

## Effects of temperature, epinephrine and $\text{Ca}^{2+}$ on the hearts of yellowfin tuna (*Thunnus albacares*)

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### Summary

Tuna are endothermic fish with high metabolic rates, cardiac outputs and aerobic capacities. While tuna warm their skeletal muscle, viscera, brain and eyes, their hearts remain near ambient temperature, raising the possibility that cardiac performance may limit their thermal niches. We used an *in situ* perfused heart preparation to investigate the effects of acute temperature change and the effects of epinephrine and extracellular  $\text{Ca}^{2+}$  on cardiac function in yellowfin tuna (*Thunnus albacares*). Heart rate showed a strong temperature-dependence, ranging from 20 beats  $\text{min}^{-1}$  at 10 °C to 109 beats  $\text{min}^{-1}$  at 25 °C. Maximal stroke volume showed an inverse temperature-dependence, ranging from 1.4  $\text{ml kg}^{-1}$  at 15 °C to 0.9  $\text{ml kg}^{-1}$  at 25 °C. Maximal cardiac outputs were

27  $\text{ml kg}^{-1} \text{min}^{-1}$  at 10 °C and 98  $\text{ml kg}^{-1} \text{min}^{-1}$  at 25 °C. There were no significant effects of perfusate epinephrine concentrations between 1 and 100  $\text{nmol l}^{-1}$  at 20 °C. Increasing extracellular  $\text{Ca}^{2+}$  concentration from 1.84 to 7.36  $\text{mmol l}^{-1}$  at 20 °C produced significant increases in maximal stroke volume, cardiac output and myocardial power output. These data demonstrate that changes in heart rate and stroke volume are involved in maintaining cardiac output during temperature changes in tuna and support the hypothesis that cardiac performance may limit the thermal niches of yellowfin tuna.

Key words: temperature, epinephrine,  $\text{Ca}^{2+}$ , cardiac function, heart, yellowfin tuna, *Thunnus albacares*.

### Introduction

Tuna are renowned for their migratory movements, endothermy and high aerobic performance (Block and Stevens, 2001). They are pelagic predators at the top of their food web, whose ecological success is due in large part to their exceptional physiology. Recent studies with electronic tags demonstrate that bluefin tuna (*Thunnus thynnus*) migrate tens of thousands of kilometers in a single year (Block et al., 2001; Gunn and Block, 2001; Kitagawa et al., 2001) powered by a suite of morphological and physiological specializations that includes a high metabolic rate with a concomitant high cardiac output (Carey and Teal, 1969; Altringham and Block, 1997; Brill and Bushnell, 2001; Korsmeyer and Dewar, 2001).

While the oxidative slow-twitch muscle, viscera, brain and eyes are warmed by conservation of metabolic heat with retia mirabilia (Carey and Teal, 1969; Linthicum and Carey, 1972), the coronary circulation receives blood directly from the gills to supply the myocardium and must be at ambient temperature. Temperatures in the lumen of the heart are determined by the efficiency and control of heat exchange in the retia and are assumed to approach ambient water temperatures (Carey et al., 1984). No measurements of myocardial temperatures in free-swimming fish have been made. Electronic tagging studies indicate that bluefin, bigeye

(*Thunnus obesus*) and yellowfin (*Thunnus albacares*) tuna range into surface waters as warm as 25–30 °C. Bluefin and bigeye tuna experience waters between 2 and 30 °C during annual migrations and dives below the thermocline (Carey and Lawson, 1973; Block et al., 1998; Brill et al., 1999; Gunn and Block, 2001; Marcinek et al., 2001). In contrast, yellowfin are primarily restricted to surface water temperatures that range from 17 to 30 °C, despite occasional brief dives to temperatures as low as 11 °C (Block et al., 1997). During the prolonged dives of Atlantic bluefin, the heart is exposed to low ambient temperatures for 12 h or more while internal visceral temperatures remain at 20–25 °C or higher (Block et al., 2001). Bluefin have been recorded in surface temperatures of 8–12 °C for weeks at high latitudes (Block et al., 2001). The heart must pump blood in support of the high metabolic rate of the bluefin's endothermic tissues while operating across this wide range of ambient temperatures. This raises the possibility that cardiac performance limits thermal niche utilization (Korsmeyer et al., 1996; Brill et al., 1999; Marcinek, 2000; Brill and Bushnell, 2001). While cold surface waters and deep dives impose serious challenges on the heart's capacity to supply oxygen to a warm body, the metabolic demands of giant tuna on the warm temperate and

tropical breeding grounds may impose the most strenuous challenges for the cardiovascular system. Recent archival tagging data indicate that bluefin internal temperatures exceed 29 °C in the Gulf of Mexico breeding ground (Block et al., 2001).

