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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 d. 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 Q_{10} 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 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 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.

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

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;

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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 warmer temperatures 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

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dynamic action (SDA)'; Carey et al., 1984; Gunn et al., 2001; Walli, 2007; Clark et al., 2008b; Clark et al., 2010).

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 f_H scope has been proposed to limit the vertical distribution of yellowfin, skipjack and juvenile bigeye tunas to temperatures above 15^{o}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^{o}C and maintain a consistent presence in the mixed layer of the cool but productive California Current waters (14-21^{o}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., 2007). 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.

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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. Due to 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 increase in $\dot{M}_{O_2}$ with decreasing temperature.

MATERIALS AND METHODS

A full description of the materials and methods is given in *Supplemental material*. Briefly, two juvenile Pacific bluefin tunas (*Thunnus orientalis*) were caught off the coast of Mexico from the fishing vessel *Shogun*. Fish were transported to the Tuna Research and Conservation Center of Stanford University, CA, USA, where they were held at 20^{o}C until archival tags measuring ECG and temperature were surgically implanted into the visceral cavity ~35 d prior to experiments. 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.

Temperature change experiments were conducted in the holding tank with an aim to quantify the swimming behaviour (swimming speed and tail beat-frequency) and heart rate (f_H) of fasted fish as they experienced firstly a rapid (~3^{o}C h^{-1} down to 14.5^{o}C) and then a slower (~0.3^{o}C h^{-1} down to 15^{o}C) change in water temperature (Fig. 1). The rapid temperature change was repeated when fish were digesting a satiation meal to determine whether HIF buffered changes in visceral temperature. Towards the end of the experimental period, Tuna 2 was transferred from the holding tank at 20^{o}C to an adjacent holding tank at 23.5^{o}C for 2 d prior to the completion of the study.

Tuna 1 was used to examine oxygen consumption rates ($\dot{M}_{O_2}$) simultaneously with measurements from the archival tag during temperature challenges. The fish swam in a tunnel respirometer for 32 h to reach an acclimated state prior to 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 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}.

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) x tissue oxygen extraction, where the latter is related to the difference in oxygen content of arterial (C_aO_2) and venous (C_vO_2) blood). Further data analysis techniques are outlined in *Supplementary material*.

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 an average (±SD) T_V

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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 an average 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 (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 (Fig. S1). The increase in swimming speed was likely a consequence of elevated tail beat amplitude, since there was no detectable systematic change in tail beat-frequency with temperature (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 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 (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 (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 (Figs. S2A, S2D), indicating that the HIF associated with digestion

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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 (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 around 50-60 beats min^{-1} at 20^{o}C and falling to 30-40 beats min^{-1} at 14.5^{o}C (Fig. S2). Upon rewarming to a T_a of 20^{o}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^{o}C (Fig. S2).

Thermal challenges in respirometer

Tuna 1 was used to provide insight into the cardiac responses associated with thermally-dependent changes in $\dot{M}_{O_2}$. Heart rate of Tuna 1 in the respirometer after 32 h of acclimation at a T_a of 20^{o}C was about 60 beats min^{-1} (Fig. 2). Decreases in T_a in the respirometer caused similar qualitative responses to those seen in the fish in the holding tank. Heart rate decreased with T_a down to about 14^{o}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 15-20^{o}C and only 1.3 between 10-15^{o}C. The increase in TBF did not translate to enhanced visceral heat retention, as T_x remained similar at all ambient temperatures from 10-20^{o}C (Fig. 2A, 2H). 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^{o}C. Regressions 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 r^2=0.84, respectively) than T_V (r^2=0.71 and r^2=0.56, respectively), while $\dot{M}_{O_2}$ and TBF are similarly correlated with T_a (r^2=0.60 and r^2=0.59, respectively) and T_V (r^2=0.62 and r^2=0.62, respectively).

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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 d. The research on free-swimming, ECG-instrumented Pacific and southern bluefin tunas indicates that these species possess routine f_H that is 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).

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 d of records from southern bluefin and Pacific bluefin tunas in large pens or tanks at 18-20°C measured with similar tags. 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).

