

## ***In situ* cardiac performance of Pacific bluefin tuna hearts in response to acute temperature change**

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### **Summary**

**This study reports the cardiovascular physiology of the Pacific bluefin tuna (*Thunnus orientalis*) in an *in situ* heart preparation. The performance of the Pacific bluefin tuna heart was examined at temperatures from 30°C down to 2°C. Heart rates ranged from 156 beats min<sup>-1</sup> at 30°C to 13 beats min<sup>-1</sup> at 2°C. Maximal stroke volumes were 1.1 ml kg<sup>-1</sup> at 25°C and 1.3 ml kg<sup>-1</sup> at 2°C. Maximal cardiac outputs were 18.1 ml kg<sup>-1</sup> min<sup>-1</sup> at 2°C and 106 ml kg<sup>-1</sup> min<sup>-1</sup> at 25°C. These data indicate that cardiovascular function in the Pacific bluefin tuna exhibits a strong temperature dependence, but cardiac function is**

**retained at temperatures colder than those tolerated by tropical tunas. The Pacific bluefin tuna's cardiac performance in the cold may be a key adaptation supporting the broad thermal niche of the bluefin tuna group in the wild. *In situ* data from Pacific bluefin are compared to *in situ* measurements of cardiac performance in yellowfin tuna and preliminary results from albacore tuna.**

Key words: Pacific bluefin tuna, *Thunnus orientalis*, *in situ* heart preparation, temperature, citrate synthase.

### **Introduction**

Tunas are apex predators renowned for their thunniform swimming mode, high metabolic rates and ability to conserve metabolic heat (Carey and Teal, 1969; Dewar and Graham, 1994a,b; Block and Stevens, 2001). The capacity to conserve metabolic heat in the slow-twitch muscle, viscera, brain and eyes and to elevate body tissue temperatures occurs along a continuum among the *Thunnus* species. Tropical species such as yellowfin tuna maintain only a small elevation above ambient temperatures while young and mature bluefin display significant thermal gradients between core (peritoneal) body temperature, swimming muscle and ambient water temperatures (Carey and Teal, 1969; Marcinek et al., 2001a; Kitigawa et al., 2002; Block et al., 2001). Measurements of metabolic performance *in vivo* have been made in tropical tunas but not as yet in any species from the bluefin tuna group. The relationships between elevated metabolic rate, cardiac output, increased heat generation and thermal heat conservation remain elusive among the *Thunnus* species. However, electronic tagging data indicates that the bluefin group has the largest capacity for maintaining large thermal gradients between core body temperatures and ambient water temperatures for prolonged periods (Block et al., 2001; Gunn and Block, 2001).

Current knowledge of physiological performance in tunas is primarily based on research conducted on juvenile yellowfin and skipjack tunas, as both species have been routinely

maintained in land-based facilities (Brill and Bushnell, 2001; Farwell, 2001). Standard metabolic rates measured in juvenile yellowfin tuna are at least twofold higher than those of active teleosts, including scombrids such as bonito *Sarda chiliensis* and other species such as sockeye salmon *Oncorhynchus nerka* and yellowtail *Seriola quinqueradiata* (Brett and Glass, 1973; Yamamoto et al., 1981; Dewar and Graham, 1994a; Korsmeyer and Dewar, 2001). Albacore tuna placed in shipboard respirometers shortly after capture have exhibited similarly high metabolic rates (Graham and Laurs, 1982).

The elevated metabolic rates of tunas are supported by a number of distinctive morphological, biochemical and physiological specializations that are unique to the tribe *Thunnini*. Large gill surface areas and thin gill epithelia enhance oxygen uptake from the environment (Muir and Hughes, 1969), while well vascularized tissues (Mathieu-Costello et al., 1996) with high levels of myoglobin (Giovane et al., 1980; Marcinek, 2000) and aerobic enzymes (Dickson, 1995; Freund, 1999) enhance oxygen extraction by the tissues (Bushnell and Brill, 1992). High metabolic rates also demand high cardiac outputs, which tunas achieve through high maximal heart rates and high stroke volumes (Brill and Bushnell, 2001).

To date, cardiovascular performance has primarily been studied in small yellowfin, skipjack and albacore tunas. In yellowfin and skipjack tunas, cardiac outputs of up to

84–132 ml kg<sup>-1</sup> min<sup>-1</sup> are among the highest recorded in teleosts (Bushnell and Brill, 1992; Farrell et al., 1992; Brill and Bushnell, 2001; Blank et al., 2002; Mercier et al., 2002). No comparable studies have been conducted on closely related ectothermic taxa such as bonito. Heart rates in spinally blocked yellowfin tuna at 25°C range from 90 to 130 beats min<sup>-1</sup> (Bushnell and Brill, 1992), although heart rates in unstressed free-swimming yellowfin tuna are lower, ranging from 67 to 100 beats min<sup>-1</sup> (Bushnell and Brill, 1991; Korsmeyer et al., 1997). These lower rates have been shown to result from tonic cholinergic input (Keen et al., 1995). Maximal heart rates of 119 and 180 beats min<sup>-1</sup> recorded at 25°C in yellowfin and skipjack tuna, respectively (Keen et al., 1995), exceed those of most other teleosts. Stroke volumes of 1.1–1.3 ml kg<sup>-1</sup> in yellowfin and skipjack tunas are similar to maximal values in rainbow trout and two- to threefold higher than routine values in teleosts including rainbow trout and yellowtail (Yamamoto et al., 1981; Bushnell and Brill, 1992; Farrell and Jones, 1992). It is unclear whether values recorded in spinally blocked tunas represent routine or maximal cardiac performance. Shipboard experiments on freshly caught albacore tunas have yielded heart rates similar to those of the tropical tunas while operating at 15–19°C, but produced threefold lower cardiac outputs due to lower stroke volumes (Lai et al., 1987; White et al., 1988).

