et al., 1994). The heart was then returned to the control perfusion conditions for a 15 min recovery and equilibration with a new adrenaline concentration (1 µmol l⁻¹) in the perfusate, which was then used to assess the effect of maximum adrenergic stimulation of the heart (see Mercier et al., 2000). The two adrenaline concentrations used (5 nmol l⁻¹ and 1 µmol l⁻¹) span the range for circulating catecholamine levels observed in resting and stressed trout, respectively (Milligan et al., 1989; Randall and Perry, 1992; Gamperl et al., 1994). An in-line Transonic flow probe (Transonic Systems, Ithaca, NY, USA) was used to record \dot{Q} (= ventral aortic flow in the output cannula). Pressures in the sinus venosus (input) and ventral aorta (output) were measured using DP6100 pressure transducers (Medizintechnik, Dusslingen, Germany), through saline-filled tubes placed at the

Table 2. Mean critical swimming speed (U_{crit}) and cardiovascular variables in adult rainbow trout that had been screened as good or poor swimmers 9 months earlier

	Good swimmers	Poor swimmers
Absolute U_{crit} (cm s ⁻¹)	123±5	89±10*
Relative U_{crit} (BL s ⁻¹)	2.93±0.12	2.14±0.25*
IMR (µmol O ₂ min ⁻¹ kg ⁻¹)	35±1	37±4
AMR (µmol O ₂ min ⁻¹ kg ⁻¹)	181±11	147±12*
Aerobic scope (µmol O ₂ min ⁻¹ kg ⁻¹)	145±11	110±12*
\dot{Q}_{max} (ml min ⁻¹ kg ⁻¹)	68.0±5.2	47.3±4.7*
f_{Hmax} (beats min ⁻¹)	98±1	92±5
V_{Smax} (ml)	0.77±0.09	0.66±0.09
$R_{sys,min}$ (kPa ml ⁻¹ min ⁻¹ kg ⁻¹)	0.064±0.005	0.087±0.006*
Ca_{O_2} (µmol ml ⁻¹)	5.81±0.38	5.36±0.60
\dot{T}_{O_2max} (µmol O ₂ min ⁻¹ kg ⁻¹)	387±88	238±83*

The subscripts 'max' or 'min' denote the maximum or minimum values (respectively) for the variable measured during the U_{crit} swim test (see text for details).

Values are mean ± S.E.M., $N=6$ in all cases.

*Significant difference between groups (Student's t -test; $P<0.05$).

tip of the cannulae. The pressure transducers were calibrated against a static water column for each preparation. Pressure and flow signals were amplified and filtered using a Model MP100A-CE data acquisition system (BIOPAC Systems Inc., Santa Barbara, CA, USA). The acquired signals were then analysed and stored using Acknowledge Software (BIOPAC Systems Inc., Santa Barbara, CA, USA) installed on a Dell laptop computer.

Myocardial power output (mW g⁻¹ ventricle mass) was calculated from the product of [\dot{Q} (ml min⁻¹) × (output–input pressure) (kPa) × (0.0167 min s⁻¹)] / ventricular mass (g). Ventricle mass was determined at the conclusion of the experiment when the cannulae were checked for correct positioning.

Physiological saline and chemicals

The physiological saline used for the perfused heart preparations (pH 7.8 at 15°C) contained (in mmol l⁻¹): NaCl 124, KCl 3.1, MSO₄·7H₂O 0.93, CaCl₂·2H₂O 2.52, glucose, 5.6 Tes salt 6.4 and Tes acid 3.6 (Keen and Farrell, 1994). The saline was equilibrated with 100% oxygen for at least 30 min prior to experimentation. The coronary artery, which supplies the outer compact myocardium of the ventricle, was not perfused and so oxygenated saline was used to ensure that a sufficient amount of oxygen diffused from the ventricular lumen to the compact myocardium. The oxygen gradient from the lumen to the myocardium of the perfused heart was at least 20-times greater than that *in vivo*. Preliminary experiments have shown that this rainbow trout heart preparation can perform maximally even when the oxygen tension is reduced to ~8 kPa. Adrenaline bitartrate was purchased from Sigma-Aldrich (St Quentin-Fallavier, France).

