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RESEARCH ARTICLE

Heart rate responses to temperature in free-swimming Pacific bluefin tuna (*Thunnus orientalis*)

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SUMMARY

The bluefin tuna heart remains at ambient water temperature (T_a) but must supply blood to warm regions of the body served by countercurrent vascular heat exchangers. Despite this unusual physiology, inherent difficulties have precluded an understanding of the cardiovascular responses to T_a in free-swimming bluefin tunas. We measured the heart rate (f_H) responses of two captive Pacific bluefin tunas (*Thunnus orientalis*; 9.7 and 13.3 kg) over a cumulative period of 40 days. Routine f_H during fasting in the holding tank at a T_a of 20°C was 45.1±8.0 and 40.7±6.5 beats min^{-1} for Tuna 1 and Tuna 2, respectively. f_H decreased in each fish with a Q_{10} temperature coefficient of 2.6 (Tuna 1) and 3.1 (Tuna 2) as T_a in the tank was slowly decreased to 15°C (~0.4°C h^{-1}), despite a gradual increase in swimming speed. The same thermal challenge during digestion revealed similar thermal dependence of f_H and indicated that the rate of visceral cooling is not buffered by the heat increment of feeding. Acutely decreasing T_a from 20 to 10°C while Tuna 1 swam in a tunnel respirometer caused a progressive increase in tail-beat frequency and oxygen consumption rate ($\dot{M}_{O_2}$). f_H of this fish decreased with a Q_{10} of 2.7 as T_a decreased between 20 and 15°C, while further cooling to 10°C saw a general plateau in f_H around 35 beats min^{-1} with a Q_{10} of 1.3. A discussion of the relationships between f_H, $\dot{M}_{O_2}$ and haemoglobin–oxygen binding sheds further light on how bluefin cardiorespiratory systems function in a changing thermal environment.

Supplementary material available online at http://jeb.biologists.org/cgi/content/full/216/17/3208/DC1

Key words: ambient temperature, visceral temperature, cardiorespiratory, cardiovascular, oxygen consumption rate, tail beat-frequency, swimming speed, thermal biology.

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INTRODUCTION

Bluefin tunas (*Thunnus orientalis*, *T. thynnus* and *T. maccoyii*) are large, powerful predators that possess a suite of exceptional specialisations to enable a high-performance lifestyle throughout the oceans of the world (Block and Finnerty, 1994; Graham and Dickson, 2004; Block et al., 2005; Patterson et al., 2008; Boustany et al., 2010). Bluefin are renowned for their capacity to use entire ocean basins for a home range, encountering broad thermal gradients both in their latitudinal migrations and during vertical diving (Block et al., 2001; Marcinek et al., 2001; Lawson et al., 2010). The acute thermal changes experienced by bluefin tunas would be lethal to many fishes, raising interesting questions about how these tunas are physiologically specialised to cope. Bluefin tunas benefit from the presence of extensive countercurrent vascular heat exchangers (retia mirabilia) that allow metabolic heat conservation in specific regions of the body to buffer from ambient temperature fluctuations (termed 'regional heterothermy' or 'regional endothermy'). Heat exchangers in bluefin tunas are associated with the circulation to the eyes, brain, viscera and slow-oxidative muscles (Carey and Teal, 1969; Carey et al., 1984; Fudge and Stevens, 1996; Dickson and Graham, 2004), and subsequently they are hypothesised to enhance visual acuity, neural processing, digestion and skeletal muscle contraction frequencies during periods in cooler waters (Carey et al., 1984; Altringham and Block, 1997; Dickson and Graham, 2004).

Although much of the bluefin tuna's body is maintained at temperatures warmer than the ambient water, the heart is not served by heat exchangers, it is positioned close to the gills, and it receives a large coronary blood supply, thus ensuring that the heart remains very close to ambient temperature at all times (Fudge and Stevens, 1996; Brill and Bushnell, 2001). Consequently, a physiological situation exists in bluefin tunas where a heart at ambient temperature must maintain blood and oxygen supply to warm tissues. The differential between ambient and body core temperatures is particularly pronounced during foraging dives below the thermocline (Block et al., 2001; Walli et al., 2009; Lawson et al., 2010). Furthermore, the temperature differential between the heart and the visceral organs is greatest during digestion when the visceral cavity undergoes a thermal increment that is dependent on meal mass [termed heat increment of feeding (HIF) or specific dynamic action (SDA)] (Carey et al., 1984; Gunn et al., 2001; Walli, 2007; Clark et al., 2008b; Clark et al., 2010a).

