Moving with the beat: heart rate and visceral temperature of free-swimming and feeding bluefin tuna

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Owing to the inherent difficulties of studying bluefin tuna, nothing is known of the cardiovascular function of free-swimming fish. Here, we surgically implanted newly designed data loggers into the visceral cavity of juvenile southern bluefin tuna (*Thunnus maccoyii*) to measure changes in the heart rate (f_H) and visceral temperature (T_V) during a two-week feeding regime in sea pens at Port Lincoln, Australia. Fish ranged in body mass from 10 to 21 kg, and water temperature remained at 18–19°C. Pre-feeding f_H typically ranged from 20 to 50 beats min^{-1} . Each feeding bout (meal sizes 2–7% of tuna body mass) was characterized by increased levels of activity and f_H (up to 130 beats min^{-1}), and a decrease in T_V from approximately 20 to 18°C as cold sardines were consumed. The feeding bout was promptly followed by a rapid increase in T_V , which signified the beginning of the heat increment of feeding (HIF). The time interval between meal consumption and the completion of HIF ranged from 10 to 24 hours and was strongly correlated with ration size. Although f_H generally decreased after its peak during the feeding bout, it remained elevated during the digestive period and returned to routine levels on a similar, but slightly earlier, temporal scale to T_V . These data imply a large contribution of f_H to the increase in circulatory oxygen transport that is required for digestion. Furthermore, these data oppose the contention that maximum f_H is exceptional in bluefin tuna compared with other fishes, and so it is likely that enhanced cardiac stroke volume and blood oxygen carrying capacity are the principal factors allowing superior rates of circulatory oxygen transport in tuna.

Keywords: digestion; heat increment of feeding; specific dynamic action; *Thunnus maccoyii*; southern bluefin tuna; cardiovascular

1. INTRODUCTION

'It is impossible to use completely intact, unanesthetized, resting fish to make even the simplest cardiorespiratory measurements using techniques commonly employed with other species' (Brill & Bushnell 2001). This statement encapsulates the inherent difficulties associated with examining cardiorespiratory function in the most active and highly evolved predatory fishes in the ocean, the tuna. Tuna can grow to 680 kg in mass and 3.5 m in length, they must swim continuously to ram ventilate their gills and maintain hydrodynamic lift, they can reach burst swimming speeds of approximately 70 km h^{-1} , and they use vascular heat exchangers to maintain regions of their body at temperatures up to 20°C above ambient seawater (Walters & Fierstine 1964; Carey & Gibson 1983; Collette & Nauen 1983; Wardle *et al.* 1989; Brill *et al.* 1994; Dewar *et al.* 1994; Fudge & Stevens 1996). The difficulties of working with tuna are compounded because they are expensive to acquire, difficult to maintain

in captivity, and relatively few are available for study (Brill & Bushnell 2001). Exceptional among the tuna are the species of bluefin tuna, *Thunnus thynnus*, *Thunnus maccoyii* and *Thunnus orientalis*. Bluefin tuna grow the largest, they have higher internal temperatures, greater relative heart masses, elevated metabolic rates, and are more difficult to handle than tropical species (Poupa *et al.* 1981; Carey & Gibson 1983; Blank *et al.* 2007a). From a conservation perspective, intense fishing pressure over the past 50 years has driven all three species of bluefin towards extinction (Safina 1993; Block *et al.* 2005; ICCAT 2006).

Given the inherent complexities associated with studying tuna, there is much speculation and few long-term measurements on the metabolic and cardiovascular functions of these animals. Early attempts at measuring the metabolic rate (=oxygen consumption rate) of anaesthetized and immobilized tuna suggested that values were much higher than in other fishes (Stevens 1972; Brill 1979; Brill 1987; Bushnell *et al.* 1990; Bushnell & Brill 1992). Recent advances in tuna transportation and in the construction of holding facilities have enabled some long-term (more than 20 hours) metabolic measurements

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of tuna while swimming at relatively low, but steady speeds (Dewar & Graham 1994; Blank *et al.* 2007a,b; Fitzgibbon *et al.* 2007, 2008). These studies support the presence of an elevated routine metabolic rate in tuna, albeit lower than first suggested, but it is the presumed maximum metabolic rate that gives tuna their highly aerobic and athletic reputation. Although no study has successfully quantified maximum rates of oxygen consumption in unstressed tuna swimming at steady state (i.e. calm and well recovered from handling), very high rates of $42 \text{ mg min}^{-1} \text{ kg}^{-1}$ for 2 kg skipjack (*Katsuwonus pelamis*) and $22 \text{ mg min}^{-1} \text{ kg}^{-1}$ for 2 kg yellowfin (*Thunnus albacares*) have been measured in freshly caught and handled fish (Gooding *et al.* 1981; Dewar & Graham 1994).

