

## Temperature effects on metabolic rate of juvenile Pacific bluefin tuna *Thunnus orientalis*

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### Summary

Pacific bluefin tuna inhabit a wide range of thermal environments across the Pacific ocean. To examine how metabolism varies across this thermal range, we studied the effect of ambient water temperature on metabolic rate of juvenile Pacific bluefin tuna, *Thunnus thynnus*, swimming in a swim tunnel. Rate of oxygen consumption ( $\dot{M}_{O_2}$ ) was measured at ambient temperatures of 8–25°C and swimming speeds of 0.75–1.75 body lengths (BL) s<sup>-1</sup>. Pacific bluefin swimming at 1 BL s<sup>-1</sup> per second exhibited a U-shaped curve of metabolic rate vs ambient temperature, with a thermal minimum zone between 15°C to 20°C. Minimum  $\dot{M}_{O_2}$  of 175±29 mg kg<sup>-1</sup> h<sup>-1</sup> was recorded at 15°C, while both cold and warm temperatures resulted in

increased metabolic rates of 331±62 mg kg<sup>-1</sup> h<sup>-1</sup> at 8°C and 256±19 mg kg<sup>-1</sup> h<sup>-1</sup> at 25°C. Tailbeat frequencies were negatively correlated with ambient temperature. Additional experiments indicated that the increase in  $\dot{M}_{O_2}$  at low temperature occurred only at low swimming speeds. Ambient water temperature data from electronic tags implanted in wild fish indicate that Pacific bluefin of similar size to the experimental fish used in the swim tunnel spend most of their time in ambient temperatures in the metabolic thermal minimum zone.

Key words: endothermy, metabolic rate, temperature, thermoregulation, tuna.

### Introduction

The four genera (*Euthynnus*, *Katsuwonus*, *Auxis* and *Thunnus*) of tunas share a suite of physiological and morphological traits enhancing swimming and metabolic performance, but each species of tuna differs in cardiovascular function, energetics and endothermic capacity (Blank et al., 2004; Block et al., 1993; Brill and Bushnell, 2001). Tunas have evolved vascular countercurrent heat exchangers, *retia mirabilia*, to conserve metabolic heat and maintain elevated temperatures in slow-twitch muscles, viscera, eyes and brain (Carey and Teal, 1966; Linthicum and Carey, 1972). Substantial thermal gradients are often present within the body of an individual fish (Carey and Teal, 1966) while some tissues, including the heart and gills, operate at ambient water temperature. Data from electronic tags indicate that Pacific bluefin (*Thunnus orientalis*) and yellowfin tuna (*T. albacares*) range through a thermally variable environment (Block et al., 2001; Block et al., 1997; Kitagawa et al., 2007; Marcinek, 2000; Schaefer et al., 2007), resulting in fluctuating tissue temperatures that are affected by species, body size, ambient water temperature, feeding status and activity level. The relationships of the metabolic rates of tunas to ambient temperature are potentially complex and may be distinct from those of ectothermic fish or terrestrial endotherms.

Measurements of oxygen consumption rates ( $\dot{M}_{O_2}$ ) in tunas are complicated by their large size and need to swim continuously to ventilate the gills and generate hydrodynamic lift (Magnuson, 1973). To date, most studies of tuna metabolism have examined tropical or warm temperate species, and only a few studies have examined acute temperature changes (Korsmeyer and Dewar, 2001). Standard metabolic rates of 250–500 mg O<sub>2</sub> kg<sup>-1</sup> h<sup>-1</sup> have been measured at 25°C in 0.5–4 kg kawakawa (*Euthynnus affinis*), yellowfin, and skipjack tuna (*Katsuwonus pelamis*) (Boggs and Kitchell, 1991; Brill, 1979; Brill, 1987; Bushnell and Brill, 1992; Dewar and Graham, 1994; Gooding et al., 1981; Korsmeyer et al., 1997). In swimming yellowfin tuna, acute temperature changes between 18 and 30°C yielded a mean Q<sub>10</sub> of 1.67 for  $\dot{M}_{O_2}$  (Dewar and Graham, 1994). Anesthetized yellowfin, skipjack and kawakawa  $\dot{M}_{O_2}$  exhibited Q<sub>10</sub> values of 2.3, 2.4 and 3.2, respectively between 20 and 25°C (Brill, 1987). While these Q<sub>10</sub> values are similar to those of ectothermic teleosts, there has been little research on the relationship of metabolic rate to ambient water temperature in the cold–temperate tunas such as albacore (*Thunnus alalunga*) or bluefin tunas (*T. thynnus*, *T. orientalis* and *T. maccoyii*). Shipboard measurements of albacore metabolic rates have been limited to a 3°C temperature range or less (Graham and Laurs, 1982; Graham et al., 1989).

Recent measurements of  $\dot{M}_{O_2}$  in swimming 7–10 kg Pacific bluefin tuna indicate that Pacific bluefin have higher metabolic rates than yellowfin tuna of similar size at 20°C (Blank et al., 2007). Minimal metabolic rates of fasted bluefin and yellowfin tunas in the swim tunnel were  $222 \pm 24$  mg  $O_2$   $kg^{-1}$   $h^{-1}$  and  $162 \pm 19$  mg  $O_2$   $kg^{-1}$   $h^{-1}$ , respectively, at 20°C. Rates up to  $498 \pm 55$  mg  $O_2$   $kg^{-1}$   $h^{-1}$  were recorded in bluefin at  $1.8$   $BL$   $s^{-1}$ . Routine metabolic rate in southern bluefin tuna (*Thunnus maccoyii*) swimming freely in a circular mesocosm was recently measured and reported as mean metabolic rate of  $460$  mg  $O_2$   $kg^{-1}$   $h^{-1}$  for fasted 20 kg fish in 19°C water (Fitzgibbon et al., 2006). Feeding resulted in elevated  $\dot{M}_{O_2}$  for a period of 20–45 h accompanied by increases in voluntary swimming speed (Fitzgibbon et al., 2007).

