J. exp. Biol. **171**, 139–148 (1992)
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SEXUAL MATURITY CAN DOUBLE HEART MASS AND CARDIAC POWER OUTPUT IN MALE RAINBOW TROUT

BY CRAIG E. FRANKLIN* AND PETER S. DAVIE

Department of Physiology and Anatomy, Massey University, Palmerston North, New Zealand

Accepted 2 July 1992

Summary

Mature male rainbow trout have significantly higher relative ventricle masses (RVM, ventricle mass as a percentage of body mass) than do immature males or females. Hatchery-reared maturing male trout had a mean RVM of 0.139%, whereas females had an RVM of only 0.074%. Moreover, as males matured and their testes grew from 0.07 to 3.92% of body mass, RVM more than doubled. In female trout no such heart growth occurred; RVM remained unchanged during the period of ovary growth.

Cardiac performance was assessed using an *in situ* perfused heart preparation. Mature male trout have larger ventricles and could generate significantly greater maximum cardiac power output per kilogram body mass than could immature males or females. This enhanced cardiac performance by the mature males was attributable to delivery of greater cardiac outputs (through larger stroke volumes) and an increased ability of the heart to work against higher output pressures. Power output per gram ventricle mass was similar in both sexes.

Introduction

Trout anglers have observed that jacks are more athletic when hooked than hens, and that the strongest fighters during capture are fish that are migrating upstream to spawn. Athleticism in salmonids plays an important part in their survival and reproduction, e.g. during upstream spawning migrations and fierce territorial disputes between males over spawning sites. Although the changes in body condition and gonad morphology in preparation for spawning are well described (see Nagahama, 1983), we were interested in changes in cardiac morphology and cardiac performance relative to reproductive readiness.

Sexual dimorphism of heart mass is common in vertebrates. In adult human males the heart weighs 280–340 g (0.45% of body mass) whereas women's hearts

* Present address: Department of Zoology, University of Queensland, Brisbane 4072, Australia.

Key words: rainbow trout, sex, sexual maturity, relative ventricle mass, cardiac performance, *Oncorhynchus mykiss*.

weigh 230–280 g (0.40 % of body mass) (Williams and Warwick, 1980). Northrup *et al.* (1957) found that the relative heart mass (RVM, ventricle mass as a percentage of body mass) of dogs increases with age, the increase being more marked in males.

Fish RVMs have been recorded for a number of species (see Santer and Greer-Walker, 1970), but few workers have recorded sex and reproductive status along with RVM. Luk'yanenko and Raspopov (1972) found that the RVM of migratory male Russian sturgeons (*Ascipenser guldenostadti*) was significantly greater than that of females, and Krykhtin (1976) found proportionally larger hearts in the biggest male Kaluga sturgeons (*Huso dauricus*). Rainbow trout show seasonal changes in RVM (Farrell *et al.* 1988): RVM increases during winter. However, Farrell *et al.* (1988) report an increase in the size of standard errors associated with this seasonal increase in heart mass (Fig. 4 of Farrell *et al.* 1988). This suggested to us that sexual dimorphism in heart size at spawning was present in their sample which included both males and females.

Temperature affects relative heart mass in teleosts (Kent and Prosser, 1985; Goolish, 1987; Graham and Farrell, 1989, 1990). Tsukuda *et al.* (1985) showed that very small goldfish (around 6 g), increase cardiac mass by about 40 % upon cold-acclimation. Rainbow trout acclimated to 5 °C (winter) had much greater RVMs than 15 °C (summer) trout (Graham and Farrell, 1989, 1990). As a result of the increase in cardiac mass in the cold-acclimated trout, their heart was able to generate greater maximum stroke volumes (0.91 ml kg^{-1} body mass) than the hearts of summer trout (0.69–0.74 ml kg^{-1} body mass; Graham and Farrell, 1989, 1990).

The current investigation was designed to determine the relationships between sex and sexual maturity, and heart size and cardiac performance in rainbow trout. Trout were reared at a hatchery until they reached sexual maturity and RVM was measured from both sexes at different stages of gonad development. These values were then compared to data collected from wild trout caught in the same river system from which the hatchery stock were derived. Isolated heart preparations from mature and immature hatchery fish were used to assess cardiac performance.

Materials and methods

Maintenance of animals and determination of heart mass

Experimental male and female trout were raised together at the National Trout Centre, Turangi, New Zealand, from a single mating of one female and three male fish. The parent stock were wild fish caught in the tributary of the Tongariro River, which flows past the hatchery. They were maintained for more than 2 years in Burrows raceways (spring water, 10±0.3 °C all year) and fed a commercial trout feed (Northern Roller Milling, Tauranga, New Zealand). The same group of fish was used for both anatomical and physiological studies. Wild trout were obtained from anglers who fished the Tongariro river during the months of July and August

1990 (water temperature 10°C). The capture and death of these wild fish was not part of the experiment but offered an opportunity to examine the morphology of wild specimens of similar genetic stock and age to the cultured trout.

