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RESEARCH ARTICLE

Effects of body mass on physiological and anatomical parameters of mature salmon: evidence against a universal heart rate scaling exponent

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SUMMARY

The influence of body mass (M_b) on the physiology of large, adult fish is poorly understood, in part because of the logistical difficulties of studying large individuals. For the first time, this study quantified the influence of M_b on the resting heart rate (f_H), blood properties and organ masses of adults of a large-growing fish species, the Chinook salmon (*Oncorhynchus tshawytscha*). Surgically implanted biologgers measured f_H and acceleration activity in sexually mature, male fish ranging in M_b from 2.7 to 16.8 kg while they roamed freely in a controlled water body at ~8°C. Blood parameters (at surgery and at death) and body organ masses (at death) were measured to investigate interrelationships with M_b. The scaling exponents for both f_H and acceleration activity were not significantly different from zero. The lack of scaling of f_H with M_b contrasts with the situation for birds and mammals. All blood parameters were independent of M_b, while the masses of the compact myocardium, ventricle and spleen each scaled near-isometrically with M_b. These data raise the possibility that blood oxygen carrying capacity, mass-specific cardiac output and cardiac power output are maintained across M_b in adult Chinook salmon. Biologging and biotelemetry should advance investigations into the effects of M_b on the physiology and behaviour of large fish, where current knowledge lags far behind that of birds and mammals.

Key words: allometry, allometric, biologging, biotelemetry, cardiorespiratory, cardiovascular, Chinook salmon, data logging, Pacific salmonids, teleost fishes.

INTRODUCTION

Fish undergo more extreme changes in body mass (M_b) than any other vertebrate, with some species increasing in M_b by more than a millionfold in their lifetime (e.g. Wardle et al., 1989; Kaji et al., 1996). Body mass can affect the rate of all biological processes from cellular metabolism to population dynamics (Schmidt-Nielsen, 1989). Indeed, physiological rate processes often vary with M_b according to the relationship aM_b^b, where a is the scaling factor (which defines the height or elevation of the curve) and b is the scaling exponent (which defines the shape and direction of the curve).

While there remains some debate about whether tissue metabolism drives circulatory oxygen and nutrient transport or *vice versa* (e.g. Coulson, 1986; Agutter and Wheatley, 2004), West and colleagues proposed a universal model for animals with closed circulatory systems (space-filling fractal networks of branching tubes) of the mass dependence of oxygen and nutrient transport (West et al., 1997). The model predicted that oxygen consumption rate ($\dot{M}_{O_2}$) and heart rate (f_H) should universally scale with M_b with exponents of 0.75 and –0.25, respectively. Intra- and inter-specific scaling of $\dot{M}_{O_2}$ subsequently has received additional attention in a range of vertebrates, sparking debate over the existence of a universal $\dot{M}_{O_2}$ scaling exponent (e.g. Clarke and Johnson, 1999; Frappell et al., 2001; White et al., 2006; Killen et al., 2007; Moran and Wells, 2007; Glazier, 2009; White, 2010). However, few studies have examined cardiovascular scaling in vertebrates (e.g. Brody, 1945; Stahl, 1967; Lindstedt and Calder, 1981), with the data being particularly scant for fish.

The limited data set for fish is in part technical. The inherent complexities of tethering large fish to recording equipment have precluded many cardiovascular measurements in fish over ~3 kg (Farrell, 1991; Lillywhite et al., 1999; White and Seymour, 2011). To date, an obvious inter-specific f_H scaling exponent for fish has not emerged (Farrell, 1991; Lillywhite et al., 1999; White and Seymour, 2011), although caution is required when interpreting data from multiple sources because of the large range of methodologies, water temperatures and post-handling recovery times used between studies. Regarding intra-specific f_H scaling in fish, existing data are equally limited and often conflicting (see Mirkovic and Rombough, 1998; Barrionuevo and Burggren, 1999). Indeed, intra-specific scaling exponents for f_H and $\dot{M}_{O_2}$ have been shown to vary throughout development (e.g. Barrionuevo and Burggren, 1999; Wuenschel et al., 2004; Moran and Wells, 2007), thus precluding extrapolation of scaling exponents to adult fish and highlighting the need to account for ontogeny and maturity when examining M_b scaling.

To circumvent some of the above constraints, the present study used implantable biologging technology (see Clark et al., 2008c; Clark et al., 2010) and took advantage of the diverse life history of Chinook salmon (*Oncorhynchus tshawytscha*), the largest of the Pacific salmonids (Groot and Margolis, 1991). After emerging from the egg with a M_b of <1 g (Kinnison et al., 1998), juvenile Chinook salmon typically remain in freshwater for around 1 year prior to commencing an ocean migration to grow and mature. Whereas the majority of individuals spend around 3 years in the ocean and return to freshwater spawning grounds as 4 year olds, some individuals leave the ocean as 2 or 3 year olds (primarily males), or late as 5 or 6 year olds. These different intra-specific life history strategies,

particularly for males, give rise to nearly an order of magnitude range in M_b among individuals at the same level of sexual maturity, which provides a novel opportunity to examine the intra-specific effects of M_b on the physiology and behaviour of wild fish.