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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, which 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 due to 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 $Hb\text{-}O_2$ binding was reported in the blood of southern bluefin tuna between 23 and 36°C, while a reverse temperature effect (left shift in $Hb\text{-}O_2$ 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 $Hb\text{-}O_2$ 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. Since 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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REFERENCES

Altringham, J. D. and Block, B. A. (1997). Why do tuna maintain elevated slow muscle temperatures - power output of muscle isolated from endothermic and ectothermic fish. *J. Exp. Biol.* **200**, 2617-2627.

The Journal of Experimental Biology – ACCEPTED AUTHOR MANUSCRIPT

Blank, J. M., Morrissette, J. M., Davie, P. S. and Block, B. A. (2002). Effects of temperature, epinephrine and Ca^{2+} on the hearts of yellowfin tuna (*Thunnus albacares*). *J. Exp. Biol.* **205**, 1881-1888.

Blank, J. M., Morrissette, J. M., Farwell, C. J., Price, M., Schallert, R. J. and Block, B. A. (2007). Temperature effects on metabolic rate of juvenile Pacific bluefin tuna *Thunnus orientalis*. *J. Exp. Biol.* **210**, 4254-4261.

Blank, J. M., Morrissette, J. M., Landeira-Fernandez, A. M., Blackwell, S. B., Williams, T. D. and Block, B. A. (2004). *In situ* cardiac performance of Pacific bluefin tuna hearts in response to acute temperature change. *J. Exp. Biol.* **207**, 881-890.

Block, B. A., Dewar, H., Blackwell, S. B., Williams, T. D., Prince, E. D., Farwell, C. J., Boustany, A., Teo, S. L. H., Seitz, A., Walli, A. et al. (2001). Migratory movements, depth preferences, and thermal biology of Atlantic bluefin tuna. *Science* **293**, 1310-1314.

Block, B. A. and Finnerty, J. R. (1994). Endothermy in fishes - a phylogenetic analysis of constraints, predispositions, and selection pressures. *Env. Biol. Fishes* **40**, 283-302.

Block, B. A., Teo, S. L. H., Walli, A., Boustany, A., Stokesbury, M. J. W., Farwell, C. J., Weng, K. C., Dewar, H. and Williams, T. D. (2005). Electronic tagging and population structure of Atlantic bluefin tuna. *Nature* **434**, 1121-1127.

Boustany, A. M., Matteson, R., Castleton, M., Farwell, C. and Block, B. A. (2010). Movements of Pacific bluefin tuna (*Thunnus orientalis*) in the Eastern North Pacific revealed with archival tags. *Prog. Oceanogr.* **86**, 94-104.

Brill, R. W., Block, B. A., Boggs, C. H., Bigelow, K. A., Freund, E. V. and Marcinek, D. J. (1999). Horizontal movements and depth distribution of large adult yellowfin tuna (Thunnus albacares) near the Hawaiian Islands, recorded using ultrasonic telemetry: implications for the physiological ecology of pelagic fishes. *Mar. Biol.* **133**, 395-408.

Brill, R. W. and Bushnell, P. G. (1991). Metabolic and cardiac scope of high energy demand teleosts, the tunas. *Can. J. Zool.* **69**, 2002-2009.

Brill, R. W. and Bushnell, P. G. (2001). The cardiovascular system of tunas. In *Tuna: Physiology, Ecology, and Evolution*, vol. 19 Fish Physiology eds. B. A. Block and E. D. Stevens), pp. 79-120. San Diego: Academic Press.

Bushnell, P. G. and Brill, R. W. (1992). Oxygen transport and cardiovascular responses in skipjack tuna (*Katsuwonus pelamis*) and yellowfin tuna (*Thunnus albacares*) exposed to acute hypoxia. *J. Comp. Physiol. B* **162**, 131-43.

Carey, F. G., Kanwisher, J. W. and Stevens, E. D. (1984). Bluefin tuna warm their viscera during digestion. *J. Exp. Biol.* **109**, 1-20.

Carey, F. G. and Teal, J. M. (1969). Regulation of body temperature by the bluefin tuna. *Comp. Biochem. Physiol. A* **28**, 205-213.

