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RESEARCH ARTICLE

Repeatability of standard metabolic rate, active metabolic rate and aerobic scope in young brown trout during a period of moderate food availability

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SUMMARY

Standard metabolic rate (SMR) and active metabolic rate (AMR) are two fundamental physiological parameters providing the floor and ceiling in aerobic energy metabolism. The total amount of energy available within these two parameters confines constitutes the absolute aerobic scope (AAS). Previous studies on fish have found SMR to closely correlate with dominance and position in the social hierarchy, and to be highly repeatable over time when fish were provided an *ad libitum* diet. In this study we tested the temporal repeatability of individual SMR, AMR and AAS, as well as repeatability of body mass, in young brown trout (*Salmo trutta* L.) fed a moderately restricted diet (0.5–0.7% fish mass day^{-1}). Metabolism was estimated from measurements of oxygen consumption rate ($\dot{M}_{O_2}$) and repeatability was evaluated four times across a 15-week period. Individual body mass was highly repeatable across the entire 15 week experimental period whereas residual body-mass-corrected SMR, AMR and AAS showed a gradual loss of repeatability over time. Individual residual SMR, AMR and AAS were significantly repeatable in the short term (5 weeks), gradually declined across the medium term (10 weeks) and completely disappeared in the long term (15 weeks). We suggest that this gradual decline in repeatability was due to the slightly restricted feeding regime. This is discussed in the context of phenotypic plasticity, natural selection and ecology.

Key words: repeatability, standard metabolic rate, active metabolic rate, absolute aerobic scope, body mass, brown trout, *Salmo trutta*, diet.

INTRODUCTION

Two physiological parameters that have received a great deal of attention from ecologists and physiologists alike are standard metabolic rate (SMR) and active metabolic rate (AMR), usually estimated from measurements of oxygen consumption rate ($\dot{M}_{O_2}$). Because SMR is defined as the minimal maintenance metabolic rate of a post-absorptive resting ectotherm, below which physiological function is impaired (Brett and Groves, 1979; Priede, 1985), it is in fact the basic cost of living at a certain temperature and therefore of major functional importance. In the other end of the metabolic scale, AMR, the maximal aerobic metabolic rate at a given temperature, provides the upper boundary for aerobic energy metabolism. Subtracting the minimal from the maximal metabolic rate provides a measure of the total amount of aerobic energy available to the animal, the absolute aerobic scope (AAS). In salmonid fish, a high SMR has been interpreted as an advantage, despite the increased cost of living, because of its positive correlation with dominance, aggression and growth rate (Metcalfe et al., 1995; Cutts et al., 1998).

In order to ascribe certain properties to an individual animal on the basis of a single physiological measurement, an in-depth understanding of the temporal consistency (repeatability) of the trait under investigation is essential. If a trait is not repeatable over time, a single measure or estimate of the trait may not be representative of future physiological performance. Repeatability studies differ greatly in the time frame over which consistency has been evaluated. Commonly, repeatability is high when evaluated over relatively short periods of time but tends to decrease with increasing time between measurements (van Berkum et al., 1989; Chappell et al., 1996; Rønning et al., 2005). Because repeatability estimates provide a measure of the consistency of individual differences within a population (Dohm, 2002), and potentially an estimate (upper limit) of heritability (e.g. Sadowska et al., 2005), a balance exists between the period of time over which the trait is repeatable and the life span (or a certain part of ontogeny) of the animal under investigation. In other words, repeatability must persist across a significant part of an animal's life, or across some part of ontogeny, in order for selection to affect the trait, provided that the trait is heritable.

Nespolo and Franco reviewed literature studies on the repeatability of metabolic rates and concluded that, in biological terms, metabolic rate could be considered a repeatable trait and that no additional studies on repeatability were needed (Nespolo and Franco, 2007). These authors assessed repeatability from whole-animal metabolic rates but, as emphasized by Konarzewski et al. (Konarzewski et al., 2005), whole-animal metabolic rate is intimately associated with body mass, artificially producing high and consistent repeatability of metabolism because of a reflection of body mass repeatability. The conclusion drawn by Nespolo and Franco (Nespolo and Franco, 2007) is based on 44 studies, of which only two are on fish. A few additional studies on fish exist, but in these the repeatability of metabolic rate has been estimated mainly as consistency of SMR (McCarthy, 2000; O'Connor et al., 2000; Cutts et al., 2001; Seppänen et al., 2010; Maciak and Konarzewski, 2010) whereas AMR has received little attention (Reidy et al., 2000). Although Seppänen et al. (Seppänen et al., 2010) were only partially able to show consistency in SMR, the other studies found repeatability of SMR (and AMR) to be quite high over periods of time up to 5 months.

In the present study, we provide estimates of repeatability of SMR, AMR and AAS, as well as body mass, in a salmonid fish, the brown trout (*Salmo trutta* Linnaeus 1758). In contrast to other repeatability studies on fish that have provided food *ad libitum* [or, as in the study by O'Connor et al. (O'Connor et al., 2000), totally deprived the fish of food] we assessed repeatability during a period of moderate food availability, a situation that most resembles food availability in the wild.