The present study used surgically implanted biologgers to measure f_H in adult wild Chinook salmon with M_b ranging from 2.7 to 16.8 kg. A focus on male fish removed the potential for any confounding effects relating to sex-specific differences, which are common among salmonids (e.g. Clark et al., 2009; Sandblom et al., 2009). Long-term measurements allowed an examination of the effects of M_b on f_H while fish roamed freely in a large, controlled water body. The biologgers were also equipped with 2D accelerometers, which helped to quantify the interrelationships between f_H, M_b and activity levels. Additionally, in the light of previous findings for other vertebrates (e.g. Lindstedt and Schaeffer, 2002; Kjeld and Ólafsson, 2008), blood variables and organ masses were measured to examine for relationships with M_b. By providing the first insight into the effects of M_b on f_H among adults of any large-growing fish species, the present study helps to test the applicability of a universal f_H scaling exponent of –0.25.

MATERIALS AND METHODS

Animals

Wild, male Chinook salmon (*Oncorhynchus tshawytscha*, Walbaum 1792) from a single genetic stock (Harrison) were used during October and November 2008. Nine fish (M_b range= 2.7–16.8 kg) were caught with a beach seine net from the Harrison River, BC, Canada, near the completion of their 140 km upriver migration to spawn. Fish were transported by road (~10 min) to the Fisheries and Oceans Canada Chehalis River Hatchery, where they were placed into a large concrete holding raceway with flow-through river water and allowed to recover from handling for 4–7 days before being used. None of the fish had spawned, although all were sexually mature as evidenced by the release of milt when gently squeezed.

All experiments were conducted with the approval of the Animal Care Committee of the University of British Columbia, in accordance with the Canadian Council on Animal Care.

Surgical implantation of biologgers

The functioning and surgical implantation of biologgers have been detailed previously (Clark et al., 2008c; Clark et al., 2009; Clark et al., 2010). On 27 October 2008, individual fish were dip-netted and placed into an anaesthetic bath containing 100 mg l^{-1} tricaine methanesulfonate (MS222; Sigma, St Louis, MO, USA) and 200 mg l^{-1} sodium bicarbonate ($NaHCO_3$) to buffer pH. Once the fish lost equilibrium (<2 min), a 2 ml blood sample was taken by caudal puncture into a heparinised vacutainer and the sample was stored on ice for subsequent analyses. Upon complete anaesthesia, the fish was removed from the anaesthetic bath, weighed and placed ventral side down on a surgery bench where the gills were continuously irrigated with chilled water containing a maintenance dose of anaesthetic (60 mg l^{-1} MS222 buffered with 120 mg l^{-1} $NaHCO_3$). An identification tag (Peterson discs; Floy Tag, http://www.floytag.com) was attached dorsally before rolling the fish supine to implant a biologger. A sterilised biologger (iLogR, mass 23 g in air; B. D. Taylor, La Trobe University, Melbourne, Australia) was inserted into the visceral cavity through a 30–40 mm ventral midline incision anterior to the ventral fins. The biologger was loosely secured with one suture to the inside visceral wall and associated tissue, and the incision was closed with 6–9 silk sutures. Following the 20 min surgical procedure, the fish was placed into an experimental holding channel (L×W×D=10×5×2 m; water depth ~80 cm), where recovery was assisted until the fish could maintain equilibrium (<10 min).

Every 10 min, the biologger recorded the date, time and temperature (visceral temperature), immediately followed by a 10.14 s recording at 200 Hz (i.e. every 5 ms) of the electrocardiogram (ECG) and the acceleration in the *X*- and *Y*-axes. The orientation of the biologger was such that the *X*-axis recorded lateral and dorso-ventral acceleration (including lateral acceleration associated with tail beats), and the *Y*-axis recorded rostro-caudal acceleration (i.e. any backward or forward acceleration). The acceleration data were packaged as described previously (Clark et al., 2010). Consequently, a single *X*- and a single *Y*-value were archived to the memory of the biologger to provide an index of total acceleration in each axis throughout each 10.14 s measurement period. This derivation of acceleration from each axis is herein referred to as 'acceleration activity'.

Experimental protocol

The primary objective of this study was to acquire reliable measurements of f_H and acceleration activity from unstressed and untethered Chinook salmon that spanned the largest range in M_b available for these adult fish. Consequently, we limited human observations of the fish to two 15 min periods per day, when general behavioural patterns and physical condition were assessed. However, following 8–9 days of data logging (6 November), experimenters had to enter the holding channel for 20 min of equipment maintenance, which startled the fish and induced high levels of burst swimming activity.

