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Maximum cardiac performance and adrenergic sensitivity of the sea bass *Dicentrarchus labrax* at high temperatures

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Summary

We examined maximum cardiac performance of sea bass *Dicentrarchus labrax* acclimated to 18°C and 22°C, temperatures near the optimum for growth of this species. Our aim was to study whether cardiac performance, especially the effect of adrenergic stimulation, differed when compared to salmonids. Sea bass and salmonids are both athletic swimmers but their cardiac anatomy differs markedly. The sea bass ventricle does not receive any oxygenated blood *via* a coronary circulation while salmonids have a well-developed arterial supply of oxygen to the compact layer of the ventricle. Using *in situ* perfused heart preparations, maximum cardiac performance of 18°C-acclimated sea bass (i.e. cardiac output=90.8±6.6 ml min^{-1} kg^{-1} and power output=11.41±0.83 mW g^{-1}) was found to be comparable to that previously reported for rainbow trout *Oncorhynchus mykiss* and brown trout *Salmo trutta* at similar temperatures and with tonic adrenergic (5 nmol l^{-1} adrenaline) stimulation. For 22°C-acclimated sea bass, heart rate was significantly higher, but maximum stroke volume was reduced by 22% (1.05±0.05 ml kg^{-1}) compared with 18°C (1.38±0.11 ml kg^{-1}). As a result, maximum cardiac output (99.4±3.9 ml min^{-1} kg^{-1}) was not significantly different at 22°C. Instead, maximum power output was 27% higher at 22°C (14.95±0.96 mW g^{-1}) compared with 18°C, primarily because of the smaller relative ventricular mass in 22°C-acclimated sea bass. Compared with tonic adrenergic stimulation with 5 nmol l^{-1} adrenaline, maximum adrenergic stimulation of the sea bass heart produced only modest stimulatory effects at both temperatures (12–13% and 14–15% increases in maximum cardiac output and power output, respectively, with no chronotropic effect). Adrenergic stimulation also increased the cardiac sensitivity to filling pressure, with the maximum left-shift in the Starling curve being produced by 50–100 nmol l^{-1} adrenaline at 18°C and 10–50 nmol l^{-1} adrenaline at 22°C. We show that the sea bass, which lacks a coronary arterial oxygen supply to the ventricle, has a powerful heart. Its maximum performance is comparable to a salmonid heart, as is the modest stimulatory effect of adrenaline at high temperature.

Key words: sea bass, heart, cardiac output, heart rate, temperature, adrenaline.

Introduction

Temperature is regarded as an 'ecological master factor' for fish because of its influence on physiological processes, including those related to swimming performance and metabolism (Brett, 1965). As such, temperature optima exist for maximum oxygen uptake, aerobic scope and maximum prolonged swimming speed (i.e. critical swimming speed, U_{crit}), but these may vary among fish species and perhaps even among different stocks within a species (Brett, 1971; Beamish, 1978; Randall and Brauner, 1991; Johnston and Ball, 1997; Taylor et al., 1996; Lee et al., 2003a; Claireaux et al., 2005). Given that temperature influences fish distributions and their migrations, there is great interest in understanding why swimming performance declines at high temperature, particularly in an era of global climate change and warming of rivers, lakes and seas.

The discovery of parallel declines in maximum oxygen uptake, aerobic scope and U_{crit} for salmon led to the suggestion of a cause–effect relationship between declining oxygen delivery and swimming performance (Brett, 1971). Mechanistically, Brett suggested that this relationship results from a failure of gill ventilation to deliver sufficient water to compensate for the decrease in water oxygen content as temperature increases (Brett, 1971). Accordingly, decreases in oxygen content of arterial and venous blood occur with increasing water temperature in resting rainbow trout

Oncorhynchus mykiss (Heath and Hughes, 1971). In addition, this study revealed cardiac arrhythmias as venous oxygen content fell towards zero, an observation that leads to the alternative suggestion that the decrease in oxygen tension in venous blood limits oxygen supply to the cardiac muscle and that this cardiac oxygen deficiency contributes to the decrease in performance of salmonids at high temperature (Farrell, 1997). Others have suggested that the problem may lie with oxygen delivery to tissues (Taylor et al., 1996; Pörtner, 2002; Farrell, in press).

While it remains difficult to separate out the proximate cause(s) for the failure of performance, temperature optima for maximum cardiac performance have been clearly defined for salmonids (Brett, 1971; Farrell et al., 1996; Taylor et al., 1996; Mercier et al., 2002). However, we know little of the temperature sensitivity of maximum cardiac performance in non-salmonids. Recently, temperature optima were shown for routine cardiac performance and oxygen uptake in Atlantic cod *Gadus morhua*, with cardiac output collapsing near the critical thermal maximum (Gollock et al., 2006). These recent findings extend considerably on earlier work with Atlantic cod (Lannig et al., 2004) that provided evidence for a constraint on blood flow at high temperature.