How the hearts of tuna maintain function across this wide range of ambient temperatures remains unknown. Studies of cardiac muscle strips in yellowfin tuna indicate that peak force increases as temperature drops from 25 to 20 °C; however, optimal and peak frequencies decrease with falling temperature, lowering overall power output (Freund, 1999). Shiels et al. (1999) reported a drop in peak force produced by yellowfin atrial strips as temperature increased from 15 to 25 °C. Korsmeyer et al. (1997a) measured relative changes in cardiovascular parameters of swimming yellowfin tuna at temperatures ranging from 18 to 28 °C, and found that an increase in stroke volume accompanied a decrease in heart rate as temperature was reduced, resulting in a net drop in cardiac output. Thus, taken together, the cardiac strip and whole-animal performance experiments indicate that, as the temperature drops, heart rate falls and the stroke volume of the tuna heart increases.

To measure the effects of temperature on yellowfin tuna hearts, this study used an *in situ* perfused preparation exposed to a range of temperatures that yellowfin may experience in the wild (10–25 °C). *In situ* perfused heart preparations have been used successfully to study cardiac performance in a variety of fish species (Farrell et al., 1985, 1989; Farrell, 1987). In perfused rainbow trout (*Oncorhynchus mykiss*) hearts, power production in the isolated preparation can match the maximum power production achieved *in vivo* (Milligan and Farrell, 1991). However, only one study has applied this technique to tuna, producing values of cardiac output similar to those determined in spinally blocked fish at 25 °C (Farrell et al., 1992). By using this preparation on fish of 2.5–3.8 kg, we provide data on cardiac performance parameters over a wide range of temperatures.

Many factors affect cardiac cell function and can influence cardiac performance *in vivo*. Ca<sup>2+</sup>, epinephrine and temperature are all known to play a role in modulating cardiac performance. Influx of extracellular Ca<sup>2+</sup> is essential for direct activation of the myofibrils and for Ca<sup>2+</sup>-induced Ca<sup>2+</sup> release from the sarcoplasmic reticulum (Fabiato, 1983). Previous perfused heart preparations in yellowfin and skipjack tuna have employed Ringer's solutions containing 1.9 mmol l<sup>-1</sup> Ca<sup>2+</sup>; however, the blood Ca<sup>2+</sup> concentration in skipjack tuna (*Katsuwonis pelamis*) has been reported to be as high as 7.6 mmol l<sup>-1</sup> (Sather and Rogers, 1967). Blood Ca<sup>2+</sup> concentrations measured in captive yellowfin tuna vary with handling and sampling methods, ranging from 3.2 mmol l<sup>-1</sup> in relatively undisturbed fish to 3.4 mmol l<sup>-1</sup> in net-captured fish (S. Fletcher, T. Williams and B. A. Block, unpublished data). Blood Ca<sup>2+</sup> concentrations of 4.7 mmol l<sup>-1</sup> have been recorded in wild bluefin tuna caught by hook and line (Cooper et al., 1994). Thus, low Ca<sup>2+</sup> concentrations may have depressed performance in previous perfused heart preparations in tuna.

Several studies of tuna cardiac function have used spinally blocked fish (Brill, 1987; Bushnell et al., 1990; Bushnell and Brill, 1992; Jones et al., 1993; Lai et al., 1987; Brill et al., 1998), which may have resulted in high levels of circulating epinephrine during the experiment. Epinephrine influences cardiac contractility by increasing the open probability of L-type Ca<sup>2+</sup> channels, thus increasing Ca<sup>2+</sup> influx into the myocytes (Reuter et al., 1986). Blockade of adrenergic receptors produces small (6%) decreases in heart rate and ventral aortic pressure in skipjack and yellowfin, suggesting that resting levels of epinephrine have little effect on the performance of tuna heart (Keen et al., 1995). However, experiments on isolated atrial strips from skipjack tuna indicate that contractile force can increase up to twofold with increasing epinephrine concentrations up to 10<sup>-5</sup> mol l<sup>-1</sup> (Keen et al., 1992).

In the present study, we investigate the temperature-dependence of heart rate, stroke volume, cardiac output and myocardial power output of yellowfin tuna hearts *in situ*. In addition, we measure the response of these cardiac parameters to variation in Ca<sup>2+</sup> and epinephrine concentrations. Together, these data indicate the scope of cardiac performance in yellowfin tuna over a range of conditions likely to be encountered in the wild.

## Materials and methods

### Fish

Yellowfin tuna *Thunnus albacares* were caught by hook and line off San Diego, CA, USA, and held aboard the F/V *Shogun* in large wells flooded with sea water prior to transport by truck to the Tuna Research and Conservation Center (TRCC) in Pacific Grove, CA, USA. Fish were held in a 109 m<sup>3</sup> tank at 20 °C and fed a diet of squid, sardines and enriched gelatin until needed for experiments, as described previously (Altringham and Block, 1997). All fish were feeding prior to experiments and were used between 6 and 35 days after arrival at the TRCC. Preliminary experiments were successful with fish of up to 9.4 kg; however, the mean body mass of fish used in this study was 3.16±0.38 kg (mean ± s.d., N=9; range 2.54–3.76 kg).