On 8 November, all fish were corralled, individually dip-netted and killed with a sharp blow to the head to retrieve the biologgers. A second blood sample was taken, as above, within 30 s of death. M_b and fork length (FL) were measured for each fish, followed by dissection to obtain mass measurements of the liver, spleen and emptied ventricle. Ventricles were subsequently prepared as described previously (Farrell et al., 2007) for quantification of the two (compact and spongy) ventricular myocardial layers.

Blood analyses

Haematocrit (Hct) was determined using micro-haematocrit capillary tubes spun at 10,000 ***g*** for 4 min. Haemoglobin concentration ([Hb]) was determined with a handheld haemoglobin analyser (HemoCue 201^+, Ängelholm, Sweden) calibrated for fish blood (Clark et al., 2008a). The mean cell haemoglobin concentration (MCHC) was calculated as [Hb]/(Hct/100). Remaining blood was spun and the plasma was collected and first frozen in liquid nitrogen before being placed at –80°C for subsequent analyses. Plasma cortisol, testosterone, lactate, glucose, osmolality, chloride, sodium and potassium were measured according to the methods outlined elsewhere (Farrell et al., 2001; Clark et al., 2010). Osmolality was measured only for the blood samples taken at the time of death.

Data analyses and statistics

The text file from each biologger was imported into LabChart software (ADInstruments, Sydney, Australia) for subsequent analyses. A rate-meter function was applied to the ECG data to calculate instantaneous f_H, and all data were inspected manually to ensure accurate values. Data were used only following 24 h of recovery from surgery. Data from each fish were averaged in 3 h blocks, where indicated herein, for the analysis of resting values. To account for minor changes in water temperature during the experimental period (range ~7–9°C), f_H data were standardised to a common temperature of 8°C using a Q_{10} of 2.3 (Clark et al., 2010).

Acceleration activity refers to the sum of *X*-axis and *Y*-axis data herein, unless otherwise indicated.

Statistical analyses were performed with the programs SigmaStat (Build 3.01.0, Systat Software, San Jose, CA, USA), SPSS (Build 16.0, Chicago, IL, USA) and Microsoft Excel (Redmond, WA, USA). Data were $\log_{10}$-transformed before statistical analyses. Regression analyses were used to determine the relationships between M_b and other measured variables. Additionally, correlation analyses (Spearman's rank order) were used to examine for statistically significant correlations between M_b and each blood variable. Monte Carlo simulation was used to examine the validity of the calculated f_H scaling exponents, given the relatively small sample size (N=9) and M_b range (0.8 orders of magnitude) (see Results). Statistical significance was considered at $P<0.05$. Values given are means ± s.e.m. unless otherwise indicated.

RESULTS

Body mass, morphometrics and blood variables

Adult Chinook salmon generally do not feed once they commence their coastal and river migration. Consequently, the fish in the present study lost an average of 5.7±0.5% M_b during the experimental period (Table 1) such that M_b and FL of the fish at death were 2.5–15.9 kg and 62–105 cm, respectively. Body mass was assumed to decrease linearly when calculating daily M_b during the experiment. Masses of the compact myocardium, ventricle and spleen each scaled with M_b (at death) with an exponent close to isometric (Fig. 1). The scaling exponent for liver mass was 0.84 (Fig. 1), indicating a relatively smaller liver mass with increasing M_b. All blood variables, both at the time of biologger implantation (initial) and at death (final), were independent of M_b. All blood variables differed significantly between the initial and final measurements, with the exception of [Hb] (Table 1).

Fish behaviour, heart rate and acceleration activity

Fish observed in the holding channel had few interactions and no obvious signs of aggression, possibly due to the absence of females. Consistent with these visual observations, biologger data revealed stable f_H and acceleration activity for most of the day and night, interspersed with only brief periods (~2–3 per day) of elevated activity (e.g. Fig. 2). Conversely, when equipment maintenance on 6 November triggered burst swimming activity, f_H increased up to ~60 beats min^{-1} in some individuals and took ~10 h to recover (Fig. 2). No attempt was made to correlate M_b with the elevated levels of f_H because the intensity of burst swimming could not be accurately quantified across individuals.

Analyses of 3 h blocks of f_H data revealed low variability in f_H around the mean for the 3 h period (e.g. Fig. 2, top panel), and so these blocks of data were analysed to determine (1) the lowest 3 h mean value obtained for each fish within a single day (29 October; Fig. 3A) and (2) the lowest 3 h mean value obtained for each fish over the entire experimental period (Fig. 3C). These analyses revealed f_H scaling exponents that were not significantly different from zero (0.056 and 0.085, respectively), rather than the expected value of –0.25 (cf. bold and dotted lines in Fig. 3A,C). The scaling exponents for acceleration activity ranged from –0.119 to –0.104 (Fig. 3B,D) and were primarily driven by a linear decrease in *X*-axis rather than *Y*-axis acceleration (Fig. 3, insets), but they were not significantly different from zero. Frequency histograms illustrate an increasing modal f_H and decreasing modal acceleration activity with increasing M_b (Fig. 4).