The response of the tuna heart to temperature change has been studied in free-swimming yellowfin, *in situ* preparations, and myocardial strip preparations (Korsmeyer et al., 1997; Freund, 1999; Shiels et al., 1999; Blank et al., 2002). Measurements on instrumented yellowfin tuna swimming in a flume indicate a trade-off between reduced heart rate and increased stroke volume with falling temperatures, resulting in a decrease of cardiac output (Korsmeyer et al., 1997). *In situ* heart preparations exhibit a similar trade-off between heart rate and stroke volume, indicating that these responses are intrinsic to the heart (Blank et al., 2002). Heart rates of yellowfin tuna *in situ* dropped from 109 beats min<sup>-1</sup> at 25°C to 19.8 beats min<sup>-1</sup> at 10°C, while experimental maximal stroke volumes rose from 0.91 at 25°C to 1.33 ml kg<sup>-1</sup> at 10°C (Blank et al., 2002). This results in a drop in cardiac output from 98 ml kg<sup>-1</sup> min<sup>-1</sup> at 25°C to 28 ml kg<sup>-1</sup> min<sup>-1</sup> at 10°C. Experiments on isolated atrial and ventricular strips from yellowfin tuna produced similar results, indicating that falling temperatures increase contractile force but reduce sustainable contraction frequencies (Freund, 1999; Shiels et al., 1999).

In contrast to data on metabolic rate and cardiovascular function in yellowfin, skipjack and albacore tunas, no measurements of heart performance or metabolic rate have been made in any species of bluefin tuna either *in vivo* or *in situ*. This is in large part due to the challenges of maintaining captive bluefin tuna in land-based facilities. Several studies have investigated cardiac morphology and metabolic biochemistry of the Atlantic and Pacific bluefin tuna, *Thunnus thunnus* and *Thunnus orientalis* (Basile et al., 1976; Maresca et al., 1976; Balestrieri et al., 1978; Tota, 1978; Gemelli et al., 1980; Greco et al., 1982; Marcinek et al., 2001b). These studies have shown that Atlantic bluefin tuna hearts are proportionally

larger than those of skipjack and yellowfin tunas (Poupa et al., 1981), exhibit a thick layer of compact myocardium (Tota, 1978) and have extraordinarily high levels of myoglobin (Giovane et al., 1980; Marcinek, 2000). Experiments on the spongy and compact layers of Atlantic bluefin ventricle indicate that the spongy layer has higher mitochondrial enzyme activities (Basile et al., 1976; Greco et al., 1982), more pronounced temperature dependence of oxidative enzymes (Maresca et al., 1976), and greater capacity to metabolize lactate (Gemelli et al., 1980), apparently reflecting the fact that spongy tissue is perfused by luminal venous blood and compact tissue receives arterial blood from the coronary artery (Tota, 1978).

Recently, the thermal physiology of free-swimming bluefin tunas, yellowfin tuna and big-eye tuna has been examined by the deployment of electronic tags on wild fish (Kitagawa et al., 2000; Block et al., 2001; Gunn and Block, 2001; Schaefer and Fuller, 2002; Musyl et al., 2003). Archival tags indicate that all bluefin species experience a wide range of environmental temperatures, with a maximal range of 2.8–31°C recorded thus far in Atlantic bluefin. To date, there is little data from large Pacific bluefin tuna, but young Pacific bluefin tuna similar in size to the animals studied in this paper have been acoustically and archivally tracked in the eastern Pacific and experience temperatures from 1.8°C to 22°C (Marcinek et al., 2001a; Kitagawa et al., 2002; B. A. Block et al., unpublished data). Archival tagging of juvenile Pacific bluefin tuna in the western Pacific indicates that these fish primarily encounter temperatures of 12–22°C but dive into waters as cold as 5°C (Kitagawa et al., 2000, 2001, 2002). In the eastern Pacific, where juveniles of both yellowfin and bluefin tuna have been tracked, acoustic tracks indicate that yellowfin tuna primarily occupy waters above 17°C. Periodic dives below this ambient temperature to 11°C lasted for only a few minutes and the tuna quickly returned to the surface, suggesting a physiological limitation (Block et al., 1997).

Bluefin tunas conserve metabolic heat and face a unique physiological challenge when diving below the thermocline or encountering cold surface waters at high latitude. The heart rapidly equilibrates to cold ambient temperatures (Brill, 1987; Brill et al., 1994), but vascular countercurrent heat exchangers defend the temperature of the brain, eyes, swimming muscles and viscera, maintaining warm tissues which demand high cardiac outputs (Brill et al., 1994). This raises the possibility that cardiac performance in cold ambient conditions limits the tuna's thermal niche and behavioral performance (Brill et al., 1998, 1999; Block et al., 1997; Marcinek et al., 2001a). What has not been directly addressed in many studies is how and why this physiological limitation occurs.

In this study, the *in situ* heart preparation of Farrell et al. (1992) was used to measure cardiac performance, including heart rate, stroke volume and cardiac output in bluefin tuna across a range of temperatures likely to be encountered in the wild (2–30°C). *In situ* preparations have been used successfully to study heart function in a variety of fish species (Farrell et al., 1983, 1988), including yellowfin and skipjack

tuna (Farrell et al., 1992). The use of the *in situ* technique allows comparison of the data presented here with similar measurements of temperature sensitivity in yellowfin tuna hearts *in situ* (Blank et al., 2002). The *in situ* preparation was also applied to one albacore tuna *Thunnus alalunga* that became available during the course of the experiments, and data are reported here for comparison. Because aerobic capacity is an important component of cardiac performance, citrate synthase activity, a commonly used index of tissue aerobic capacity, was measured in homogenates of Pacific bluefin tuna atrium and ventricle.

## Materials and methods

### Fish

Pacific bluefin tuna (*Thunnus orientalis* Temminck and Schlegel 1844) were captured by hook and line off San Diego, CA, USA, held on board the fishing vessel in wells filled with flowing seawater, and transported by truck to the Tuna Research and Conservation Center (TRCC) in Pacific Grove, CA, USA. Fish were held in 109 m<sup>3</sup> circular tanks containing 20±1.5°C seawater and fed a diet of squid and enriched gelatin, as previously described (Farwell, 2001). All fish were feeding prior to experiments and were used within 60 days of arrival at the TRCC. The mean body mass of bluefin tuna used for *in situ* preparations was 6.75±0.18 kg (mean ± S.E.M., *N*=9, range 6.04–7.47 kg). A single albacore tuna (5.30 kg) was also studied. The mean body mass of fish used for citrate synthase (CS) assays was 15.9±5.8 kg (mean ± S.E.M., *N*=6, range 6.3–36.7 kg). Tissue samples used for CS assays were taken at sea or within 5 days of arrival at the TRCC. All procedures were conducted in accordance with Stanford University institutional animal use protocols.