While we are beginning to gain a better understanding of the routine metabolism of free-swimming unstressed tuna, measurements of the cardiovascular adjustments associated with these long-term metabolic measurements are more difficult and have eluded researchers. Only short-term (less than 5 hours) studies have been possible owing to the need to keep the fish healthy while maintaining it within a confined area such that it can be tethered to recording equipment. Bushnell & Brill (1991) trailed electrocardiogram (ECG) electrode wires from small (less than 3.5 kg) yellowfin and skipjack and measured respective routine heart rates (f_H) of 68 and 77 beats min^{-1} during a 50 min experiment while the fish swam in a shallow tank at 25°C. Korsmeyer *et al.* (1997a,b) instrumented small (less than 2.5 kg) yellowfin with various combinations of ECG electrodes, Doppler blood flow probes and pre- and post-branchial catheters to measure a range of cardiorespiratory variables while tuna swam in a water tunnel. They showed large inter-individual variation in the effects of swimming speed on f_H , but reported a routine f_H of approximately 65 beats min^{-1} at approximately 25°C. Korsmeyer *et al.* (1997a) helped to confirm the suggestion that changes in cardiac stroke volume are somewhat limited in tuna and therefore cardiac output is almost exclusively regulated by f_H (e.g. Farrell 1996; Brill & Bushnell 2001). Additionally, Korsmeyer *et al.* (1997a) provided f_H data to support the fact that the tuna heart is outside the area warmed by vascular heat exchangers and is thus directly influenced by ambient water temperature (e.g. Carey *et al.* 1984; Brill *et al.* 1994; Fudge & Stevens 1996).

Although these studies have contributed to our understanding of tuna cardiovascular physiology, they are limited to small (approx. 3 kg) tropical species (skipjack and yellowfin), they use external instrumentation that increases drag and can rapidly become damaged or fouled, and the short recovery periods were unlikely to have allowed complete recovery from handling stress to provide data that can be applied to tuna in the wild (metabolic recovery from handling stress in Pacific bluefin may take more than 24 hours; Blank *et al.* 2007b). There have been no successful attempts at measuring any cardiovascular variables in larger free-swimming bluefin. Previous experiments using acoustic temperature transmitters in the stomach, or intraperitoneally implanted archival temperature tags, determined that bluefin tuna display a thermal increment during digestion (known as the heat increment of feeding, HIF or specific dynamic action, SDA) (Carey *et al.* 1984; Gunn *et al.* 2001). This is

possible in bluefin tuna because the visceral heat derived from digestion is conserved by discrete visceral heat exchangers rather than being lost via peripheral blood vessels and gills that remain at ambient water temperature (Carey *et al.* 1971; Carey 1973; Stevens & Carey 1981; Stevens & McLeese 1984; Fudge & Stevens 1996). More recent work has shown that this thermal increment appears closely associated with an increase in oxygen consumption rates (Fitzgibbon *et al.* 2007). There is an expectation that the increases in circulatory oxygen transport and visceral blood perfusion required for digestion in bluefin tuna are largely regulated by an increase in f_H , although no study has measured cardiovascular function during ingestion and digestion of food in any tuna species to investigate this. Furthermore, it may be hypothesized that bluefin tuna maintain higher routine heart rates than other tuna species in order to supply their enhanced metabolic demands (Blank *et al.* 2007a).

Using juvenile southern bluefin tuna (*T. maccoyii*; 10–21 kg) within sea pens at Port Lincoln, Australia, the present study used a newly designed and surgically implantable data logger to measure f_H and visceral temperature (T_V) in free-swimming and untethered tuna. The study aimed to determine the typical range in f_H displayed by free-swimming *T. maccoyii* at a constant water temperature, and to quantify the dynamics in f_H and T_V associated with ingestion and digestion of sardine meals.