### Fish handling and surgery

Fish were captured in a nylon sling, transported out of the tank in an envelope of sea water and killed by pithing. In some preparations, the spinal cord was ablated by insertion of a 25 cm piece of 120 kg test monofilament to eliminate *post-mortem* swimming motions. Surgical procedures were similar to those of Farrell et al. (1992). The dorsal hepatic vein was ligated, and the sinus venosus was cannulated and perfused *via* the central hepatic vein. A second cannula was inserted into the ventral aorta to receive output from the heart. In some preparations, the coronary artery was cannulated with a small polyethylene tube and perfused with oxygenated Ringer. In all preparations, the pericardium was kept intact. After surgery, the entire fish was transferred to a 75 l insulated water bath

filled with saline at 20 °C. The input cannula was connected to three 500 ml perfusate reservoirs, and recycling of perfusate was initiated by moving the output tubing back to the perfusate reservoirs, which set output pressure at approximately 6 kPa.

#### Solutions

For the temperature experiments, the perfusate consisted of (in mmol l<sup>-1</sup>) 185.7 NaCl, 1.1 MgCl<sub>2</sub>, 7.0 KCl, 3.22 CaCl<sub>2</sub>, 10 sodium pyruvate and 10 Hepes. The pH was adjusted to 7.8 at 20 °C by addition of NaOH. Epinephrine was maintained in the solution at 1 nmol l<sup>-1</sup>. For the epinephrine experiments, the perfusate contained 1.84 mmol l<sup>-1</sup> CaCl<sub>2</sub>, and the epinephrine concentration in the perfusate was varied between 1 and 100 nmol l<sup>-1</sup>. For the Ca<sup>2+</sup> experiments, the nominal Ca<sup>2+</sup> concentration was 1.84, 3.68 or 7.36 mmol l<sup>-1</sup>, and epinephrine concentration was maintained at 1 nmol l<sup>-1</sup>. Saline for the 75 l bath was made up as a 1:3 mixture of sea water with tap water (with ice as needed). The perfusate was bubbled with 100 % oxygen throughout the experiments.

#### Cardiac performance tests

Once the fish had been placed into the saline bath and the heart was successfully recycling fluid to the reservoir, a set of tests was completed: measurements under standard conditions, at maximum flow, at maximum output pressure, at maximum power and again under standard conditions. Standard conditions entailed an input pressure of 0–0.05 kPa and an output pressure of approximately 6 kPa. To determine the maximum flow the heart could produce, input pressure was elevated to 0.6 kPa and cardiac output was allowed to stabilize. Input pressure was returned to 0 kPa and output pressure was elevated in steps of 1–2 kPa until the heart could no longer beat rhythmically or cardiac output was reduced by 50 %. Following a brief recovery period, input pressure was again elevated to 0.6 kPa and output pressure was simultaneously increased to approximately 10 kPa and elevated in additional 1 kPa steps to estimate maximum power production. Standard conditions were intended to approximate *in vivo* conditions for a fish in a relaxed state, while conditions of maximal flow and pressure were intended to evoke the maximal performance of the heart, thus simulating a high-activity state.

#### Temperature experiments

With input and output pressures at standard conditions, the temperatures of the bath saline and perfusate were simultaneously adjusted over a period of 3–5 min, and the preparation was allowed to equilibrate for 3–5 min prior to measurements at the new temperature. Control tests at 20 °C were completed between measurements at each test temperature. In cases in which cardiac output under standard conditions declined by more than 10 % from the initial control test value (up to 31 %), data at the test temperature were normalized by the ratio of initial values to control values bracketing the test temperature. Experiments were completed within 90–180 min following surgery.

#### Epinephrine and Ca<sup>2+</sup> experiments

Following surgery, initial measurements were made using a perfusate containing 1.84 mmol l<sup>-1</sup> CaCl<sub>2</sub> and 1 nmol l<sup>-1</sup> epinephrine. Following the set of performance tests described above, epinephrine was added to the perfusate to a final concentration of 10 or 100 nmol l<sup>-1</sup>. After 3–5 min, the performance tests were repeated. Following the epinephrine tests, the perfusate reservoirs were drained, and the fluid was replaced with fresh Ringer's solution containing 1 nmol l<sup>-1</sup> epinephrine for control tests. The performance tests were repeated, and CaCl<sub>2</sub> was then added (from a 1.84 mol l<sup>-1</sup> stock) to 3.68 mmol l<sup>-1</sup> or 7.36 mmol l<sup>-1</sup>. Performance tests were repeated at each of these Ca<sup>2+</sup> concentrations. The reservoirs were again drained, and the perfusate was replaced with Ringer containing 1.84 mmol l<sup>-1</sup> CaCl<sub>2</sub> and 1 nmol l<sup>-1</sup> epinephrine for final control tests. Data were corrected for the decline in performance of the preparation as described above.