### Fish handling and surgical procedures

Fish were captured in a nylon sling, transported out of the tank in an envelope of sea water and euthanized by pithing. The spinal cord was ablated by insertion of a 30 cm piece of 120 kg test monofilament to eliminate *post-mortem* swimming motions. Surgical procedures were identical to those described previously (Blank et al., 2002). The peritoneal cavity was opened ventrally and the sinus venosus was cannulated and perfused with oxygenated Ringer's solution *via* a hepatic vein. A second cannula was inserted into the ventral aorta to receive output from the heart. The coronary artery was cannulated and perfused with oxygenated Ringer's successfully in seven of nine preparations. The pericardium was kept intact. After surgery, the entire fish was transferred to a 75 l insulated water bath filled with saline at 15°C. The input cannula was connected to insulated 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. At the completion of each experiment, the atrium and ventricle were removed, cut open, blotted dry on paper towels and weighed. Mean atrial and ventricular masses of Pacific bluefin tuna were 3.85±0.66 g and 21.8±3.0 g

(0.057±0.009% and 0.322±0.025% of body mass), respectively. Atrial and ventricular masses of one albacore tuna were 2.01 g and 11.27 g (0.038% and 0.213% of body mass), respectively.

### Cardiac performance tests

Once the fish was placed into the saline bath and the heart was successfully recycling fluid to the reservoir, a set of tests was completed, comprising measurements at standard conditions, maximum flow, maximum power, and standard conditions again. Standard conditions were defined as input pressure of 0–0.05 kPa and output pressure of approximately 6 kPa. To determine the maximum flow that the heart could produce, input pressure was elevated to ~2.4 kPa and cardiac output was allowed to stabilize (maximal flow conditions). Following a brief recovery period, input pressure was again elevated to ~2.4 kPa and output pressure was simultaneously increased to ~10 kPa and then elevated in additional 1 kPa steps until power no longer increased (maximal power conditions). 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.

### 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 15°C were completed between measurements at each test temperature. In cases where cardiac output at standard conditions declined by more than 10% from the initial control test, data at the test temperature were normalized by the ratio of initial values to control values bracketing the test temperature. Experiments were completed within a period of 90–150 min following surgery.

### Solutions

Ringer's solution 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 Ringer's perfusate at 1 nmol l<sup>-1</sup>. The perfusate was bubbled with 100% oxygen throughout the experiments. Saline for the 75 l bath was made up as a 1:3 mixture (v/v) of seawater and tapwater (or ice as needed).

### Instrumentation, calibrations and analysis

Flows were measured with a Transonic 6-N in-line flow probe connected to a Transonic T-106 flow meter (Transonic Systems Inc., Ithaca, NY, USA). Both input and output pressures were measured with pressure transducers from ADInstruments (Sydney, Australia). Flow and pressure signals were read by a Powerlab 8s hooked to a Macintosh G4 computer running Chart 4.0 software (ADInstruments). Flow

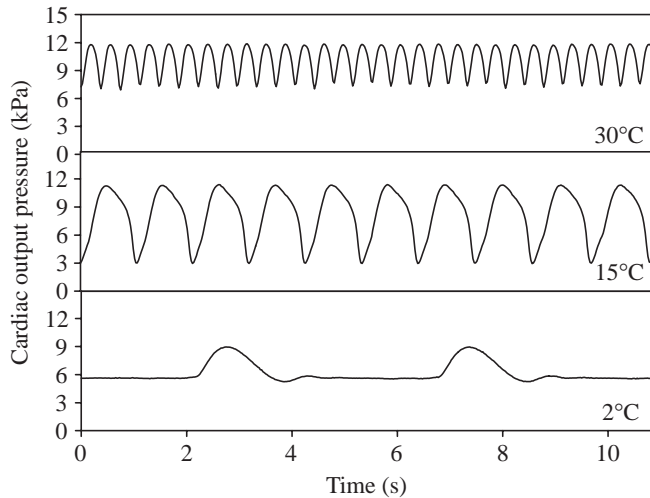


Fig. 1. Heart rate from a Pacific bluefin tuna heart perfused with Ringer's solution *in situ*. Cardiac output pressure data from a single fish at 30°C (top), 15°C (middle) and 2°C (bottom) are shown.

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 5 or 6 beats at each temperature and experimental condition using the Powerlab program. Power output is expressed as  $\text{mW g}^{-1}$  heart (ventricle+atrium) mass. Results at different temperatures within the standard or maximal condition were compared by single-factor analysis of variance (ANOVA) and Student–Newman–Keuls *post-hoc* tests. Differences between standard and maximal conditions at individual temperatures were assessed by paired *t*-tests. Linear regressions of performance at different temperatures were compared among species by ANOVA. Significance was assessed at  $P \leq 0.05$ . Data are presented as means  $\pm$  S.E.M.

#### Citrate synthase (CS) assays

Atria and ventricles were removed from wild Pacific bluefin tuna at sea or within 5 days of arrival at the TRCC and freeze-clamped with copper tongs cooled in liquid nitrogen. Crude homogenates were prepared and CS activity was determined by the reduction of 5,5'-dithiobis(2-nitrobenzoic acid) (DTNB) and the resulting change in absorbance at 412 nm using a Perkin-Elmer Lambda 6 spectrophotometer (Norwalk, CT, USA). The reaction mixture contained  $0.4 \text{ mmol l}^{-1}$  acetyl CoA,  $0.25 \text{ mmol l}^{-1}$  DTNB,  $0.5 \text{ mmol l}^{-1}$  oxaloacetate and  $50 \text{ mmol l}^{-1}$  imidazole, pH 7.83 (Hansen and Sidell, 1983). Reactions were conducted at 25°C.

#### Archival tagging

Atlantic bluefin tuna were archival tagged as reported in Block et al. (2001). Implantable archival records in Fig. 5 are from Wildlife Computers MK7 (Redmond, WA, USA) archival tags placed in bluefin tuna of 208 cm and 219 cm curved fork length in January 1999. Data were recorded at 2 min intervals for ambient external temperature, internal