Arrhythmia is commonly considered an early signal of cardiac failure in both mammals and fish (e.g. Heath and Hughes, 1971; Chatelier et al., 2005a), but the problem may be more complex than a deficient myocardial oxygen supply. Calcium handling during excitation–contraction coupling and a collapse of adrenergic stimulation, as well as extracellular acidosis and hyperkalemia, have also been implicated (Farrell, 1997; Hanson et al., 2006; Farrell, in press). With higher heart rates, there is a shorter active state at high temperature (Vornanen, 1998), and so a more rapid flux of calcium through the sarcolemmal L-type calcium channel is needed to maintain tension development. The main modulator of the open state of this channel is β-adrenergic stimulation, as shown in both fish (Vornanen, 1998; Shiels and Farrell, 2000) and mammals (Reuter, 1983). However, it has been shown in rainbow trout that this modulator becomes increasingly less effective as acclimation temperature increases, with the result that adrenergic stimulation of cardiac inotropy decreases at high temperature (Ask et al., 1980; Keen et al., 1993; Shiels and Farrell, 2000; Shiels et al., 2002; Shiels et al., 2003). In fact, the perfused rainbow trout heart becomes almost refractory to adrenaline at a temperature above 18°C, which appears to be the optimum temperature for maximum performance in this species (Farrell et al., 1996). Adrenergic stimulation is also critical in protecting the fish heart from the debilitating extracellular hypoxia, acidosis and hyperkalemia (Gesser and Jorgensen, 1982; Gesser et al., 1982; Farrell et al., 1984; Farrell, 1985; Driedzic and Gesser, 1994; Nielsen and Gesser, 2001; Andersen et al., 2004) associated with maximal performance such that the rainbow trout heart can perform maximally at a lower oxygen tension with maximum adrenergic stimulation than without it (Hanson et al., 2006). This protective adrenergic effect diminishes at high temperature (Hanson and Farrell, in press), and is further compounded by rainbow trout swimming with a greater anaerobic locomotory effort at high temperature (Jain and Farrell, 2003; Lee et al., 2003b) that then heightens the extent of the extracellular acidosis and hyperkalemia. We were interested in discovering whether non-salmonids also show limited adrenergic inotropy at high temperature, knowledge that would be important in our general understanding of why maximum cardiac performance in fish is limited at high temperature. Consequently, the present study assessed maximum cardiac capacity and adrenergic stimulation at high temperatures in the European sea bass *Dicentrarchus labrax*. Sea bass lack a coronary circulation, and so we could be certain that changes in venous blood composition directly influence the entire myocardium, unlike in salmonids where the presence of a coronary circulation confounds matters.

Sea bass are a representative of the *Moronidae,* which are found in fresh and brackish water, as well as in coastal marine areas of eastern North America, Europe and northern Africa. Sea bass are found throughout the Mediterranean Sea and along the eastern coast of Europe from Portugal to Norway. They are active predators (feeding on fish and crustaceans) and live in small shoals as juveniles. As adults (up to 100 cm; 12 kg), sea bass are powerful swimmers that inhabit dynamic depths ranging from 1 to 100 m. Temperature but not salinity (Chatelier et al., 2005b) has a strong influence on their performance. For instance, between 10°C and 20°C, standard and active metabolic rates increase 2.5- and 5.5-fold, respectively (Claireaux and Lagardere, 1999). Optimal temperatures for metabolic scope and U_{crit} are 22°C and 27°C, respectively (Claireaux et al., 2006; Claireaux and Lagardere, 1999). Temperatures below 9–10°C are too cold for reproduction and, in the English Channel, adult sea bass over-winter offshore, while non-maturing juveniles remain inshore (Pickett and Pawson, 1994). Similar to salmonids, sea bass are athletic swimmers but their cardiac anatomy is markedly different. The sea bass ventricle does not receive any oxygenated blood *via* a coronary circulation while salmonids have a well-developed arterial supply of oxygen to the compact myocardium (Axelsson and Farrell, 1993; Gamperl et al., 1995). The aim of the present study was to investigate whether maximum cardiac performance and the effect of adrenergic stimulation differ in sea bass compared to salmonids.

Materials and methods

Animal handling and care

Sea bass *Dicentrarchus labrax* L. were obtained from a commercial fish farm (Ferme Marine des Baleines, Ile de Ré, France) and transported to the Centre de Recherche sur les Ecosystèmes Marins et Aquacoles (UMR 010, CNRS-Ifremer) in L'Houmeau, France, where experiments were conducted. Fish were randomly assigned to an experimental group and were acclimated for at least 3 weeks to either 18°C or 22°C indoors in 1 m^3 tanks supplied with recirculated and biofiltered

seawater (flow: 8 l min^{-1}; salinity: 28–30‰; oxygen: >85% air-saturation). Fish were exposed to natural photoperiod and were fed a commercial diet (Bar D Perform Natura 4.5, SICA du Gouessant, BP 228, Lamballe 22402, France). Cardiac assessments were made at these two acclimation temperatures to examine phenotypic plasticity.