2. MATERIAL AND METHODS

(a) Animals

Twelve juvenile southern bluefin tuna (*T. maccoyii*) were purchased from a commercial farmer in Port Lincoln, South Australia, in January 2007. The fish had been captured by purse seine net in the Great Australian Bight and towed to Port Lincoln in a transport net at a speed of 1 knot. They were housed in a sea pen (12 m diameter and 8 m deep) prior to experiments and fed, usually once per day, on partially or completely thawed Australian sardines (*Sardinops sagax*).

(b) Data loggers

Data loggers (iLogRs) were constructed in the Department of Zoology at La Trobe University, Melbourne, Australia. The body of the logger comprised a printed circuit board ($L \times W \times D = 50 \times 30 \times 8 \text{ mm}$), on which the components included a microprocessor, lithium battery, clock and temperature sensor. A flexible lead (3 mm diameter $\times$ 180 mm long) extended from the body of the logger and terminated at a piezoelectric electrode ($L \times W \times D = 8 \times 2 \times 1 \text{ mm}$) that functioned to measure the ballistic shock wave mechanically propagated through the body from the heart with each contraction. The signal from the piezoelectric electrode was amplified prior to being written to the memory and was used to calculate the heart rate in beats min^{-1} . The body of the logger was coated in beeswax and then the entire logger including the lead was coated with four to five layers of biocompatible silicon (type 3140, Dow Corning, North Ryde, NSW, Australia). The final mass of each logger in air was $19 \pm 2 \text{ g}$ (0.1–0.2% of tuna body mass). The loggers were programmed to record for 10 s in every 5 min, whereas in the meantime they remained in a sleep mode to conserve battery power. Thus, every 5 min, the logger wrote to memory the time, date and temperature,

as well as recording the signal from the piezoelectric electrode at 200 Hz for 10 s. Recordings were made for two weeks. Using data loggers set to the same specifications as here, preliminary experiments on Murray cod (*Maccullochella peelii peelii*), Rosenberg's goanna (*Varanus rosenbergi*) and chickens (*Gallus gallus*) proved the capacity of the loggers to determine heart rates as low as 12 beats min^{-1} and at least as high as 250 beats min^{-1} , with a theoretical maximum exceeding 500 beats min^{-1} (B. D. Taylor 2007, unpublished observation).

(c) *Surgical implantation of data loggers*

All 12 fish were implanted with an iLogR in April 2007. They were individually caught from the sea pen with a baited hook and brought onto the deck of a boat that was anchored beside the sea pen. The eyes of the fish were immediately covered with a wet cotton cloth, one or two ribbon identification tags were inserted in the dorsal muscle using a 3 mm diameter stainless steel trocar, and then the fish was placed supine in a vinyl-covered foam cradle without gill irrigation. A local anaesthetic (1 mg kg^{-1} lignocaine, Troy Laboratories, NSW, Australia) was injected intramuscularly into the ventral midline approximately halfway between the ventral fins and the vent. A 50–70 mm long incision was made through the ventral tissue using a sharp knife and the peritoneum perforated. An iLogR that had been soaking in iodine antiseptic (Betadine, Faulding, Australia) was clasped at the piezoelectric electrode with a pair of uterine forceps (length 250 mm) and inserted through the incision with the aim of sliding the electrode close to the internal wall of the abdomen and over the ventral surface of the liver to position it adjacent to the heart immediately outside of the pericardial cavity. Once the lead was in place, the forceps were removed and the body section of the iLogR was inserted through the incision and into the peritoneum such that it lay adjacent to the pyloric caecum. Thus, the location of the body of the logger enabled measurements of visceral temperature (T_V) near the pyloric caecum. A 2.5 ml dose of broad-spectrum antibiotic (Amoxil, GlaxoSmithKline, www.gsk.com) was injected into the abdominal cavity before the incision was closed with one or two sutures (Vicryl 1-0, Ethicon, www.ethicon.com) and the fish was released into one of three sea pens (details above; four fish in each pen, and no non-experimental fish were present). The tissue at the point of the incision was of a thickness (40–50 mm) that caused the wound to press closed tightly even prior to the suture(s) being used. The procedure took 3–4 min from the time the fish took the baited hook until it was released into the sea pen. All fish remained relatively calm during the procedure and began swimming immediately when returned to the water.