Table 1. Temperature effects on cardiac parameters

Parameter	Temperature (°C)	Conditions		N
		Standard	Maximal	
Heart rate (beats $\text{min}^{-1}$ )	2	16.1 $\pm$ 0.6 <sup>a</sup>	13.3 $\pm$ 0.9 <sup>a</sup>	3
	5	23.1 $\pm$ 1.4 <sup>a</sup>	21.1 $\pm$ 1.4 <sup>a</sup>	6
	10	36.4 $\pm$ 2.4 <sup>b</sup>	33.4 $\pm$ 3.4 <sup>b</sup>	6
	15	54.7 $\pm$ 2.2 <sup>c</sup>	55.3 $\pm$ 3.5 <sup>c</sup>	8
	20	78.2 $\pm$ 3.1 <sup>d</sup>	83.8 $\pm$ 5.2 <sup>d</sup>	7
	25	100.1 $\pm$ 2.7 <sup>e</sup>	104.5 $\pm$ 6.5 <sup>e</sup>	6
Stroke volume ( $\text{ml kg}^{-1}$ )	2	0.86 $\pm$ 0.01 <sup>a</sup>	1.32 $\pm$ 0.10	3
	5	0.86 $\pm$ 0.07 <sup>a</sup>	1.24 $\pm$ 0.07	5
	10	0.57 $\pm$ 0.05 <sup>b</sup>	1.21 $\pm$ 0.03	4
	15	0.37 $\pm$ 0.02 <sup>c</sup>	1.23 $\pm$ 0.04	6
	20	0.23 $\pm$ 0.02 <sup>d</sup>	1.11 $\pm$ 0.03	3
	25	0.18 $\pm$ 0.02 <sup>d</sup>	1.06 $\pm$ 0.07	4
Cardiac output ( $\text{ml kg}^{-1} \text{ min}^{-1}$ )	2	14.2 $\pm$ 0.9	18.1 $\pm$ 1.7 <sup>a</sup>	3
	5	19.4 $\pm$ 2.0	26.7 $\pm$ 3.0 <sup>a</sup>	5
	10	20.6 $\pm$ 2.6	40.8 $\pm$ 5.0 <sup>b</sup>	4
	15	20.5 $\pm$ 1.3	67.5 $\pm$ 3.8 <sup>c</sup>	6
	20	17.0 $\pm$ 2.3	89.8 $\pm$ 7.5 <sup>d</sup>	3
	25	18.1 $\pm$ 2.1	106.2 $\pm$ 5.1 <sup>e</sup>	4
Power ( $\text{mW g}^{-1}$ )	2	0.46 $\pm$ 0.03	0.43 $\pm$ 0.05 <sup>a</sup>	3
	5	0.61 $\pm$ 0.08	0.74 $\pm$ 0.11 <sup>a</sup>	5
	10	0.67 $\pm$ 0.11	1.41 $\pm$ 0.19 <sup>a</sup>	4
	15	0.65 $\pm$ 0.06	2.75 $\pm$ 0.30 <sup>b</sup>	6
	20	0.50 $\pm$ 0.07	3.59 $\pm$ 0.76 <sup>b,c</sup>	3
	25	0.65 $\pm$ 0.09	4.38 $\pm$ 0.68 <sup>c</sup>	4

Values are means  $\pm$  S.E.M.

Values within a column that do not share a letter are significantly different ( $P < 0.05$ ); where no letters appear, no significant differences were detected.

Standard conditions: input pressure 0–0.05 kPa, output pressure ~6 kPa; maximal conditions: input pressure ~2.4 kPa, output pressure >10 kPa. See text for details.

temperature and pressure records, and obtained when the tuna were recaptured and the data downloaded from recovered tags.

## Results

The *in situ* perfused heart preparation of Farrell et al. (1992) was used to measure heart rate, stroke volume, cardiac output and myocardial power output in spontaneously beating Pacific bluefin tuna hearts perfused with Ringer's solution at temperatures ranging from 30°C to 2°C. Raw data records indicating heart rate at three temperatures are shown in Fig. 1. All preparations continued to beat rhythmically at temperatures from 30°C to 2°C; however, cardiac parameters showed significant effects of temperature (Table 1). Decreasing temperatures induced pronounced bradycardia, with heart rate falling from  $105 \pm 6.5 \text{ beats min}^{-1}$  at 25°C to  $13.3 \pm 0.9 \text{ beats min}^{-1}$  at 2°C ( $Q_{10} = 2.5$ ,  $P < 0.001$ ) (Fig. 2A). Heart rate was unaffected by changes in input pressure or

output pressure except at 2°C. Warming Pacific bluefin tuna hearts to 25°C worked well but mixed results occurred at 30°C. This is most likely due to the limitations of the *in situ* preparation at the highest temperatures where oxygen demand is the greatest. When warming the heart to 30°C, function could be retained only if input pressure was elevated to produce maximal flow conditions prior to warming. Warming the heart to 30°C under standard conditions of ambient input pressure typically resulted in arrhythmia or failure of the heart. Heart rates recorded in three preparations at 30°C were  $156 \pm 9.4$  beats  $\text{min}^{-1}$ .

Cardiac output and power output were unaffected by temperature when the heart was perfused under standard conditions (Fig. 2C,D, open circles). Although heart rate decreased with decreasing temperature, there was a compensatory rise in stroke volume, which increased significantly from  $0.18 \pm 0.019$  ml  $\text{kg}^{-1}$  at 25°C to  $0.86 \pm 0.012$  ml  $\text{kg}^{-1}$  at 2°C ( $P < 0.001$ , Fig. 2B). Cardiac outputs were  $20.6 \pm 2.6$  ml  $\text{kg}^{-1} \text{min}^{-1}$  and power outputs were  $0.67 \pm 0.11$  mW  $\text{g}^{-1}$  at 10°C. Stroke volumes and cardiac outputs at 30°C are not reported due to failure of the preparations to return to control conditions at 15°C.

In contrast to standard conditions, maximal cardiac output and power output increased directly with temperature due to increases in heart rate combined with constant stroke volume. Stroke volumes were greater at maximal flow conditions relative to standard conditions at all temperatures ( $P < 0.05$ ). This difference was more pronounced at warmer temperatures, where maximal stroke volume at 25°C showed a nearly sixfold increase over standard conditions. However, maximal stroke volume showed no significant effects of temperature, rising only slightly from  $1.06 \pm 0.07$  ml  $\text{kg}^{-1}$  at 25°C to  $1.32 \pm 0.10$  ml  $\text{kg}^{-1}$  at 2°C (Fig. 2B, filled circles). Maximal cardiac output showed a strong temperature effect, increasing from  $18.1 \pm 1.7$  ml  $\text{kg}^{-1} \text{min}^{-1}$  at 2°C to  $106 \pm 5.1$  ml  $\text{kg}^{-1} \text{min}^{-1}$  at 25°C ( $Q_{10} = 2.0$ ) ( $P < 0.001$ , Fig. 2C, filled circles). Maximal power outputs also increased significantly at higher temperatures, rising from  $0.43 \pm 0.05$  mW  $\text{g}^{-1}$  at 2°C to  $4.38 \pm 0.68$  mW  $\text{g}^{-1}$  at 25°C ( $Q_{10} = 2.75$ ) ( $P < 0.001$ , Fig. 2D, filled circles).