MATERIALS AND METHODS

Fish

All brown trout used in this experiment were offspring of wild trout caught by electrofishing in the River Skjern, Denmark. Fertilised eggs from parent fish were kept in freshwater trays and hatched in March 2009 at The Danish Centre for Wild Salmon (Skjern, Denmark). Immediately after hatching, fish were held in hatchery troughs. Exogenous feeding commenced in late April/early May, after which the fish were transferred to circular flow-through tanks, kept at ambient temperature and photoperiod, and fed commercial trout pellets. In October 2009, at a size of 95–110 mm, 260 brown trout were relocated to holding facilities at Aarhus University, Denmark, where they were held in a 720 l freshwater aquarium at 15±0.5°C and 9 h:15 h light:dark photoperiod. Water was continually recirculated through a filter and exchanged at a rate of 700 l week^{-1}. Fish were fed daily with BioMar (Brande, Denmark) INICIO Plus trout pellets from an automatic feeder, mounted above the aquarium, at an amount corresponding to 0.5–0.7% fish mass per day. On 4 November 2009, 50 fish were randomly chosen from the population and individually marked using small implantable FDX-B passive integrated transponder (PIT) tags encapsulated in a bio-compatible glass tube (LoligoSystems, Tjele, Denmark). The tags weighed 0.1 g with dimensions of 13.5×2.1 mm and provided each fish with a unique 15-digit code. PIT tags were surgically implanted into the abdominal cavity of anaesthetised fish (0.1 g l^{-1} benzocain) through a small incision on the ventrolateral side just posterior to the pectoral fin. After marking, the aquarium was divided with a rigid plastic net (mesh size 5×5 mm) providing full separation of marked and unmarked fish while allowing for complete mixing of water throughout the aquarium. Only the marked fish were used in this experiment. Effects of PIT-tag marking on growth and survival of brown trout have been found to be negligible on fish larger than 55–57 mm (Ombredane et al., 1998; Acolas et al., 2007). In the present study, no fish died or exhibited reduced growth as a consequence of the tagging procedure. Tag retention was 100%. Of the 50 marked fish, 36 were randomly chosen for respirometric measurements.

Feeding regime

As this study focused on the repeatability of aerobic energy metabolism in fish fed a moderately restricted diet, appropriate ration sizes, eliciting growth of brown trout in between maintenance and *ad libitum* feeding rates, were predicted prior to the experiment according to Elliott (Elliott, 1975a; Elliott, 1975b). At the time of trial 1 (see Experimental protocol), fish in the present study were fed average rations corresponding to 34.2% of *ad libitum* satiation rations provided to brown trout of similar size by Elliott (Elliott, 1975a). At the time of trials 2, 3 and 4, average diets constituted 36.1, 38.2 and 38.6% of satiation rations, respectively. These rations corresponded to 0.5–0.7% trout mass day^{-1} in the present experiment. In the studies by Elliott (Elliott, 1975a; Elliot, 1975b), fish were fed the crustacean *Gammarus pulex* whereas fish in the present study were fed the energetically more nutritious trout pellets.

Respirometry

Respirometry was performed using automated intermittent closed respirometry. The experimental setup comprised six acrylic respirometer chambers submerged in an ambient tank containing 115 l fully aerated tap water at 15±0.2°C. This setup allowed for simultaneous oxygen consumption measurements of six fish. Water in the tank was recirculated through a UV steriliser to minimise bacterial respiration. Each respirometer chamber was equipped with two sets of gas-proof tubing. One set recirculated water through the chamber past a galvanic oxygen electrode by means of a pump, while the other set flushed the chamber at a rate of 750 ml min^{-1} by sucking in water from the ambient tank and returning it through a tube elevated above the water surface. Prior to the experiment, O_2 electrodes were calibrated against an anoxic solution of sodium sulphite in 0.01 mol l^{-1} sodium tetraborate and fully aerated water from the ambient tank. Oxygen saturation was assured by vigorous bubbling with atmospheric air, and water oxygen tension (P_{wO_2}) was calculated as $P_{wO_2}=F_{O_2}(P_{BAR}-P_{H_2O})$, where F_{O_2} is the fraction of oxygen in the atmosphere (0.2095), P_{BAR} is the barometric pressure and P_{H_2O} is the water vapour pressure at given temperature and salinity. The flushing period replenished the respirometer chamber with fully aerated water while at the same time removing metabolites. Total volume of one respirometer chamber was 540 ml. The recirculation system was activated at all times whereas the flush system was controlled by the computer and alternately turned on and off (i.e. producing an open and closed phase, respectively). Changes in P_{wO_2} due to fish respiration were continuously monitored at 1 Hz by AutoResp^{TM} software (LoligoSystems, Tjele, Denmark), but only data for the closed phase of the cycle (referred to as the measurement period) were used for later analysis.

Experimental protocol

To evaluate the repeatability of AMR, SMR and AAS, the $\dot{M}_{O_2}$ of all fish was measured in four trials (mid December 2009, late January 2010, early March 2010 and early April 2010) over a period of 15 weeks, incremented at 5 week intervals. On each day of the experiment, six fish were randomly caught by hand netting, lifted out of the holding aquarium and individually placed in an oval 90 l tub containing 30 l of fully aerated tap water at 15±0.8°C. Prior to this, fish had not been fed for 23 h. To measure AMR, fish in the tub were chased by hand until signs of total exhaustion were evident (i.e. the fish became unresponsive). This occurred within 2 min of chasing. The chasing protocol consistently produced short bursts of very high activity and was believed to induce maximal oxygen consumption rate as described by Cutts et al. (Cutts et al., 2002). Following the 2 min chase, fish were lifted out of the tub into the air and immediately placed in the respirometer chambers where the measurement period commenced after a maximum of 10 s from cessation of chasing. $\dot{M}_{O_2}$ was evaluated for 60 s, after which the chamber was flushed for 210 s, allowing enough time to exchange >99% of the water inside (Steffensen, 1989). This first measurement of $\dot{M}_{O_2}$ (that was always higher than subsequent measurements) was used as an estimate of AMR. Following measurements of AMR, the software was reprogrammed and measurement periods were increased to 105–120 s depending on fish size. Periods of flush remained the same. Half of the tank was covered with black plastic sheeting to minimise disturbance and the system was left unattended for the next 20 h (until 08:00 h the following morning). With a full cycle of flush and measurement of 330 s (maximum), this produced over 218 measurements of $\dot{M}_{O_2}$ per fish. Through pilot experiments, respiration from bacteria was found to be negligible over the short

periods of measurement and was disregarded in further analysis. When experiments were terminated in the morning, fish were mildly anaesthetised in benzocain and weighed to the nearest 0.1 g before being returned to the holding aquarium (which had been divided with an additional mesh screen to avoid mixing of fish that had already been, or were about to be, screened for $\dot{M}_{O_2}$). After each day, all experimental equipment was disassembled, disinfected and thoroughly cleaned before new fish were introduced.