Despite the exceptional conditions under which the tuna heart must function, little is known of the *in vivo* cardiovascular responses of tunas to changes in water temperature due to the inherent difficulties of studying free-swimming tunas. Current knowledge is limited to *in situ* and *in vitro* heart preparations, or tethered or immobilised tunas shortly after handling and instrumentation (Dizon et al., 1974; Bushnell and Brill, 1992; Korsmeyer et al., 1997a; Blank et al., 2002; Blank et al., 2004).

A temperature-mediated decrease in heart rate (f_H) scope has been proposed to limit the vertical distribution of yellowfin, skipjack and juvenile bigeye tunas to temperatures above 15°C (Holland et al., 1990; Brill et al., 1999; Brill and Bushnell, 2001), although large yellowfin can occasionally withstand much cooler temperatures for short periods (Dagorn et al., 2006; Schaefer et al., 2011). In contrast, other research indicates that the cardiac function of Pacific bluefin tunas is more tolerant of cold temperatures such that they can routinely dive into waters less than 10°C and maintain a consistent presence in the mixed layer of the cool but productive California Current waters (14–21°C) (Block et al., 2001; Blank et al., 2004; Kitagawa et al., 2007; Galli et al., 2009; Boustany et al., 2010; Galli et al., 2011).

Swimming speed is temperature dependent in some tuna species (Dizon et al., 1977; Malte et al., 2007), and juvenile Pacific bluefin tunas increase tail-beat frequency and oxygen consumption rate ($\dot{M}_{O_2}$) with decreasing water temperature while swimming in a tunnel respirometer (Blank et al., 2007b). This increase in $\dot{M}_{O_2}$ with decreasing water temperature is unusual for a fish, and thus the question arises as to how bluefin tunas are able to increase circulatory oxygen transport in the face of decreasing water temperature. Moreover, in the absence of direct measurements of cardiovascular data from free-swimming and untethered individuals at different temperatures, it remains unclear how thermally driven changes in swimming activity might interact with cardiovascular function.

In light of these knowledge gaps, the present study used innovative archival tag technology to provide the first insight into the cardiovascular responses of free-swimming, untethered and well-recovered Pacific bluefin tunas faced with acute changes in water temperature. Because of the inherent difficulties of performing such measurements on large fish, this study includes data from only two individuals. We aimed to identify how f_H is regulated in response to acute changes in water temperature in free-swimming tunas, and investigate the cardiovascular responses underlying the known increase in $\dot{M}_{O_2}$ with decreasing temperature.

MATERIALS AND METHODS

Animals

Wild juvenile Pacific bluefin tunas were caught with several conspecifics using barbless hooks off the coast of Mexico from the fishing vessel *Shogun*. The studies were conducted over a period of two collections. One experimental fish used in these studies was caught in 2006 and another in collections made in 2007. The fish were brought aboard the fishing vessel and remained in oxygenated seawater wells for up to 3 days prior to transport to the shore and then transfer by vinyl sling to a container aboard a truck. The truck took approximately 9 h to move fish by road to the Tuna Research and Conservation Center (TRCC), Stanford University, CA, USA. Seven other tunas were housed in the same tank as the experimental fish for the duration of the experiments presented here. Fish were kept in a 109 m^3 circular tank (~10 m diameter) as described previously (Farwell, 2001), and under a photoperiod that was routinely adjusted to match the local conditions (the light:dark photoperiod generally ranged from 10 h:14 h to 12 h:12 h across seasons; dim lighting remained during 'dark' hours). They remained in the holding tank at 20±0.3°C until January 2008 (time in captivity ~180 days for Tuna 1 and ~530 days for Tuna 2) prior to surgery and were fed daily on a diet of thawed sardines (*Sardinops sagax*), squid (*Loligo opalescens* and *Illex* sp.) and gelatine blocks with supplementary vitamins and nutrients (see Farwell, 2001). Body mass (M_b) and straight fork length (FL) for Tuna 1 and Tuna 2 at the time of experiments were 9.7 kg and 77 cm, and 13.3 kg and 80 cm, respectively.