#### Instrumentation, calibrations and analysis

Flows were measured with a 4 mm Zepeda electromagnetic cannulating flow probe connected to a Zepeda SWF 5 flow meter. Input and output pressures were measured with Statham P23XL pressure transducers through a Neurolog NL900-424 preamplifier (Neurolog DC preamplifier, Digitimer, UK). Flow and pressure signals were read by a Maclab 8s hooked to a PowerMacintosh (1400cs) computer running Maclab 3.5.4/s software (AD Instruments, Sydney, Australia). Flow signals were calibrated by weighing the saline output over a measured time. Pressure signals were calibrated with a water manometer. Mean flow, pressures, power and heart rate were calculated from five or six beats using the Powerlab program. Power output is expressed as mW g<sup>-1</sup> heart mass (ventricle plus atrium). Single-factor analyses of variance (ANOVAs) and regression analysis were performed with temperature, epinephrine concentration or Ca<sup>2+</sup> concentration as the independent variable for each set of conditions. Significance was assessed at  $P \leq 0.05$ .

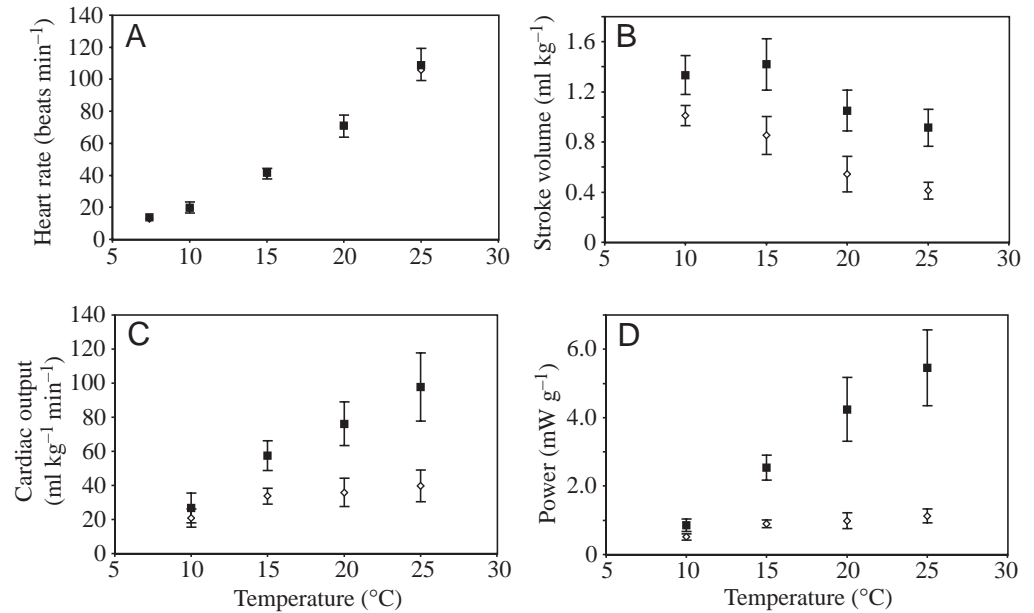
## Results

#### Effects of temperature

All cardiac parameters were affected by temperature. Heart rate increased significantly at warmer temperatures (Fig. 1A; Table 1). Q<sub>10</sub> values ranged from 2.23 (20–25 °C) to 4.36 (10–15 °C) over 5 °C temperature ranges. Heart rates were unaffected by changes in input and output pressures, except at maximal output pressure, at which arrhythmias occasionally developed. All preparations continued to beat rhythmically at 10 °C, and one yellowfin tuna heart was cooled to 7.4 °C with no signs of arrhythmia.

Stroke volume was inversely affected by temperature (Fig. 1B; Table 1). Mean stroke volume increased from 0.41 ml kg<sup>-1</sup> at 25 °C to 1.01 ml kg<sup>-1</sup> at 10 °C under standard conditions. However, under maximal cardiac output conditions, stroke volume was greatest at 15 °C (1.42 ml kg<sup>-1</sup>).

Fig. 1. Values of cardiac parameters recorded from spontaneously beating yellowfin tuna hearts *in situ* at temperatures of 10 to 25 °C. (A) Heart rate, (B) stroke volume, (C) cardiac output, (D) myocardial power output. Standard conditions are shown as open symbols, maximal conditions as filled symbols.  $N=5$  at 15 and 20 °C,  $N=4$  at 10 and 25 °C. Values are means  $\pm$  s.d. One value is given for heart rate at 7.4 °C.



and showed no further increase at 10 °C. Stroke volume varied among individual fish, which probably reflects differences in the success of the surgery. Coronary perfusion had no observable effect on cardiac performance.

The increase in stroke volume with decreasing temperature was insufficient to compensate for the decline in heart rate. As a result, cardiac output was reduced significantly between 15 and 10 °C under standard conditions and with each decrease in temperature under maximal flow conditions (Fig. 1C;

Table 1). The highest cardiac output ( $97.6 \pm 20.0$  ml kg<sup>-1</sup> min<sup>-1</sup>) was recorded under maximal flow conditions at 25 °C.  $Q_{10}$  values under standard conditions ranged from 1.14 (15–20 °C) to 2.58 (10–15 °C). The decline in cardiac output at lower temperatures was more pronounced when filling pressure was elevated to achieve maximal flow condition, with  $Q_{10}$  values ranging from 1.64 (20–25 °C) to 4.59 (10–15 °C). All fish showed a similar response to temperature; however individual values of cardiac output ranged from 69 to 115 ml kg<sup>-1</sup> min<sup>-1</sup> at 25 °C.