(d) *Protocol*

Ambient water temperature during the data logging period remained at 18–19°C and was monitored continuously using archival temperature sensors (iButtons; Maxim Integrated Products, Dallas Semiconductor, California, USA) that were maintained at a water depth of 3 m. The pens remained well mixed such that there was no thermal stratification. Fresh sardines were obtained from a local commercial supplier, and then individually weighed and sorted into airtight bags before being

stored at –20°C. Sardines were thawed, sometimes not completely, in seawater as required. A subsample of sardines was analysed calorimetrically for energy content (Nutrition Research Laboratory, Roseworthy, South Australia), which was determined to be 7 MJ kg^{-1} (1673 kcal kg^{-1}). The three sea pens were moored within 10 m of one another and were accessed by boat once per day to feed the tuna. All tuna resumed normal feeding behaviour 3–5 days following the surgery, but they did not feed close enough to the water surface for accurate identification of food intake until 2–3 days later. For subsequent days, sardines were offered to the tuna one at a time such that the meal size of each tuna could be quantified (ribbon identification tags used to distinguish individual tuna). Feeding of each pen took approximately 15 min. Meal size was regulated throughout the experiment to induce varying digestive challenges, but with an aim to satisfy daily nutritional requirements. Although the memory of the iLogRs was full after two weeks of logging, we continued to feed and monitor the fish for a further two weeks to investigate any longer term effects of logger implantation. At one month after implantation, all tuna were caught using a purse seine net and euthanized using standard commercial harvesting procedures (pithed and then bled by venesection of the lateral vascular beds). Water temperature at the time of capture was 17°C. A thermocouple was used to determine the temperature of the lateral bleed cut and the heart of each fish as soon as possible after death (within 3 min), and then body mass and length measurements were made.

(e) *Data handling and statistics*

Data were read from each logger with a customized interface (La Trobe University, Melbourne, Australia), stored as a tab-separated text file with corresponding dates and times, and then imported into Chart software (ADInstruments, Australia) for subsequent analysis. A rate meter was applied to the data from the piezoelectric electrode to give the heart rate in beats min^{-1} , and all data were manually viewed to confirm accurate values. Periods of noise (10 s blocks) were excluded when a rhythmic heart beat could not be detected. Values are reported as means $\pm$ s.e.m. Statistical tests are reported in the text. A significant difference was assumed at $p < 0.05$.

3. RESULTS

(a) *Data logger output and surgical recovery*

Representative traces from the piezoelectric electrode of a data logger clearly illustrate the rhythmic pulses associated with the beating heart (figure 1). All but one fish completely recovered from the surgery and recommenced feeding within 3–5 days. However, due to a combination of iLogR malfunction and incorrect placement of the piezoelectric electrode, f_H and T_V data were obtained from five and nine fish, respectively. Some fish displayed a bradycardia, but maintenance of T_V for up to 18 hours post-surgery before f_H increased and stabilized typically at approximately 20–50 beats min^{-1} (figure 2). All fish remained healthy for the one-month experimental period and their behaviour was indistinguishable from their non-experimental counterparts in the commercial sea pens located throughout Port Lincoln (T. D. Clark 2007, personal observation).

Figure 1. Representative traces obtained from the piezoelectric sensor on the data logger at three different heart rates. (a) 63.2 beats min^{-1} , (b) 85.8 beats min^{-1} and (c) 109.7 beats min^{-1} .

(b) Feeding and digestion

Periods of feeding on cold sardines (and probably some water ingestion) were characterized by rapid decreases in T_V by 4–11%, which were typically associated with increases in f_H of 55–76% that resulted from the high activity levels of fish as they competed for food (figures 2 and 3; table 1). These periods were promptly followed by rapid rates of heating that indicated the beginning of the HIF (figures 2 and 3). Maximum heating rates ($^{\circ}\text{C h}^{-1}$) were calculated from the linear, middle section of the visceral temperature curve that immediately followed a feeding event (shown in figure 3), and ranged from 0.5 to 2.6 $^{\circ}\text{C h}^{-1}$ across individual fish. Although the range of meal sizes, as a percentage of tuna body mass, was similar across individuals of different body mass (regression analysis, $p=0.367$), smaller tuna (10–11 kg) displayed a higher rate of heating than larger tuna (15–21 kg) when heating rate was standardized for meal size and tuna body mass (0.11–0.58 versus 0.04–0.22 $^{\circ}\text{C h}^{-1} \text{ kg sardines}^{-1} \text{ kg tuna body mass}^{-1}$, respectively; t -test, $p<0.001$). The time interval between meal consumption and peak HIF during digestion (figure 3) had a weak but significant correlation with relative meal size (figure 4; $p=0.005$). The best predictor of meal size was the time interval between meal consumption and the completion of HIF (figure 4; $p<0.001$), where the latter was marked by a fall in T_V to within 0.3 $^{\circ}\text{C}$ of the value 3 hours prior to feeding.