Cardiac anatomy

In vivo cardiac morphology were assessed for an additional 9 fish per group (good swimmers: mass=1104±47 g, length=425±7 mm; poor swimmers: mass=1167±33 g, length=423±4 mm) using echo-Doppler imaging (Esaote-Pie Medical FalcoVet 100 scanner and 7.5 MHz probe, Fontenay-sous-Bois, France; accuracy ±0.3 mm). Fish were lightly anaesthetised and placed in an operating sling where a hand-held probe provided a lateral image of the ventricle, bulbus arteriosus and ventral aorta. The image was stored and subsequently analysed. After calibration, the machine software allowed the measurement of ventricular height (H) and length (L), as well as the angle (α) subtended between the ventral aorta and the ventral surface of the ventricular wall (Fig. 1).

Data analysis and statistics

Comparisons between good and bad swimmers for single variables were performed using a Student t -test. The effect of swimming speed on *in vivo* variables was assessed and compared between the two groups using a two-way ANOVA with repeated measures. A probability less than 5% ($P < 0.05$) was taken as the limit for statistical significance.

Results

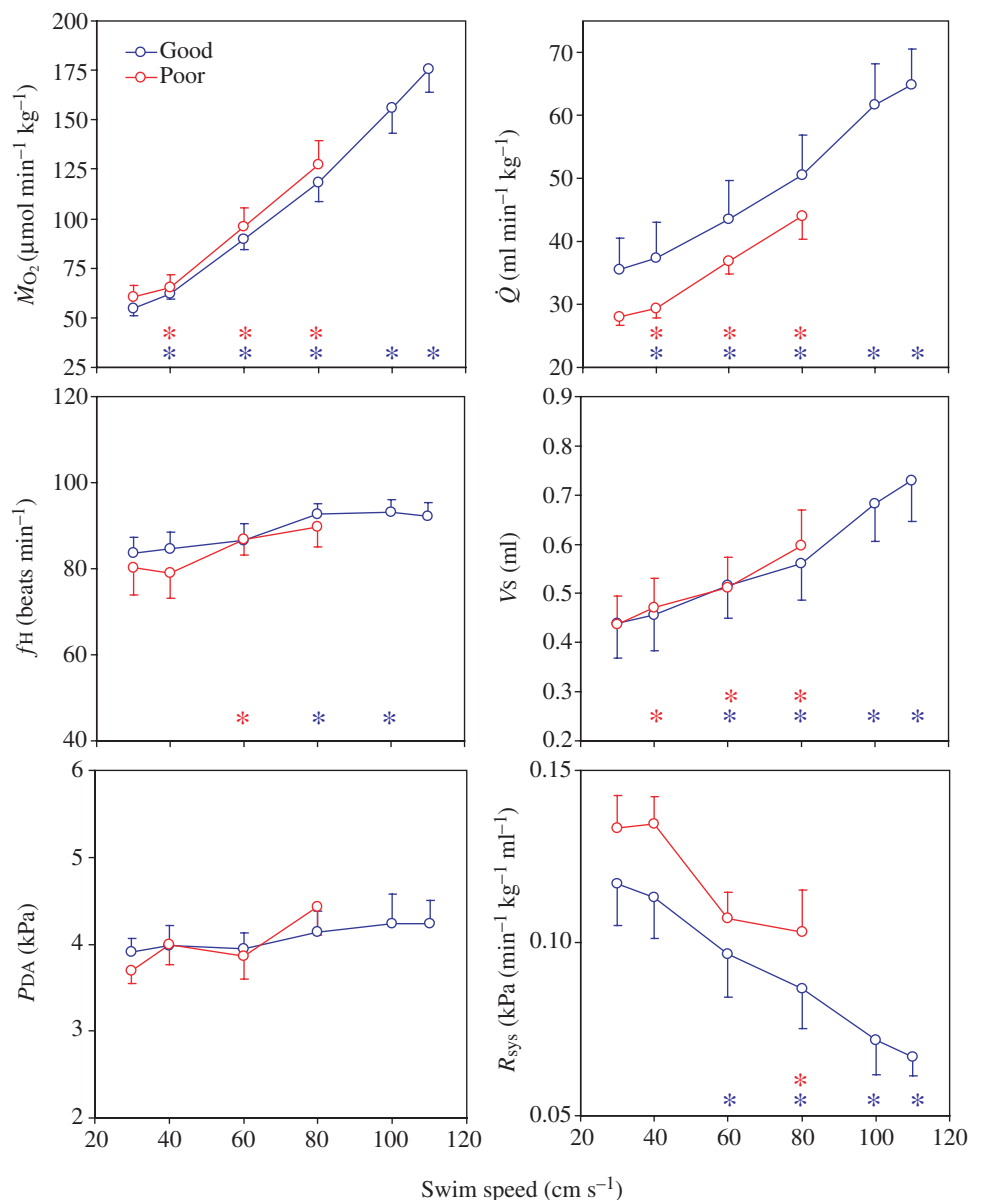
Fish doubled their length and increased their body mass eightfold during the 9-month period between screening and testing (Table 1). There was no significant difference in either body length or mass of good and bad swimmers in either January