Two fish died during January measurements because of computer failure, and one fish had to be excluded from analysis during March measurements as a consequence of a faulty O_2 electrode. This reduced the sample size to 33 fish.

Data analysis

Data were analysed using Mathematica 5.2 (Wolfram Research, Inc., Champaign, IL, USA). Linear regressions between Pw_{O_2} and time were made for each period of measurement and slopes (k) derived from these regressions were used to calculate oxygen consumption by the fish according to the equation:

$$\dot{M}_{O_2} = kV_{resp}\beta w_{O_2}, \quad (1)$$

where $\dot{M}_{O_2}$ is the oxygen consumption rate (μmol min^{-1}), k is the change in Pw_{O_2} over time (kPa min^{-1}), V_{resp} is the volume of the respirometer minus the volume of fish (l) and βw_{O_2} is the solubility coefficient of oxygen in water at given temperature and salinity (μmol l^{-1} kPa^{-1}) (Dejours, 1981). SMR was determined as the mean of the 10 lowest $\dot{M}_{O_2}$ measurements, excluding outliers, over the 20 h experimental period. To normalise data, all values of $\dot{M}_{O_2}$ and body mass were $\log_{10}$-transformed prior to analysis. Mass-independent data of $\dot{M}_{O_2}$ are expressed as residual metabolic rates (rSMR, rAMR and rAAS) calculated from least-squares linear regression of oxygen consumption rate on body mass. Residuals for AAS were calculated from the relationship between $\dot{M}_{O_2}$ expressed as AMR–SMR and body mass. Thus, fish with higher than expected $\dot{M}_{O_2}$ have positive residuals and fish with lower than expected $\dot{M}_{O_2}$ have negative residuals.

Growth rates were calculated as specific growth rate (SGR, % day^{-1}) according to the equation:

$$SGR = [(\ln M_f - \ln M_i)t^{-1}] \times 100, \quad (2)$$

where M_i and M_f are body mass (g) of fish at the start and end of the experiment, respectively, and t is the time (days) it took the fish to grow from M_i to M_f.

Statistical analyses were performed in SigmaPlot® 11 (Systat Software Inc., San Jose, CA, USA). Repeatability of body mass and residual metabolic rates was assessed from Kendall's coefficient of concordance (W) calculated according to Zar (Zar, 1996). Where overall concordance proved significant (i.e. the null hypothesis of independence were rejected), pairwise Spearman rank-order correlations (r_S) among variables were computed as suggested by Legendre (Legendre, 2005) to assess the congruence of individual trials. The level of significance was set to $P<0.05$. If not stated otherwise, values presented are means ± s.e.m.

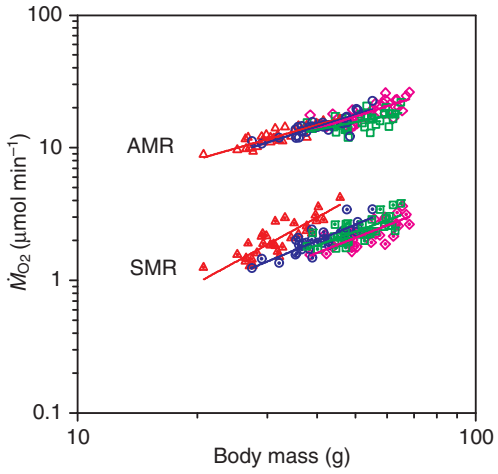

Fig. 1. Relationship between active and standard rate of oxygen consumption ($\dot{M}_{O_2}$, μmol min^{-1}) at 15°C and body mass (g) of brown trout from the four trials. Red triangles, trial 1; blue circles, trial 2; green squares, trial 3; pink diamonds, trial 4. Data are presented on double logarithmic axes with fitted lines representing the power function $\dot{M}_{O_2}=aM^b$, where a and b are constants and M is body mass. For $\log_{10}$-transformed regression equations, see Table 2. AMR, active metabolic rate; SMR, standard metabolic rate.

RESULTS

Metabolic rates, estimated from measurements of $\dot{M}_{O_2}$ as described above, were divided into two groups: active and standard metabolic rates (Fig. 1). Minimum, maximum and mean $\dot{M}_{O_2}$ values from the four trials are listed in Table 1. Body mass of fish ranged from 20.7 to 45.7 g (mean=32.3±0.96 g), 27.4 to 55.1 g (mean=40.9±1.13 g), 37.7 to 64.9 g (mean=51.6±1.28 g) and 38.4 to 68.2 g (mean=54.0±1.39 g) in trials 1, 2, 3 and 4, respectively.