The sample of hatchery-reared trout came from a fish kill caused by vandalism. The carcasses were weighed and their the ventricles and gonads removed and weighed to determine the RVM and gonadosomatic index (GSI, gonad mass as a percentage of body mass).

Heart preparation

The heart preparation was similar to the one used by Farrell *et al.* (1986); the heart remaining within an intact pericardium. After anaesthesia in 0.04 % aqueous benzocaine (Sigma) and exposure of the caudal aspect of the pericardium, an input cannula supplied with physiological saline containing 10 nmol l^{-1} adrenaline [modified Cortland's, pH 7.9 (Wolf, 1963); see Farrell *et al.* 1986] was inserted into the sinus venosus through the hepatic vein to which it was firmly tied. The common cardinal veins were tied off. An output cannula was tied into the ventral aorta and the heart preparation was then transferred to a saline bath maintained at 15°C. The input cannula was connected to a constant pressure device (Farrell *et al.* 1982) and the output pressure cannula was connected to a pressure head. Input and output pressures were recorded (Statham P23XL pressure transducers) from side-arms off the cannulae (and later corrected for cannulae resistances) and cardiac output was measured with an electromagnetic flow probe and meter (Zepeda Instruments, Seattle, WA). Flow and preamplified (Neurolog, Digitimer, UK) pressure signals were directed to a Gould chart recorder (Gould, Ohio) and to a personal computer (IBM XT compatible) where the analog signals were digitised (ADDA-12, Flytech Productions, Taiwan). Cardiac power output was calculated using ANALAB software (Massey University, New Zealand) and was displayed on-screen in real time (see Davie and Franklin, 1992).

Experimental protocol

Cardiac performance was assessed by determining the following variables. (1) Maximum cardiac output. This was measured after input pressure had been raised until there was no further increase in flow (output pressure was kept at 4.9 kPa). (2) Maximum sustainable output pressure. With the output pressure initially set at 4.9 kPa, cardiac output was set at 17.6 ml min^{-1} kg^{-1} body mass (Kiceniuk and Jones, 1977) by adjusting input pressure. Output pressure was then increased in 0.5- to 1.0-kPa increments until cardiac power output decreased (determined from the real time computer display). The output pressure recorded immediately before this drop in power output was deemed to be the maximum sustainable output pressure. (3) Maximum cardiac power output. This was measured by observing the computer display of cardiac power output. Whilst the heart was being maximally filled, output pressure was adjusted until there was a peak in power production.

Analysis

Stroke volume (V_S) was calculated by dividing flow by heart rate. Cardiac power output (mW) was calculated as [output pressure (kPa)−input pressure (kPa)]× cardiac output ($ml\,s^{-1}$). Stroke volume, cardiac output and power output were normalised per kilogram body mass and power output was also normalised per gram ventricle mass (wet mass). Results are presented as means±S.E.M. and significant differences determined using a Student's *t*-test. Correlation analysis was used to determine the relationship between gonadosomatic index and RVM in male and female trout. The lines fitted to the data are described by equations (see Fig. 1) and were fitted by the least-squares method.

Results

Relative ventricle mass and reproductive status

The relative ventricle masses (RVMs) of maturing or mature male rainbow trout were significantly greater than the RVMs of immature males or females in both captive and wild samples (Fig. 1; Table 1). Mature males had larger hearts and there was a highly significant correlation between RVM and gonadosomatic index