Clark, T. D., Brandt, W. T., Nogueira, J., Rodriguez, L. E., Price, M., Farwell, C. J. and Block, B. A. (2010). Postprandial metabolism of Pacific bluefin tuna (*Thunnus orientalis*). *J. Exp. Biol.* **213**, 2379-2385.

Clark, T. D. and Seymour, R. S. (2006). Cardiorespiratory physiology and swimming energetics of a high-energy-demand teleost, the yellowtail kingfish (*Seriola lalandi*). *J. Exp. Biol.* **209**, 3940-3951.

Clark, T. D., Seymour, R. S., Wells, R. M. G. and Frappell, P. B. (2008a). Thermal effects on the blood respiratory properties of southern bluefin tuna, *Thunnus maccoyii*. *Comp. Biochem. Physiol. A* **150**, 239-246.

The Journal of Experimental Biology – ACCEPTED AUTHOR MANUSCRIPT

Clark, T. D., Taylor, B. D., Seymour, R. S., Ellis, D., Buchanan, J., Fitzgibbon, Q. P. and Frappell, P. B. (2008b). Moving with the beat: heart rate and visceral temperature of free-swimming and feeding bluefin tuna. *Proc. Roy. Soc. B: Biol. Sci.* **275**, 2841-2850.

Dagorn, L., Holland, K., N. , Hallier, J.-P., Taquet, M., Moreno, G., Sancho, G., Itano, D., G., Aumeeruddy, R., Girard, C., Million, J. et al. (2006). Deep diving behavior observed in yellowfin tuna (Thunnus albacares). *Aquat. Living Resour.* **19**, 85-88.

Dickson, K. A. and Graham, J. B. (2004). Evolution and consequences of endothermy in fishes. *Physiol. Biochem. Zool.* **77**, 998-1018.

Dizon, A. E., Neill, W. H. and Magnuson, J. J. (1977). Rapid temperature compensation of volitional swimming speeds and lethal temperatures in tropical tunas (Scombridae). *Env. Biol. Fishes* **2**, 83.

Dizon, A. E., Stevens, E. D., Neill, W. H. and Magnuson, J. J. (1974). Sensitivity of restrained skipjack tuna (*Katsuwonus pelamis*) to abrupt increases in temperature. *Comp. Biochem. Physiol. A* **49**, 291-299.

Fudge, D. S. and Stevens, E. D. (1996). The visceral retia mirabilia of tuna and sharks: an annotated translation and discussion of the Eschricht & Müller 1835 paper and related papers. *Guelph Ichthyology Reviews* **4**, 1-53.

Galli, G. L. J., Lipnick, M. S. and Block, B. A. (2009). Effect of thermal acclimation on action potentials and sarcolemmal K^+ channels from Pacific bluefin tuna cardiomyocytes. *Am. J. Physiol. - Reg. I.* **297**, R502-509.

Galli, G. L. J., Lipnick, M. S., Shiels, H. A. and Block, B. A. (2011). Temperature effects on Ca^{2+} cycling in scombrid cardiomyocytes: a phylogenetic comparison. *J. Exp. Biol.* **214**, 1068-1076.

Gordon, M. S. (1968). Oxygen consumption of red and white muscles from tuna fishes. *Science* **159**, 87-90.

Graham, J. B. and Dickson, K. A. (2004). Tuna comparative physiology. *J. Exp. Biol.* **207**, 4015-4024.

Gunn, J., Hartog, J. and Rough, K. (2001). The relationship between food intake and visceral warming in southern bluefin tuna (*Thunnus maccoyii*). In *Electronic Tagging and Tracking in Marine Fisheries*, vol. 1 eds. J. R. Sibert and J. L. Nielsen), pp. 1009-1130: Dordrecht: Kluwer Academic Publishers.

Holland, K. N., Brill, R. W. and Change, R. K. C. (1990). Horizontal and vertical movements of yellowfin and bigeye tuna associated with fish aggregating devices. *Fish. Bull.* **88**, 493-507.