Fig. 2. A comparison of mean \dot{M}_{O_2} and cardiovascular variables during sustained swimming in adult rainbow trout that had been screened as good or poor swimmers 9 months earlier. The mean values are shown only for those speeds at which measurements were collected for all of the animals in each group, i.e. up to 80 cm s⁻¹ in poor swimmers and up to 110 cm s⁻¹ in good swimmers. Values are means ± S.E.M., $N=6$. Asterisks above the abscissa denote a significant change in the variable relative to a swimming speed of 30 cm s⁻¹ within either the good (blue) or bad (red) swimmers. There were no significant differences in any variable when compared between good and bad swimmers at any common swimming speed.

or September. However, condition factor (CF) was significantly 5% higher for poor swimmers than good swimmers (Table 1).

In vivo performance

Fish that swam poorly in the screening test had a significantly (27%) lower U_{crit} (both in absolute and relative terms) 9 months later (Table 2). Fig. 2 compares \dot{M}_{O_2} and cardiovascular variables during the U_{crit} protocol for good and poor swimmers. There was no difference in derived IMR (Table 2). Oxygen uptake increased significantly with each increase in swimming speed in both groups, and at common speeds there were no significant differences between the good and poor swimmers (Fig. 2). However, the good swimmers achieved a significantly 19% higher AMR by achieving a higher U_{crit} and, consequently, had a significantly 24% higher aerobic scope (Table 2).



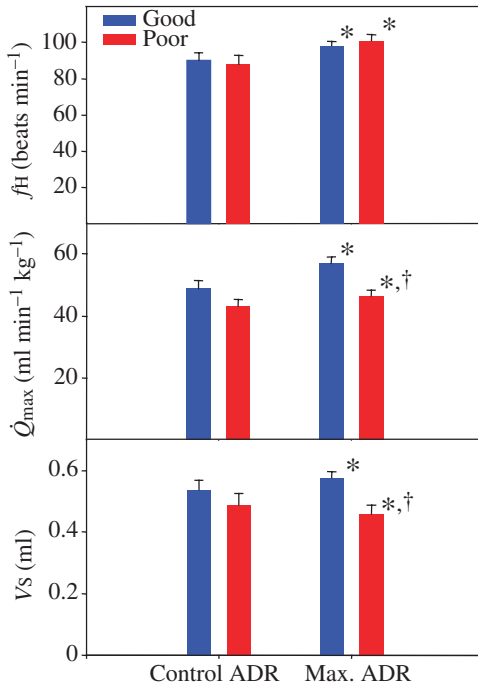


Fig. 3. Comparison of maximum cardiac performance of *in situ* perfused hearts from rainbow trout that had been screened as good or poor swimmers 9 months earlier. *A significant effect of increasing adrenaline (ADR) within a group; †significant difference ($P < 0.05$) between two groups of fish under common conditions. Values are means \pm s.e.m., $N=8$ good swimmers; $N=7$ poor swimmers.