Maximal power output was highest at 25 °C and was  $5.5 \pm 1.1$  mW g<sup>-1</sup> heart tissue. Myocardial power output showed a significant temperature-dependence, decreasing at lower temperatures under both conditions (Table 1). This effect was most pronounced under maximal power conditions (Fig. 1D), when both input and output pressures were elevated.

#### Effects of Ca<sup>2+</sup> and epinephrine

Increasing the concentration of perfusate Ca<sup>2+</sup> from 1.84 to 3.68 and 7.36 mmol l<sup>-1</sup> by addition of a concentrated Ca<sup>2+</sup> stock solution produced significant increases in stroke volume, cardiac output and myocardial power output under maximal flow and maximal power conditions (Fig. 2). Values recorded under standard conditions were unaffected.

Increasing epinephrine concentration from 1 to 100 nmol l<sup>-1</sup> had no significant effect on any cardiac parameter (Fig. 3). Epinephrine trials took place prior to Ca<sup>2+</sup> trials using the same fish, so values of cardiac parameters recorded at maximal [epinephrine] and maximum [Ca<sup>2+</sup>] are not directly comparable.

#### Discussion

This paper presents measurements of the effects of temperature, Ca<sup>2+</sup> and epinephrine on cardiac performance *in*

Table 1. Effects of temperature on cardiac parameters

Parameter	Temperature (°C)	Standard conditions	Maximum flow
Heart rate (beats min <sup>-1</sup> )	10	19.6 $\pm$ 3.2 <sup>a</sup>	19.8 $\pm$ 3.8 <sup>e</sup>
	15	40.9 $\pm$ 3.1 <sup>b</sup>	41.6 $\pm$ 2.8 <sup>f</sup>
	20	70.8 $\pm$ 6.9 <sup>c</sup>	70.9 $\pm$ 6.6 <sup>g</sup>
	25	105.8 $\pm$ 6.5 <sup>d</sup>	108.9 $\pm$ 10.3 <sup>h</sup>
Stroke volume (ml kg <sup>-1</sup> )	10	1.01 $\pm$ 0.08 <sup>a</sup>	1.33 $\pm$ 0.16 <sup>e</sup>
	15	0.85 $\pm$ 0.15 <sup>a</sup>	1.42 $\pm$ 0.20 <sup>e</sup>
	20	0.54 $\pm$ 0.14 <sup>b</sup>	1.05 $\pm$ 0.16 <sup>f</sup>
	25	0.41 $\pm$ 0.07 <sup>b</sup>	0.91 $\pm$ 0.15 <sup>f</sup>
Cardiac output (ml kg <sup>-1</sup> min <sup>-1</sup> )	10	20.9 $\pm$ 5.3 <sup>a</sup>	26.8 $\pm$ 8.8 <sup>e</sup>
	15	33.7 $\pm$ 4.6 <sup>b</sup>	57.4 $\pm$ 8.8 <sup>f</sup>
	20	35.9 $\pm$ 8.4 <sup>b</sup>	76.2 $\pm$ 12.8 <sup>g</sup>
	25	39.7 $\pm$ 9.2 <sup>b</sup>	97.6 $\pm$ 20.0 <sup>h</sup>
Power (mW g <sup>-1</sup> )	10	0.51 $\pm$ 0.09 <sup>a</sup>	0.86 $\pm$ 0.18 <sup>e</sup>
	15	0.90 $\pm$ 0.11 <sup>b</sup>	2.54 $\pm$ 0.36 <sup>f</sup>
	20	0.99 $\pm$ 0.23 <sup>c</sup>	4.24 $\pm$ 0.94 <sup>g</sup>
	25	1.12 $\pm$ 0.20 <sup>d</sup>	5.46 $\pm$ 1.11 <sup>h</sup>

Values are means  $\pm$  s.d.;  $N=4$  at 10 and 25 °C,  $N=5$  at 15 and 20 °C.

Different letters indicate significant differences within a column and within a parameter.

*situ* in yellowfin tuna. The overall cardiac performance of the yellowfin tuna heart preparations at 25 °C matched that of previous studies, indicating that the experimental protocol is appropriate for fish of the size examined (2.5–3.8 kg). Heart rates recorded at 25 °C were within the range of values recorded in previous studies (Bushnell et al., 1990; Bushnell and Brill, 1991, 1992; Farrell et al., 1992; Jones et al., 1993; Korsmeyer et al., 1997a). Mean values of stroke volume ( $0.91 \pm 0.15 \text{ ml kg}^{-1}$ ) and cardiac output ( $97.6 \pm 20.0 \text{ ml kg}^{-1} \text{ min}^{-1}$ ) measured under maximal flow conditions at 25 °C in this study fell within the range of values measured in previous studies of spinally blocked and anesthetized yellowfin (Bushnell and Brill, 1992; Jones et al., 1993) and were slightly lower than values previously recorded *in situ* in smaller fish (Farrell et al., 1992). Korsmeyer et al. (1997b) estimated similar values for cardiac output in swimming yellowfin tuna using the Fick equation, but calculated higher stroke volumes.