Adult Atlantic bluefin tuna maintain large elevations in muscle temperature (Carey and Teal, 1969) and juvenile Pacific bluefin (12–20 kg) have the capacity to maintain relatively stable muscle temperatures 6–8°C above ambient water temperatures (Marcinek et al., 2001). Visceral temperatures of wild Pacific bluefin exhibit diel cycles of warming associated with specific dynamic action, resulting in elevations of 4–12°C above ambient water temperature for 6–24 h following feeding (Kitagawa et al., 2007). In contrast to the warm muscle and viscera of swimming bluefin tunas, the heart remains at or near ambient water temperature in all tuna species (Brill et al., 1994; Carey et al., 1984), resulting in pronounced effects of ambient temperature on heart function (Blank et al., 2002; Blank et al., 2004; Korsmeyer et al., 1997; Landeira-Fernandez et al., 2004).

The availability of Pacific bluefin tuna in captivity and the ability to maintain tunas in the swim tunnel for extended periods of up to 6 days provides an unprecedented opportunity to study the response of Pacific bluefin tuna metabolic rates to changes of ambient water temperatures and swimming speeds. Measurements at 8–25°C indicate that metabolic rates of bluefin tuna swimming at low speed reach a thermal minimum zone (TMZ) that corresponds to the preferred sea surface temperatures of similar sized Pacific bluefin tunas in the wild.

## Materials and methods

### Fish

Pacific bluefin tuna *Thunnus orientalis* (Temminck & Schlegel, 1844) were captured on barbless circle hooks off San Diego, CA, USA at latitudes 31°20' to 31°30'N, and longitudes 117°24' to 117°30'W in 20–20.1°C sea surface temperature. Following capture, the tuna were held on board the fishing vessel in wells filled with aerated seawater for 1–4 days. Tuna were transferred in seawater-filled vinyl slings to a 10 600 l tank at 15°C and transported by truck to the Tuna Research and Conservation Center (TRCC) in Pacific Grove, CA, USA. Bluefin tuna were held in 109 m<sup>3</sup> circular tanks containing  $20 \pm 1.0$ °C seawater and fed a diet of squid, anchovies and enriched gelatin, as previously described (Farwell, 2001). Fish were held for 58–162 days prior to use in swim tunnel experiments and archival tags were implanted in the visceral cavity during this period. Mean curved fork length (CFL) of 9 bluefin used in the swim tunnel was  $76 \pm 4$  cm (range 70–84 cm), and mass was  $8.8 \pm 0.8$  kg (range 7.4–9.9 kg). All fish were feeding prior to experiments. Food

was withheld from the entire tank for 45–72 h prior to the introduction of a fish into the swim tunnel.

### Swim tunnel

Bluefin tuna swam in a swim tunnel consisting of an 870 l acrylic respirometer chamber with a square 45 cm × 45 cm × 135 cm working section contained in a 1500 l external tank for thermal insulation (Loligo Systems, Denmark). Turbulence was minimized by two honeycomb sections upstream of the working section and additional flow straighteners. The sides of the working section were marked with vertical stripes of black tape at 10 cm intervals to assist the fish in maintaining position in the tank. Water velocities were calibrated by video analysis of dye injections and corrected for solid blocking effects for each fish (Bell and Terhune, 1970). Fish cross-sectional area ranged from 6 to 10% of working section area and blocking correction ranged from 5 to 11% of uncorrected velocity. Seawater was supplied to the respirometer from a 20 000 l reservoir, which was continuously filtered and aerated. Temperature of the swim tunnel was adjusted to the target temperature  $\pm 0.1$ °C by a 6800 W heat pump and addition of warm or cold filtered seawater to the 20 000 l reservoir. The entire swim tunnel was enclosed by black plastic sheeting to minimize light fluctuations and access to the building was limited while the experiment was in progress to minimize disturbance. A video camera and a mirror mounted at a 45° angle above the working section provided an overhead view of the swimming fish, allowing continuous monitoring of its swimming behavior.

### Introduction of the fish

Each tuna was captured in a water-filled nylon sling and a measurement of its CFL was taken. The sling holding the fish was lifted from the holding tank and carried to the swim tunnel. The front end of the sling was submerged in seawater flowing at  $\sim 1$   $BL$   $s^{-1}$  in the working section and opened. One researcher manually guided the fish from the sling into the flowing water, while the other researcher removed the sling. The lid of the working section was used to cover the front of the working section as the fish was introduced and then moved to cover the rest of the working section. One researcher positioned a hand at the rear of the working section to prevent the fish from contacting the rear grate as it adjusted to the swim tunnel. Once the fish was swimming steadily, approximately 30–45 min after introduction, the lid of the working section was secured with wing nuts and the pumps supplying aerated seawater to the swim tunnel were set to alternate between flushing the swim tunnel with aerated seawater and closing for measurements of  $\dot{M}_{O_2}$ . Fish that did not swim steadily were removed from the swim tunnel and returned to the holding tank.