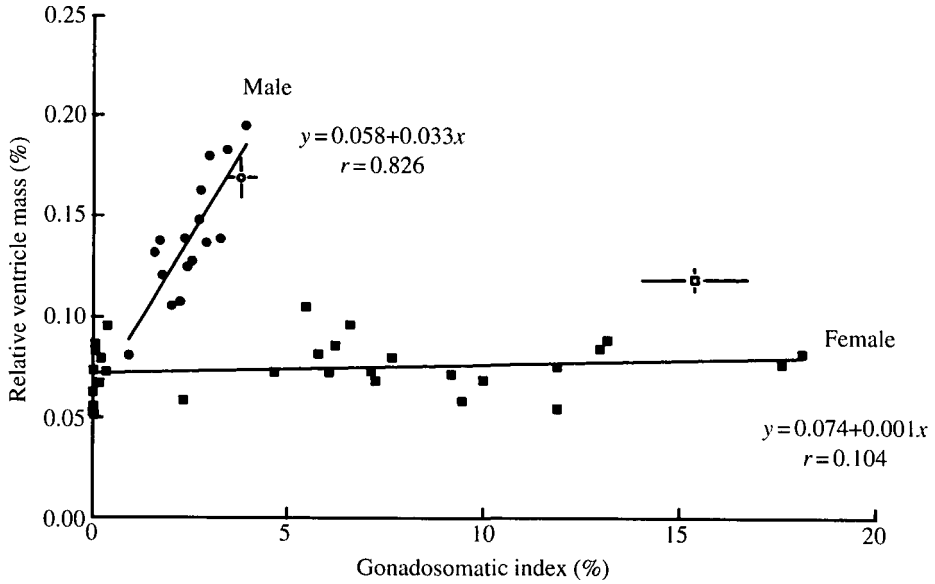

Fig. 1. Gonadosomatic index (GSI) and relative ventricle mass (RVM) in male (circles) and female (squares) hatchery-reared rainbow trout. Open symbols are means for mature wild trout (N=12 males; N=13 females). There was a highly significant correlation between the RVM and GSI of males (r=0.826, P<0.01) but not of females (r=0.104, P=0.622). Wild trout were significantly heavier (body mass, mean±S.E., 2.21±0.34 kg, N=27) than hatchery-reared trout (mean±S.E., 1.32±0.24 kg, N=46) (P<0.01). The origins of the trout and hatchery rearing conditions are described in the text.

Table 1. *Body mass, gonadosomatic index and relative ventricle mass of male and female hatchery-reared and wild rainbow trout*

	Body mass (kg)	Gonadosomatic index (%)	Relative ventricle mass (%)
Hatchery-reared trout			
Maturing males (N=16)	1.22±0.06	2.48±0.191	0.139±0.008
Maturing females (N=30)	1.37±0.04	5.83±1.01*	0.074±0.002**
Wild trout			
Maturing males (N=10)	2.05±0.11†	3.78±0.39†	0.169±0.010†
Immature males (N=4)	2.47±0.20	0.31±0.05**	0.117±0.005**
Females (N=13)	2.27±0.08†	15.4±1.40**†	0.119±0.005**†

Significant differences (Student's t-tests) between males (mature or immature in the case of wild fish) and females, from the hatchery or the river, are indicated by * $P<0.05$, ** $P<0.001$.
† indicates significant differences between hatchery-reared and wild trout ($P<0.05$).

(GSI) in the males (Fig. 1; r=0.826, $P<0.01$). Ventricle mass more than doubled as the testes developed (RVM was 0.07 % at a GSI of 0.93 %, and 0.195 % at a GSI of 3.92 %) (Fig. 1). No such correlation existed for the females (Fig. 1), in which RVM did not change significantly as GSI increased from 0.07 % to 18 %.

Cardiac performance

A consequence of the greater RVM in mature male trout was an enhancement in the fish's cardiac performance, as assessed from isolated heart preparations, but not in the performance of the heart tissue *per se* (Table 2). A higher RVM in mature males resulted in a significant elevation in cardiac output because of increases in V_S (Table 2). The ability of the heart to maintain work against higher output pressures was also enhanced in mature males (Student's t-test, $P<0.05$, Table 2). Because the hearts from mature males had larger V_S values and a greater ability to work against higher output pressures, there was an overall increase in cardiac power output per kilogram body mass (Table 2). However, if the cardiac power values are expressed per gram ventricle mass there is no difference between the cardiac tissue of mature males and immature males and females (Table 2).

Discussion

Relative ventricle mass and reproductive status

Cardiac growth with sexual maturation has not previously been recorded in trout. Although it is clear that male sturgeons develop larger hearts during the breeding season (Luk'yanenko and Raspopov, 1972), data for salmonids are limited. Of the four 'old freshwater remigrating fish' (Atlantic salmon, *Salmo salar*) studied by Poupa *et al.* (1974), the three largest had RVMs between 0.14 and 0.16, whereas the smallest mature fish had an RVM of 0.21. It is tempting to speculate that the three largest were females and the smallest of this group was a

Table 2. *Cardiac performance of* in situ *perfused hearts from hatchery-reared, mature male and immature male and female rainbow trout*

	Body mass (kg)	Relative ventricle mass (% of BM)	Maximum cardiac output ($ml\,min^{-1}\,kg^{-1}$ BM)	Maximum stroke volume ($ml\,kg^{-1}$ BM)	Maximum sustainable output pressure (kPa)	Maximum cardiac power output	
						($mW\,g^{-1}$ VM)	($mW\,kg^{-1}$ BM)
Mature males	0.68±0.05	0.138±0.011	54.1±3.9	0.774±0.037	8.90±0.37	3.29±0.43	4.42±0.46
Immature males and females	0.87±0.07	0.076±0.003***	36.3±2.7**	0.532±0.031**	7.67±0.35*	3.52±0.14	2.68±0.14**

N=5; mean±standard error; BM, body mass; VM, ventricular mass.