In situ perfused heart studies

Cardiac performance studies were conducted on six fish at both 18°C (body mass=252±4 g; ventricular mass=0.252±0.009 g; relative ventricular mass=0.100±0.003%) and 22°C (body mass=318±6 g; ventricular mass=0.248±0.005 g; relative ventricular mass=0.078±0.001%). Maximum cardiac performance was assessed with the Farrell *in situ* fish heart preparation (see Farrell et al., 1986), with the modifications outlined by Farrell et al. (Farrell et al., 1988) and Mercier et al. (Mercier et al., 2002). Fish were first anaesthetised by 0.05 g l^{-1} tricaine methane sulphonate (MS222), buffered with 0.05 g l^{-1} $NaHCO_3$, and placed in an operating sling where the gills were irrigated with a lower concentration of anaesthetic (0.02 g l^{-1} MS222 buffered with 0.02 g l^{-1} $NaHCO_3$). The heart was isolated in terms of saline input and output by securing stainless steel input and output cannulae into the sinus venosus *via* a hepatic vein and the ventral aorta, respectively, while leaving the heart undisturbed and pericardium intact. Cardiac perfusion was started immediately using oxygenated saline containing a tonic level of adrenaline (5 nmol l^{-1} AD; adrenaline bitartrate, Sigma-Aldrich, St Quentin-Fallavier, France). The preparation was then immersed in a saline-filled, temperature-controlled organ bath at the appropriate acclimation temperature (18°C or 22°C), with the input and output cannulae attached to constant pressure heads. A 15–20 min period of control perfusion preceded any assessment, during which the filling (input) pressure of the heart was set to give a routine cardiac output ($\dot{Q}$=ventral aortic flow in the output cannula) of 25 ml min^{-1} kg^{-1} body mass and mean output pressure was set at ~5 kPa to simulate routine *in vivo* ventral aortic blood pressures. Maximum $\dot{Q}$ was established with stepwise increases in filling pressure (i.e. a Starling response) until $\dot{Q}$ reached a plateau. Diastolic output pressure was then increased with the heart pumping maximally until a maximum cardiac power output was reached. The heart was then returned to the control perfusion conditions for a 15 min recovery and an equilibration with a new perfusate adrenaline concentration (10 nmol l^{-1}, 50 nmol l^{-1}, 100 nmol l^{-1} and 500 nmol l^{-1}). The adrenaline concentration range spanned that observed in plasma of resting and stressed rainbow trout (e.g. Milligan et al., 1989; Gamperl et al., 1994) and therefore resulted in a tonic through to a maximal adrenergic stimulation of the sea bass heart. With the perfused sea bass heart, great care was needed to prevent excessive increases in output pressure. Occasionally at 18°C and more frequently at 22°C, an excessive output pressure produced irreversible cardiac failure that was not alleviated by either restoring control conditions or increasing the adrenaline concentration. These partial data sets were not used for the general data analysis presented here, although the heart had performed well up to this point. An in-line Transonic flow probe (Transonic Systems, Ithaca, NY, USA) was used to record $\dot{Q}$. Pressures in the sinus venosus (input) and ventral aorta (output) were measured with pressure transducers (model DPT-6100, pvb Medizintechnik, Germany), through saline-filled tubes placed at the tip of the cannulae. The pressure transducers were calibrated against a static water column with each preparation. Pressure and flow signals were amplified (4ChAmp amplifier, Somedic, Sweden) and stored with a custom-made data acquisition program, General Acquisition (Labview version 6.01, National Instruments, USA). The perfusate (pH 7.8 at 15°C) contained (in mmol l^{-1}): NaCl, 124; KCl, 3.1; $MgSO_4.7H_2O$, 0.93; $CaCl_2.2H_2O$, 2.52; glucose, 5.6 TES (N-tris[hydroxymethyl]methyl-2-aminoethanesulphonic acid) salt, 6.4; and TES acid, 3.6 (Keen and Farrell, 1994). The TES buffer system simulated the buffering capacity and temperature dependency (dpK_a/dT=0.016 pH units $°C^{-1}$) of rainbow trout plasma. By equilibrating the saline with 100% O_2, the oxygen gradient between the saline and the myocardium was at least 20-times greater than the *in vivo* oxygen gradient between venous blood and the myocardium. Sea bass do not have a coronary circulation.

Data analysis and statistics

Myocardial power output (mW g^{-1} ventricle mass) was calculated from the product of $[\dot{Q}\times(P_{out}-P_{in})\times(0.0167 \text{ min } s^{-1})]$/ventricular mass (g), where $\dot{Q}$ is in ml min^{-1}, P_{out} is output pressure and P_{in} is input pressure (in Pa). Ventricular mass was determined at the conclusion of the experiment when the cannulae were checked for correct positioning. The relationships between cardiac filling pressure and cardiac stroke volume (V_{SH}) and $\dot{Q}$ (Starling curves), and between P_{out} and P_{in} (power output curves), were derived by fitting curves to the data for each fish. This permitted an interpolation of values of V_{SH}, $\dot{Q}$ and power output for standardized levels of filling pressure and output pressure among fish so that the Starling and power output curves could be based on mean values derived at each acclimation temperature for six fish (see Figs 2 and 3). Individual fish differed somewhat in their sensitivity to filling and output pressures and so there are slight numerical differences for the maximum values presented in the figures (based on interpolations) and in Table 1 (based on recorded values). Comparisons of the tonic and maximal effects of adrenaline were performed with a paired *t*-test. A probability of less than 5% (P<0.05) was taken as the limit for statistical significance.