### Respirometry procedures

Oxygen consumption was measured by stopped-flow respirometry as detailed by Steffensen (Steffensen, 1989). The swim tunnel was closed for 10 min for measurement of  $\dot{M}_{O_2}$  and then flushed with aerated seawater for 10 min to restore dissolved oxygen (DO<sub>2</sub>). These steps were repeated throughout the experiment. DO<sub>2</sub> remained above 80% of air saturation levels throughout each experiment and was generally above

90% saturation except at 25°C. Temperature and oxygen content of the seawater in the swim tunnel were logged at 10 or 15 s intervals by a temperature-compensated multiprobe (Yellow Springs Instruments Model 556, Yellow Springs, OH, USA) and  $\dot{M}_{O_2}$  was calculated from the rate of decline in  $DO_2$  during each closed period. The  $O_2$  electrode was calibrated in air-saturated seawater at 20°C prior to each experiment, and the calibration was found to drift by less than 1% over the duration of each experiment.

#### Series I

Six bluefin tuna (mass=9.1±0.6 kg, range 8.1–9.9 kg) were used in the initial series of measurements. Three of these fish were previously used in separate experiments on different dates (Blank et al., 2007) and were reintroduced to the swim tunnel for this study. Once metabolic rate measurements were initiated, the swim speed was adjusted to 1.0  $BL\ s^{-1}$  and the fish was allowed to acclimate to the respirometer overnight while temperature was maintained at 20°C, matching the acclimation temperature in the holding tank. Following the initial acclimation period, or following speed tests conducted in three individuals according to the previously described protocol (Blank et al., 2007), a series of at least 12 consecutive  $\dot{M}_{O_2}$  measurements was completed at 20°C. Temperature was then adjusted and at least 12 measurements were taken at 15°C, followed by 10°C, 8°C and 25°C (Fig. 1). Attempts to measure  $\dot{M}_{O_2}$  at temperatures below 8°C were unsuccessful, as some fish stopped swimming or began to lose righting responses when the swim tunnel was cooled further. Each test temperature was held constant for a minimum of 4 h throughout the measurement and flush cycles. If the behavior or  $\dot{M}_{O_2}$  of the fish was irregular, this period was extended to increase the number of measurements taken.

Attempts were made to test all fish at all temperatures; however, we were only able to complete measurements on five of the six fish at 8°C and at 25°C. Fish were maintained in the swim tunnel for a total of 2–6 days in order to complete acclimation, temperature changes and  $\dot{M}_{O_2}$  measurements. In some experiments, a 12 h:12 h light:dark cycle (with dim light at night) was maintained, matching the cycle in the holding tanks. In most cases, the lighting changes appeared to startle the fish and constant dim light was used instead. Control measurements at 20°C were repeated at or near the end of the

experiment to confirm the repeatability of the baseline metabolic rate.  $\dot{M}_{O_2}$  at 20°C changed by –4 to +17% (mean=9%) over the period of the experiment. When all measurements were completed, the fish was removed from the swim tunnel and weighed on a cushioned vinyl V-board with a damp cloth over its eyes. The length of the fish was verified and the fish was returned to its holding tank. After each experiment, the swim tunnel was sealed and background respiration measured. In all cases, background respiration was negligible.

#### Series II

In a second series of experiments on a different group of three fish (mass=8.1±0.6 kg, range 7.4–8.4 kg), the effects of swimming speed on  $\dot{M}_{O_2}$  were compared at three different temperatures. Each fish was introduced to the swim tunnel as described above and allowed to acclimate to the swim tunnel for 2–3 h while swimming at 1.0  $BL\ s^{-1}$  at 20°C. The fish was then presented with a practice series of speed changes in which speed was increased in 0.15  $BL\ s^{-1}$  increments during each flush period up to a maximum of 1.75  $BL\ s^{-1}$ . Following completion of this ‘practice speed test’, the speed was reduced to 1.0  $BL\ s^{-1}$  and the fish was allowed to acclimate to the swim tunnel overnight for a minimum of 15 h. On the following day, speed was elevated or lowered in increments of 0.10 or 0.15  $BL\ s^{-1}$  and a series of at least four consecutive  $\dot{M}_{O_2}$  measurements was completed at each speed setting from 0.75  $BL\ s^{-1}$  to 1.75  $BL\ s^{-1}$ . Fish were observed to swim closer to the back of the swim tunnel at faster speeds, so speeds above 1.75  $BL\ s^{-1}$  were not tested to minimize the risk of damage to the caudal fin of the fish. No attempt was made to measure maximum swimming speed or  $\dot{M}_{O_{2max}}$ . After the initial speed test, the temperature of the swim tunnel was adjusted to 8 or 25°C, the fish was allowed to equilibrate for at least 4 h, and the entire speed test was repeated at the new temperature. Speed tests were conducted at each temperature on successive days and control measurements were taken at 20°C before the fish was removed from the swim tunnel, as described above.

#### Data analysis

While in the swim tunnel, the fish was monitored continuously *via* closed circuit video and any aberrant swimming behaviors or external disturbances were noted. Individual measurements of  $\dot{M}_{O_2}$  and other variables associated with disturbances such as earthquakes, power outages, air bubbles in the respirometer and spontaneous attempts by the fish to turn around in the working section were excluded from further analysis. The first 60 min of data following each