In Fig. 3, cardiac performance measured with the *in situ* perfused heart technique is compared in three species of tuna. The maximal cardiac performance of a single albacore tuna tested *in situ* from 10°C to 20°C is shown in comparison with data from bluefin tuna and yellowfin tuna tested with an

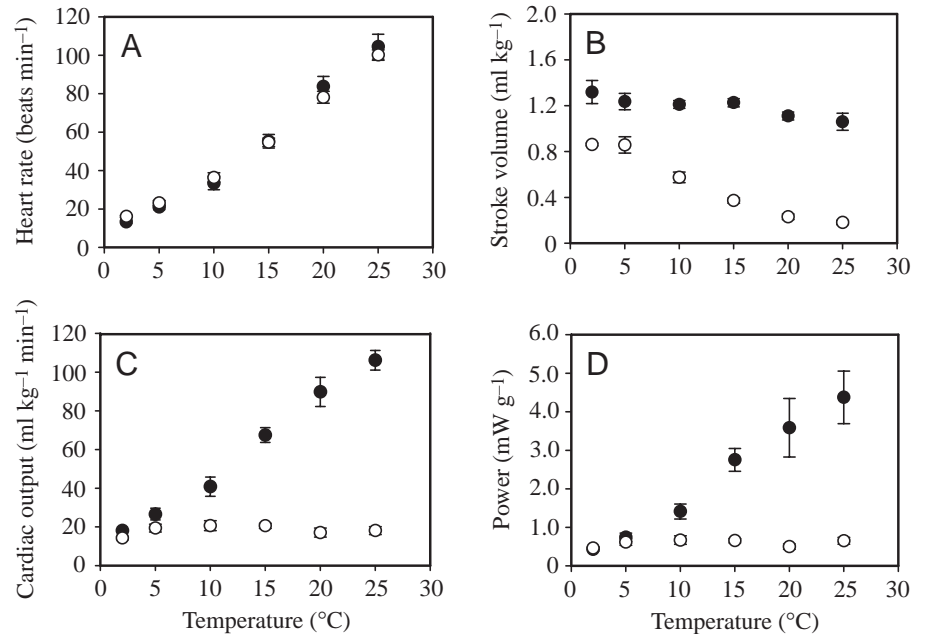


Fig. 2. Cardiac performance in bluefin tuna *in situ*. Values of cardiac parameters were recorded in spontaneously beating bluefin tuna hearts perfused *in situ* with oxygenated Ringer's solution at temperatures of 2–25°C. At each test temperature, values were recorded at standard conditions (open circles) of ambient input pressure and then input pressure was elevated to maximize stroke volume and cardiac output (A–C, filled circles). Input and output pressures were then elevated until power output was maximized (D, filled circles). (A) Heart rate, (B) stroke volume, (C) cardiac output, (D) power. Values are means  $\pm$  S.E.M.;  $N=3$  (2°),  $N=4$  (10°, 20°, 25°),  $N=5$  (5°),  $N=6$  (15°). Significant differences between standard and maximal conditions ( $P < 0.05$ ) occur in A at 2°C only, in B at all temperatures, in C at all temperatures  $\geq 5^\circ\text{C}$  and in D at all temperatures  $\geq 10^\circ\text{C}$ . Significant differences between temperatures are indicated in Table 1. For details of standard and maximal conditions, see Materials and methods.

identical preparation (Blank et al., 2002). All three species show a strong temperature-dependence of heart rate with an apparent difference in the lower limit. Heart rate of the single albacore tuna decreased from 88 beats  $\text{min}^{-1}$  at 20°C to 38 beats  $\text{min}^{-1}$  at 10°C (Fig. 3A). For all three species, stroke volume is less sensitive to temperature than heart rate. In albacore tuna, stroke volume rose from  $0.93$  ml  $\text{kg}^{-1}$  at 20°C to  $1.43$  ml  $\text{kg}^{-1}$  at 10°C (Fig. 3B). Cardiac output fell from  $82$  ml  $\text{kg}^{-1} \text{min}^{-1}$  at 20°C to  $54$  ml  $\text{kg}^{-1} \text{min}^{-1}$  at 10°C (Fig. 3C). Power output was greater in the albacore tuna than in bluefin tuna when normalized to the smaller heart mass of the albacore (Fig. 3D).

To examine the aerobic capacity of the Pacific bluefin heart, CS activity was measured in atria and ventricles at 25°C. CS activities in atria and ventricles were  $77 \pm 3.1$  and  $95 \pm 3.1$  U  $\text{g}^{-1}$  wet mass, respectively.

## Discussion

This paper presents the first measurements of cardiac performance in the intact heart of the Pacific bluefin tuna *Thunnus orientalis*. *In situ* heart preparations perfused with Ringer's solution yielded data on heart rate, stroke volume,