Significant differences between mature male and immature male and female trout are indicated by * $P<0.05$, ** $P<0.01$, *** $P<0.001$; Student's t-test.

male. Other studies presenting RVMs for salmonids have not noted sex and reproductive condition (Farrell *et al.* 1988).

Sexual dimorphism of the heart of mammals can be induced by elevated plasma androgen levels (Koenig *et al.* 1982). Growth is induced in a variety of myocardial tissue components including myocytes, sarcoplasmic reticulum and connective tissue (Shuling *et al.* 1990) and may be caused by a direct action of steroids on myocardial receptors or *via* a range of hormonal or neural routes (see Stumpf, 1990).

Rainbow trout are seasonal breeders and have peak steroid levels during the spawning season. Scott and Sumpter (1989) recorded testosterone levels up to about $80\,ng\,ml^{-1}$ and 11-ketotestosterone levels up to $120\,ng\,ml^{-1}$ in maturing male rainbow trout. Females have similar testosterone levels to males (Scott *et al.* 1980) but lower 11-ketotestosterone levels. Interestingly, seasonal-breeding mammals, such as sheep, have peak steroid levels about an order of magnitude lower than those of trout. Schanbacher and Ford (1976) report peak plasma testosterone levels in the ram of about $9.81\,ng\,ml^{-1}$ and sexual dimorphism of the heart is much less pronounced than in trout. The higher levels of circulating steroids in trout compared to those in mammals may induce exaggerated cardiac hypertrophy in the former. In mammals, plasma steroid levels approaching those of trout are found only in humans who are taking anabolic steroids in the hope of improving their athletic performance (Lamb, 1984). Left ventricular hypertrophy has been recorded in one case of a bodybuilder taking anabolic steroids (McKillop *et al.* 1986).

Cold-acclimation increases relative heart mass in goldfish (Tsukuda *et al.* 1985), carp (Goolish, 1987) and probably trout (Graham and Farrell, 1989). Small goldfish were almost certainly not reproductively active and showed cardiac hypertrophy with cold-acclimation (RVM at 10°C 0.23, RVM at 25°C 0.18; Tsukuda *et al.* 1985). Cardiac growth in cold-acclimated winter trout studied by Graham and Farrell (1989) may have been affected by sexual maturation as well as cold-acclimation. The trout used by Graham and Farrell weighed around 500 g and were probably 2+ year fish. The acclimations to different temperatures were performed at different times of the year and, in the winter, cold-acclimated group, precocious males could be expected to show gonad development since salmonids spawn in autumn and winter. Moreover, a preponderance of males in both their winter (15 males, 2 females) and summer samples (15 males, 5 females) would exaggerate the effect of any reproductive maturation as it is only the males that show an increase in RVM with maturation.

Cardiac performance

Greater RVM enhanced the overall performance of hearts from mature male trout (Table 2), principally as a result of increased stroke volume. The ability of hearts of mature males to maintain work against higher output pressures was also enhanced and, as a result of both of these changes, there was an overall increase in measured cardiac power output per kilogram fish mass. The very significant

increase in power output available to mature male trout as a result of increased heart mass is a strategy used by tunas to increase cardiac performance (Farrell *et al.* 1992). The power output per gram ventricular mass is not significantly different in the larger hearts of mature trout from that of the hearts of mature or immature females or immature males.

The significance of our observations to fish physiologists interested in cardiovascular function is apparent when we realise that the benchmark study of oxygen transport during sustained exercise in trout used barren females (Kiceniuk and Jones, 1977). In the light of our results, we might have expected the cardiac outputs (and $\dot{V}s$ values) of females with relatively small hearts to be on the low side when compared to other studies. However, stroke volumes were identical to those of Wood and Shelton (1980), who measured cardiac output directly. Kiceniuk and Jones (1977) used the Fick method for estimating cardiac output (and hence $\dot{V}s$), a technique known to give high values (Johansen and Pettersen, 1981). This appears to have compensated for the small heart size in female trout.

The significance of cardiac hypertrophy in maturing male trout is not immediately apparent. We might expect that increased cardiac scope facilitates active behaviour associated with territoriality and spawning. Exercise alone, however, induces no change in cardiac mass (Farrell *et al.* 1990) and growth of the heart is slow during upstream migration (Poupa *et al.* 1974). It is apparent that there is a complex web of interactions occurring between the cardiovascular and reproductive systems during sexual maturation in trout about which much remains to be understood.

In summary, seasonal changes in trout in preparation for breeding not only effect changes in reproductive status but also modify the scope of the cardiovascular system. We have found that, as the testes of male rainbow trout grow in readiness for spawning, the ventricle mass doubles. No comparable increase in ventricle mass occurs in maturing female trout or in any other vertebrate species, male or female, so far as we can tell. Cardiac performance of mature males was nearly twice that of females and immature males. We believe that the high levels of circulating steroids present in male trout during sexual maturation stimulate heart growth.

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