Monte Carlo simulation was used to test the idea that a relatively small sample size and M_b range masked a true f_H scaling exponent of –0.25. Following Garland (Garland, 1984), coefficients of variation (CV) were calculated as the standard deviation of residuals from the relationship between $\log M_b$ and $\log f_H$ as 5.3% for the data in Fig. 3A and 9.3% for the data in Fig. 3C. Hypothetical f_H data

Table 1. Mean values±s.e.m. (ranges in parentheses) for body mass (M_b) and blood variables for mature male Chinook salmon (*Oncorhynchus tshawytscha*) at the time of biologger implantation (Initial) and at death (Final)

Variable	Initial	Final	%Change	Significance
M_b (kg)	8.7±1.7	8.2±1.6	–5.7±0.5	**0.006**
	(2.7 to 16.8)	(2.5 to 15.9)	(–8.8 to –3.9)	
Haemoglobin concentration (g l^{-1})	113±3	116±8	0.4±4.1	0.851
	(98 to 127)	(95 to 144)	(–15.3 to 12.8)	
Haematocrit (%)	62±3	53±5	–13.5±3.3	**0.008**
	(50 to 73)	(41 to 69)	(–25.9 to –5.6)	
MCHC (g l^{-1})	187±8	222±7	16.2±2.3	**<0.001**
	(149 to 224)	(206 to 252)	(6.0 to 21.9)	
Glucose (mmol l^{-1})	10.1±0.9	19.6±1.7	111.6±22.7	**0.002**
	(7.5 to 16.2)	(12.7 to 24.1)	(50.1 to 186.5)	
Lactate (mmol l^{-1})	4.3±0.3	6.8±0.8	58.8±21.1	**0.021**
	(3.5 to 5.6)	(4.1 to 9.1)	(8.5 to 157.3)	
Chloride (mmol l^{-1})	116±2	83±10	–27.9±8.3	**0.018**
	(108 to 124)	(51 to 113)	(–53.6 to –1.2)	
Sodium (mmol l^{-1})	154±5	118±8	–23.9±4.9	**0.005**
	(136 to 175)	(96 to 143)	(–35.2 to –6.4)	
Potassium (mmol l^{-1})	1.1±0.3	0.5±0.2	–51.5±12.8	**0.014**
	(0.4 to 3.0)	(0.1 to 1.3)	(–84.5 to –11.7)	
Osmolality (mOsm kg^{-1})	–	272±12	–	–
	–	(242 to 312)	–	
Cortisol (ng ml^{-1})	369±43	799±148	114.3±16.7	**0.007**
	(206 to 567)	(397 to 1217)	(73.1 to 189.3)	
Testosterone (ng ml^{-1})	46±4	30±8	–39.6±14.0	**0.049**
	(25 to 68)	(5 to 53)	(–88.6 to 2.3)	

MCHC, mean cell haemoglobin concentration.

%Change was calculated for each individual as Final/Initial×100–100. Significant differences between Initial and Final values were determined using paired *t*-tests (*P*-values given; $P<0.05$ in bold font).