Although f_H generally decreased to some degree after its peak during the feeding bout, it remained elevated during the digestive period and returned to routine levels on a similar, but slightly earlier, temporal scale to T_V (figures 2 and 3; table 1). Rates of cooling towards the end of HIF were varied and not as consistent as heating curves. Nonetheless, the slopes of the regressions in figure 4 indicate that the time interval from peak HIF to the completion of HIF increased with meal size, i.e. the cooling time was longer with larger meals (cf. 5.7 hours and 14.9 hours after a meal size of 2 and 7% of tuna body mass, respectively). Frequency histograms of f_H for five fish across the entire experimental period illustrate a range of approximately 20–130 beats min^{-1} , with modal values of approximately 20–50 beats min^{-1} (figure 5). Minimum f_H (taken as the minimum f_H that was held by each fish for more than 10% of the experimental period) was 29.2 ± 3.6 beats min^{-1} , while maximum f_H (maximum f_H achieved by each fish) was 117.0 ± 4.6 beats min^{-1} . Frequency histograms of T_V indicate a range of 17–26 $^{\circ}\text{C}$ with modal values between 19 and 22 $^{\circ}\text{C}$ (figure 5).

(c) Mass, length and acute temperature measurements

Post-absorptive body mass (M_b) ranged from 10 to 21.2 kg (mean $\pm$ s.e.m. = 14.8 ± 1.2 kg) and fork length (FL) ranged from 773 to 1012 mm (900 ± 24 mm). The two variables were related according to $M_b = 0.047 \times \text{FL} - 27.63$ ($r^2=0.96$; $N=11$). Freshly killed, post-absorptive fish taken from water of 17 $^{\circ}\text{C}$ had a heart temperature of $18.6 \pm 0.2^{\circ}\text{C}$ and a lateral bleed-cut temperature of $21.7 \pm 0.7^{\circ}\text{C}$. The highest temperatures (heart 19.1 $^{\circ}\text{C}$ and bleed cut 26.4 $^{\circ}\text{C}$) were recorded from a fish that escaped the seine net on the first attempt, underwent a high level of activity, and apparently generated more heat.

4. DISCUSSION

(a) Cardiovascular limits

Given that our measurements of f_H in *T. maccoyii* were taken over a broad temporal scale, and that voluntary feeding is an excellent indicator of fish health, we have confidence that our values of f_H are representative of healthy, wild fish. Conversely, much of the data on tuna cardiovascular function have been recorded in somewhat unusual circumstances, such as total neuromuscular blockade with forced ventilation (Stevens 1972; White *et al.* 1988) or spinal blockade with fish in a water stream (Bushnell & Brill 1992; Brill & Bushnell 2001). Of the few studies that have used intact swimming tuna, most give reason to believe that the fish were stressed and not resting, primarily owing to short recovery periods after instrumentation, confinement to a small area, external instrumentation and/or tethering to recording equipment (Kanwisher *et al.* 1974; Bushnell & Brill 1991, 1992; Korsmeyer *et al.* 1997a,b). Indeed, the bradycardia displayed by *T. maccoyii* for up to 18 hours post-surgery (figure 2) is a clear indication of the need for sufficient recovery periods.

Routine heart rates of 62–97 beats min^{-1} at 25 $^{\circ}\text{C}$ were regarded as the most reflective of unstressed skipjack and yellowfin in a review by Brill & Bushnell (2001).