Surgery

Archival tags (mass 23 g in air; B. D. Taylor, Melbourne, Australia) were implanted into the visceral cavity using methods similar to those used previously (Clark et al., 2008b), and in accordance with approved vertebrate surgical protocols from Stanford University IACUC. The archival tags measured visceral temperature (T_V) and the electrocardiogram (ECG) at 200 Hz for 10 s every 10 min (for details, see Clark et al., 2010b). On the day of surgery, the water depth of the holding tank was lowered to ~1 m (from 1.7 m) before a team of researchers entered the tank and caught the targeted tuna in a vinyl sling. The fish was positioned ventral side down between two vinyl-covered foam pads on a surgery bench with continuous gill irrigation and an identification tag was inserted into the dorsal tissue. The fish was then rolled into a supine position and a 4 cm incision was made with a stainless steel scalpel just laterally of the ventral midline and immediately behind the tip of a pectoral fin. A path for the archival tag around the visceral organs was created with an alcohol-rinsed and betadine-coated trochar that was inserted through the incision into the peritoneal cavity. The betadine-soaked electronic tag was inserted through the incision into the forward portion of the visceral cavity using a pair of introducing forceps with an aim to position the ECG leads ventral to the liver and as close as possible to the pericardial cavity. The incision was closed with three to four monofilament sutures (Ethicon, Bridgewater, NJ, USA) and the fish was released back into the holding tank. Both fish immediately recovered normal swimming behaviours, resumed feeding within 3 days, and were given at least 35 days to recover from surgery prior to experimentation. Experiments were conducted throughout February and March 2008.

Experimental protocol

Holding tank

The experiments in the holding tank aimed to quantify the swimming behaviour (swimming speed and tail-beat frequency) and f_H of the fish as they experienced firstly a rapid and then a slower change in water temperature. Fish swimming behaviours throughout the experiments were monitored using a video camera mounted vertically above one edge of the holding tank approximately 4 m above the water surface. Fish were distinguished by their dorsal identification tags. Video footage was stored on VHS tapes for subsequent analysis of swimming speed and tail-beat frequency using slow-motion playback. The nine fish typically swam as a group and most often circled the tank approximately 1 m from the perimeter wall. The video camera was positioned such that footage was recorded from directly above the fish when they swam through the field of view (typically once per lap of the tank, i.e. every ~20 s), and such that the swimming path was typically linear across the centre of the field of view.

The experimental protocol for changing the temperature of the holding tank is illustrated in Fig. 1. All modifications to the holding tank (water temperature and depth) were achieved by adjusting a series of valves on the filtration system located at least 5 m from the tank, which allowed the exchange of water between all tanks within the facility without visually disturbing the fish. A rapid temperature decrease was achieved by first lowering the tank water depth to approximately 1.2 m, and then refilling the tank with cooler water (12.5°C) from an adjacent tank. This process was repeated seven times to achieve the desired holding tank temperature of approximately 14.5°C (~2 h, ~3°C h^{-1}). The water temperature was

maintained at around 14.5°C for 2.5 h before the tank was re-warmed using warm water (24°C) from another adjacent tank (~3 h, ~1.8°C h^{-1}). A slow temperature change from 20 to 15°C at ~0.4°C h^{-1} was achieved using the same protocol, but at a slower rate of water exchange and without the need to modify water depth in the holding tank. The slow temperature decrease and subsequent increase took approximately 45 h in total.

To examine how the postprandial visceral thermal increment may influence f_H and the rate of T_V change during changes in water temperature, the rapid temperature change protocol was repeated 12 h following ingestion of sardines to satiation [1.1–1.2 kg consumed per tuna; HIF is close to maximum in southern bluefin tunas under similar conditions (Clark et al., 2008b)].

Towards the end of the experimental period, Tuna 2 was transferred from the holding tank at 20°C to an adjacent holding tank at 23.5°C for 2 days prior to the completion of the study.

Swim respirometer

Tunas must be trained first to swim in the tunnel respirometer, a process of a month or more prior to conducting an experiment. Not all tunas will persist through this period of training. A single tuna was trained (Tuna 1) and in the respirometry experiment we aimed to measure the f_H responses of the tuna while following protocols similar to those used previously (Blank et al., 2007a; Blank et al., 2007b). The swim respirometer, its functioning, and the process of fish capture and introduction to the respirometer have been detailed previously (Blank et al., 2007a; Blank et al., 2007b; Clark et al., 2010a). Briefly, the fish (fasted for 3 days) was transported to the working section (45×45×135 cm, depth × width × length) of an 861 l respirometer, which was housed behind black plastic curtains to minimise light fluctuations and visual disturbance (light conditions were maintained constant throughout experiments). The lid of the respirometer remained open and an experimenter remained with the fish for the first 2.5 h as a precaution in case the fish stopped swimming or tried to turn around. When the fish appeared calm, the lid was sealed and the experimenter left the enclosure around the swim tunnel. Subsequently, the fish was monitored using a video camera that was focused on a diagonally mounted mirror above the swim respirometer, the footage from which was displayed on a monitor in a nearby office. Measurements of oxygen concentration (mg l^{-1}) and temperature of the respirometer water were archived every 10 s with a temperature-compensated oxygen meter (Yellow Springs Instruments, model 556 MPS; www.ysi.com). A flush pump refreshed the respirometer water for 10 min in every 20 min, and oxygen consumption rates were calculated from the decline in water oxygen levels between flushes. The fish swam in the respirometer for 32 h to reach an acclimated state prior to the commencement of the temperature challenges. The first temperature challenge was a stepwise decrease in water temperature (~2°C every 2 h) to ~14°C followed by a return to 20°C, while the second temperature challenge was a more rapid decrease to ~10°C for 2 h and a subsequent return to 20°C. The fish remained in the respirometer for 60 h in total and water speed was maintained at 1 FL s^{-1} during the temperature challenges.