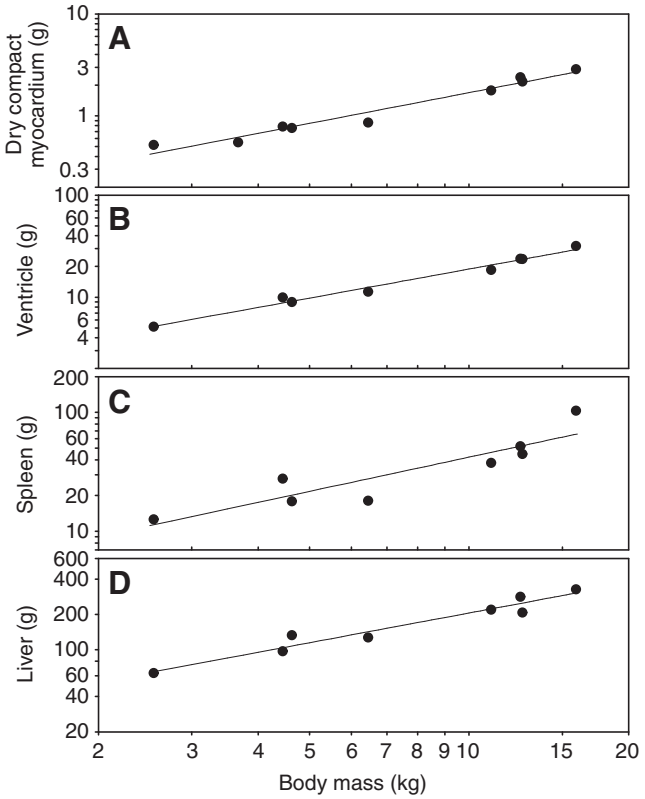

Fig. 1. Relationships between body mass (M_b) and each of (A) dry compact myocardium mass, (B) wet ventricle mass, (C) wet spleen mass and (D) wet liver mass for mature male Chinook salmon (*Oncorhynchus tshawytscha*) at the end of the experimental period (N=8–9; measurements for B–D were not made for one fish). Horizontal and vertical axes are presented on $\log_{10}$ scales. Regression lines are described by: (A) dry compact myocardium mass=$0.168M_b^{1.002}$ (r^2=0.96, P<0.001); (B) ventricle mass=$2.14M_b^{0.95}$ (r^2=0.98, P<0.001); (C) spleen mass=$4.67M_b^{0.95}$ (r^2=0.81, P=0.002); (D) liver mass=$29.66M_b^{0.84}$ (r^2=0.95, P<0.001).

were calculated as $-0.25\log M_b$ for nine 'fish' with masses equal to those measured on 29 October. For each value of f_H, a randomly generated normal deviate with a mean of zero and standard deviation equal to the CV of the appropriate relationship (5.3% or 9.3%) was then added to simulate variation between individuals. The slope of the relationship between $\log M_b$ and $\log f_H$ was then calculated, and the process repeated 10,000 times. For CVs of 5.3% and 9.3%, none of the 10,000 relationships had an exponent greater than zero; 95% of the 10,000 exponents were lower than –0.20 and –0.17 for CVs of 5.3% and 9.3%, respectively. Even with the CV increased to 30%, the percentage of resulting exponents greater than zero was ~6%. As such, the probability of finding scaling exponents of 0.056 and 0.085 when the 'true' scaling exponent is –0.25 is extremely remote.

DISCUSSION

Critique of methods

The present study represents the first use of biologging technology to investigate the effects of M_b on f_H in any fish. By minimising the stress of tethering and confinement [which can reduce vagal tone, increase adrenergic tone and consequently elevate resting f_H (Olson and Farrell, 2006)], the resting values for f_H measured in this study are among the lowest recorded for salmonids at similar temperatures (Clark et al., 2009; Sandblom et al., 2009; Clark et al., 2010). Although the fish were free to roam, acceleration activity was minimal [cf. fig. 4 in Clark et al. (Clark et al., 2010)] and did not correlate significantly with M_b. Thus, activity did not compromise investigations into the effects of M_b on resting f_H, and it is likely that allowing this schooling species to roam freely with conspecifics helped to minimise stress.

We also removed the confounding effects of sex, species and sexual maturity on the analysis of M_b scaling, but could not distinguish between the effects of M_b and age because these two parameters co-varied. It could be argued that resting f_H increased with fish age and therefore masked an underlying negative scaling exponent for f_H. However, this possibility is highly unlikely in the light of current knowledge of the effects of ageing on the vertebrate cardiovascular system, where resting f_H has been found to remain constant or decrease with adult age (Ringer et al., 1957; Fleg et al., 1995). Also, the blood variables suggested that ageing was not associated with other significant physiological alterations that might change cardiovascular function (e.g. neither testosterone nor cortisol was dependent on M_b). The (mass-independent) differences between the properties of the initial and final blood samples were probably consequences of advanced maturity [e.g. increased cortisol (see Sandblom et al., 2009)] as well as the corralling procedure immediately prior to death (e.g. increased lactate).