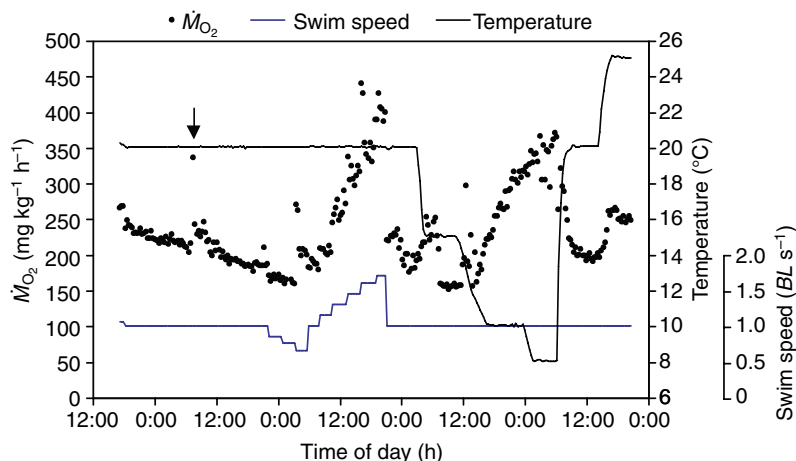


Fig. 1. Time course of respirometry in a bluefin tuna. Oxygen consumption ( $\dot{M}_{O_2}$ , filled circles) was calculated from the slope of ambient  $[O_2]$  measured during 10 min periods with the swim tunnel sealed. An overnight acclimation period preceded tests of swim speed (blue line, in  $BL\ s^{-1}$ ) and temperature (black line) effects on  $\dot{M}_{O_2}$ . The arrow during the acclimation period indicates elevated  $\dot{M}_{O_2}$  measurements following a power surge, which startled the fish. Speed changes are described elsewhere (Blank et al., 2007) and were not a part of the present study.

ambient temperature change were also excluded to allow for thermal equilibration. The mean of all remaining measurements at a given speed and/or temperature was then taken as the  $\dot{M}_{O_2}$  for that condition for subsequent calculations and statistical analysis (minimum  $N=5$ ).

Tailbeat frequency was measured by an observer watching the live video display of the overhead view of the swimming fish. Sixty tailbeats were timed with a stopwatch 3 times and the computed tailbeats  $\text{min}^{-1}$  from the three counts were averaged. This process was repeated at least two times for each fish at each speed. Gross cost of transport (GCOT) was calculated from  $\dot{M}_{O_2}$  and swimming speed using an oxy-calorific coefficient of  $14.1 \text{ J mg O}_2^{-1}$  for a mixed diet (Videler, 1993). For series I, data collected at different temperatures were compared by one-way analysis of variance (ANOVA) using Systat 11.0. *Post-hoc* comparisons were made with Tukey's HSD and significance was assessed at  $P<0.05$ . For series II, two-way ANOVA was used to assess the combined effects of temperature and swimming speed on  $\dot{M}_{O_2}$ , tailbeat frequency and GCOT. GCOT data were log-transformed to assure normality. Data are presented as means  $\pm$  s.d.

#### Archival tagging

Two models of archival tags (Lotek LTD 1400 and LTD 2310, Lotek Wireless, Inc., Newmarket, Ontario, Canada) were implanted in fish to record visceral temperatures at 4 or 8 s intervals as the fish swam in the respirometer. For tag implantation, the water level in the holding tank was lowered to 90 cm and a bluefin tuna was gently guided into a water-filled nylon sling. The fish was placed ventral-side up, its eyes covered with a moist cloth, and its gills irrigated with a hose placed in its mouth. A sterile #22 surgical blade was used to make a small incision in the ventral body wall and the tag was inserted in the peritoneal cavity with the sensor stalk protruding from the ventral body wall. The incision was sutured shut and the fish was released to recover in the tank for a minimum of 7 days. Fish were observed to feed as soon as 2 days after tag implantation and all tagged fish had recovered prior to use in swim tunnel experiments. Tags were programmed to record visceral temperature (both tags) and/or ambient water temperature (LTD 2310 only) at intervals of 4 or 8 s. An additional Lotek 2310 tag was synchronized and placed in the swim tunnel to record ambient temperature. All fish were tagged, and tags were recovered from three fish used in Series I experiments *post-mortem* and time series data were downloaded. Each of the three tags was embedded in the visceral mass in contact with the pyloric caecum. The mean visceral and ambient temperatures during the last hour at each ambient test temperature were considered to represent steady state and used for analysis of thermal excess.

Wild bluefin ( $N=10$ ,  $CFL=75.2\pm 2.8$  cm) were tagged offshore of San Diego, California using previously published procedures (Block et al., 1998; Kitagawa et al., 2007). Tags were programmed to collect pressure, light and temperature data every 4, 8, 16 or 32 s. Since the LTD2310 tags were implanted in the peritoneal cavity of the tuna, recapture of the fish and recovery of the tag were necessary in order to

recover the collected data. The tagged bluefin tuna were recaptured after  $241\pm 131$  days at liberty and data was downloaded from the tags. For each day, we extracted pressure, ambient water temperature, and body temperature data.

All surgical, tagging and other experimental procedures were conducted in accordance with Stanford University institutional animal use protocols.