Keen, J. E., Aota, S., Brill, R. W., Farrell, A. P. and Randall, D. J. (1995). Cholinergic and adrenergic regulation of heart rate and ventral aortic pressure in two species of tropical tunas, *Katsuwonus pelamis* and *Thunnus albacares*. *Can J Zool* **73**, 1681-1688.

Kitagawa, T., Kimura, S., Nakata, H. and Yamada, H. (2007). Why do young Pacific bluefin tuna repeatedly dive to depths through the thermocline? *Fisheries Sci.* **73**, 98-106.

Korsmeyer, K. E., Lai, N. C., Shadwick, R. E. and Graham, J. B. (1997a). Heart rate and stroke volume contributions to cardiac output in swimming yellowfin tuna - response to exercise and temperature. *J. Exp. Biol.* **200**, 1975-1986.

Korsmeyer, K. E., Lai, N. C., Shadwick, R. E. and Graham, J. B. (1997b). Oxygen transport and cardiovascular responses to exercise in the yellowfin tuna *Thunnus albacares*. *J. Exp. Biol.* **200**, 1987-1997.

Landeira-Fernandez, A. M., Castilho, P. C. and Block, B. A. (2012). Thermal dependence of cardiac SR Ca2+-ATPase from fish and mammals. *J. Therm. Biol.* **37**, 217-223.

The Journal of Experimental Biology – ACCEPTED AUTHOR MANUSCRIPT

Lawson, G. L., Castleton, M. R. and Block, B. A. (2010). Movements and diving behavior of Atlantic bluefin tuna *Thunnus thynnus* in relation to water column structure in the northwestern Atlantic. *Mar. Ecol. Prog. Ser.* **400**, 245-265.

Malte, H., Larsen, C., Musyl, M. and Brill, R. (2007). Differential heating and cooling rates in bigeye tuna (*Thunnus obesus* Lowe): a model of non-steady state heat exchange. *J. Exp. Biol.* **210**, 2618-2626.

Marcinek, D. J., Blackwell, S. B., Dewar, H., Freund, E. V., Farwell, C., Dau, D., Seitz, A. C. and Block, B. A. (2001). Depth and muscle temperature of Pacific bluefin tuna examined with acoustic and pop-up satellite archival tags. *Mar. Biol.* **138**, 869-885.

Patterson, T. A., Evans, K., Carter, T. I. and Gunn, J. S. (2008). Movement and behaviour of large southern bluefin tuna (Thunnus maccoyii) in the Australian region determined using pop-up satellite archival tags. *Fish. Oceanogr.* **17**, 352-367.

Rossi-Fanelli, A., Antonini, E. and Giuffre, R. (1960). Oxygen equilibrium of myoglobin from *Thunnus thynnus*. *Nature* **186**, 896.

Schaefer, K. M., Fuller, D. W. and Block, B. A. (2011). Movements, behavior, and habitat utilization of yellowfin tuna (*Thunnus albacares*) in the Pacific Ocean off Baja California, Mexico, determined from archival tag data analyses, including unscented Kalman filtering. *Fisheries Res.* **112**, 22-37.

Walli, A., Teo, S. L. H., Boustany, A., Farwell, C. J., Williams, T., Dewar, H., Prince, E. and Block, B. A. (2009). Seasonal movements, aggregations and diving behavior of Atlantic bluefin tuna (*Thunnus thynnus*) revealed with archival tags. *PLoS ONE* **4**, e6151.

Walli, A. G. (2007). On the movements, aggregations and the foraging habitat of bluefin tuna (*Thunnus thynnus* & *orientalis*). Santa Cruz, California: Ph.D. dissertation, University of California Santa Cruz.

FIGURE LEGENDS

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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 seven 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 two-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 kg 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 Figs. S1 and S2 are indicated.

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 Panel 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}. Regressions lines are described by (B) $y = 867.02x^{-0.53}$, $r^2=0.60$; (C) $y = 1429.50x^{-0.68}$, $r^2=0.62$; (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) $y = -3.17x + 162.15$, $r^2=0.59$; (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).