Within trials, the two lines from regression equations describing the relationship between SMR and AMR and body mass (Table 2)

Table 1. Minimum, maximum and mean oxygen consumption rates ($\dot{M}_{O_2}$, μmol min^{-1}) measured at 15°C in each of the four trials

Metabolic rate	Trial	N	Minimum	Maximum	Mean	CV
SMR	1	36	1.25	4.20	2.13±0.12	32.5
	2	34	1.24	3.43	2.06±0.09	23.9
	3	33	1.71	3.81	2.43±0.09	21.6
	4	33	1.57	3.54	2.31±0.09	22.4
AMR	1	36	8.89	16.46	12.32±0.36	16.6
	2	34	10.71	22.48	14.72±0.45	17.5
	3	33	11.97	22.10	15.91±0.41	14.7
	4	33	14.34	26.27	18.60±0.61	18.7
AAS	1	36	7.63	13.10	10.18±0.26	14.4
	2	34	9.26	19.05	12.66±0.39	17.7
	3	33	10.00	18.28	13.49±0.37	15.6
	4	33	12.04	23.62	16.30±0.55	19.6

AAS, absolute aerobic scope; AMR, active metabolic rate; CV, coefficient of variation (%); N, number of fish in each trial; SMR, standard metabolic rate. Means are presented ±s.e.m.

Table 2. Parameters from least-squares linear regression of $\log_{10}$-transformed oxygen consumption rates ($\dot{M}_{O_2}$, μmol min^{-1}) *versus* $\log_{10}$-transformed body mass (M, g), $\log\dot{M}_{O_2}=\log a+b\log M$, for the four trials

Metabolic rate	Trial	N	log$a$	b	r^2	P
SMR	1	36	−1.956±0.263	1.507±0.175	0.705	<0.0001
	2	34	−1.615±0.219	1.194±0.136	0.713	<0.0001
	3	33	−1.297±0.315	0.979±0.184	0.477	<0.0001
	4	33	−1.565±0.286	1.110±0.166	0.592	<0.0001
AMR	1	36	−0.195±0.123	0.852±0.082	0.778	<0.0001
	2	34	−0.225±0.161	0.864±0.100	0.705	<0.0001
	3	33	0.262±0.252	0.547±0.148	0.307	0.0008
	4	33	−0.268±0.255	0.886±0.147	0.539	<0.0001
AAS	1	36	−0.060±0.125	0.708±0.083	0.700	<0.0001
	2	34	−0.203±0.190	0.809±0.118	0.602	<0.0001
	3	33	0.328±0.288	0.467±0.168	0.199	0.0093
	4	33	−0.272±0.290	0.854±0.168	0.455	<0.0001

Constants log$a$ and b are presented ±s.e.m.

significantly differed in slopes in trial 1 (ANCOVA, $F_{1,62}$=11.51, P=0.001) but not in trial 2 (ANCOVA, $F_{1,62}$=3.82, P=0.055), trial 3 (ANCOVA, $F_{1,62}$=3.35, P=0.072) or trial 4 (ANCOVA, $F_{1,62}$=1.02, P=0.316). Elevation differed in all trials (ANCOVA, trial 2, $F_{1,63}$=5174.03, P<0.0001; trial 3, $F_{1,63}$=3054.06, P<0.0001; trial 4, $F_{1,63}$=4089.61, P<0.0001), and AMR was always significantly higher than SMR. Because slopes differed in trial 1, differences in the elevation of data points between AMR and SMR were tested by comparison of means from mass-standardised $\dot{M}_{O_2}$ values [standardized to the mean body mass of fish in trial 1 according to $\dot{M}_{O_2(32.3g)}=\dot{M}_{O_2observed}$ $(M/32.3\,g)^{(1-b)}$, where b is the slope for the respective regression equation (AMR or SMR) listed in Table 2]. This showed that AMR was significantly higher than SMR (t-test, t_{64}=57.25, P<0.0001), as was expected.

Residual (body-mass-corrected) metabolic rates for the 33 surviving fish from trials 1, 2, 3 and 4, respectively, were calculated from regression equations in Table 2, and distributed as 20–/13+, 20–/13+, 19–/14+ and 18–/15+ for SMR; 18–/15+, 15–/18+, 17–/16+ and 18–/15+ for AMR; and 18–/15+, 17–/16+, 17–/16+ and 17–/16+ for AAS, with minus signs denoting the number of fish with lower than expected metabolic rates and plus signs denoting the number of fish with higher than expected metabolic rates.

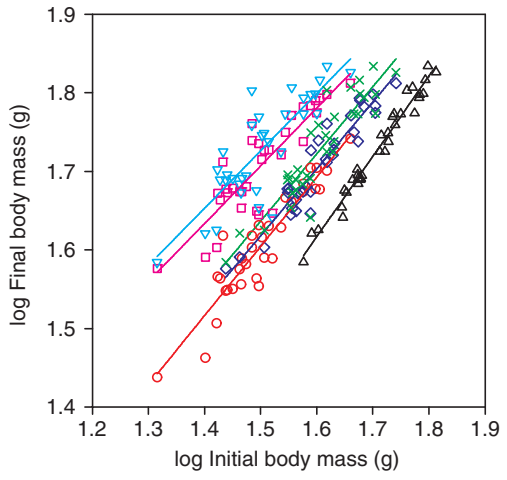

Fig. 2. Repeatability of body mass of brown trout for all combinations of trials. Red circles, trial 1 *vs* 2; blue diamonds, 2 *vs* 3; black triangles, 3 *vs* 4; pink squares, 1 *vs* 3; green crosses, 2 *vs* 4; turquoise triangles, 1 *vs* 4. See Table 3 for statistics.

Because metabolism was estimated four times over 15 weeks, six combinations of either body mass or residual metabolic rates are possible, presenting repeatability over three 5-week periods, two 10-week periods and one 15-week period. Individual body mass was strongly correlated between all trials (Fig. 2) and repeatability was very high (overall W=0.912, P<0.0001), with Spearman correlation coefficients ranging from 0.786 to 0.973 for pairwise combinations of trials (Table 3).