Data analyses

ECG data from the archival tags were imported into LabChart software (ADInstruments, Sydney, Australia) and f_H was calculated as an average for each 10 s period (data shown in Fig. 1). Routine f_H from the holding tank at a T_a of 20±0.3°C was calculated after excluding data associated with feeding/digesting events and data from different ambient temperatures (leaving ~63 h of data per tuna). Maximal f_H for each tuna was calculated as the highest f_H achieved in any 10 s period after confirming values by manually viewing the raw ECG traces. Furthermore, histograms were formulated for the f_H and T_V data to examine frequency distributions in bins of 10 beats min^{-1} and 1°C, respectively. Oxygen pulse for the fish in the respirometer was calculated as $\dot{M}_{O_2}/f_H$, and represents the amount of oxygen extracted by the tissues per heart beat [i.e. cardiac stroke volume (V_S) × tissue oxygen extraction, where the latter is related to the difference in oxygen content of arterial (Ca_{O_2}) and venous (Cv_{O_2}) blood].

Tail-beat frequency (beats min^{-1}) for the fish in the respirometer was calculated periodically from the video footage by measuring the time (min) taken for 60 tail beats (TBF = 60/time). Tail-beat frequencies in the holding tank were calculated during periods when the fish were in the field of view of the video camera. Swimming speeds of the fish in the holding tank were determined from the same video footage as the TBF measurements, and were calculated as FL s^{-1} based on the time taken for the tail tip of a fish to cover a 3 m distance that was in the field of view of the video camera (a 1 m grid of black lines on the bottom of the holding tank provided a reference point). TBF and swimming speeds of the fish in the holding tank were calculated as the average of three independent measurements occurring within 10 min (i.e. three separate instances when the fish passed through the field of view of the video camera).

RESULTS

Routine measurements at constant water temperature

Tunas in the holding tank were generally fed three times per week on alternate days, and once the SDA events were completed the fish were considered to be in a fasted, ‘resting’ state. These fasted bluefin tunas at a T_a of 20±0.3°C maintained a thermal excess, where Tuna 1 (9.7 kg) had a mean ± s.d. T_V of 22.6±0.6°C with the most (66.1%) records occurring between 22 and 23°C, while the larger Tuna 2 (13.3 kg) had a mean T_V of 23.3±0.4°C with the most (52.0%) records occurring between 23 and 24°C (Fig. 1). During the same periods (~63 h per tuna), routine f_H of Tuna 1 averaged 45.1±8.0 beats min^{-1} with the most (48.3%) records occurring between 40 and 50 beats min^{-1}, while routine f_H of Tuna 2 averaged 40.7±6.5 beats min^{-1} with the most (44.5%) records occurring between 30 and 40 beats min^{-1} (Fig. 1). The tunas were fed once in the holding tank during the course of these records at a T_a of 20°C, with the feeding and digestion event associated with elevated f_H up to 75–90 beats min^{-1} (Fig. 1).

Thermal challenges in holding tank

A slow drop in T_a in the holding tank from 20 to 15°C was mirrored by similar absolute decreases in T_V of the fasted fish (supplementary material Fig. S1A). Heart rate decreased with T_a, although an increase in swimming speed at the coolest temperatures appeared to reduce the influence of T_a on f_H (supplementary material Fig. S1). The increase in swimming speed was likely a consequence of elevated tail beat amplitude, as there was no detectable systematic change in tail-beat frequency with temperature (supplementary material Fig. S1). Using only the data during the decrease in T_a, Q_{10} for f_H was 2.6 for Tuna 1 and 3.1 for Tuna 2 between 20 and 15°C.