Results

Effects of adrenergic stimulation on maximum cardiac performance at 18°C

The maximum performance of 18°C-acclimated sea bass hearts is presented in Table 1 for adrenaline concentrations of 5 nmol l^{-1} (tonic level) and 50 nmol l^{-1}. Increasing the adrenaline concentration improved cardiac inotropy without

Table 1. *The maximum performance of hearts from 18°C- and 22°C-acclimated sea bass with 5 nmol l^{-1} and 50 nmol l^{-1} adrenergic stimulation*

Adrenaline concentration (nmol l^{-1})	Maximum $\dot{Q}$ (ml min^{-1} kg^{-1} body mass)	Maximum V_{SH} (ml kg^{-1} body mass)	Maximum power output (mW g^{-1} ventricular mass)
18°C			
5	90.8±6.6	1.38±0.11	11.41±0.83
50	101.3±5.7	1.51±0.11	13.16±0.84
22°C			
5	99.4±3.9	1.05±0.05*	14.95±0.96*
50	108.8±9.4	1.18±0.05*	16.69±1.15*

Values are mean ± s.e.m. (N=6).

$\dot{Q}$, cardiac output; V_{SH}, cardiac stroke volume.

For 18°C, maximum $\dot{Q}$ and V_{SH} values were obtained typically with an input pressure of 0.25 kPa (occasionally 0.20 kPa) and maximum power output values were obtained typically with an output pressure of 8.0 kPa (occasionally 7.5-9.0 kPa).

For 22°C, maximum $\dot{Q}$ and V_{SH} values were obtained typically with an input pressure of 0.15 kPa (occasionally 0.20 kPa) and maximum power output values were obtained typically with output pressures ranging from 6.5-9.0 kPa.

Asterisks denote a significant difference between temperature acclimation groups (Students t-test; P<0.05).

significantly affecting heart rate (66–69 min^{-1}; Fig. 1). Adrenergic stimulation produced only a modest improvement in maximum cardiac performance. The maximum responses of $\dot{Q}$ (12% increase; Fig. 2A) and power output (15% increase; Fig. 2C) occurred with 50–100 nmol l^{-1} adrenaline. Adrenaline also shifted the Starling curve to the left (Fig. 2B), making the heart more sensitive to filling pressure. As a result, to generate a V_{SH} of 1.35 ml kg^{-1}, a filling pressure of only 0.15 kPa was required with 100 nmol l^{-1} adrenaline compared with 0.25 kPa with 5 nmol l^{-1} adrenaline (Fig. 2B). Most of this shift in stretch sensitivity was observed between 5 nmol l^{-1} and 10 nmol l^{-1} adrenaline.

Effects of adrenergic stimulation on maximum cardiac performance at 22°C

The maximum performance of 22°C-acclimated sea bass hearts is presented in Table 1. Adrenergic stimulation produced results similar to those observed at 18°C; cardiac inotropy was modestly improved without changing heart rate (Fig. 1). The peak response of $\dot{Q}$ (14% increase) occurred with 10–50 nmol l^{-1} adrenaline (Fig. 3A) and power output (14% increase) with 10–50 nmol l^{-1} adrenaline (Fig. 3C). Adrenaline also caused a left-shift in the Starling curve (Fig. 3B).

The fragility of hearts from 22°C-acclimated sea bass was demonstrated with two additional heart preparations: one that failed after 10 nmol l^{-1} adrenaline and another that failed after 50 nmol l^{-1} adrenaline. These hearts initially had an exceptionally high performance with the tonic adrenaline concentration (maximum $\dot{Q}$ of 158 and 138 ml min^{-1} kg^{-1} and maximum power output of 16 and 19 mW g^{-1}), and thus perhaps we had overtaxed these hearts. In addition, several experiments were attempted at perfusion temperatures of 23°C and 24°C, but the hearts performed inconsistently and poorly.

Comparison of cardiac performance at 18°C and 22°C

Heart rate was significantly higher at 22°C compared with 18°C although there was no chronotropic effect of adrenaline at either acclimation temperature. Q_{10} values for heart rate ranged between 2.27 and 2.63, depending on the adrenaline concentration (calculated from data in Fig. 1). Maximum power output was also 27–31% higher for 22°C-acclimated sea bass (Fig. 1; Table 1), but this was primarily because relative ventricular mass was 22% smaller for 22°C-acclimated sea bass (see Materials and methods). However, maximum $\dot{Q}$ was unchanged by temperature acclimation because maximum V_{SH} was significantly reduced by 22–24% for 22°C- *vs* 18°C-acclimated sea bass (Fig. 1; Table 1).