#### Temperature

Changes in ambient temperatures had profound effects on all cardiac parameters. Heart rate showed a linear dependence on temperature, falling to  $19.6 \pm 3.2 \text{ beats min}^{-1}$  at 10 °C under standard conditions. No arrhythmias were noted at the lowest temperatures tested (10 and 7 °C), which match the lowest ambient water temperatures encountered in acoustic and archival pop-up satellite tracks of yellowfin tuna (Block et al., 1997; K. Weng, M. J. W. Stokesbury, A. M. Boustany and B. Block, in preparation). Stroke volume increased as temperature decreased, such that decreasing temperatures had little effect on cardiac output until temperature dropped below 15 °C under standard conditions. The increase in stroke volume with decreasing temperature is consistent with results from ventricular strips (Freund, 1999) and direct measurements of stroke volume changes in swimming yellowfin (Korsmeyer et al., 1997a). The results from whole-animal and tissue studies indicate that changes in stroke volume are likely to be an important factor in maintaining cardiac output in tuna during ambient temperature changes, as is the case for other teleosts including rainbow trout (Farrell et al., 1996).

Importantly, the maximal cardiac output generated at each temperature dropped sharply, with a  $Q_{10}$  of 1.6 (20–25 °C) to 4.6 (10–15 °C) (Table 1) under maximal flow conditions. This result indicates that the scope for increase in cardiac output is greatly reduced at low temperatures, as shown in Fig. 4. This lack of scope *in situ* supports the hypothesis that temperature-related reductions in cardiac output may be responsible for the thermal limitation seen in acoustic and archival tag recordings of yellowfin tuna (Block et al., 1997; Brill et al., 1999; Marcinek, 2000). Tuna range through a thermally variable

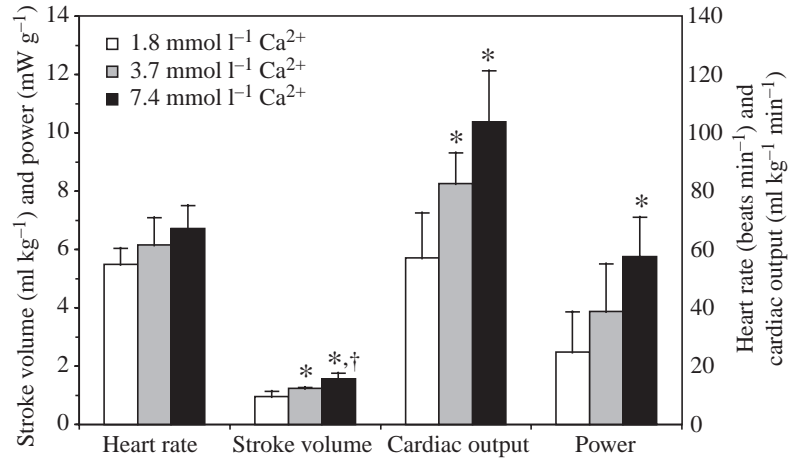


Fig. 2. Effects of external  $[\text{Ca}^{2+}]$  on cardiac parameters recorded from spontaneously beating yellowfin tuna hearts *in situ* at 20 °C. Data shown are for standard conditions (Heart rate), maximum flow (Stroke volume, Cardiac output) and maximum power (Power). Values are means + S.D.  $N=4$ . An asterisk indicates a value significantly different from the value at  $1.8 \text{ mmol l}^{-1} \text{ Ca}^{2+}$ . A dagger indicates a value significantly different from the value at  $3.7 \text{ mmol l}^{-1} \text{ Ca}^{2+}$ .

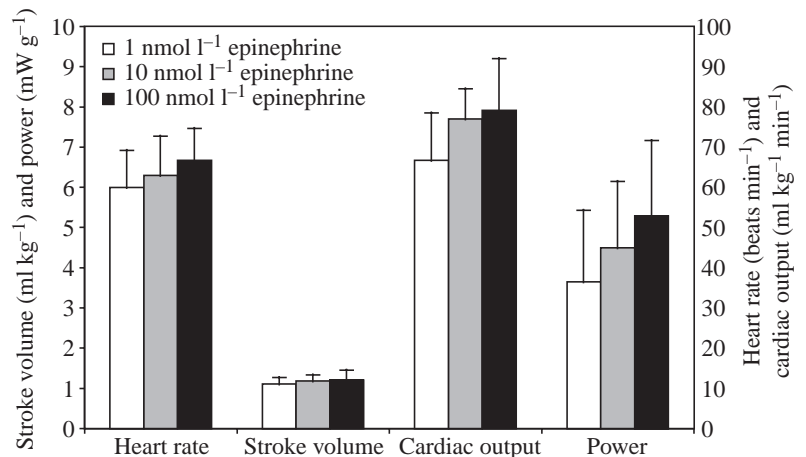


Fig. 3. Effects of perfusate [epinephrine] on cardiac parameters recorded from spontaneously beating yellowfin tuna hearts *in situ* at 20 °C. Data shown are for standard conditions (Heart rate), maximum flow (Stroke volume, Cardiac output) and maximum power (Power). Values are means + S.D.  $N=4$ . There are no significant differences among data points for a given parameter.