#### Results

Metabolic rates of Pacific bluefin tuna swimming at  $1.0 \text{ BL s}^{-1}$  exhibited a U-shaped metabolic curve with respect to ambient water temperatures, with  $\dot{M}_{O_2}$  values ranging from  $175\pm 29 \text{ mg kg}^{-1} \text{ h}^{-1}$  to  $331\pm 62 \text{ mg kg}^{-1} \text{ h}^{-1}$  (Fig. 2A). An apparent thermal minimum zone was reached between 15 and  $20^\circ\text{C}$  with similar  $\dot{M}_{O_2}$  ( $P=0.95$ ,  $15^\circ\text{C}$  vs  $20^\circ\text{C}$ ) and a  $Q_{10}$  of only 1.25 in this temperature range. Bluefin tuna swimming at a constant speed of  $1.0 \text{ BL s}^{-1}$  increased  $\dot{M}_{O_2}$  significantly from  $175\pm 29 \text{ mg kg}^{-1} \text{ h}^{-1}$  at  $15^\circ\text{C}$  to a maximum of  $331\pm 62 \text{ mg kg}^{-1} \text{ h}^{-1}$  at  $8^\circ\text{C}$  ( $P<0.001$ ), resulting in a mean  $Q_{10}$  of 0.41 between 8 and  $15^\circ\text{C}$ . Tailbeat frequency increased from  $103\pm 5$  tailbeats  $\text{min}^{-1}$  at  $25^\circ\text{C}$  to  $127\pm 11$  tailbeats  $\text{min}^{-1}$  at  $8^\circ\text{C}$  (Fig. 2B,  $P=0.006$ ). At  $8\text{--}10^\circ\text{C}$ , swimming behavior became noticeably erratic. Bluefin periodically made contact with the front honeycomb or front corner of the swim tunnel's working section and produced bursts of rapid tailbeats. From  $20\text{--}25^\circ\text{C}$ ,

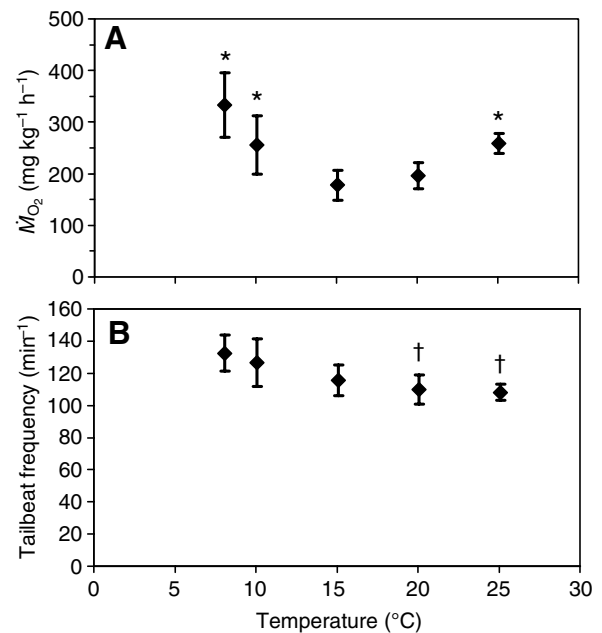


Fig. 2. Temperature effects on swimming bluefin tuna. (A)  $\dot{M}_{O_2}$  (mean  $\pm$  s.d.) of six bluefin tuna swimming at a constant speed of  $1.0 \text{ BL s}^{-1}$  in the swim tunnel was significantly affected by temperature (ANOVA,  $P<0.001$ ).  $\dot{M}_{O_2}$  of each fish at each temperature was calculated as the mean of six or more  $\dot{M}_{O_2}$  measurements taken after an equilibration period of at least 1 h at the stated temperature ( $\pm 0.1^\circ\text{C}$ ). \* Significant difference from  $\dot{M}_{O_2}$  at  $15^\circ\text{C}$  ( $P<0.05$ , Tukey's HSD). (B) Mean tailbeat frequency ( $\pm$  s.d.) of six bluefin tuna swimming at  $1.0 \text{ BL s}^{-1}$  in the swim tunnel was significantly affected by temperature (ANOVA,  $P<0.01$ ). † Significant difference from TBF at  $8^\circ\text{C}$ .

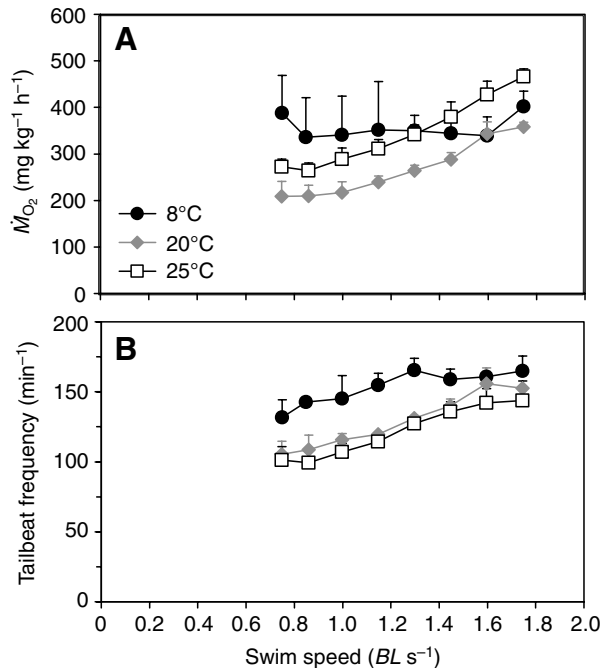


Fig. 3. Effects of ambient temperature and speed on bluefin tuna  $\dot{M}_{O_2}$  and tailbeat frequency (TBF). (A)  $\dot{M}_{O_2}$  measurements at swim speeds of 0.75–1.75  $BL\ s^{-1}$  were repeated at three steady-state temperatures on three consecutive days. A significant interaction of temperature and speed effects on  $\dot{M}_{O_2}$  was observed (ANOVA,  $P < 0.05$ ).  $\dot{M}_{O_2}$  increased with swimming speed at 20 and 25°C ( $P < 0.001$ ), but was unaffected by speed at 8°C ( $P = 0.89$ ). Data shown are means  $\pm$  s.d. of  $\dot{M}_{O_2}$  measurements from three individual bluefin tuna (mass = 8.1  $\pm$  0.6 kg). (B) Tailbeats were counted as bluefin swam at speeds of 0.75–1.75  $BL\ s^{-1}$  at temperatures of 8, 20 and 25°C. Both temperature and speed had significant effects on TBF ( $P < 0.001$ ). Data shown are means  $\pm$  s.d. of TBF counts from three individual bluefin tuna (mass = 8.1  $\pm$  0.6 kg).

bluefin tuna  $\dot{M}_{O_2}$  increased from 193  $\pm$  25 to 256  $\pm$  19  $mg\ kg^{-1}\ h^{-1}$  ( $Q_{10}$  of 1.8; Fig. 2A).