The similarity of the Starling response curves for 18°C- and 22°C-acclimated sea bass is illustrated in Fig. 4. While the curves for both tonic and maximum stimulation were largely independent of acclimation temperature, there were two exceptions. The 18°C-acclimated heart responded to a higher input pressure (producing a significantly higher maximum V_{SH}) and maximum adrenergic stimulation was produced with 10–50 nmol l^{-1} adrenaline at 22°C *vs* 50–100 nmol l^{-1} adrenaline at 18°C (Fig. 4). Thus, the relatively smaller and faster beating 22°C-acclimated sea bass heart had a smaller maximum V_{SH} and a higher maximum power output, while the stimulatory effects of adrenaline remained modest (⩽15% improvements) at both acclimation temperatures.

Discussion

The main objective of the present study was to assess maximum cardiac performance in the sea bass. This has not been done previously and only limited *in vivo* cardiovascular measurements with sea bass are available for comparison with the present data. *In vivo* heart rates measured at 22°C during swimming [103 min^{-1} (Sandblom et al., 2005)] compare favourably with those measured here (95–100 min^{-1}). Likewise, the Q_{10} of 2.1 reported (Sandblom et al., 2005) for *in vivo* heart rate [51 min^{-1} at 16°C (Axelsson et al., 2002)] is

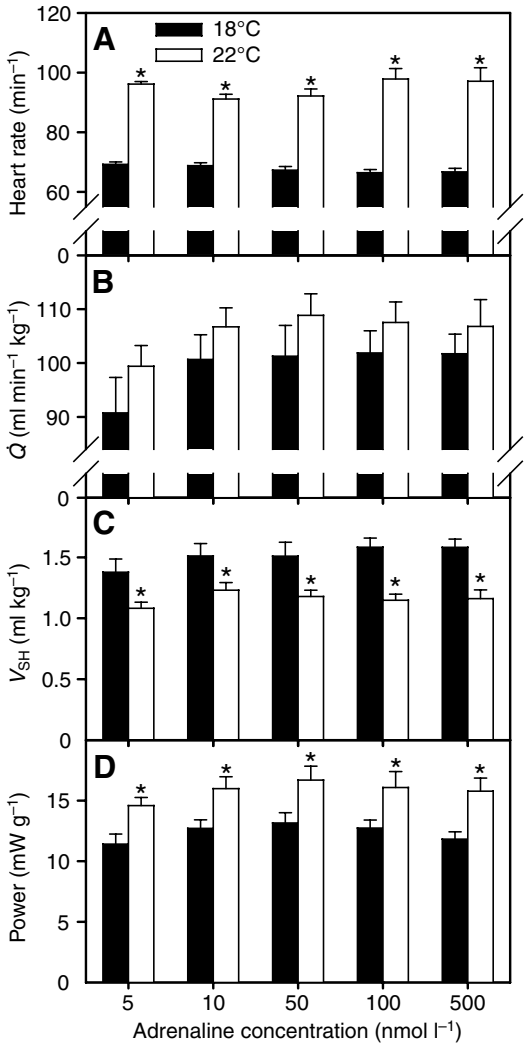

Fig. 1. A comparison of (A) heart rate, (B) cardiac output $\dot{Q}$, (C) stroke volume V_{SH} and (D) power output of perfused sea bass hearts from 18°C- and 22°C-acclimated fish and their responses to increasing concentrations of adrenaline in the perfusate. Values are means ± s.e.m. for fish acclimated to 18°C (N=6) and 22°C (N=6). Asterisks indicate a significant difference (P<0.05) between acclimation temperatures.