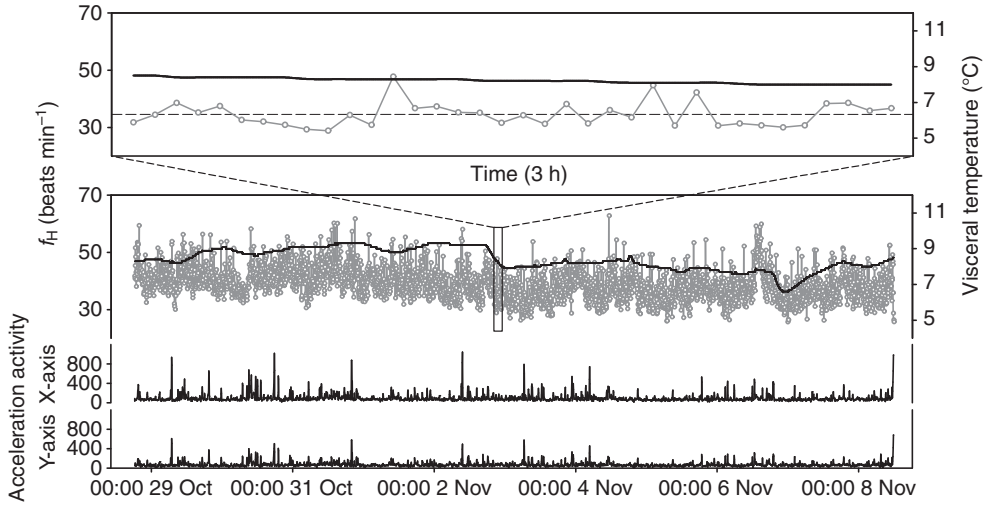

Fig. 2. Representative traces for an individual male Chinook salmon of biologged heart rate (f_H; open circles and grey line), visceral temperature (black line) and *X*- and *Y*-axis acceleration over the entire experimental period (bottom panel) and over a 3 h period where f_H was at a minimum (top panel). Horizontal dashed line in the top panel indicates the mean f_H of 34.5 beats min^{-1} obtained over the 3 h period. Heart rate data from the top panel contributed to Fig. 3C following standardisation to a common temperature of 8°C. Human disturbance on 6 November caused a prolonged elevation in f_H (see Materials and methods). The fish was 6.8 kg at the time of biologger implantation and decreased to 6.5 kg over the ~10 day experimental period.

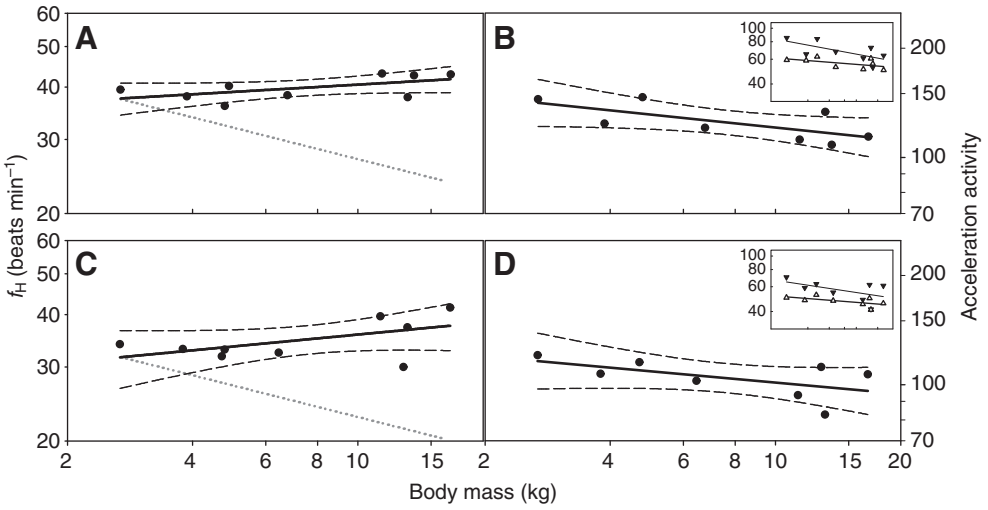

Fig. 3. Relationships between M_b and (A,C) f_H and (B,D) acceleration activity (AA) for mature male Chinook salmon (N=9). All horizontal and vertical axes are presented on $\log_{10}$ scales. (A,B) Lowest 3 h mean value obtained for each fish within a single day (29 October 2008), (C,D) lowest 3 h mean value obtained for each fish over the entire experimental period. Acceleration data were not obtained from one individual. To account for small changes in water temperature throughout the experimental period, f_H data were standardised to a common temperature of 8°C using a Q_{10} of 2.3 (Clark et al., 2010). Regression lines (standard errors in parentheses) are described by: (A) f_H=35.563(±1.066)×$M_b^{0.056(\pm0.031)}$ (r^2=0.32, P=0.111); (B) AA=158.855(±1.112)×$M_b^{-0.119(\pm0.050)}$ (r^2=0.49, P=0.054); (C) f_H=29.309(±1.215)×$M_b^{0.085(\pm0.054)}$ (r^2=0.26, P=0.162); (D) AA=128.529(±1.135)×$M_b^{-0.104(\pm0.060)}$ (r^2=0.33, P=0.135). Dashed lines are 95% confidence intervals. Dotted grey lines in A and C represent theoretical regression lines assuming a scaling exponent of –0.25. Insets in B and D display the acceleration for the X- (inverted filled triangles) and Y-axes (upright open triangles).