Swimming significantly increased \dot{Q} and, at any common speed, \dot{Q} was similar in both good and poor swimmers (Fig. 2). However, maximum \dot{Q} was significantly (30%) higher for the good swimmers (Table 2). f_H and V_s increased significantly in both groups during swimming and in both groups the increase in \dot{Q} during swimming was predominantly a result of increased V_s rather than f_H (Fig. 2). Nevertheless, the maximum values of f_H and V_s were not significantly different between the good and poor swimmers (Table 2). Both groups of fish maintained P_{DA} during exercise (Fig. 2), but the decrease in R_{sys} induced by exercise (Fig. 2) was significantly (35%) greater in good vs poor swimmers (Table 2).

The oxygen content of arterial blood did not differ significantly between good and poor swimmers and was unchanged during the exercise protocol when measured at 40 cm s^{-1} and 80 cm s^{-1} , which was just prior to fatigue for the poor swimmers. The resting and two exercise values for Ca_{O_2} were averaged for each fish prior to calculating the group mean for Ca_{O_2} (Table 2). Maximum \dot{T}_{O_2} was significantly (39%) higher in good swimmers compared with poor swimmers, as a direct result of the former group's higher maximum \dot{Q} (Table 2).

In vitro performance

Under tonic adrenergic stimulation, maximum \dot{Q} was not statistically different between the good and poor swimmers ($48.0 \pm 2.7 \text{ ml min}^{-1} \text{kg}^{-1}$ and $42.2 \pm 2.3 \text{ ml min}^{-1} \text{kg}^{-1}$,

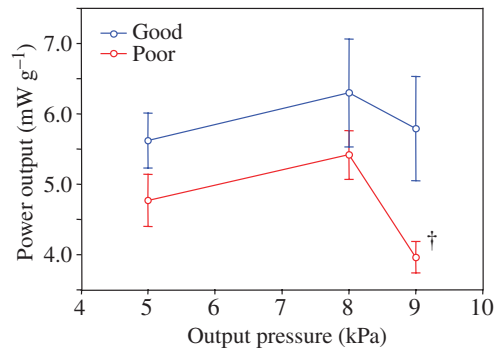


Fig. 4. Comparison of maximum power output as output pressure was raised in rainbow trout *in situ* perfused hearts from rainbow trout that had been screened as good or poor swimmers 9 months earlier. Both swim group and output pressure are significant determinants of power output (ANOVA; $P < 0.05$) and no interaction between the factors was found. †Pairwise multiple comparisons confirmed the significant difference between good (blue) and poor (red) swimmers at the highest output pressure (9 kPa) as well as the significant drop in power output between 8 and 9 kPa in the poor swimmer group. Values are means \pm s.e.m., $N=8$ good swimmers; $N=7$ poor swimmers.

respectively; Fig. 3). Maximum stimulation with adrenaline ($1 \mu\text{mol l}^{-1}$) significantly increased maximum \dot{Q} in both good and poor swimmers, but that increase was significantly greater in good swimmers than in poor swimmers (maximum \dot{Q} increased to $56.4 \pm 2.3 \text{ ml min}^{-1} \text{kg}^{-1}$ and $45.9 \pm 1.9 \text{ ml min}^{-1} \text{kg}^{-1}$, respectively). Under control (tonic adrenergic stimulation) conditions, f_H was similar for good and poor swimmers, as was the modest elevation in f_H produced by maximum adrenergic stimulation (an increase from $87.1 \pm 5.4 \text{ beats min}^{-1}$ to $100.9 \pm 3.9 \text{ beats min}^{-1}$ in poor swimmers and from $89.1 \pm 4.4 \text{ beats min}^{-1}$ to $97.5 \pm 2.8 \text{ beats min}^{-1}$ in good swimmers). Similarly, V_s was not statistically different between the two swim groups under tonic adrenergic stimulation ($0.54 \pm 0.03 \text{ ml}$ in good swimmers vs $0.49 \pm 0.03 \text{ ml}$ in poor swimmers). However, under maximum adrenergic stimulation, V_s increased significantly in good swimmers ($0.58 \pm 0.02 \text{ ml}$), whereas it decreased significantly in poor swimmers ($0.46 \pm 0.03 \text{ ml}$). Thus, the maximum cardiac pumping ability of poor swimmers was significantly (26%) lower than that of good swimmers.