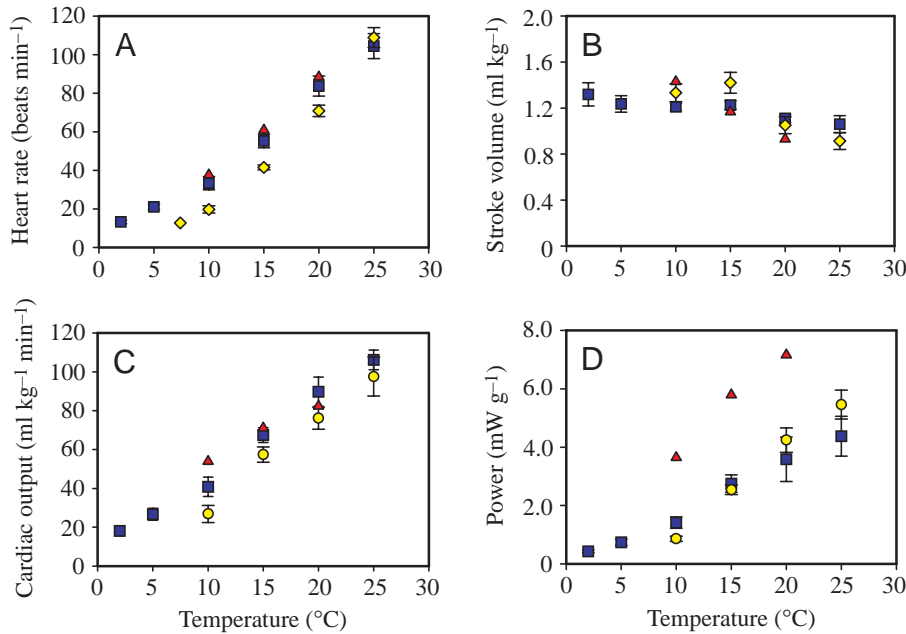


Fig. 3. Comparison of cardiac performance *in situ* in three tuna species. Maximal values of cardiac parameters recorded in spontaneously beating hearts of albacore tuna (red triangles), bluefin tuna (blue squares) and yellowfin tuna (yellow diamonds) are presented. (A) Heart rate, (B) stroke volume, (C) cardiac output, (D) power output. Sample sizes for bluefin tuna are as in Fig. 3. Values are means  $\pm$  S.E.M.; for yellowfin tuna,  $N=4$  (10°, 25°),  $N=5$  (15°, 20°); for albacore tuna,  $N=1$ .

cardiac output and myocardial power output in Pacific bluefin tuna ranging in size from 6.0 to 7.5 kg. Overall, cardiac performance was comparable to published data on juvenile yellowfin tuna (1–3 kg) (Bushnell et al., 1990; Bushnell and Brill, 1991, 1992; Farrell et al., 1992; Jones et al., 1993; Korsmeyer et al., 1997; Blank et al., 2002); however, several significant differences were observed, which may represent important distinctions in cardiovascular physiology among *Thunnus* species.

#### Temperature effects on *in situ* cardiac performance

Pacific bluefin tuna heart rates ranged from  $156 \pm 9.4$  beats  $\text{min}^{-1}$  at 30°C to  $13 \pm 0.9$  beats  $\text{min}^{-1}$  at 2°C (Table 1; Fig. 2A). Heart rates of albacore tuna (Fig. 3A) were similar to those of bluefin tuna from 10°C to 20°C and slightly lower than those previously recorded in anesthetized albacore tuna (Lai et al., 1987; White et al., 1988). While the absolute values of Pacific bluefin tuna heart rate were within the range of heart rates previously reported in yellowfin tuna at 25°C, Pacific bluefin heart rates were significantly higher than those of yellowfin tuna at temperatures from 15°C to 2°C. The most important distinction among the species is that the Pacific bluefin tuna heart rates showed a significantly lower temperature dependence than those of yellowfin across the tested temperatures ( $P < 0.0001$ , Fig. 3A). The  $Q_{10}$  for Pacific bluefin heart rate was 2.1 between 25°C and 10°C compared to 3.1 for yellowfin across the same temperatures (Blank et al., 2002). Maximal stroke volumes of  $1.1$ – $1.3$  ml  $\text{kg}^{-1}$  measured in Pacific bluefin tuna were not significantly affected by temperature change between 25° and 2°C and were similar to those of yellowfin tuna. Albacore tuna stroke volumes exceeded those previously recorded in albacore tuna more than threefold, resulting in over twofold higher maximal cardiac outputs (Lai et al., 1987; White et al., 1988). The albacore heart yielded similar stroke volume and cardiac output to that of

bluefin tuna despite a smaller relative heart mass (0.21% relative ventricular mass). This resulted in greater myocardial power output when normalized to heart mass. The maximal cardiac outputs of  $106.2 \pm 5.1$  ml  $\text{kg}^{-1} \text{min}^{-1}$  in Pacific bluefin tuna at 25°C were within the range previously recorded in yellowfin and skipjack tuna (Bushnell and Brill, 1992; Farrell et al., 1992; Jones et al., 1993; Blank et al., 2002). Importantly, maximal cardiac outputs of Pacific bluefin tuna were significantly less temperature sensitive than those of yellowfin tuna across the measured temperature range ( $P < 0.01$ ), resulting in significantly higher cardiac outputs at low temperatures (Fig. 3C) (Blank et al., 2002).

In Pacific bluefin tuna as in yellowfin tuna, there is a substantial decrease in cardiac performance at low temperature due to decreases in heart rate. This cold-induced bradycardia is accompanied by a low cardiac scope, or ability to increase cardiac output from standard to maximal conditions through changes in stroke volume at lower temperatures (Fig. 4). The calculated value for scope indicates the cardiac response to increased filling pressure (i.e. the Starling effect). It is possible that cardiac performance at maximal conditions would be further increased by adrenergic stimulation, although only small effects of epinephrine were seen in yellowfin tuna hearts *in situ* (Blank et al., 2002). In addition, perfusion with Ringer's solution rather than blood may impose an oxygen limitation on the heart, particularly at higher temperatures, where workload increases while oxygen supply decreases. At maximal conditions, improved luminal perfusion accompanying increases in cardiac output may help to offset such oxygen limitation. This might explain the ability of bluefin hearts to function at 30°C at our maximal flow conditions but not at standard conditions. Whether our maximal *in situ* conditions succeed in reproducing maximal *in vivo* cardiac output is unclear due to the absence of *in vivo* measurements of cardiac output in bluefin tuna. In our previous study (Blank et al., 2002), *in situ* values for maximal cardiac output in yellowfin tuna matched values recorded in spinally blocked fish. However, maximal cardiac outputs of both yellowfin and bluefin tuna are similar to those measured in recent *in situ* studies in triploid brown trout (Mercier et al., 2002), despite the difference in metabolic rates between trout and tunas

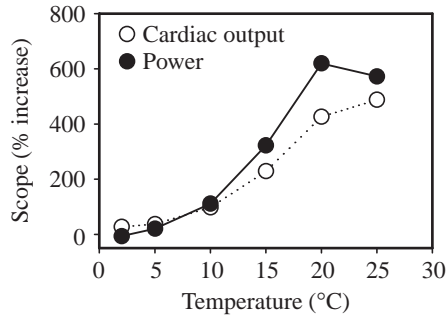


Fig. 4. Scope for increase in cardiac output (open symbols) and myocardial power output (filled symbols) of *in situ* perfused bluefin tuna hearts at temperatures of 2–25°C. Scope was calculated from data in Table 1 as the percentage difference between mean values of cardiac power output at standard conditions vs. maximal flow (cardiac output) or maximal power (power output) conditions.

(Altimiras et al., 2002). This discrepancy suggests that tuna hearts may be capable of greater cardiac outputs than those revealed by *in situ* preparations.