f_H scaling in fish

Contrary to our current understanding of birds and mammals (Stahl, 1967; Lindstedt and Calder, 1981; West et al., 1997; Lindstedt and Schaeffer, 2002), f_H of adult Chinook salmon did not scale with $M_b^{-0.25}$ (Fig. 3). While examining the intra-specific effects of M_b in adult fish offers many advantages, including an ability to maintain identical experimental conditions across individuals, an examination of inter-specific effects has the potential to allow a greater range in M_b across which to identify scaling relationships. Nevertheless, measurements of f_H from adult fish are primarily limited to small species, and examinations of inter-specific f_H scaling have yielded similar results to those of the present study, where b≈0 (e.g. Farrell, 1991; Lillywhite et al., 1999). Although this topic requires significantly more attention, there does not appear to be a clear allometric relationship between f_H and M_b in fish. Indeed, a comparison of the data from the present study with those in fig. 1 of Mirkovic and Rombough (Mirkovic and Rombough, 1998) (assuming 8°C) indicates similar heart rates in adult Chinook salmon (30–40 beats min^{-1}) to those in juveniles of the related rainbow trout (*Oncorhynchus mykiss*) at a M_b of 0.02–0.05 g (50–60 beats min^{-1}). The difference in f_H would be approximately 1000 beats min^{-1} if b=–0.25, such as in birds and mammals. An intra-specific examination of the influence of M_b on the activity of cardiac pacemaker cells would be interesting in this context.

Salmonids apparently have an upper limit to f_H of approximately 2 Hz, which is purported to be related to calcium handling by cardiomyocytes during excitation–contraction coupling (Farrell, 1991). Indeed, a limited involvement of the sarcoplasmic reticulum in delivering activator calcium is associated with fish cardiomyocytes retaining a large surface area to volume ratio by undergoing hyperplasia to a greater degree than hypertrophy during cardiac growth (Farrell et al., 1988; Sun et al., 2009). Consequently, salmon maintain a near-constant cardiomyocyte surface area to volume ratio despite isometric ventricular growth, a situation that contrasts diametrically with the situation in mammalian hearts, where postnatal growth of cardiomyocytes is largely through hypertrophy, resulting in a progressively smaller surface area to volume ratio with isometric cardiac growth. Thus, a fascinating possibility is that the constant, small size of salmon cardiomyocytes leads to an independence of f_H from M_b, something that could be explored with future studies on isolated cardiomyocytes.

Body mass and the circulatory system of Chinook salmon

Sufficient data now exist to permit an investigation of the effects of M_b on multiple aspects of the circulatory system of adult Chinook salmon. Similar levels of [Hb], Hct and MCHC across M_b (Table 1) suggest that maximum blood oxygen carrying capacity is mass independent, at least when water temperature is optimal. At high temperature extremes, there is some evidence that larger individuals cannot maintain arterial oxygen saturation (Clark et al., 2008b). Gill surface area generally has a scaling exponent of b≈0.8 (Gray, 1954; Hughes, 1966; Oikawa and Itazawa, 1985) and therefore may play some role in this finding, as might allometric scaling of gas diffusion distances across the gill epithelium. In terms of tissue oxygen delivery, capillary density decreased in the red skeletal muscle of rainbow trout with increasing M_b, whereas mass-specific myoglobin content scaled positively (b=0.7) and oxidative enzymes were essentially independent of M_b (b=–0.01 to –0.02) (Young and Egginton, 2009). Thus, the scaling of f_H in Chinook salmon is more similar to that of the oxidative enzymes of red muscle in the related rainbow trout.

As with resting f_H in Chinook salmon (Fig. 3), mass-specific citrate synthase activity and mitochondrial density of the rainbow trout ventricle are thought to be mass independent (Rodnick and Williams, 1999). However, ventricular and compact myocardial mass of Chinook salmon scaled isometrically with M_b (Fig. 1). As ventricular volume and mass are determinants of cardiac stroke volume and cardiac power output, the possibility exists that these