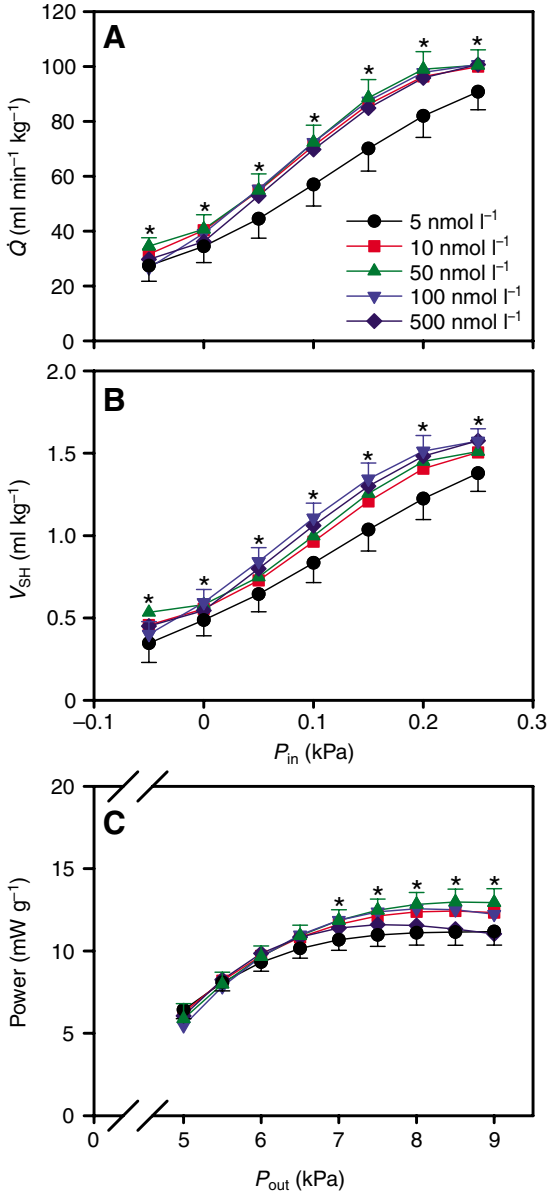

Fig. 2. The responses of (A) cardiac output $\dot{Q}$ and (B) cardiac stroke volume V_{SH} to increasing filling pressure P_{in}, and (C) cardiac power output to increasing output pressure P_{out}, for the perfused 18°C-acclimated sea bass heart preparation with different concentrations of adrenaline (5–500 nmol l^{-1}) in the perfusate. Values are means ± s.e.m. for the control and maximal stimulation for fish acclimated to 18°C (N=6). Asterisks indicate a significant difference (P<0.05) between control and maximal stimulation.

similar to the Q_{10} values determined here for perfused hearts. This means that the increase in heart rate (from 80 to 103 min^{-1}) during swimming (at 2 body lengths s^{-1}) for 22°C-acclimated sea bass is likely through release of vagal tone. Swimming also increased central venous blood pressure from 0.11 kPa to 0.16 kPa (Sandblom et al., 2005), a range that lies well within the filling pressures for the Starling curves produced here for perfused hearts. Ventral aortic pressure has not been measured in sea bass, but by assuming a loss of about one third of arterial blood pressure to gill resistance, the *in vivo* dorsal aortic blood pressures of 2.9–3.8 kPa (Sandblom et al., 2005) can be used to estimate that ventral aortic pressure would increase from 3.9 kPa to 5.1 kPa during moderate swimming. Again, this estimate of ventral aortic blood pressure range is well within the output pressure range used here for the perfused heart. Thus, this limited comparison suggests that the present data on maximum cardiac performance of the perfused sea bass heart are highly relevant to the *in vivo* situation.

The present study also measured the effect of adrenergic stimulation of the sea bass heart with the aim of comparing maximum cardiac performance with that of salmonids. This comparison is best done using a common acclimation temperature across the species, i.e. 18°C. We discovered that maximum cardiac performance with maximum adrenergic

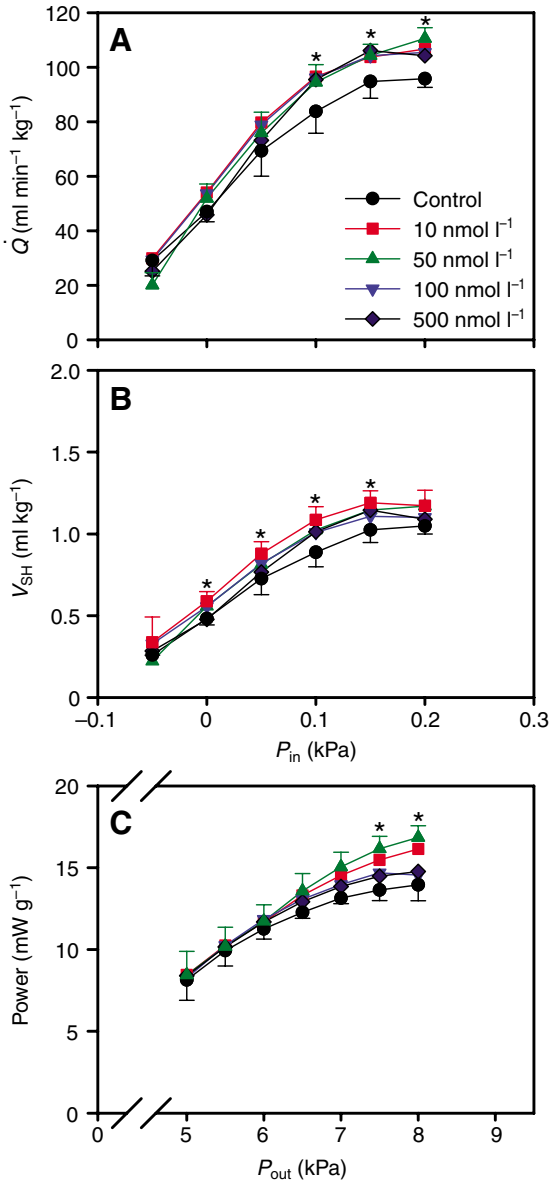

Fig. 3. The responses of (A) cardiac output and (B) cardiac stroke volume V_{SH} to increasing filling pressure P_{in}, and (C) cardiac power output $\dot{Q}$ to increasing output pressure P_{out}, for the perfused 22°C-acclimated sea bass heart preparation with different concentrations of adrenaline (10–500 nmol l^{-1}) in the perfusate. Values are means ± s.e.m. for the control and maximal stimulation for fish acclimated to 22°C (N=6). Asterisks indicate a significant difference (P<0.05) between control and maximal stimulation.