Overall concordance coefficients for rSMR (W=0.542, P<0.0001), rAMR (W=0.485, P=0.0011) and rAAS (W=0.517, P=0.0004) indicated significant associations amongst trials for all three energetic parameters. To determine the influence of each of the trials on the overall significance, pairwise Spearman rank-order comparisons were performed. For rSMR (Fig. 3), correlations indicated significant repeatability in all trials 5 weeks apart (Table 3). This significant relationship also persisted over the 10-week period from trial 2 to trial 4 but not from trial 1 to trial 3. Over 15 weeks, no correlation existed between rSMR. The same pattern was observed for rAMR (Fig. 4), although repeatability at the significance level of P<0.05 was absent in the first of the three 5-week periods (Table 3). rAAS was significantly repeatable across both 5 and 10 weeks, although correlations were weaker for the 10-week periods (Table 3). As for rSMR and rAMR, no significant repeatability of rAAS was found across 15 weeks. In other words, repeatability of rSMR, rAMR and rAAS tended to gradually decline across the entire experimental period (Fig. 5).

Mean SGRs for trout across the various trial periods ranged from 0.13±0.01 to 0.62±0.02% day^{-1} (Table 4). For the full combination of trials, we observed either a significant negative correlation between individual SGR and rSMR at the start of the growing period or no correlation at all (Table 4), implying that fish with higher than expected SMRs either grew less than or the same as conspecifics with lower than expected SMRs.

DISCUSSION

In this study, the repeatability of body-mass-corrected SMR, AMR and AAS gradually decreased over time, being highest over periods of 5 weeks (SMR, r_S=0.52–0.58; AMR, r_S=0.32–0.38; AAS, r_S=0.39–0.43), decreasing over 10 weeks (SMR, r_S=0.16–0.42; AMR, r_S=0.25–0.35; AAS, r_S=0.35–0.36) and disappearing altogether over 15 weeks (SMR, r_S=0.09; AMR, r_S=0.23; AAS, r_S=0.21). Gradual decline in repeatability of $\dot{M}_{O_2}$ is commonly found in studies on birds, mammals and lizards (De Vera and Hayes, 1995; Chappell et al., 1995; Chappell et al., 1996; Broggi et al., 2009), although the magnitude of the decrease often is slight and the trait

Table 3. Repeatability of body mass, residual standard and active metabolic rate, and residual absolute aerobic scope for pairwise combinations of all trials

		M		rSMR		rAMR		rAAS	
Trial	Interval (weeks)	r_S	*P*	r_S	*P*	r_S	*P*	r_S	*P*
1 *vs* 2	5	0.894	<0.0001	0.569	0.0006	0.316^{NS}	0.073	0.390	0.025
2 *vs* 3	5	0.920	<0.0001	0.579	0.0004	0.362	0.038	0.413	0.017
3 *vs* 4	5	0.973	<0.0001	0.517	0.002	0.380	0.030	0.425	0.014
1 *vs* 3	10	0.824	<0.0001	0.163^{NS}	0.363	0.253^{NS}	0.155	0.345	0.049
2 *vs* 4	10	0.897	<0.0001	0.416	0.016	0.346	0.048	0.359	0.040
1 *vs* 4	15	0.786	<0.0001	0.093^{NS}	0.604	0.225^{NS}	0.206	0.206^{NS}	0.249

M, body mass; rAAS, residual absolute aerobic scope; rAMR, residual active metabolic rate; rSMR, residual standard metabolic rate.
NS, not significant.

under investigation still significantly repeatable. For fish, estimates of repeatability often have been based on only two time points across the experimental period (McCarthy, 2000; Reidy et al., 2000; Virani and Rees, 2000; Maciak and Konarzewski, 2010) or, in those studies including three or four time points, repeatability has been evaluated as one overall measure (O'Connor et al., 2000; Cutts et al., 2001), making an assessment of any gradual decline difficult. McCarthy found repeatability of rSMR in Atlantic salmon (*Salmo salar*) to be 0.68 over a 16 week period (McCarthy, 2000). For the spined loach (*Cobitis taenia*), Maciak and Konarzewskifound repeatabilities of rSMR of 0.68 and 0.73 in normoxia and hypoxia, respectively, over a 5 month period (Maciak and Konarzewski, 2010). These values are at best comparable to repeatabilities found across the 5 week intervals in the present study, but not over longer time periods where repeatability disappeared altogether. In a study by O'Connor et al. (O'Connor et al., 2000), rSMR of Atlantic salmon was found to be repeatable over a period of 8 weeks (r=0.40), even though the fish had been starved in the middle of the period. This repeatability of 0.40 is similar to values from the present study across the same time frame.

Reidy et al. (Reidy et al., 2000) provide estimates of repeatability of $\dot{M}_{O_2}$ in swimming Atlantic cod (*Gadus morhua*), but only include values at swimming speeds below critical swimming speed (U_{crit}), which otherwise could have been analogous to AMR in the present study. Nevertheless, these authors present a repeatability of $\dot{M}_{O_2}$ of 0.83 over 3 months at a swimming speed of 50 cm s^{-1}, the speed closest to the mean U_{crit} of 58.4 cm s^{-1} found in their study. This value of repeatability seems quite high compared with repeatabilities of AMR from our study, which at best reached 0.38 over a 5 week period. The high repeatability reported for the cod could be due to the different, less conservative, mass-independent standardisation procedure used, where $\dot{M}_{O_2}$ values for fish weighing between 0.67 and 2.66 kg were adjusted to a standard mass of 1 kg using a mass exponent of 0.8.