To investigate the simultaneous impact of digestion and temperature on f_H, fish were given a thermal challenge 12 h after feeding. The feeding event was associated with abrupt increases in swimming activity and f_H. Heart rate remained elevated and T_V increased progressively following the feeding event at 20°C, with f_H reaching a maximum of 90 beats min^{-1} for Tuna 1 and

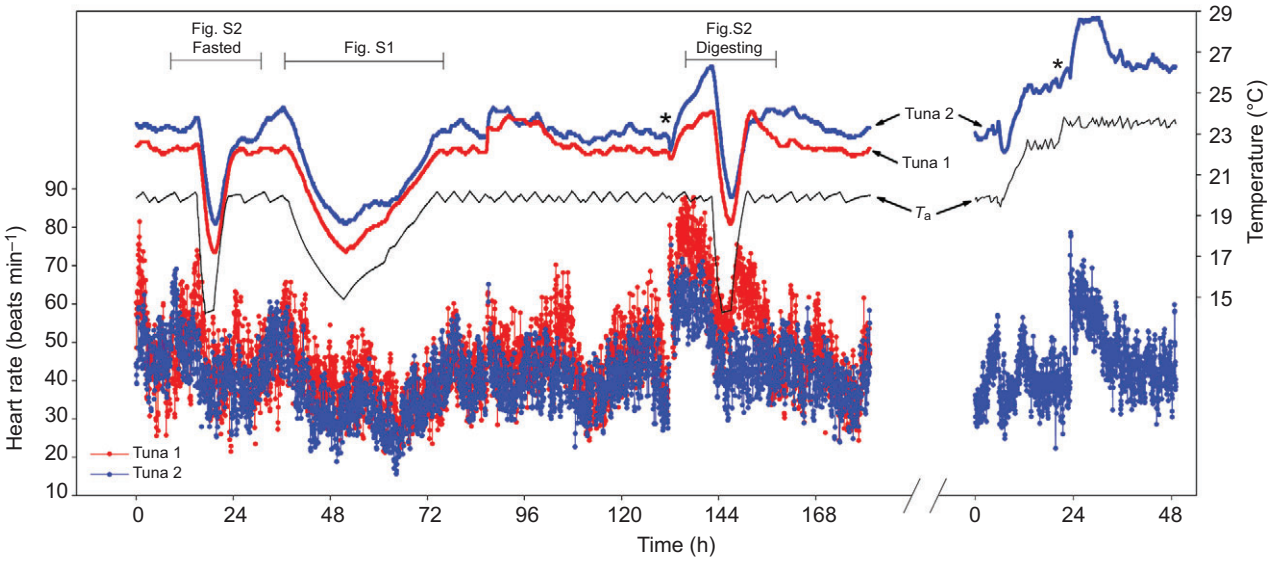

Fig. 1. Visceral temperature (T_V) and heart rate (f_H) of two captive Pacific bluefin tunas (*Thunnus orientalis*) in a large holding tank swimming with seven conspecifics when faced with a series of ambient water temperature (T_a) challenges over approximately 7 days (Tuna 1 body mass 9.7 kg, straight fork length 77 cm; Tuna 2, 13.3 kg, 80 cm). From left to right, the temperature challenges were (1) rapid while the tunas were in a fasted state, (2) slow while the tunas were in a fasted state and (3) rapid while the tunas were digesting a satiation meal of sardines. Also included on the right side of the figure is a 2-day period where Tuna 2 was transferred to another tank at 22.5°C and subsequently further warmed to 23.7°C. Asterisks indicate feeding periods. Meal sizes were 1.1 and 1.2 kg for Tuna 1 and Tuna 2, respectively, at 20°C, and 1.0 kg for Tuna 2 at 23.7°C. The data used to generate supplementary material Figs S1 and S2 are indicated.

75 beats min^{-1} for Tuna 2 (Fig. 1). Both f_H and T_V were higher in digesting fish than in fasted fish at the commencement of the rapid temperature challenge (supplementary material Fig. S2). A rapid decrease in tank T_a from 20°C to 14.5°C caused a predictable decrease in T_V regardless of whether the fish were in fasted or digesting states (supplementary material Fig. S2). Rates of T_V change (up to 0.4°C min^{-1} for Tuna 1, up to 0.3°C min^{-1} for Tuna 2) were not different between fasted and digesting states (supplementary material Fig. S2A,D), indicating that the HIF associated with digestion did not afford any buffer against heat loss rates in the holding tank. Nevertheless, digesting fish maintained a higher T_V at all times due to the higher thermal excess (T_x) afforded by the HIF (supplementary material Fig. S2). The decrease in T_a caused a parallel drop in f_H from 70–80 beats min^{-1} down to 40–55 beats min^{-1} in digesting fish. The response in f_H to the decrease in T_a was not as obvious in fasted fish, with f_H starting at approximately 50–60 beats min^{-1} at 20°C and falling to 30–40 beats min^{-1} at 14.5°C (supplementary material Fig. S2). Upon rewarming to a T_a of 20°C, f_H and T_V of Tuna 1 in a digesting state remained elevated for ~9 h in comparison with the same fish in a fasted state, while Tuna 2 seemed to have almost completed the digestive process by the time T_a returned to 20°C (supplementary material Fig. S2).