In a second series of experiments, the influence of swimming speed on Pacific bluefin tuna metabolism was compared at three ambient temperatures (Fig. 3). Warming from 20°C to 25°C increased  $\dot{M}_{O_2}$  at each swim speed. At both 20 and 25°C, increasing speed from 1.0 to 1.75  $BL\ s^{-1}$  resulted in a 1.7-fold increase in  $\dot{M}_{O_2}$  ( $P < 0.001$ ). In contrast, bluefin tuna cooled to 8°C showed no change of  $\dot{M}_{O_2}$  with swim speed ( $P = 0.89$ ). A significant interaction of temperature and swim speed was detected ( $P = 0.015$ ). Tailbeat frequencies recorded at 8°C were higher than those at 20 and 25°C ( $P < 0.001$ , Fig. 3B). GCOT calculated from  $\dot{M}_{O_2}$  at each speed showed a significant interaction of speed and temperature ( $P = 0.023$ ), with a maximum GCOT of 2.8  $\pm$  0.5  $J\ kg^{-1}\ m^{-1}$  at 0.75  $BL\ s^{-1}$  and 8°C (Fig. 4).

Visceral temperatures recorded from fasted fish with an implanted archival tag in Series I experiments followed, but lagged behind, ambient water temperature (Fig. 5A). Steady-state thermal excess (Tx), measured during the last hour spent by the fish at each ambient water temperature (following thermal equilibration), ranged from 1.1 to 1.8°C and differed significantly across ambient temperatures ( $P < 0.01$ , Fig. 5B).

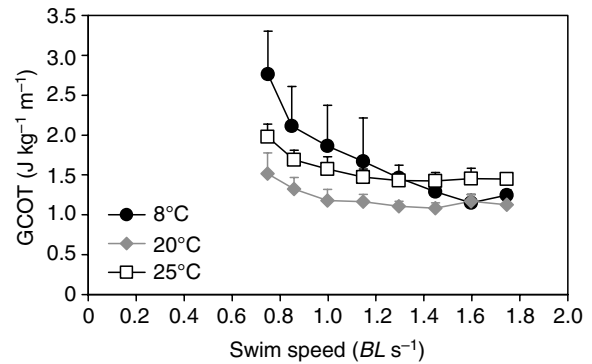


Fig. 4. Effects of ambient temperature and swimming speed on gross cost of transport (GCOT) of bluefin tuna. GCOT was calculated from measured  $\dot{M}_{O_2}$  and swimming speed using an oxycaloric coefficient of 14.1  $J\ mg\ O_2^{-1}$ . A significant interaction of speed and temperature was observed ( $P = 0.023$ ). Values are means  $\pm$  s.d. of  $\dot{M}_{O_2}$  measurements from three individual bluefin tuna (mass = 8.1  $\pm$  0.6 kg).

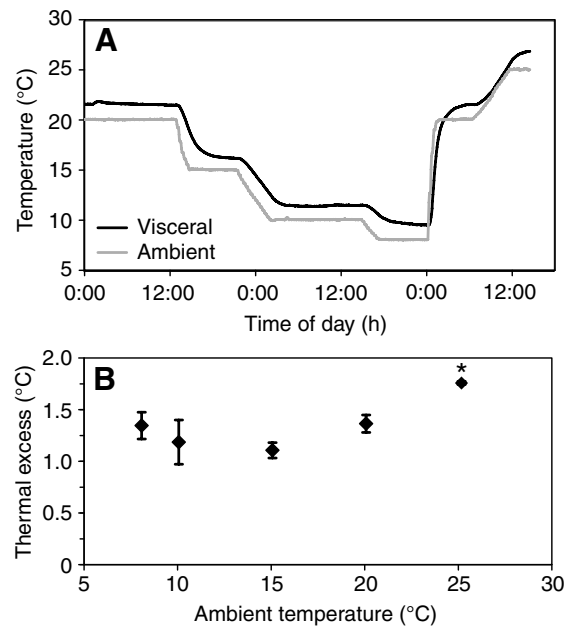


Fig. 5. Visceral temperatures of bluefin tuna in the respirometer. (A) Visceral (black) and ambient water temperature (gray) data were logged at 4 s or 8 s intervals by archival tags implanted in the peritoneal cavity of the fish prior to the experiment and recovered *post-mortem*. Swim speed was held constant at 1.0  $BL\ s^{-1}$  throughout the experiment. Thermal inertia is evident as a time lag between changes in ambient and visceral temperatures. (B) Visceral thermal excess (Tx) of bluefin tuna swimming in the respirometer. Steady-state Tx was calculated as the difference between visceral and ambient temperatures of fish swimming at constant speeds of 1.0  $BL\ s^{-1}$  following equilibration to the designated ambient temperature for at least 2.5 h. Temperature data were logged at 4 s or 8 s resolution by archival tags implanted in the peritoneal cavity prior to experiments. Values shown are mean  $\pm$  s.d. for  $N = 3$  bluefin tuna ( $N = 2$  at 25°C).