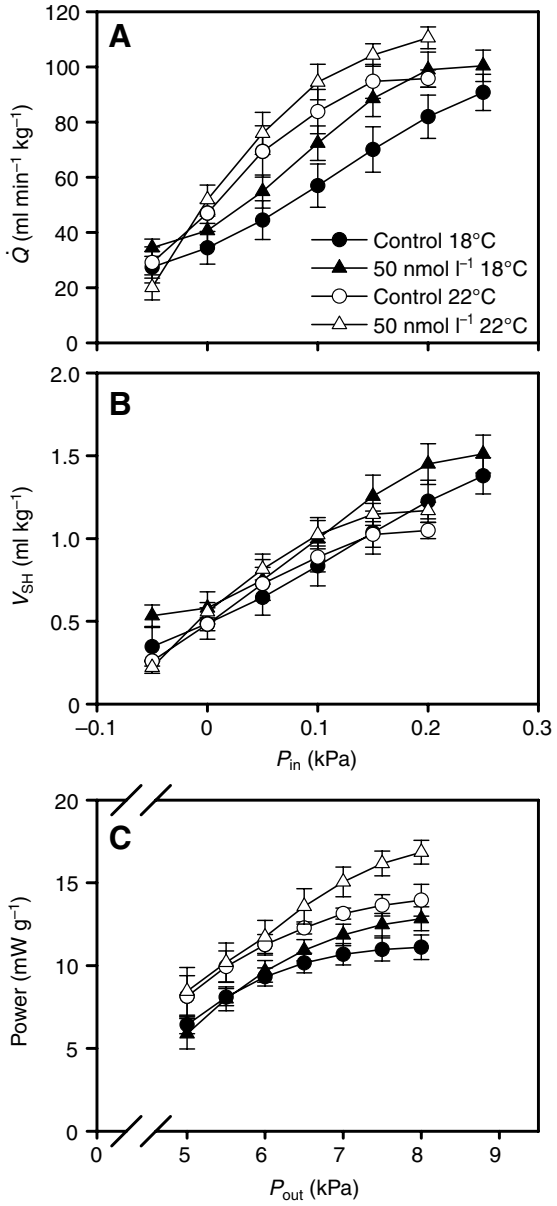

Fig. 4. A comparison of temperature acclimation on the tonic and maximum adrenergic stimulation of the perfused sea bass heart preparation at 22°C (100 nmol l^{-1} adrenaline; N=6) and 18°C (50 nmol l^{-1} adrenaline; N=6). Relationships are presented for (A) cardiac output and (B) cardiac stroke volume V_{SH} to increasing filling pressure P_{in}, and (C) cardiac power output to output pressure P_{out}. Values are means ± s.e.m. (N=6). For clarity, significant differences are not included here, but are given in the other figures.

stimulation for 18°C-acclimated sea bass (i.e. $\dot{Q}$=90.8 and 101.3 ml min^{-1} kg^{-1}, and power output=11.4 and 13.2 mW g^{-1} for tonic and maximum adrenergic stimulation, respectively) is at least equivalent to if not better than maximum cardiac performance of rainbow trout and triploid brown trout *Salmo trutta*. For 18°C-acclimated triploid brown trout, maximum $\dot{Q}$ and power output reached 118.5 ml min^{-1} kg^{-1} and 12.1 mW g^{-1}, respectively, with maximum adrenergic stimulation (Mercier et al., 2002). For diploid rainbow trout, maximum cardiac performance was lower [$\dot{Q}$=66–78 ml min^{-1} kg^{-1} and power output=7.0–7.4 mW g^{-1} at 15–18°C (Farrell, 2002)] than the sea bass. Therefore, the sea bass heart is at least as powerful as the salmonid heart.

The comparison of cardiac anatomy and physiology between sea bass and salmonids can be extended a little further with an added caution that unknown culture effects among species could contribute to differences (see Gamperl and Farrell, 2004; Claireaux et al., 2005). At 18°C, heart rate was lower for sea

bass (65–70 min^{-1}) when compared with triploid brown trout and rainbow trout [between 88 and 92 min^{-1} at 18°C (Wood et al., 1979; Altimiras et al., 2002; Mercier et al., 2002; Taylor et al., 1996)]. To compensate, sea bass have up to a 50% larger maximum V_{SH} (1.4–1.5 ml kg^{-1}) compared with rainbow trout [0.9–1.1 ml kg^{-1} (Farrell et al., 1996)] and triploid brown trout [1.1–1.2 ml kg^{-1} (Mercier et al., 2002)]. Surprisingly, relative ventricular mass is similar across these three species at this common acclimation temperature. Given that sea bass lack a coronary circulation, their entirely spongy myocardium must be more compliant to accommodate a larger end-diastolic volume and, at the same time, may require a longer blood residence to extract sufficient oxygen from venous blood. Perhaps the absence of a coronary circulation necessitates slower heart rates and the resulting higher maximum V_{SH} compared with salmonids. Of course, other factors will play a role in settting maximum V_{SH} since maximum V_{SH} has been shown to decrease with increases in heart rate and acclimation temperature (present study) (Farrell et al., 1996). It has been suggested (Vornanen, 1989) that the negative effect of temperature on cardiac contractility is directly related to a shortening of the active state of the cardiomyocyte during the excitation–contraction coupling. In fact, this negative inotropic effect of temperature may be manifest as the increased susceptibility of perfused rainbow trout hearts to elevations in output pressure at high acclimation temperature (Farrell et al., 1996), a phenomenon also observed here with sea bass hearts.