#### Cellular factors influencing cardiac temperature sensitivity

The most notable feature of this study is that the Pacific bluefin tuna heart continues to function at cold ambient temperatures (2°C). The cellular adaptations that allow a bluefin tuna heart to function in the cold remain largely unknown. High aerobic capacity may be an important contributor to maintenance of ATP supplies in hearts working at very cold temperatures, although cold-acclimation has little effect on aerobic capacity in the striped bass heart (Rodnick and Sidell, 1997). Citrate synthase activities of  $77 \pm 3.1$  and  $95 \pm 3.1$  U g<sup>-1</sup> wet mass at 25°C in atrium and ventricle of Pacific bluefin tuna indicate that aerobic capacity is elevated relative to those of teleosts such as trout and striped bass (Rodnick and Sidell, 1997; Clark and Rodnick, 1998). However, these values are similar to aerobic capacities measured in endothermic and ectothermic scombrids, including yellowfin tuna, Eastern pacific bonito and Pacific mackerel (Freund, 1999), suggesting that differences in thermal sensitivity of tuna hearts are not related to the aerobic capacity of the myocardium. Several lines of evidence point to specializations in excitation–contraction (E–C) coupling proteins as a critical factor in achieving high heart rates and cold tolerance of tuna hearts (Shiels et al., 2002a; Landeira-Fernandez et al., 2004). New measurements of L-type calcium channel function in cardiac myocytes of Pacific bluefin tuna indicate that peak Ca<sup>2+</sup> current amplitudes and kinetics in the atrium are enhanced relative to those of many other teleosts (Shiels et al., 2002a). L-type calcium channel function is enhanced in atrium of bluefin tuna relative to Pacific mackerel, but the contribution of this channel to E–C coupling in other tuna species remains unexplored.

Biochemical studies of ventricles of several tuna species indicate that increased performance and cold-tolerance in Pacific bluefin tuna hearts may depend on increased levels of

sarcoplasmic reticulum (SR) proteins, in particular the SR Ca<sup>2+</sup> ATPase (Landeira-Fernandez et al., 2004) and SR calcium release channel (J. M. Morrissette and B. A. Block, unpublished data). SR contributes to cardiac function by providing an intracellular calcium store, thereby reducing diffusion distances and accelerating rates of force development and relaxation (Bers, 2002). Increased SR contribution to E–C coupling is commonly implicated in myocardial cold tolerance in studies of cold-acclimation in fish (Keen et al., 1994; Aho and Vornanen, 1998) and hibernation in mammals (Liu et al., 1997). While the SR was long thought to play a minimal role in teleost hearts (Farrell and Jones, 1992), recent work has begun to clarify its importance in a variety of fish species. Application of ryanodine to block SR function in myocardial strips has been shown to reduce force in a variety of teleost cardiac tissues (Hove-Madsen, 1992; Møller-nielsen and Gesser, 1992; Keen et al., 1994; Aho and Vornanen, 1998; Shiels et al., 1998). In addition, patch-clamp techniques and *in vitro* Ca<sup>2+</sup> uptake assays have demonstrated SR Ca<sup>2+</sup> uptake in trout myocardium (Aho and Vornanen, 1998; Hove-Madsen et al., 1998). Application of ryanodine to block SR function in myocardial strips has particularly pronounced effects on force development of yellowfin tuna heart (Shiels et al., 1999). New measurements of Ca<sup>2+</sup> uptake and Ca<sup>2+</sup> ATPase activity in SR vesicles of scombrid hearts indicate that Pacific bluefin ventricle has high SR Ca<sup>2+</sup> ATPase activities that exceed those of yellowfin and albacore tuna (Landeira-Fernandez et al., 2004). While many other factors regulate heart rate *in situ* as well as in wild fish, high ventricular SR Ca<sup>2+</sup> ATPase activity may be a critical factor permitting bluefin tuna to sustain heart rate in extraordinarily cold waters. As indicated by Landeira-Fernandez et al. (2004), the enhancement of SR Ca<sup>2+</sup> ATPase protein expression in all tunas in comparison to their sister taxa may be a key specialization underlying increased cardiac performance in *Thunnus*.

#### Ecological implications of cardiac thermal sensitivity

Our results indicate that improved cardiac performance in the cold at the cellular and organismal levels may contribute to the ability of the bluefin tuna to penetrate into a cooler thermal niche. Bluefin tunas occupy temperate and sub-polar latitudes where ambient water temperatures at the surface and at depth are cool. The ability of the bluefin tuna heart to maintain contractile performance at extremely low temperatures is critical to this ecological niche expansion. Yellowfin tuna studied with acoustic tags at the northern (coolest) portion of their range primarily occupied waters with surface temperatures greater than 17°C and occasionally dived to depths where ambient temperatures were 11°C (Block et al., 1997). Most dives below these temperatures lasted less than a minute or two and yellowfin tissue temperatures were unlikely to equilibrate to such low ambient temperatures. The physiological limitations that prevent yellowfin tuna from remaining below the thermocline to forage extensively have remained unknown. Most studies of yellowfin tuna, including tracking studies in warmer waters, show similar ‘bounce’

diving (Brill et al., 1999) where time in cool deep waters is short. Electronic tagging of juvenile Pacific bluefin tuna in the eastern Pacific demonstrated that this species makes regular migrations to cold temperate latitudes where surface waters are significantly colder (11–14°C). During these prolonged periods of feeding at high latitudes the juvenile Pacific bluefin tuna dive frequently, encountering waters as cool as 1.8–7°C for short durations (Block et al., 2001; B. A. Block, unpublished data). This differs from the western Pacific, where electronically tagged juvenile bluefin tuna primarily occupy warmer temperatures (Kitagawa et al., 2002).