A somewhat peculiar finding in the present study is the unusually high exponents for scaling of SMR with body mass. Since the study

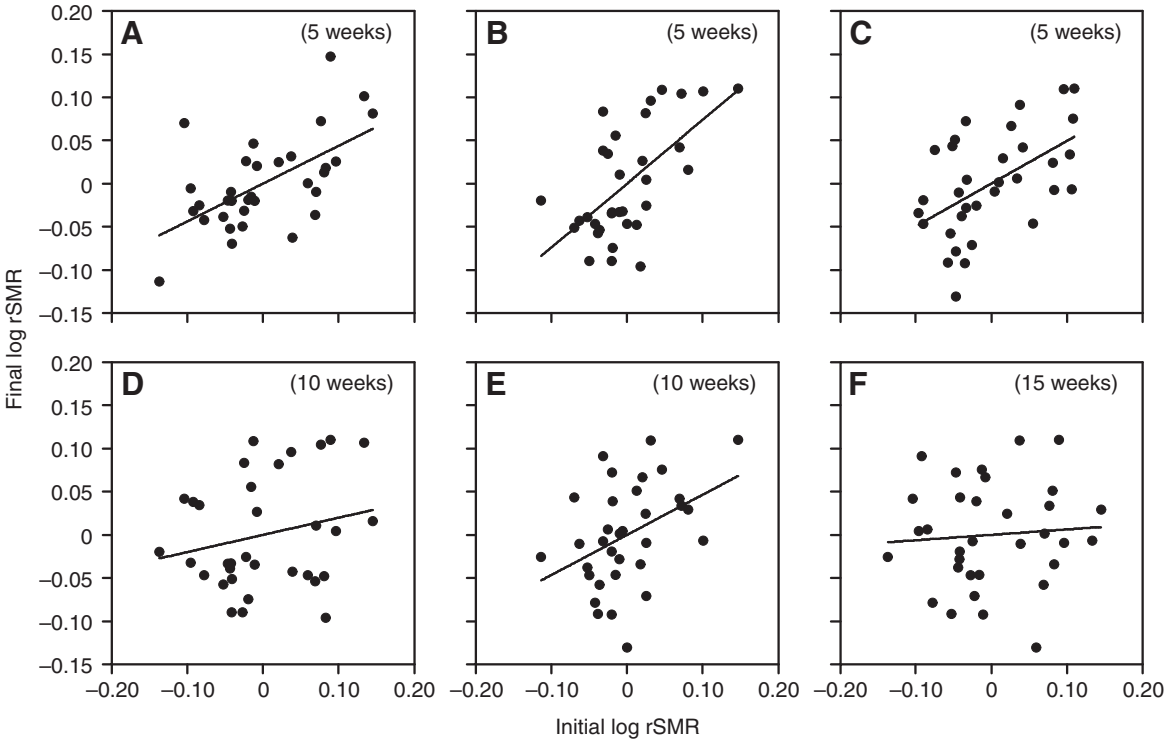

Fig. 3. Repeatability of residual (body-mass-corrected) standard metabolic rate (rSMR, μmol O_2 min^{-1}) in brown trout for the three 5-week periods (A, trial 1 *vs* 2; B, 2 *vs* 3; C, 3 *vs* 4), two 10-week periods (D, 1 *vs* 3; E, 2 *vs* 4) and one 15-week period (F, 1 *vs* 4). See Table 3 for statistics.

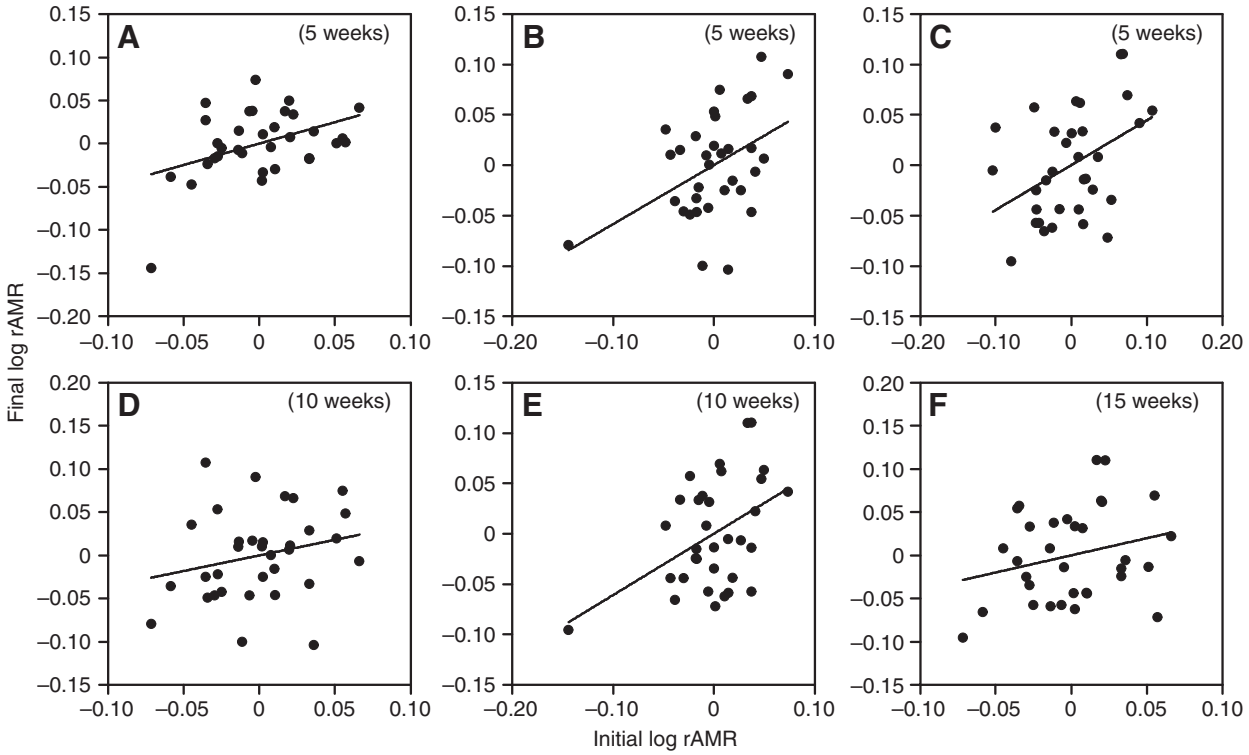

Fig. 4. Repeatability of residual (body-mass-corrected) active metabolic rate (rAMR, μmol O_2 min^{-1}) in brown trout for the three 5-week periods (A, trial 1 *vs* 2; B, 2 *vs* 3; C, 3 *vs* 4), two 10-week periods (D, 1 *vs* 3; E, 2 *vs* 4) and one 15-week period (F, 1 *vs* 4). See Table 3 for statistics.