Thermal challenges in respirometer

Tuna 1 was used to provide insight into the cardiac responses associated with thermally dependent changes in $\dot{M}_{O_2}$. The heart rate of Tuna 1 in the respirometer after 32 h of acclimation at a T_a of 20°C was approximately 60 beats min^{-1} (Fig. 2). Decreases in T_a in the respirometer caused qualitative responses similar to those seen in the fish in the holding tank. Heart rate decreased with T_a down to approximately 14°C, below which f_H tended to plateau around 35 beats min^{-1} while TBF continued to increase despite maintenance of the same water velocity through the respirometer (Fig. 2). Consequently, Q_{10} for f_H was 2.7 between 15 and 20°C and only 1.3 between 10 and 15°C. The increase in TBF did not translate to enhanced visceral heat retention, as T_x remained similar at all ambient temperatures from 10 to 20°C (Fig. 2A,H). Importantly, there was a clear increase in $\dot{M}_{O_2}$ with decreasing T_a, which resulted from a linear increase in the oxygen pulse while f_H remained essentially constant below 14°C. Regressions shown in Fig. 2 (equations in caption) suggest that f_H and oxygen pulse are more strongly correlated with T_a (r^2=0.86 and 0.84, respectively) than T_V (r^2=0.71 and 0.56, respectively), while $\dot{M}_{O_2}$ and TBF are similarly correlated with T_a (r^2=0.60 and 0.59, respectively) and T_V (r^2=0.62 and 0.62, respectively).

DISCUSSION

The ability to maintain captive Pacific bluefin tunas provided the opportunity to explore thermal effects on cardiorespiratory parameters in routinely swimming fish equipped with surgically implanted archival tags. We observed that two Pacific bluefin tunas at a holding temperature of 20°C generally maintained a routine f_H of 40–45 beats min^{-1} (typical range 25–50 beats min^{-1}) during non-feeding and non-digesting periods (Fig. 1; squares and triangles in Fig. 2), which is within the range of routine f_H reported for southern bluefin tuna held in a sea pen at 18–19°C (Clark et al., 2008b). These heart rates are similar if not lower than those reported for *in situ* heart preparations of Pacific bluefin (Blank et al., 2004). Although the instrumented Pacific bluefin in the present study are few in number, the data from the archival tags presented here are from a cumulative period of 40 days. The research on free-swimming, ECG-instrumented Pacific and southern bluefin tunas indicates that these species possess routine f_H that are not markedly higher than other active teleosts (Brill and Bushnell, 1991; Korsmeyer et al., 1997a; Korsmeyer et al., 1997b; Brill and Bushnell, 2001; Clark and Seymour, 2006; Clark et al., 2008b).