Cardiac power output was calculated from the product of \dot{Q} and output pressure. The effect of increasing output pressure while the heart was pumping maximally is shown in Fig. 4. Overall, cardiac power output in good swimmers was significantly higher than in poor swimmers. When output pressure was progressively raised from 8 kPa to 9 kPa, power output was unchanged in good swimmers. In contrast, the same increase in output pressure from 8 kPa to 9 kPa in poor swimmers resulted in a significant decrease in power output from $4.77 \pm 0.97 \text{ mW g}^{-1}$ to $3.96 \pm 0.58 \text{ mW g}^{-1}$, which was 32% lower than the 5.97 mW g^{-1} for good swimmers. Thus, the hearts from poor swimmers were less able to tolerate a high

Table 3. Ventricular and aortic morphometrics performed using echo-Doppler imaging from adult rainbow trout that had been screened as good or poor swimmers 9 months earlier

	Good swimmers	Poor swimmers
Angle (deg.)	154±4	153±5
Length (cm)	1.17±0.04	1.06±0.04
Width (cm)	1.16±0.04	1.21±0.05
Length/width ratio	1.01±0.01	0.88±0.04*

Values are mean ± S.E.M., N=9.
*Significant difference between groups (Student's *t*-test; *P*<0.05).

cardiac afterload compared with good swimmers, as well as having a lower maximum \dot{Q} .

Cardiac anatomy

The ventricle was significantly longer in good swimmers compared with poor swimmers, although there were no differences in ventricular width or the subtended angle (Table 3). As a result, the ventricular length to width ratio was smaller in the poor swimmers (Table 3).

Discussion

To our knowledge, this is the first combined *in vivo* and *in vitro* examination of maximum cardiac performance in fish as it relates to swimming ability and cardiac anatomy. Earlier studies have trained fish and then measured cardiac performance either *in vivo* during U_{crit} tests (Gallaughan et al., 2001) or *in vitro* with perfused heart preparations (Farrell et al., 1991), but never together. The present study provided both *in vivo* and *in vitro* measurements of maximum \dot{Q} , showing that trout screened as good swimmers had a larger cardiac pumping capacity compared with poor swimmers from the same population. Furthermore, good and poor swimmers also differed in ventricular dimensions and the heart's ability to pump against a high resistance, with the implication that the more rounded ventricle of the poor swimmers was a weaker heart.

The *in vivo* values for maximum \dot{Q} in the current study are consistent with other reports for rainbow trout exercising to U_{crit} (Kiceniuk and Jones, 1977; Taylor et al., 1996). Kiceniuk and Jones (1977) measured \dot{Q} indirectly by the Fick equation and found a maximum \dot{Q} value of 51 ml min⁻¹ kg⁻¹ at 11°C. Similarly, Thorarensen et al. (1996a) and Brodeur et al. (2001) report maximum \dot{Q} values for rainbow trout of 49 ml min⁻¹ kg⁻¹ at 10°C and 65 ml min⁻¹ kg⁻¹ at 12°C, respectively. Taylor et al. (1996) also measured blood flow indirectly, with microspheres, and found that maximum \dot{Q} was very sensitive to temperature, being 20 ml min⁻¹ kg⁻¹ at 4°C, 69 ml min⁻¹ kg⁻¹ at 11°C and decreasing to 42 ml min⁻¹ kg⁻¹ at 18°C. Thus, both the good and the poor swimmers in the current study had maximum \dot{Q} values at 16°C that were intermediate between those for 11°C and 18°C reported by Taylor et al. (1996).