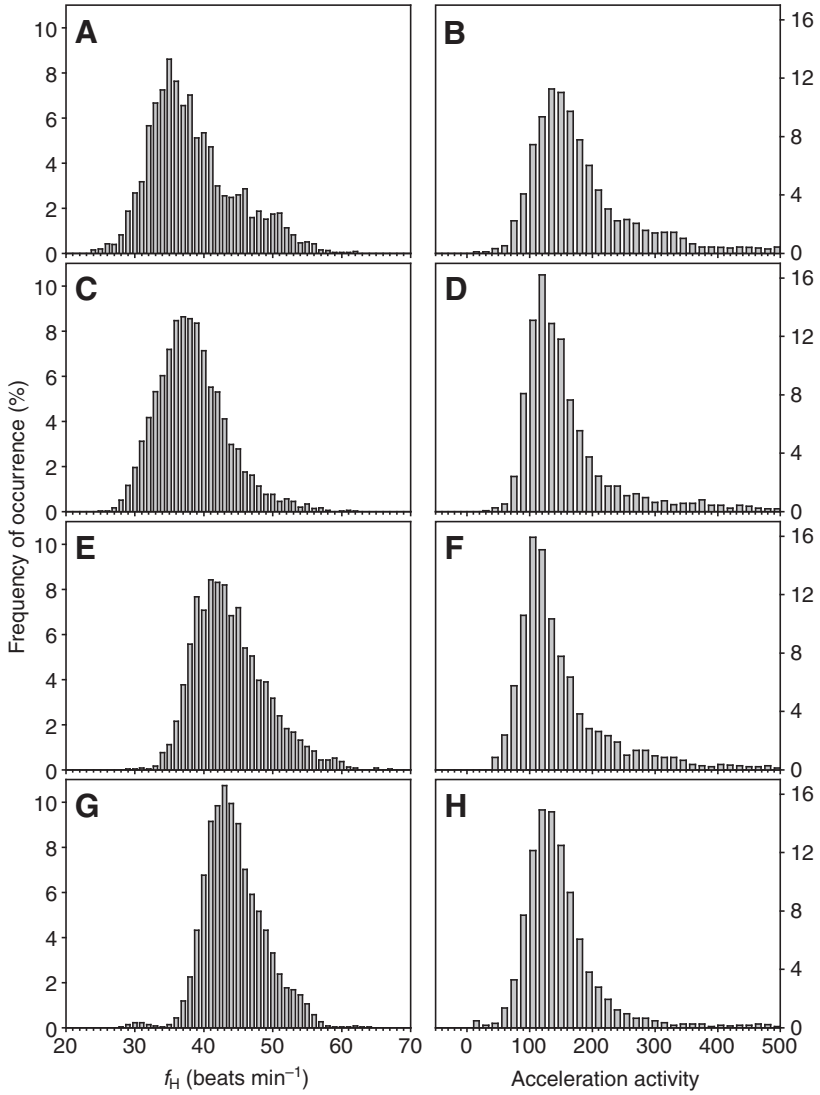

Fig. 4. Frequency histograms for four male Chinook salmon of biologged f_H and acceleration activity (sum of *X*- and *Y*-axes) over the entire experimental period (10.14 s recording every 10 min). To account for small changes in water temperature throughout the experimental period, f_H data were standardised to a common temperature of 8°C using a Q_{10} of 2.3 (Clark et al., 2010). Body masses of fish at biologger implantation were (A,B) 4.9 kg, (C,D) 6.8 kg, (E,F) 13.7 kg and (G,H) 16.8 kg.

physiological variables also increase isometrically with M_b. Furthermore, if f_H is mass independent and cardiac stroke volume scales isometrically with M_b, cardiac output should also scale isometrically. This suggestion is supported by the only *in vivo* measurements of cardiac output in adult, resting Chinook salmon, where cardiac output and stroke volume at 13°C remained at ~29 ml min^{-1} kg^{-1} and ~0.49 ml beat^{-1} kg^{-1}, respectively, across a M_b range of 2.1–5.4 kg [rearranged data from Clark et al. (Clark et al., 2008b)]. Combined with reports of standard metabolic rate scaling with a high exponent in fish (b=0.88) (White et al., 2006), these ideas should prompt further intra-specific studies of the interrelationships between circulatory and metabolic scaling to determine the mechanisms underlying the disparity in scaling exponents of fish compared with birds and mammals.

CONCLUSIONS

It is unlikely that the existence of intra- and inter-specific f_H scaling in fish can be reliably determined by compiling existing data (e.g. Farrell, 1991; Lillywhite et al., 1999). Instead, there is a requirement for future empirical studies of the effects of M_b on cardiorespiratory parameters, where particular attention is paid to minimising confounding factors, especially handling stress, that may compromise results and obscure relationships with M_b. The present study utilised modern biologging technology and free-roaming fish to overcome many of the difficulties of measuring f_H in large individuals. The results provide evidence that f_H does not scale intra-specifically with M_b in adult fish, and suggest that there is little difference in f_H between juveniles and large adults of the *Oncorhynchus* genus (Mirkovic and Rombough, 1998). Considering that fish represent more than half of all living vertebrates, and that M_b varies intra- and inter-specifically by a greater degree in fish than in any other vertebrate group (Wardle et al., 1989; Wieser, 1995; Kaji et al., 1996; Nelson, 2006), the possibilities for examining the influence of M_b on physiological processes are exceptional, but remain poorly explored. Indeed, the inability of large Chinook salmon to cope with an acute temperature increase in comparison with smaller individuals (see Clark et al., 2008b) suggests an influence of M_b on the tolerance of Chinook salmon to environmental perturbations. With the current trend in the global climate (e.g. Daufresne et al., 2009), there is a particular need to understand the interrelationships between M_b and the physiology of fish when faced with stressful perturbations, including changes in water temperature, pH and salinity when undergoing different activities.

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