Electronic tagging data from Atlantic and Pacific bluefin tunas indicate that larger bluefin maintain significantly larger temperature differentials between mean body temperature and ambient water temperature while foraging in cold seas (Block et al., 2001). This most likely results from increased thermal inertia, the increased mass of aerobic muscle and viscera contributing to whole body heat production, and the extensive *retia mirabilia*. Thus far, the only internal peritoneal temperature data from bluefin tuna exceeding 150 kg have come from the Atlantic Ocean. Mature Atlantic bluefin tuna (200–400 kg) tracked with archival tags spend up to 7 months of the year north of 50°N latitude, feeding in waters with surface temperatures as cool as 7–9°C (Block et al., 2001, 2003). Such long-term residency in temperate and sub-polar seas by Atlantic bluefin tuna poses a challenge for the heart that only mature bluefin tuna appear to have solved. Some, but not all, mature Atlantic bluefin tuna (150 kg or larger) display a repetitive diving pattern where peritoneal temperature remains constant, despite repetitive dives into cool waters (Fig. 5). This diving pattern suggests a potential physiological limitation that may be induced by cooling of the heart, as was observed previously in electronic tracking records of big-eye tuna (Brill et al., 1998, 1999). Tunas foraging in cold seas face the challenge of maintaining oxygen delivery to warm aerobic tissues while heart temperature declines. Although metabolic rate has not been measured in any species of the bluefin tuna group, it is likely that bluefin tuna have metabolic rates similar to, or higher than, tropical tunas of similar size. The high level of heat production and heat conservation in adult bluefin (Carey and Teal, 1969; Carey et al., 1984) is likely to maintain high rates of oxygen consumption in the slow-oxidative muscle and visceral organs and high tissue metabolic rates even in the face of cold ambient temperatures. It has been suggested that high myoglobin levels in tuna slow-twitch axial muscle may serve as an oxygen store during brief periods of cold-induced bradycardia in bluefin tuna, in a manner analogous to the oxygen reserve of marine mammals (Marcinek, 2000). While the adaptations for enhanced performance lead to impressive cold tolerance in giant Atlantic bluefin, thermal limits are still apparent. Atlantic bluefin tuna encountering cold conditions (ambient waters <4°C) may be unable to elevate cardiac output sufficiently to support strenuous feeding activities at depth for extended periods and may be forced to return continuously to the surface where warmer ambient waters contribute to higher heart rates, higher cardiac outputs, and reoxygenation of the

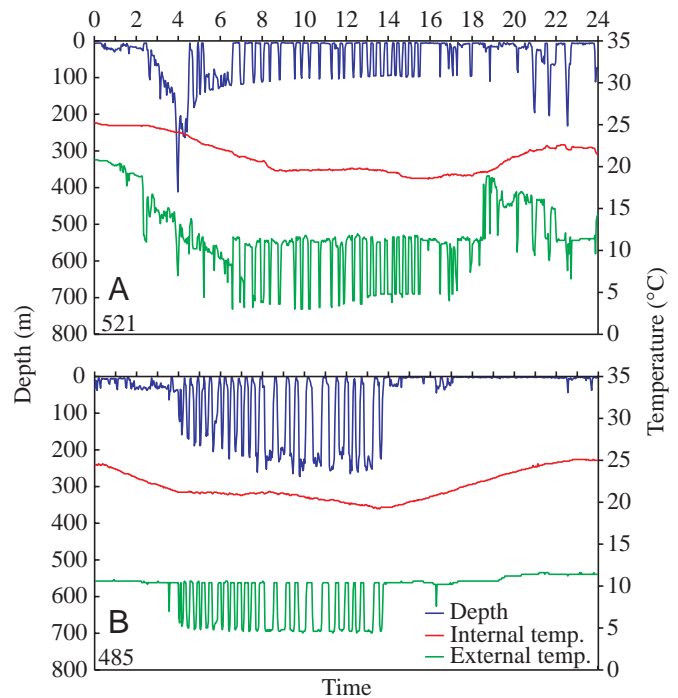


Fig. 5. Diving behavior of Atlantic bluefin tunas recorded with electronic tags. Data on ambient and internal temperature and pressure are recorded at 2 min intervals for two Atlantic bluefin tuna in the North Atlantic. (A) Fish 521; 219 cm, 179 kg; (B) Fish 485; 208 cm, 154 kg at time of release. Days shown are representative of behaviors observed daily in the Gulf of Maine (A) and Flemish Cap (B). Both fish show a pattern of rapid diving into cool water and regular returns to the surface. Conservation of internal body temperature is also shown. The lack of any temperature effect (significant cooling) on the peritoneal cavity suggests that the continuous resurfacing may be associated with physiological requirements for rewarming of the heart and reoxygenation of the tissues.

tissue and myoglobin stores. This ‘bounce’ diving behavior is less apparent in giant bluefin tuna of large size encountering similar conditions in sub-polar and polar seas, suggesting that they have somehow overcome this limitation.

While cold tolerance is clearly important for allowing bluefin tuna to exploit prey resources at higher latitudes and in deep waters, the ability to maintain function in warm waters is also important. All tunas of the genus *Thunnus* breed in warm waters ranging from 23°C to 31°C (Schaefer, 2001; Block et al., 2001; S. L. Teo and B. A. Block, unpublished data). These warm waters may constitute another major physiological challenge for giant adult bluefin. In adult bluefin tunas, peritoneal temperatures as high as 33°C have been recorded (Block et al., 2001; B. A. Block, unpublished data), suggesting that extraordinarily high tissue oxygenation demands occur during breeding in warm waters with relatively low oxygen content. Giant bluefin tunas breeding in warm waters may approach the limits of their cardiac capacity. As heart rate reaches an upper thermal maximum, the tendency for stroke volume to fall with increasing temperatures imposes a limit to increases in cardiac



output in fish encountering acute high temperatures (Farrell et al., 1996). Falling stroke volumes at higher temperatures result from a combination of factors, including the negative force–frequency curve of most teleost ventricular tissues in combination with the Starling (length–tension) effect (Shiels et al., 2002b). The low temperature-dependence of maximal stroke volume in Pacific bluefin tuna hearts suggests that they may have the ability to maintain contractile force at higher frequencies associated with higher temperatures. As a result, oxygen delivery can be maintained while the fish are on the warm spawning grounds. Although heart rate continues to increase at temperatures up to 30°C, there may be an upper limit associated with calcium cycling enzyme kinetics in the bluefin myocyte that result in a ceiling above which performance declines. Ultimately, this may limit cardiac performance and oxygen delivery. Evidence for such an upper limit may be seen in the high mortality associated with capture (a sympathetic stress) of giant bluefin on scientific longlines in the Gulf of Mexico (A. M. Boustany and B. A. Block, unpublished data). Improving techniques to allow measurement of cardiac performance at high temperatures should elucidate the critical upper limit of cardiac performance.

Maintenance of high cardiac outputs at high as well as low temperatures are likely to play a crucial role in increasing the thermal niche of bluefin tunas. Understanding how the bluefin tuna heart performs over this wide temperature range should provide important information about vertebrate cardiac performance.

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