The present study, which used individual diversity in swimming performance as a means of segregating two groups of fish, shows similarities with earlier studies in which fish were exercise-trained in an attempt to improve their aerobic capacity. For example, training of rainbow trout at 50% of U_{crit} for 1 month increased maximum \dot{Q} and power output by 17% and 26%, respectively, as measured in perfused hearts (Farrell et al., 1991). In chinook salmon *Oncorhynchus tshawytscha*, training at 1.5 BL s⁻¹ did not improve either U_{crit} or AMR (Thorarensen et al., 1993), but a more vigorous training protocol that involved them swimming to U_{crit} on alternate days for 4 months did elicit a significant 50% increase in AMR (Gallaughan et al., 2001). In the present study, similar differences in maximum \dot{Q} between good and poor swimmers (26% *in vitro* and 30% *in vivo*) were associated with a 19% higher AMR and a 27% higher U_{crit} . Given this quantitative agreement, it appears that the extremes of inherent individual diversity in maximum \dot{Q} and associated swimming ability, within a large group of hatchery-raised rainbow trout, are approximately equivalent to the effects of intensive and prolonged training protocols aimed at remodelling salmonid cardiac and aerobic performance.

The finding that rainbow trout retained a swimming performance trait over a 9 month period was not surprising. Numerous studies have demonstrated that swimming performance is a repeatable trait in salmonid and non-salmonid fishes, both in the short term (Randall et al., 1987; Brauner et al., 1994; Kolok and Farrell, 1994; Jain et al., 1998; Farrell et al., 2003) and the long term (Kolok, 1992; Martinez et al., 2002). Although individual diversity in swimming performance has been related to muscle biochemistry (Kolok, 1992; Martinez et al., 2002), we are unaware of any previous linkages of individual diversity in maximum \dot{Q} , AMR, aerobic scope and U_{crit} , as revealed in the present study. While the basis for this diversity awaits further study of potential genetic, environmental or even social influences, we believe that the *in vitro* work, by providing definitive information about maximum cardiac pumping capacity, lends direct support for the contention that rainbow trout utilise their maximum cardiac pumping ability at or near U_{crit} .

One concern encountered in the present study was that the fish grew faster than anticipated, which resulted in experimental fish that were larger than the preferred optimal for perfused heart work. Because approximately 30% of the outer ventricular wall receives oxygen from a coronary circulation and this was not perfused in the heart preparation, the expectation was that the oxygenated perfusate, which has an oxygen tension nearly 50-times higher than that measured in venous blood passing through the heart at U_{crit} (~1.6 kPa; Farrell and Clutterham, 2003), would provide sufficient oxygen delivery to the compact myocardium. This was certainly the case for the poor swimmers because there was an excellent agreement between the maximum \dot{Q} measured *in vivo* (47.3 ml min⁻¹ kg⁻¹) and that measured *in vitro* (45.9 ml min⁻¹ kg⁻¹). However, the agreement was not quite as good for the good swimmers, where maximum \dot{Q} *in vivo* was

68.0 ml min⁻¹ kg⁻¹ vs 56.4 ml min⁻¹ kg⁻¹ *in vitro*. This concern is unlikely to invalidate our main finding that maximum cardiac pumping ability was significantly lower in poor compared with good swimmers, since if anything we underestimated maximum \dot{Q} *in vitro*.

The difference observed in cardiac anatomy between good and poor swimmers did not translate into a significantly larger V_s for good swimmers under a condition of tonic adrenergic stimulation. However, hearts from poor swimmers were less sensitive to adrenergic stimulation, which did not increase maximum \dot{Q} *in vitro*, a fact that may underlie the lower *in vivo* maximum \dot{Q} . Mechanistically, it seems that while a modest positive chronotropic response to adrenergic stimulation was common to good and poor swimmers, the consequence of this elevated f_H was to modestly decrease maximum V_s in the poor swimmers. This indicates a limited inotropic action of adrenaline in the poor swimmers, a response that was not observed in the good swimmers. An inotropic deficiency in poor swimmers was further manifested as a lower maximum power output (i.e. maximum V_s was not maintained when either output pressure or f_H was increased). Given this shortcoming, the inability of poor swimmers to decrease R_{sys} (which sets cardiac afterload) during swimming may have contributed to their lower maximum \dot{Q} *in vivo*. A limited cardiac response to adrenergic stimulation was expected at 16°C because earlier work has shown that adrenergic sensitivity of rainbow trout hearts falls off at temperatures approaching 18°C (Farrell et al., 1986, 1996), unlike at colder temperatures when adrenergic stimulation may be critical for basic cardiac rhythmicity (Graham and Farrell, 1989) and calcium channel function (Shiels et al., 2003).