by Brett (Brett, 1965) on oxygen consumption of swimming sockeye salmon (*Oncorhynchus nerka*), exponents for SMR have usually been found to be lower than exponents for scaling of AMR. However, high degrees of variation in scaling exponents for fish have been acknowledged in several reviews. Clarke and Johnston (Clarke and Johnston, 1999) report intraspecific exponents in the range 0.40 to 1.29, both well below and above the general interspecific exponent of 0.80 in their review. For larval fish undertaking rapid growth, Giguère et al. (Giguère et al., 1988) report exponents in the range of 0.65 to 1.69, whereas Post and Lee report slopes in the range of 0.55 to 1.14 for larval and juvenile fish with a more than tenfold difference in body mass (Post and Lee, 1996). This often-observed high variation in exponents has recently led several authors to suggest that no support for a universal exponent, nor a universal model for its prediction, exists (Bokma, 2004; Glazier, 2005; White et al., 2006; White et al., 2007). In relation to the present study, we do not have a clear answer to why the scaling exponents for SMR are so high, but it is possible that the elevation is due to a combination of age (i.e. the fish still being in a stage of ontogeny where growth is prevailing, despite the present feeding regime) and reduced food availability, in conjunction with a small difference between body masses within trials. This last factor has been shown by Bokma (Bokma, 2004) to cause large variations in exponents within species. In our study, mean body mass difference within trials (difference between largest and smallest fish) was 27.4 g. For such a mass difference, Bokma show exponents that vary up to values of 1.5 (Bokma, 2004), as high as exponents found in the present study. Reduced food availability as a cause of high exponents is supported by the metabolic-level boundaries hypothesis, which predicts that decreases in metabolic rates caused by low energy availability should be accompanied by increases in scaling exponents (Glazier, 2005). Finally, scaling exponents >1 for SMR in salmonids, at similar temperatures and body masses as the present study, have been reported for both brook trout (*Salvelinus fontinalis*) (Beamish, 1964) and Atlantic salmon (Seppänen et al., 2010) and, where $\dot{M}_{O_2}$ has been measured several times in the same individuals, exponents commonly vary between trials (e.g. Cutts et al., 2001; Seppänen et al., 2010). With the exception of the somewhat low value of the scaling exponent for AMR in trial 3, these exponents

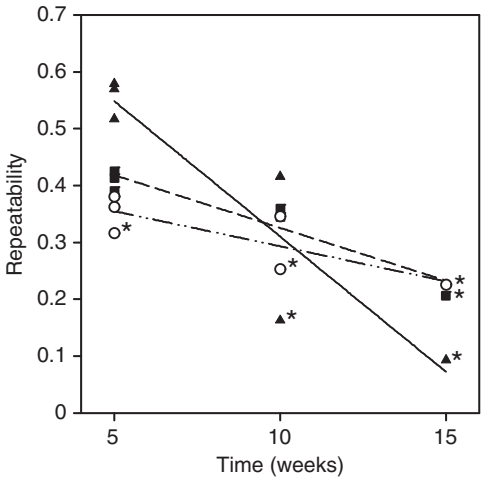

Fig. 5. Gradual decline in repeatability over time for rSMR (filled triangles, —), rAMR (open circles, –·–) and rAAS (filled squares, – –) in brown trout. Asterisks denote lack of significance (*cf.* Table 3).

Table 4. Mean specific growth rate (SGR, % day^{-1}) across trials for the 33 fish and Pearson product-moment correlation coefficients (r) for correlations of individual SGR and rSMR at the start of the growing period

Trial	Interval (weeks)	SGR	r	P
1 to 2	5	0.53±0.02	–0.364	0.037
2 to 3	5	0.62±0.02	0.031^{NS}	0.865
3 to 4	5	0.13±0.01	$–0.002^{NS}$	0.992
1 to 3	10	0.59±0.02	–0.360	0.040
2 to 4	10	0.41±0.02	$–0.060^{NS}$	0.741
1 to 4	15	0.47±0.02	–0.419	0.015

SGRs are presented as means ± s.e.m. Negative r-values indicate a negative correlation between variables.
NS, not significant.

seem representative of the generally observed values for AMR [see Glazier (Glazier, 2005) for a comprehensive review].

Even though repeatability of AMR in the present study showed the same trend as SMR, declining over time, estimates of repeatability of AMR were generally less consistent. As pointed out by Chappell et al. (Chappell et al., 1995) inconsistency in (maximal) performance may be the result of three factors, namely: (1) equipment limitations, (2) failure to elicit maximal performance of the experimental animal and (3) true physiological changes between measurements. It is possible that individual fish responded differently to the chasing protocol used to elicit maximal performance in this study, introducing inconsistency into the repeatability estimates of AMR (and thereby also AAS). This potential pitfall was also emphasised by Reidy et al. (Reidy et al., 1995), who compared different methods for eliciting exhaustion in Atlantic cod. For measurements of SMR however, inconsistency are most likely not introduced by either factor 1 or 2 and the decline in repeatability in the long term (15 weeks) should be interpreted as a true physiological change.