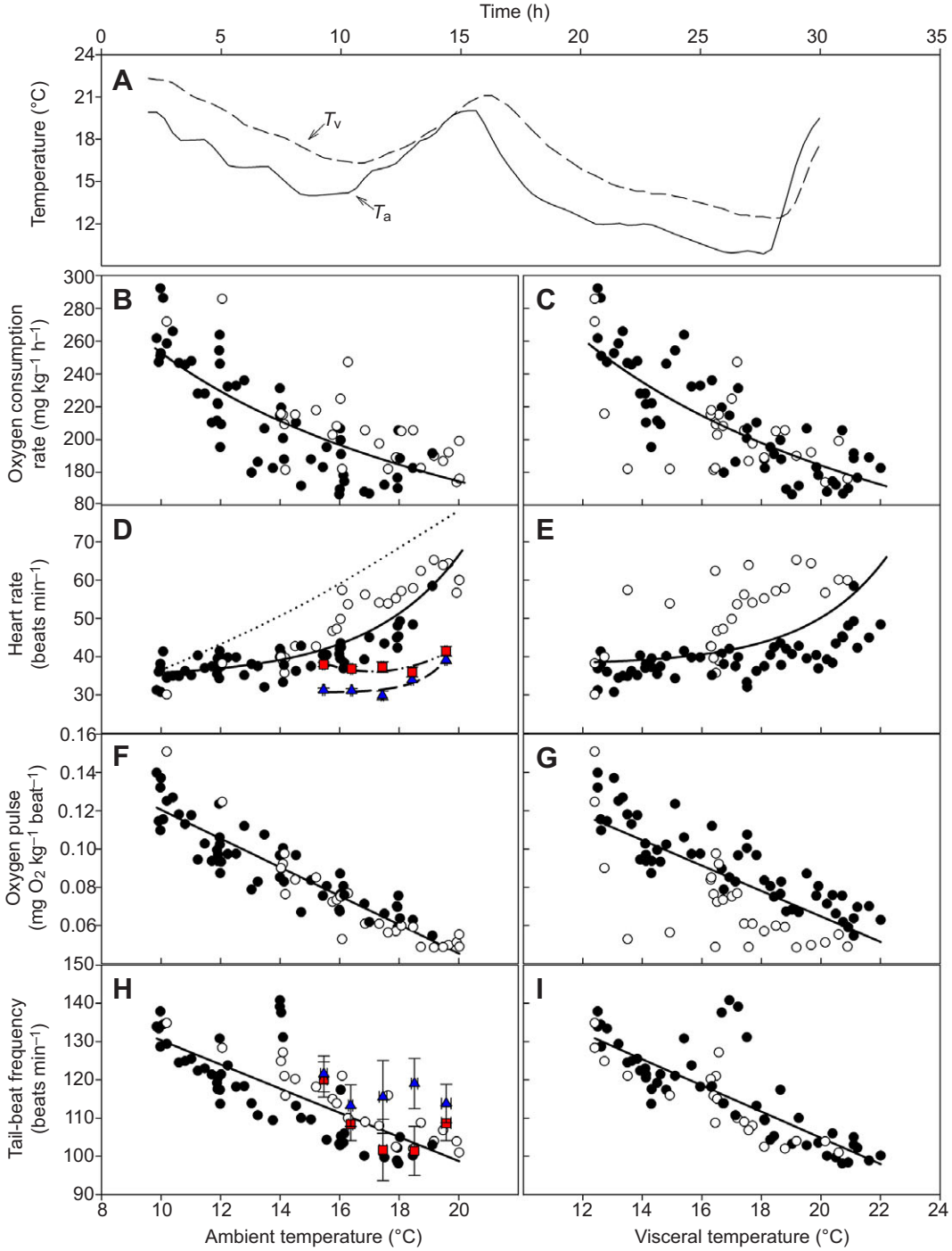

Fig. 2. Rate of oxygen consumption ($\dot{M}_{O_2}$; B,C), heart rate (f_H; D,E), oxygen pulse (F,G) and tail-beat frequency (TBF; H,I) of a Pacific bluefin tuna (*Thunnus orientalis*; Tuna 1) in a swim respirometer as a function of ambient water temperature (T_a) and visceral temperature (T_V) when undergoing the temperature challenges illustrated in A (body mass=9.7 kg, straight fork length=77 cm; P<0.001 for all regressions). Closed circles are periods of decreasing T_a; open circles are periods of increasing T_a (as in A). Water speed remained at 1 fork length s^{-1}. Regression lines are described by: (B) y=867.02$x^{-0.53}$, r^2=0.60; (C) y=1429.50$x^{-0.68}$, r^2=0.62; (D) y=34.54+0.050e$^{0.32x}$, r^2=0.86; (E) y=37.83+0.007e$^{0.37x}$, r^2=0.71; (F) y=–0.007x+0.20, r^2=0.84; (G) y=–0.007x+0.20, r^2=0.56; (H) y=–3.17x+162.15, r^2=0.59; and (I) y=–3.44x+173.81, r^2=0.62. Also shown in D and H are the heart rates and tail-beat frequencies, respectively, of this fish (Tuna 1; squares) and another fish (Tuna 2; triangles) when exposed to a slow change in T_a in a holding tank (data binned into T_a groups of 15–15.9°C, 16–16.9°C...19–19.9°C; values are means ± s.e.m.). Dotted line in D represents the standard heart rates of *T. orientalis* hearts in an *in situ* preparation (from Blank et al., 2004).

Maximal f_H observed in the present study was 90 beats min^{-1} for Tuna 1 and 75 beats min^{-1} for Tuna 2. To date, maximum f_H has not exceeded 120–130 beats min^{-1} in a total of 256 days of records from southern bluefin and Pacific bluefin tunas in large pens or tanks at 18–20°C measured with similar tags (Clark et al., 2008b; Clark et al., 2010a). These studies have not specifically tested maximum metabolic rate or f_H, nor have they exposed tunas to the highest T_a observed in archival tag records [>30°C (Walli et al.,

2009)], but in both locations the tunas were periodically excited by feeding, capture and handling throughout the experimental period that presumably elicited at least near-maximal f_H at the given holding temperatures (present study) (Clark et al., 2008b). The maximum f_H values obtained in the present study are not exceptional for fishes in general and are comparable to those obtained from *in situ* preparations of Pacific bluefin tuna hearts (Blank et al., 2004).