Between 20 and 60% of farmed triploid brown trout *Salmo trutta* have been observed with a bent aorta, depending on the origin of the fish (G. Claireaux and J. Aubin, personal observations; Poppe et al., 2003), with an α value of >100° in the worst cases. In the present work with rainbow trout, aortic deformities were not observed. Hatchery-raised salmonids are also characterised by having a more rounded ventricle than those captured from the wild (Poppe et al., 2003; Gamperl and Farrell, 2004) and this would mean that the L/H ratio for the ventricle would tend towards unity. The present finding of a reduced ventricular L/H ratio for poor swimmers is consistent with a more rounded ventricular shape. Furthermore, amongst the individual fish used in the present study, this ventricular ratio was negatively correlated with fish condition factor, i.e. the higher the condition factor, the more rounded the ventricle (Fig. 5). Moreover, when data for wild anadromous rainbow trout (steelhead) from the Clearwater River, Idaho, USA (Poppe et al., 2003) are included on this graph, it becomes clear that condition factor and ventricular shape may be more generally related. There are a variety of potential reasons why these correlations might exist and further work is needed to tease them apart. For example, there is likely an optimum condition factor for swimming performance that would lie somewhere between the states of starvation and obesity. The fish in the present study were very well fed and their condition factors indicated that they were near or above this optimum. Until the relationships

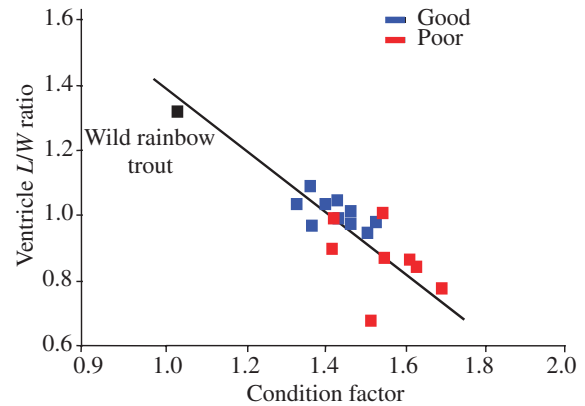


Fig. 5. Relationship between ventricle length/width ratio and condition factor (CF) in rainbow trout that had been screened as good or poor swimmers 9 months earlier. Regression line: L/W ratio = $-0.87CF + 2.23$, $r^2 = 0.78$. The black symbol for wild rainbow trout population (Poppe et al., 2003) was not included in the regression analysis.

between cardiac shape, swimming performance, condition factor, reduced maximum cardiac pumping ability and reduced cardiac sensitivity to adrenaline among cultured rainbow trout are resolved, it remains probable that specific culture conditions and practices (e.g. fast growth, lack of physical exercise, nutrition, phenotype and genotype selection, etc.) play a role in the more rounded ventricle of farmed salmonids, and within this aquaculture context, knowledge of ventricular shape in relation to condition factor may have potential to be a predictor of swimming ability. Nevertheless, future studies would do well to consider how a change in relative ventricular mass might affect ventricular shape, given that cold temperature acclimation and sexual maturation (in males) are both known to increase relative ventricular mass in rainbow trout.

List of symbols and abbreviations

AMR	active metabolic rate
BL	body length
Ca_{O_2}	arterial blood total O_2 content
CF	condition factor
DA	dorsal aorta
f_H	heart rate
H	height
Hct	hematocrit
IMR	immobile metabolic rate
L	length
\dot{M}_{O_2}	rate of oxygen uptake
P_{DA}	dorsal aortic blood pressure
\dot{Q}	cardiac output = blood flow rate in the ventral aorta
R_{sys}	systemic vascular resistance
\dot{T}_{O_2}	rate of arterial blood O_2 transport
U_{crit}	critical swimming
V_s	cardiac stroke volume
α	angle

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