The high repeatability of body mass found in this study (r_S=0.79–0.97) is in agreement with the study by Maciak and Konarzewski (Maciak and Konarzewski, 2010), who observed very similar values of 0.98 and 0.99 over a 1 month interval in spined loach exposed to normoxia and hypoxia, respectively, as well as long-term repeatability of 0.86 over an interval of 5 months for a combination of normoxic and hypoxic treatments. High repeatability of body mass over long time periods has also been found for adult birds (Broggi et al., 2009) (r=0.74–0.81) and mammals (Szafrańska et al., 2007) (r=0.93–0.95). In a study on lizards (van Berkum et al., 1989), repeatability of body mass was found to be absent across early ontogeny (age 2 weeks to 13 months), but present during later life stages (age 2–13 months). These findings could indicate that repeatability of body mass does not so much depend on the time frame over which it is estimated, but rather what part of an animals' ontogeny is assessed; once you have grown big, you tend to remain so, and *vice versa*. This of course calls for an answer to what the ultimate factors may be that allow individuals within a species to outcompete others.

In Atlantic salmon, a link between SMR and dominance and aggression has been demonstrated (Metcalfe et al., 1995; Cutts et al., 1998; Metcalfe, 1998). Fish that have what seems to be an energetic disadvantage, namely a high SMR and hence high cost of living, can outcompete individuals of their own species, thereby gaining preferential access to food through their high status in the social hierarchy. A noteworthy finding in the present study is that fish with high rSMRs grew less (or the same) than conspecifics with lower rSMRs. In other words, no apparent advantage existed in having relatively high SMRs. This somewhat contradicts the findings in Atlantic salmon where the high relative SMR, found to correlate with dominance (Metcalfe et al., 1995), also correlated with growth rate (Metcalfe et al., 1992). However, as also pointed out by Metcalfe et al. (Metcalfe et al., 1995), inflexibility in an individuals' SMR would be a disadvantage whenever food is not so abundant as to offset the extra cost of maintaining a high relative SMR. Because trout in the present study were fed a moderately restricted diet throughout the experiment (compared with the normal *ad libitum* diet in other repeatability studies), it is possible that the benefits associated with high rSMRs disappeared, causing the social hierarchy to break down and the repeatability to gradually diminish. Such loss of repeatability in $\dot{M}_{O_2}$ is supported by the study of O'Connor et al. (O'Connor et al., 2000), where a period of total food deprivation caused the rank order of SMR in Atlantic salmon to become inconsistent.

In the context of selection, temporal repeatability of a trait is often used as an estimate of heritability [see Dohm (Dohm, 2002) for discussion], i.e. the trait needs to be consistent long enough for selection to have time to work on it. In relation to the present study, this would infer that selection would not work on SMR, AMR or AAS. It has often been discussed whether repeatabilities found in laboratory settings can be transferred to animals in the wild (Rønning et al., 2005; Labocha et al., 2004; Nespolo and Franco, 2007). If loss of repeatability of rSMR (or aerobic metabolism in general) in the present study is truly a consequence of the moderately restricted diet as discussed before, this phenomenon of disintegration of physiological performance would likely have a somewhat higher resemblance to natural populations (compared with where an *ad libitum* diet has been provided) because food in the wild will most often be heterogeneously distributed, both temporally and spatially. With this in mind, it would seem odd if selection did not influence a physiological parameter as important as SMR, AMR or AAS, despite its temporal instability. An alternative possibility, as also mentioned by Rønning et al. (Rønning et al., 2005), is that selection instead would work on the flexibility of a trait, giving animals with a relatively high degree of phenotypic plasticity a selective advantage over conspecifics with more restricted metabolic capacities whenever ecological variability prevails. This was also recognised by Dohm, who stated that repeatability estimates might be problematic in terms of providing bounds for heritability when the trait under investigation is highly plastic or context dependent (Dohm, 2002).

Ecologically, it is therefore likely that our study on repeatability of aerobic performance, despite being a laboratory study, more closely mimics natural conditions than studies where fish have had unlimited access to food. Studies on correlations between metabolic rate and growth in brown trout (Álvarez and Nicieza, 2005) and between dominance and growth in Atlantic salmon (Harwood et al., 2003) in the wild also suggest that shortage of food tends to dissolve any relationships between traits, inferring no apparent advantage to high SMR or dominance.

In conclusion, we found that total body mass was highly stable over a period of 15 weeks, whereas rSMR, rAMR and rAAS did not retain repeatability. In fact, we observed a gradual disappearance of repeatability of both SMR and AMR, as well as AAS, across the entire 15-week experimental period. SGR was either uncorrelated or negatively correlated with rSMR, indicating that fish with high rSMRs were at a disadvantage, at least under the present (slightly restricted) feeding regime. We propose that the gradual disappearance of repeatability of rSMR (and possibly overall aerobic performance) is due to this feeding regime, and that such a scenario, compared with an *ad libitum* feeding regime, more closely resembles situations encountered by fish in the wild.

LIST OF SYMBOLS AND ABBREVIATIONS

AAS	absolute aerobic scope
AMR	active metabolic rate
F_{O_2}	fraction of oxygen in atmosphere
$\dot{M}_{O_2}$	oxygen consumption rate
P_{BAR}	barometric pressure
P_{H_2O}	water vapour pressure
PIT	passive integrated transponder
$P_{W_{O_2}}$	partial pressure of oxygen in water
rAAS	residual absolute aerobic scope
rAMR	residual active metabolic rate
rSMR	residual standard metabolic rate
SGR	specific growth rate
SMR	standard metabolic rate
U_{crit}	critical swimming speed
V_{resp}	volume of respirometer (minus volume of fish)
$\beta_{W_{O_2}}$	solubility coefficient of oxygen in water

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