Figure 2. Logged traces of heart rate (f_H , circles) and visceral temperature (T_V , black solid line) obtained from three *Thunnus maccoyii* throughout the experimental period beginning when the logger was implanted (data binned into 20 min means). Body mass of each fish was 10.0, 11.1 and 11.0 kg for (a–c), respectively. Meal sizes are indicated as a percentage of tuna body mass at each of the feeding bouts (ND, not determined). Downward spikes in T_V correlate with food ingestion, and this is followed by a thermal increment that is dependent on feed ration size (figure 4). Dashed grey line on each figure indicates ambient water temperature. Deviations in T_V below ambient water temperature result from feeds of partially frozen sardines.

Figure 3. Representative traces of heart rate (f_H , circles) and visceral temperature (T_V , black solid line) of *Thunnus maccoyii* during consumption and digestion of a sardine meal equivalent to 6.4% of tuna body mass. Hatched area indicates the range of routine f_H for this individual at this point in time (figure 2b). Dashed lines with arrows indicate how particular variables were calculated (see text).

Table 1. Changes in visceral temperature (T_V ; °C) and heart rate (f_H ; beats min^{-1}) associated with feeding and digestion in *Thunnus maccoyii*. Values are means $\pm$ s.e.m. in parentheses. N , number of tuna; n , number of feeds. ('At feed' is the trough in T_V that occurs during a feeding bout; 'at HIF peak' is the first instance where T_V reached a maximum during the heat increment of feeding (HIF); 'completion of HIF' is where T_V fell to within 0.3°C of the value 3 hours prior to the feeding bout. Percentage changes in T_V and f_H are given for 'at feed' and 'at HIF peak' in relation to the corresponding value at '3 hours prior to feed'. Repeated-measures ANOVA was used to determine differences within variables across rows; same superscript letters indicate statistically similar values. Too few meal sizes were under 3% of tuna body mass (figure 4), and so these data were not included here.)

meal size (percentage of body mass)	3 hours prior to feed				at feed				at HIF peak				completion of HIF			
	T_V	f_H			T_V	% change	f_H	% change	T_V	% change	f_H	% change	T_V	% change	f_H	% change
3–4 ($n=5$; $N=4$)	19.7 ^A (0.1)	58.1 ^{BC} (0.1)			18.9 ^A (0.4)		90.1 ^D (7.9)	55	22.6 (0.7)	15	76.6 ^{CD} (6.3)	32	20.0 ^A (0.1)		48.8 ^B (6.2)	
4–5 ($n=4$; $N=3$)	19.7 ^E (0.2)	57.1 ^F (5.4)			18.1 (0.5)		100.4 ^G (6.3)	76	22.1 (0.4)	12	87.8 ^G (9.7)	54	20.0 ^E (0.2)		54.1 ^F (6.0)	
5–6 ($n=4$; $N=3$)	20.0 ^H (0.2)	47.2 ^I (5.1)			18.4 (0.3)		77.3 ^J (14.4)	64	23.3 (0.6)	17	88.6 ^I (14.1)	88	20.3 ^H (0.3)		45.4 ^I (7.8)	
6–7 ($n=5$; $N=4$)	19.9 ^K (0.2)	51.3 ^L (6.8)			18.0 (0.4)		88.7 ^M (8.7)	73	22.7 (0.5)	14	79.0 ^M (7.8)	54	20.2 ^K (0.1)		51.3 ^L (6.7)

Figure 4. Effects of relative meal size on time to reach the peak of the heat increment of feeding (HIF; filled circles) and time to complete HIF (open circles; $N=9$). HIF was considered complete when visceral temperature returned to within 0.3°C of what it had been 3 hours prior to feeding. Solid lines are linear regressions as described by the given equations (dashed lines are 95% CIs). The energy equivalent of the meals ranges from 2.5 MJ at 2.1% to 8 MJ at 6.9% of tuna body mass.

Data from *T. maccoyii* in the present study indicate that routine, post-absorptive f_H is much lower (20–50 beats min^{-1} at 18–19°C, assuming a swimming speed of 1 body length s^{-1} from Fitzgibbon *et al.* 2007), although routine f_H of yellowfin at the same temperature and swimming speed is bracketed by this range (approx. 40 beats min^{-1} at 18°C after approx. 2 hours of recovery from handling and instrumentation; figure 4 in Korsmeyer *et al.* 1997a). Consideration must be given to the likely negative allometry of tuna f_H but, as yet, there are insufficient steady-state data to address this topic in any detail. It may be argued that the lowest heart rates recorded in this study (i.e. approx. 20 beats min^{-1}) are periods of spontaneous bradycardia and are not representative of steady-state f_H in resting fish. Opposing this suggestion, however, is the fact that three out of five individuals spent 20–50% of the entire experimental period with heart rates under 30 beats min^{-1} (figures 2a,c and 5). Furthermore, with greater periods of fasting, it is anticipated that f_H would be maintained at low rates for even greater proportions of time (e.g. figure 2b).