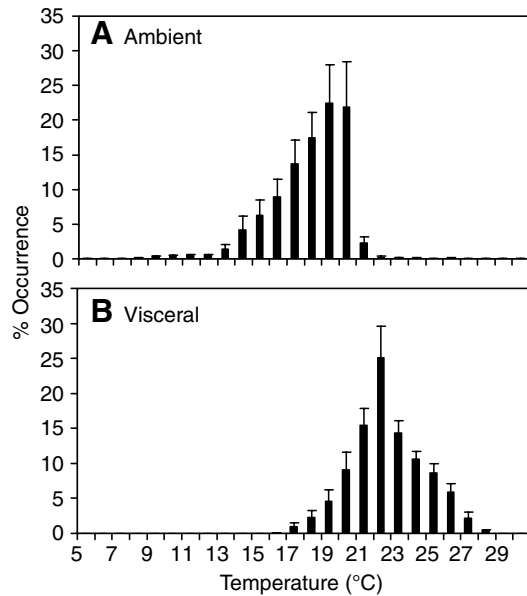


Fig. 6. Ambient water (A) and visceral temperatures (B) recorded from bluefin tuna in the wild ( $CFL=75.2\pm 2.8$  cm;  $mass=8.6\pm 0.9$  kg at time of tagging; interval between tagging and recapture= $241\pm 131$  days). Histograms indicate % occupancy of  $1^\circ\text{C}$  temperature bins. Values are mean  $\pm$  s.e.m. ( $N=10$ ).

Ambient and internal temperatures were recorded in the wild in ten Pacific bluefin tuna of similar size to the Pacific bluefin used in swim tunnel experiments (Fig. 6). The mean ambient temperature experienced by these fish in the eastern Pacific was  $18.4\pm 1.2^\circ\text{C}$ , while the mean visceral temperature was  $22.9\pm 0.9^\circ\text{C}$ . Ambient water temperatures experienced by bluefin were negatively skewed, with a mode at  $19^\circ\text{C}$  and few observations above  $21^\circ\text{C}$ . Tag data revealed periods of both feeding and fasting in wild bluefin (Fig. 7). Large daily increases in thermal excess resulting from specific dynamic action indicate regular feeding, while periods of reduced  $T_x$  indicate periods of fasting lasting several days (Gunn et al., 2001).

### Discussion

This study examines the relationship of metabolic rate to ambient water temperature in juvenile Pacific bluefin tuna swimming in a swim-tunnel respirometer. Bluefin tuna exhibited a remarkable U-shaped relationship of metabolic rate to ambient water temperature, with a 'thermal minimum zone' between  $15^\circ\text{C}$  and  $20^\circ\text{C}$  (Fig. 2A). The U-shaped relationship of bluefin  $\dot{M}_{O_2}$  to ambient temperature differs from the thermal response of most fishes, and more closely resembles the response of an endothermic animal to ambient temperature. Previous studies of metabolic rate in yellowfin and skipjack tunas have reported  $Q_{10}$  values of 1.7–3.2 (Brill, 1987; Dewar and Graham, 1994), which are within the typical range for ectothermic fish. However, these studies used small individuals of tropical tuna species at ambient temperatures that were no lower than  $18^\circ\text{C}$ . Tunas in this size range are capable of elevating muscle temperature above ambient (Dewar et al., 1994; Dickson, 1994), but maintain lower thermal excess temperatures than larger individuals and may be less capable of

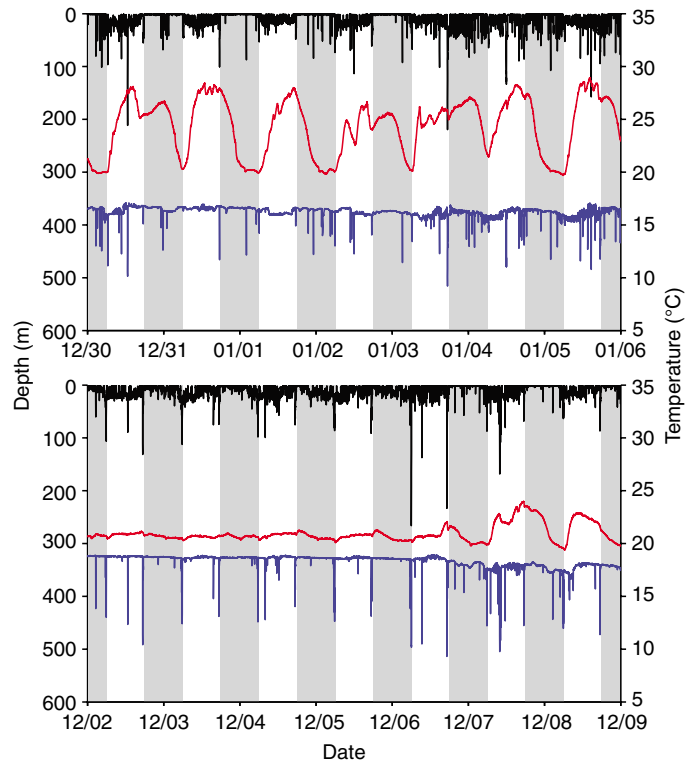


Fig. 7. Archival tag record demonstrating fasting and feeding in a wild Pacific bluefin tuna ( $\sim 16$  kg). Depth (black), peritoneal cavity temperature ( $T_b$ , red), and ambient temperature ( $T_a$ , blue) are shown. Day and night are represented as light and dark bars, respectively. In (A) regular feeding events are indicated by a rise in peritoneal cavity temperature resulting from digestion. In (B) a stable  $T_b$  elevated only slightly relative to  $T_a$  indicates the absence of feeding events for four days, followed by the resumption of feeding.

thermoregulation. Thus, they might exhibit a qualitatively different metabolic response to ambient temperature change than the Pacific bluefin in this study.