Despite the Q_{10} >2 for heart rate, the present results clearly suggest that the optimum temperature for maximum performance of the sea bass heart was reached at the acclimation temperatures of 18 and 22°C. Maximum $\dot{Q}$ was unchanged over this temperature acclimation range (the increase in heart rate was largely offset by a decrease in maximum V_{SH}). Maximum power output was higher at 22°C only as a result of a relatively smaller ventricular mass, and the heart was more sensitive to a high afterload. This optimum temperature for maximum cardiac performance in sea bass corresponds well with the optimum temperatures determined for whole animal functions [active metabolic rate and aerobic metabolic scope reach a maximum at 22–24°C (Claireaux and Lagardere, 1999), and 22–24°C is the optimal temperature for growth (Lefebvre et al., 2001; Claireaux and Lefrançois, in press) and endurance swimming (Claireaux et al., 2006)]. Furthermore, examination of the distribution pattern of sea bass in a thermally stratified water column shows that sea bass avoid water layers with a temperature above 22°C (Claireaux and Lefrançois, in press). The temperature optimum for maximum cardiac performance in triploid brown trout was found to be between 14°C and 18°C [neither heart rate, maximum power output nor isometric tension changed significantly (Mercier et al., 2002)]. Similarly, this temperature range corresponded with a decrease in aerobic scope during swimming in brown trout (Altimiras et al., 2002). Thus, for both sea bass (without a coronary circulation) and salmonids (with a coronary circulation), there is good correspondence between the temperature optima for maximum cardiac performance and those for whole animal functions.

The present study is also consistent with the suggestion that the temperature optimum for maximum cardiac performance corresponds with a decreased stimulatory effect of adrenaline (Farrell et al., 1996). In sea bass, increasing adrenergic stimulation from a tonic to a maximal level had modest effects at both acclimation temperatures (no chronotropic effect, only $\leqslant$15% increases in maximum cardiac output and power output, and a left-shift in the Starling curve without enhancing V_{SH}). Similar results were obtained with 18°C-acclimated triploid brown trout [a 30–40% increase in maximum $\dot{Q}$ and power output, a modest positive chronotropy, and an attenuation of isometric tension development in atrial and ventricular strips; (Mercier et al., 2002)]. Thus, the relationship between optimum temperature for maximum cardiac performance and reduced adrenergic stimulation now can be extended beyond salmonids to sea bass. In reaching this conclusion, we must add a note of caution. The control condition for comparison was a tonic stimulation with 5 nmol l^{-1} adrenaline and although adrenergic effects were modest, they did occur at the lowest adrenaline concentrations. Future work should measure plasma catecholamine concentrations and examine the effect of β-adrenergic antagonists on cardiac performance.

One factor contributing to reduced adrenergic effects at high acclimation temperature in salmonids is a reduction in cardiac β-adrenoceptor density (B_{max}). For rainbow trout, an almost twofold decrease in B_{max} to ~20 fmol mg $protein^{-1}$ and an unchanged K_d with warm acclimation was reported (Gamperl et al., 1994), confirming the earlier finding (Keen et al., 1993). Gamperl et al. also reported (Gamperl et al., 1998) a high B_{max} value (58 fmol mg $protein^{-1}$) for cold-acclimated chinook salmon (13°C; *Oncorhynchus tshawytscha*). However, interspecific differences for B_{max} and binding affinity (K_d) can be considerable among fish species (Olsson et al., 2000) and temperature acclimation had no effect on B_{max} of the tropical African catfish *Clarias gariepinus*, although K_d increased significantly at an acclimation temperature of 32°C compared with 15°C and 22°C (Hanson et al., 2005). Thus, while both B_{max} and K_d of ventricular β-adrenoceptors can vary with temperature acclimation within a species, no pattern has emerged across species. In fact, the present observation that the peak response to adrenaline occurs at a lower concentration at 22°C than at 18°C can be viewed as either an increased sensitivity or a constraint on the maximum effect. Consequently, the role of adrenergic stimulation in setting the optimal performance of salmonid and non-salmonid fish hearts warrants further attention, especially given the correspondence between the temperature optima for maximum cardiac performance and those for whole animal functions.

In summary, the present results clearly show that the sea bass possesses a powerful heart even though it lacks a coronary circulation. The optimum acclimation temperature for the sea bass heart appears to be between 18 and 22°C. At these acclimation temperatures maximum adrenergic stimulation has no effect on either heart rate or maximum $\dot{Q}$, but has a modest positive inotropic effect that increases maximum cardiac power output.

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