environment while relying on their metabolic and vascular specializations to maintain relatively constant temperatures for the viscera, swimming muscles and brain (Carey and Teal, 1969). Although much of the tuna body is protected from changes in ambient temperature (Marcinek et al., 2001), the heart must meet the demands of high metabolic rates in the face of sudden shifts in ambient temperature during deep dives or when crossing frontal regions.

#### Ca<sup>2+</sup>

Increases in external  $\text{Ca}^{2+}$  concentration produced significant increases in stroke volume, cardiac output and

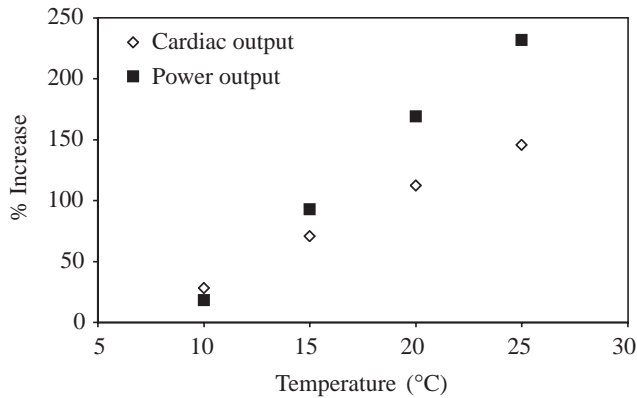


Fig. 4. Scope for increase in cardiac output (open symbols) and myocardial power output (filled symbols) of *in situ* perfused yellowfin tuna hearts at temperatures of 10–25 °C. Scope is determined as the percentage difference between mean values at standard and maximal flow (cardiac output) or maximal power (power output) conditions.

myocardial power output at conditions of maximal flow and power in yellowfin tuna hearts. These results are in accord with previous experiments showing that increasing  $\text{Ca}^{2+}$  concentration increased isometric force in atrial strips from skipjack tuna (Keen et al., 1992) and in intact hearts of a variety of other teleosts (Driedzic and Gesser, 1985). Keen et al. (1992) also reported that increased  $[\text{Ca}^{2+}]$  shortened the activation and relaxation kinetics for isolated atrial strips. However, differences in heart rate with varying  $[\text{Ca}^{2+}]$  in this study were not significant.

Contraction of cardiomyocytes is initiated by the influx of extracellular  $\text{Ca}^{2+}$  through L-type  $\text{Ca}^{2+}$  channels, which stimulates the myofibrils directly and/or opens  $\text{Ca}^{2+}$ -release channels in the sarcoplasmic reticulum (Fabiato, 1983). The relative importance of these two functions varies among taxa. Recent studies indicate that tuna myocytes rely on intracellular  $\text{Ca}^{2+}$  stores to a larger extent than myocytes of other teleosts (Freund, 1999; Shiels et al., 1999); however, extracellular  $[\text{Ca}^{2+}]$  modulates contractility. Blood  $[\text{Ca}^{2+}]$  may rise with exercise in teleosts (Ruben and Bennett, 1981), suggesting that exercise-induced changes in blood  $[\text{Ca}^{2+}]$  may play a role in increasing cardiac output. The magnitude and importance of such changes in tuna are unknown. Reported blood  $\text{Ca}^{2+}$  levels vary more than twofold among tuna species (Sather and Rogers, 1967; Cooper et al., 1994) and could influence cardiac contractility. However these differences may reflect different holding and sampling conditions rather than interspecific variation. Thus, while the potential influence of external  $[\text{Ca}^{2+}]$  levels on cardiac contractility is clear, its relevance *in vivo* remains to be determined.

#### Epinephrine

There were no significant effects of epinephrine on any cardiac parameters, although upward trends were evident in heart rate, cardiac output and power output with increasing epinephrine concentrations (Fig. 3). The lack of an epinephrine

effect is at odds with previous studies on isolated atrial strips (Keen et al., 1992). This discrepancy may result from the different epinephrine concentrations used in the two studies. We used three different epinephrine concentrations (1, 10 and 100  $\text{nmol l}^{-1}$ ), while Keen et al. (1992) saw large effects of epinephrine at 10  $\text{mmol l}^{-1}$ . Little is known about circulating epinephrine concentrations in tuna, but Keen et al. (1995) reported levels ranging from 3.5 to 55  $\text{nmol l}^{-1}$  in anesthetized, spinally blocked yellowfin. Although Watson (1990) measured millimolar levels of catecholamines in blood from stressed tuna, no values are available for free-swimming tuna, so it is possible that millimolar concentrations of epinephrine influence heart function *in vivo*.