As with resting and routine heart rates, the logistical difficulties of working with tuna have resulted in substantial controversy about their maximum attainable f_H (Farrell 1996; Brill & Bushnell 2001). Studies of immobilized skipjack have reported maximum f_H of approximately 200 beats min^{-1} at 25°C (Keen *et al.* 1995; Brill & Bushnell 2001). Maximum values for yellowfin at the same temperature are lower and similar to other high performance teleosts at approximately 120 beats min^{-1} (Keen *et al.* 1995; Korsmeyer *et al.* 1997a; Brill & Bushnell 2001; Clark & Seymour 2006), yet a f_H of 190 beats min^{-1} has been reported for yellowfin at a water temperature of 28°C (Korsmeyer *et al.* 1997a). The mean maximum f_H determined for *T. maccoyii* in the present study was 117 beats min^{-1} at 19°C, although f_H as high as 130 beats min^{-1} was recorded in some individuals (figures 2 and 5). These values are substantially higher than the maximum f_H of 84 beats min^{-1} obtained for an *in situ* perfused preparation of Pacific

Figure 5. Histograms of heart rates (f_H) and visceral temperatures (T_V) measured for individual *Thunnus maccoyii* throughout the two-week experimental period. Heart rate and T_V data are paired within each individual fish (i.e. same number of f_H and T_V measurements contribute to the histograms). Body mass: (a,b) 10 kg, (c,d) 11 kg, (e,f) 11.1 kg, (g,h) 16.9 kg and (i,j) 19.3 kg.

bluefin hearts at 20°C (Blank *et al.* 2004), which is the only previous report of bluefin f_H . Nevertheless, the maximum f_H reported for *T. maccoyii* is lower than may have been anticipated based on previous literature, and it is not exceptional for a fish (Farrell 1996; Tibbits 1996; Lillywhite *et al.* 1999; Clark & Seymour 2006). Although

we did not attempt to maximally exercise *T. maccoyii* in the present study, we suggest that the extremely high level of activity and competition that characterized a feeding bout was enough to obtain near-maximal heart rates at 19°C. Although this remains tentative without further investigation, support stems from the fact that the mean relative

scope in f_H measured for *T. maccoyii* in the present study was fourfold (29–117 beats min^{-1} ; figures 2 and 5), which is impressive among fishes and vertebrates in general (Butler *et al.* 1992, 2000; Cooke *et al.* 2003; Clark *et al.* 2005b,c, 2007; Clark & Seymour 2006). Entertaining the suggestion that 130 beats min^{-1} is the maximum f_H for *T. maccoyii* at 19°C, and using a Q_{10} of 2.3 (from Korsmeyer *et al.* 1997a), the predicted maximum f_H of *T. maccoyii* at 25°C would be just above 200 beats min^{-1} and thus similar to maximum f_H for skipjack. However, although adult *T. maccoyii* may experience water temperatures of 25°C (range approx. 5–30°C), juveniles of the size used in the current study generally remain at southern latitudes and are unlikely to encounter such warm waters in the wild or in commercial sea pens (Davis & Stanley 2002; Polacheck *et al.* 2004).

Assuming a stable cardiac stroke volume of 1.0–1.3 ml $\text{beat}^{-1} \text{kg}^{-1}$ (Farrell 1996; Korsmeyer *et al.* 1997a; Brill & Bushnell 2001; Blank *et al.* 2004) and using the extreme minimum and maximum f_H values measured in this study, it is possible to predict the limits in cardiac output (i.e. $f_H \times \text{stroke volume}$) for *T. maccoyii* at 19°C. Thus, minimum and maximum cardiac outputs are predicted as 20–26 ml $\text{min}^{-1} \text{kg}^{-1}$ (assuming f_H of 20 beats min^{-1}) and 130–169 ml $\text{min}^{-1} \text{kg}^{-1}$ (assuming f_H of 130 beats min^{-1}). These maximum values agree with previous predictions and are exceptional among fishes at the same temperature (Brill & Bushnell 1991b, 2001; Bushnell & Jones 1994; Farrell 1996;), primarily due to the large cardiac stroke volume resulting from the large relative ventricular mass of tuna (Bushnell & Jones 1994). Additionally, tuna have blood oxygen carrying capacities that rival mammalian values (Jones *et al.* 1986; Brill & Bushnell 1991a,b; Graham & Dickson 2004; Clark *et al.* 2008), and so it is evident how they may achieve extremely high rates of oxygen consumption without extraordinarily high f_H .