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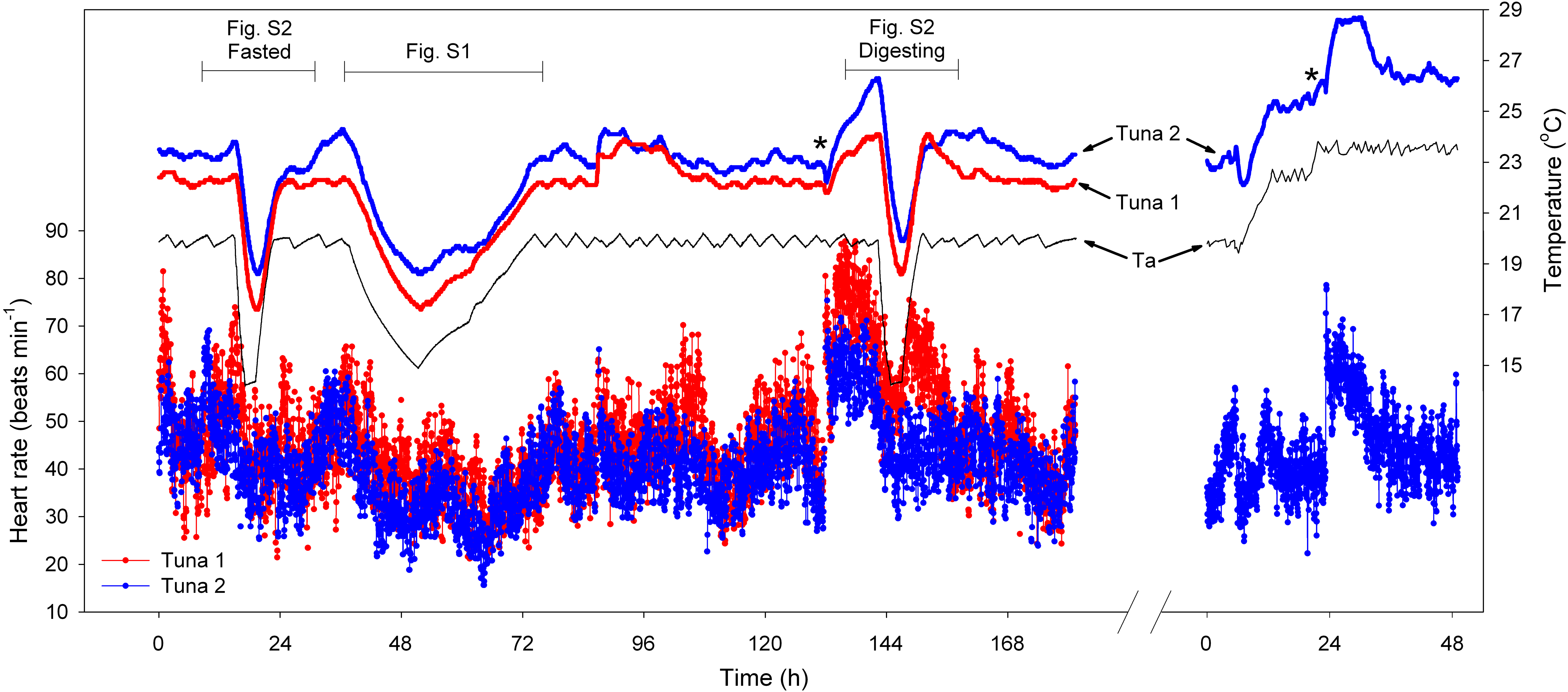
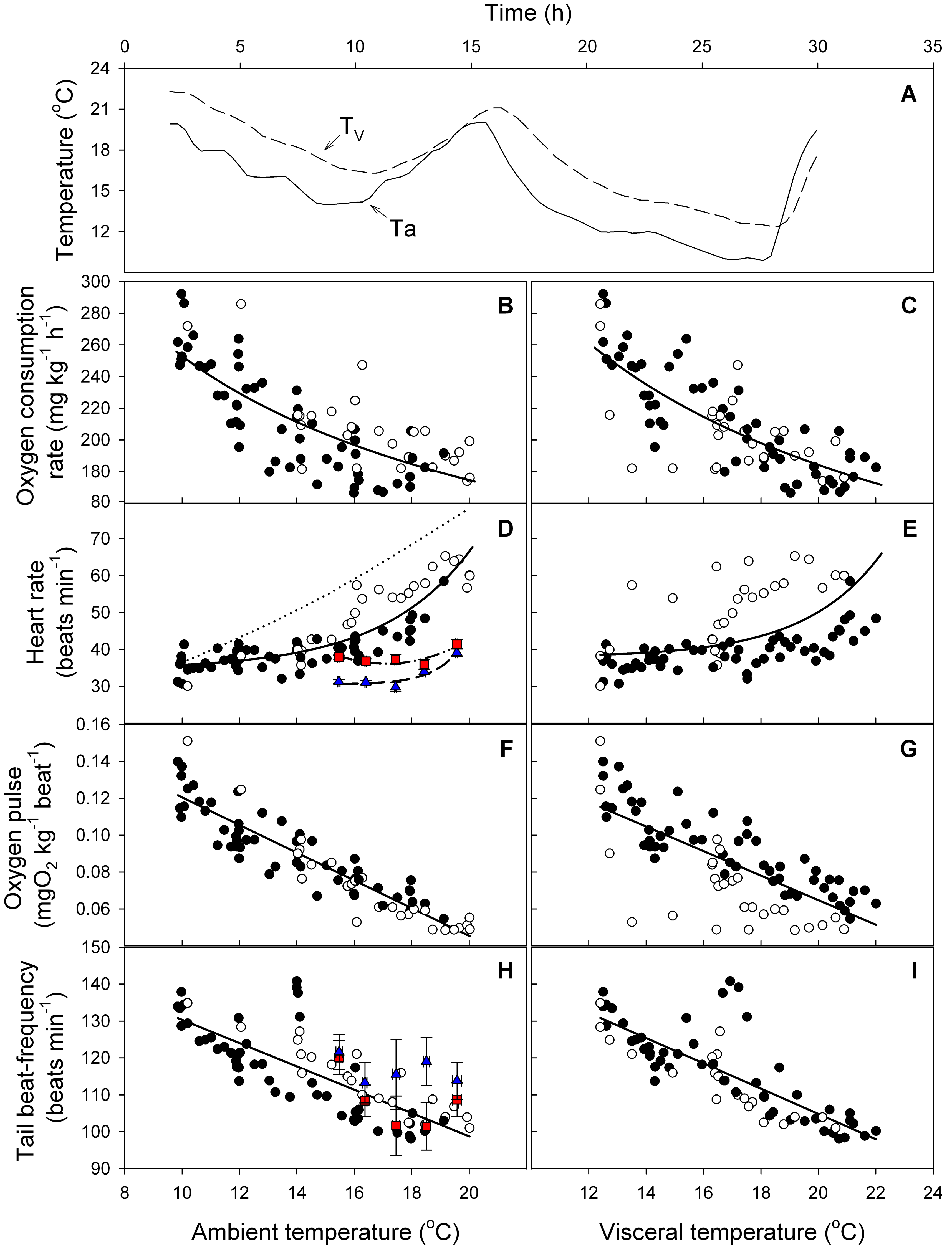


Fig. S2
Fasted
Fig. S1
Fig. S2
Digesting
*
*
Tuna 2
Tuna 1
Ta
Heart rate (beats min^{-1})
10
20
30
40
50
60
70
80
90
Temperature (°C)
29
27
25
23
21
19
17
15
Time (h)
0
24
48
72
96
120
144
168
0
24
48
Tuna 1
Tuna 2


Time (h)
0
5
10
15
20
25
30
35
Temperature (°C)
24
21
18
15
12
A
T_V
Ta
Oxygen consumption rate (mg kg^{-1} h^{-1})
300
280
260
240
220
200
180
80
B
C
Heart rate (beats min^{-1})
70
60
50
40
30
D
E
Oxygen pulse (mgO$_2$ kg^{-1} beat^{-1})
0.16
0.14
0.12
0.10
0.08
0.06
F
G
Tail beat-frequency (beats min^{-1})
150
140
130
120
110
100
90
H
I
8
10
12
14
16
18
20
22
24
Ambient temperature (°C)
Visceral temperature (°C)