The low external  $\text{Ca}^{2+}$  concentration (1.84  $\text{mmol l}^{-1}$ ) used in these preliminary experiments may have limited the effects of epinephrine, which acts to increase the open probability of voltage-gated  $\text{Ca}^{2+}$  channels. Thus, it is possible that simultaneous elevation of epinephrine and  $\text{Ca}^{2+}$  levels would exert synergistic effects on the tuna heart.

Spinally blocked tuna have mass-specific stroke volumes near our recorded maxima and show very limited scope for modulation of stroke volume (Bushnell and Brill, 1992). Our lower stroke volume values may reflect the possible limitations of perfused preparations, which include incomplete perfusion of the myocardium. In addition, hormones released from the heart into the recirculating perfusate may have exerted pharmacological effects on our preparation. Alternatively, this difference may suggest that measurements on spinally blocked fish do not represent routine values, as suggested by Korsmeyer et al. (1997a). Spinally blocked fish may operate near their maximal cardiac output because of the stress of the procedures (and adrenergic stimulation), and their hearts are therefore thus comparable with those in our maximal flow or maximal power conditions. Our *in situ* hearts set cardiac output and stroke volume at 'standard' conditions in response to adjustments of input and output pressures and reflect 'standard' performance only in so far as these pressures are physiological. In contrast to stroke volume and cardiac output, accurate ventral aortic pressures of yellowfin tuna measured *in situ* (10–11 kPa; Brill and Bushnell, 2001) and in swimming fish (12.2 kPa; Korsmeyer et al., 1997b) are similar to the pressures generated by our *in situ* hearts at maximal power outputs (output pressure at maximal power output  $12.18 \pm 2.68$  kPa; range 8.17–15.4 kPa,  $N=6$ ), but much higher than the 6 kPa set for standard conditions. Blood pressures in spinally blocked fish may represent elevated values, as with stroke volume and cardiac output. The absence of reliable measurements of cardiac output or stroke volume in free-swimming tuna prevents us from distinguishing among these hypotheses.

#### Cardiac performance in other *Thunnus* species

Electronic tagging data indicate that yellowfin tuna are restricted in their ambient temperature preferences in comparison with bluefin and big-eye tuna (Block et al., 1997, 2001; Brill et al., 1999). At the northern extent of their range, where yellowfin are likely to encounter the coolest

temperatures, acoustic tracks indicate that yellowfin venture into cool waters (to 11 °C) below the thermocline only occasionally and return to the surface after relatively short periods (<7 min) at depth (Block et al., 1997). More extensive recordings from pop-up satellite archival tags indicate similar temperature limitations for yellowfin tuna in the Gulf of Mexico (K. Weng, M. Stokesbury, A. M. Boustany and B. A. Block, personal communication). Our data supports the hypothesis that cardiac limitations restrict the thermal range of yellowfin tuna.

In contrast to yellowfin, northern and southern bluefin tuna have successfully invaded cooler waters and encounter cold temperatures while foraging for extended periods in deeper waters. Atlantic bluefin tuna migrate rapidly from waters at 22–29 °C in the breeding grounds to northern feeding areas, where they spend extended periods in cold surface waters (8–12 °C) and encounter waters as cold as 2–4 °C at depth without apparent compromise (Block et al., 2001; B. A. Block, unpublished data). This raises the question of what physiological specializations of the heart, if any, are associated with the wide thermal tolerance within the *Thunnus* lineage.

Warm extremes of ambient temperatures may pose a greater challenge to tuna than cold ambient temperatures. Warm temperatures increase the oxygen demand of aerobic tissues, requiring increased cardiac output through increased heart rate. However, the accompanying decline in stroke volume observed in the present study suggests that the ability of yellowfin tuna to elevate cardiac output is limited at high temperatures, as is the case in trout hearts near their upper lethal limit of temperature (Farrell et al., 1996). Adult bluefin tuna breed in waters of 23–29 °C (Block et al., 2001). To support the high metabolic rates of giant bluefin tuna at high ambient temperatures, high cardiac outputs and correspondingly high heart rates are required. High-frequency hearts require more rapid Ca<sup>2+</sup> cycling and increased expression of excitation/contraction coupling proteins (Lillywhite et al., 1999). Although little is known about the function of the sarcoplasmic reticulum Ca<sup>2+</sup>-release channel or the Ca<sup>2+</sup> ATPase in tuna hearts, preliminary data indicate that both the tropical yellowfin and skipjack have a significant reliance on internal sarcoplasmic-reticulum-mediated Ca<sup>2+</sup> release (Keen et al., 1992; Freund, 1999; Shiels et al., 1999). We hypothesize that the need for rapid Ca<sup>2+</sup> cycling has resulted in the evolution of increased reliance on sarcoplasmic reticulum Ca<sup>2+</sup> release and re-uptake at both warm and cold temperatures. Further investigation will be required to measure cardiac performance in a variety of tuna species and to determine the underlying cellular mechanisms enabling high performance across a range of temperatures.

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