(b) Heart rate and visceral temperature changes associated with feeding and digestion

The tuna approached the surface of the water when the feed boat arrived. Activity levels and f_H increased in anticipation of food, which is suggestive of a large release of cholinergic (vagal) inhibition (Keen *et al.* 1995). The feeding bout was characterized by even higher levels of activity and f_H , and a decrease in T_V as cold sardines were consumed, probably with some ingestion of water (figure 3). These responses are likely to be analogous to those occurring during a feeding bout in the natural environment (Dewar *et al.* 1999; Kitagawa *et al.* 2007). Within an hour after the feeding bout, T_V began to increase rapidly for approximately 1–3 hours in smaller tuna and approximately 4–7 hours in larger tuna, before the increase slowed and a plateau in T_V was reached (figures 2 and 3). The T_V profiles are consistent with stomach temperatures measured using acoustic temperature transmitters in giant bluefin in the range of 450 kg (Carey *et al.* 1984), although in those bigger fish the stomach temperature continued to rise post-feeding for 12–20 hours and it reached 15°C above ambient water temperature (cf. figure 2). It may be argued that the increase in T_V after feeding is not directly linked with HIF, but rather results from a postprandial increase in swimming activity and associated heat production at

the red muscles with conductive or convective heat transfer into the viscera. Recent work on Pacific bluefin, however, has indicated that negligible increases in T_V are associated with increases in swimming speed from 1.0 to 1.8 body lengths s^{-1} (Clark *et al.* 2008, unpublished observation), and so the postprandial increase in T_V following feeding in the present study can be directly associated with digestive processes.

Data for a range of vertebrates including fishes suggest that more than 90 per cent of HIF is associated with post-digestive stimulation of cellular protein synthesis (Jobling 1983; Blaxter 1989), and so it is reasonable to assume that the completion of HIF in *T. maccoyii* is intimately associated with the completion of all digestive processes including ingestion, digestion and assimilation. Time to complete HIF was strongly correlated with meal size. Whereas a meal size equivalent to 2 per cent of tuna body mass required approximately 10 hours to digest and assimilate, a meal of 7 per cent of tuna body mass required approximately 24 hours. Heart rate increased by 1.5–1.9-fold from pre-feeding to the peak of HIF when the tuna consumed a meal of sardines equivalent to 4–7% of their body mass (table 1). Similar meal sizes of the same sardines have been reported to cause increases in oxygen consumption rates of 1.9–2.7-fold above routine values in *T. maccoyii* (Fitzgibbon *et al.* 2007). Furthermore, a satiation feed of sardines caused a 1.9-fold increase in oxygen consumption rate in Pacific bluefin (Clark *et al.* 2008, unpublished observation). Combining these data, we suggest that the majority of the increase in circulatory oxygen transport during digestion in *T. maccoyii* is related to increases in f_H , as has been shown for other vertebrates (Hicks *et al.* 2000; Wang *et al.* 2001; Clark *et al.* 2005a; Eliason *et al.* 2008).

(c) Conclusions

This study is the first to measure f_H during feeding and digestion in any tuna species, and the first to measure any cardiovascular variables in free-swimming bluefin tuna. Contrary to expectations, juvenile *T. maccoyii* typically maintained a low routine f_H between 20 and 50 beats min^{-1} at 18–19°C. Feeding bouts were characterized by rapid increases in f_H and T_V , which remained above baseline levels throughout the digestive period. The maximum measured f_H , up to 130 beats min^{-1} , is unexceptional compared with other fish species at the same temperature, and it is therefore suggested that large cardiac stroke volume and high blood oxygen carrying capacity are the principal variables allowing superior rates of circulatory oxygen transport in tuna.

The project was conducted with the approval of the animal ethics committee of the University of Adelaide (S-024-2005).

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