The Q_{10} for f_H ranged from 2.6 to 3.1 for the two tunas between 15 and 20°C in the holding tank, but dropped to 1.3 when Tuna 1 was further cooled from 15 to 10°C in the respirometer. A previous study on free-swimming but tethered yellowfin tuna reported Q_{10} values for f_H of 2.2–2.4 across a T_a range of 18–28°C (Korsmeyer et al., 1997a). Heart rates of yellowfin in that study ranged from 40 to 190 beats min^{-1} across the temperature range, values that are higher than for Pacific bluefin in the present study but consistent once differences in T_a are considered. The study of yellowfin did not document any obvious plateau in f_H at cool temperatures like that reported here for Pacific bluefin, although this may not be surprising given the decrease in swimming speed with decreasing T_a that has been reported for yellowfin (Dizon et al., 1977) in contrast with the findings presented here. These results highlight the complexity of performance in tunas where differing capabilities for regional endothermy exist and differences in temperature-related influences on muscle function indicate variation across tuna species. Notably, it has been documented that the *in vitro* metabolism of slow- and fast-twitch muscle from skipjack and bigeye tunas is independent of temperature between 5 and 35°C (Gordon, 1968), yet slow-twitch muscle power output is highly temperature dependent in yellowfin and thus force and frequency benefit from countercurrent heat exchangers (Altringham and Block, 1997). Cardiac studies indicate that Pacific bluefin tunas outperform yellowfin tunas at cooler temperatures because of their capacity to maintain heart function, which at the cellular level has been linked to enrichment of sarcoplasmic reticulum calcium stores, enhanced calcium ATPase activity and a short action potential duration (Galli et al., 2009; Galli et al., 2011; Landeira-Fernandez et al., 2012).

A notable observation from the present study is the elevated f_H of Tuna 1 in the respirometer at 20°C in comparison with the same individual while swimming in the holding tank, despite the fact that the fish was given 32 h to adjust to the respirometer before the experiments commenced (Fig. 2D). We attribute this difference to slight adjustments in swimming gait, where the fish in the respirometer maintained a rhythmic tail beat pattern at all times while the fish in the holding tank interspersed rhythmic tail beats with short periods of 'coasting' or 'gliding'. Although the slight adjustments in swimming gait were not detected through changes in TBF by the methods used here (Fig. 2H), there is a need for future research to examine the interaction between T_a, swimming gait, TBF, $\dot{M}_{O_2}$ and cardiovascular parameters in bluefin tunas. Such experiments could be achieved with the use of accelerometry tags in combination with the ECG tags and experimental protocols used here. Importantly, testing bluefin tunas at the extreme limits of their thermal tolerance will reveal the resilience and limitations of the cardiovascular system.

Recently, temperature-independent Hb–O_2 binding was reported in the blood of southern bluefin tuna between 23 and 36°C, while a reverse temperature effect (left shift in Hb–O_2 dissociation curve with increasing temperature) was reported between 10 and 23°C (Clark et al., 2008a). As the first to simultaneously measure f_H and $\dot{M}_{O_2}$ of any bluefin tuna species, the present study helps to shed further light on the unusual oxygen transport mechanisms of these fish. We propose that the unusual Hb–O_2 binding characteristics in bluefin tunas may play some role in enhancing oxygen unloading at the muscles at cool water temperatures such that C_{vO_2} decreases (C_{aO_2}–C_{vO_2} increases) and permits the observed increase in $\dot{M}_{O_2}$ with TBF and swimming speed. Moreover, by comparing the f_H of spontaneously beating hearts in an *in situ* preparation with the findings presented here (Fig. 2D), we suggest that a greater proportion of f_H scope is utilised at cold temperature in free-swimming bluefin [perhaps promoted by a release in cholinergic tone (Keen et al., 1995), and faster swimming and/or increased TBF] such that the influence of temperature on f_H is functionally minimised. Because bluefin myoglobin has a higher affinity for oxygen than does haemoglobin (Rossi-Fanelli et al., 1960), this could potentially play a role in facilitating diffusion to tissues as the bluefins reach their thermal limits for cardiovascular oxygen delivery.

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AUTHOR CONTRIBUTIONS

T.D.C., C.J.F., L.E.R., W.T.B. and B.A.B. conducted the study. T.D.C. and B.A.B. analysed the data and wrote the paper.

COMPETING INTERESTS

No competing interests declared.

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