The observation that bluefin tuna metabolic rates are minimized at intermediate temperatures and increase at colder temperatures is unique among teleost fishes. Several potential explanations for this pattern must be considered, including stress related to confinement in the swim tunnel at low temperature, failure or decreased efficiency of the slow-twitch muscle at low temperature, and metabolic or behavioral thermoregulation.

High metabolic rates and tailbeat frequencies at low temperatures might indicate a stress or avoidance response as the fish encounter an unfamiliar or unpleasant ambient water temperature. The transient elevation of  $\dot{M}_{O_2}$  following a power outage (Fig. 1) illustrates the potential for stress to elevate recorded  $\dot{M}_{O_2}$  values (Brett, 1962; Steffensen, 1989). Observations of fish pushing against the front of the working section while producing bursts of rapid tailbeats at low ambient water temperatures ( $8\text{--}10^\circ\text{C}$ ) could be construed to reflect stress or escape behavior, but the possible role of stress at low temperatures remains unclear.

The increase in  $\dot{M}_{O_2}$  at low temperatures in Series I may be associated with a cold-induced decline of power from slow-

twitch muscle, resulting in earlier recruitment of fast-twitch muscle (Rome et al., 1984) and a potential increase in locomotor cost as the fish swam at a constant speed. Optimum frequency and power output of yellowfin tuna slow-twitch muscle are temperature dependent (Altringham and Block, 1997) and slow-twitch muscle from the endothermic salmon shark is unable to produce positive work at low temperatures (Bernal et al., 2005), raising the possibility that bluefin tuna slow-twitch muscle would be similarly impaired at cold temperatures. The decline in optimum contraction frequency at low temperature *in vitro* indicates that cold muscle should be least effective at high swimming speeds (Altringham and Block, 1997). However, cold ambient temperatures increased  $\dot{M}_{O_2}$  and GCOT only at low speeds (Fig. 3A, Fig. 4), suggesting that muscle power output was not limiting to swimming capacity at low temperature.

Alternatively, elevated  $\dot{M}_{O_2}$  at low ambient temperature may reflect metabolic or behavioral thermoregulation. Visceral thermal excess was less than 2°C at all ambient temperatures (Fig. 5A,B), reflecting the 45–72 h fasting period preceding introduction of the fish to the swim tunnel. This observation of a low visceral thermal excess in fasted bluefin tuna is in accord with large data sets from wild fish (Itoh et al., 2003; Kitagawa et al., 2001). Muscle temperature was not measured in this study to avoid potential injury associated with implantation of temperature loggers in the muscle. Therefore, regulation of muscle temperature cannot be ruled out. The bursts of rapid tailbeats and increased mean tailbeat frequencies recorded at low temperatures (Fig. 2B, Fig. 3B) provide a potential mechanism for increased metabolic heat production in the swimming muscle. A similar response was observed in restrained albacore tuna, which increased tailbeat frequency by 20% and reduced tailbeat amplitude in association with thermoregulation at ambient temperatures below 14°C (Graham and Dickson, 1981). Albacore were able to maintain stable muscle temperatures during more than 2 h of exposure to low ambient temperatures.

Experiments in which temperatures are recorded in the slow-twitch muscle of bluefin during respirometry would be required to clarify whether maintenance of muscle temperature might be related to elevation of  $\dot{M}_{O_2}$  at low temperatures in swimming bluefin. Acoustic tagging data from wild fish indicate that slow-twitch muscle temperatures are more stable than visceral temperatures in both large and small Atlantic and Pacific bluefin (Carey and Lawson, 1973; Carey and Teal, 1969; Marcinek et al., 2001). However, it is unclear whether 8–10 kg bluefin are capable of maintaining stable muscle temperatures during extended exposure to 8–10°C water.

Importantly, the 15–20°C range at which  $\dot{M}_{O_2}$  is minimized in the swim tunnel corresponds well to the modal ambient temperatures recorded in archival tag data from similar sized wild 8–10 kg Pacific bluefin (Fig. 5) (Kitagawa et al., 2006). These results suggest that juvenile fish may occupy regions with ambient water temperatures that result in the lowest metabolic costs. Small fish tracked off the west coast of North America sometimes experience lower sea surface temperatures (12–14°C) for prolonged periods (Kitagawa et al., 2007), which might entail higher energetic costs. However, numerous factors, such as thermal acclimation to colder waters, faster swimming

speeds in the wild, and specific dynamic action could conceivably shift the thermal optimum in wild fish.

Based on electronic tag data, 8–10 kg Pacific bluefin such as those in this study make occasional dives into 8–10°C water, but do not spend long periods at these temperatures. Archival tag records from both adult and juvenile Atlantic and Pacific bluefin tuna frequently show ‘bounce’ diving patterns in which the fish alternate diving into cool waters below the thermocline and returning to the warmer surface waters, typically on a time scale of minutes (Blank et al., 2004; Gunn and Block, 2001; Kitagawa et al., 2001). Bluefin encountering low temperatures at depth might adjust dive duration to maintain skeletal, cardiac muscle and/or visceral temperatures above a threshold for adequate tissue function (Blank et al., 2004). As diving below the thermocline is often associated with foraging, burst swimming activity and specific dynamic action may also contribute to metabolic heat production during dives. How metabolic rate, body temperature and diving behavior are interrelated has yet to be fully explored. Instrumentation of bluefin for muscle temperature measurement and chronic blood sampling during swim tunnel respirometry at varying ambient temperatures would provide valuable information on the physiological factors determining performance limits of bluefin tuna at high and low ambient temperatures.

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