The Journal of Experimental Biology 213, 2379-2385

doi:10.1242/jeb.043455

Postprandial metabolism of Pacific bluefin tuna (*Thunnus orientalis*)

T. D. Clark[1,2,*], W. T. Brandt[1], J. Nogueira[1], L. E. Rodriguez[1], M. Price[1], C. J. Farwell[1] and B. A. Block[1]

[1]Tuna Research and Conservation Center, Stanford University, Hopkins Marine Station/Monterey Bay Aquarium, Pacific Grove, CA 93950, USA and [2]Faculty of Land and Food Systems, University of British Columbia, Vancouver, Canada, V6T 1Z4

*Author for correspondence (timothy.clark.mail@gmail.com)

Accepted 7 April 2010

SUMMARY

Specific dynamic action (SDA) is defined as the energy expended during ingestion, digestion, absorption and assimilation of a meal. This study presents the first data on the SDA response of individual tunas of any species. Juvenile Pacific bluefin tunas (*Thunnus orientalis*; body mass 9.7–11.0 kg; *N*=7) were individually fed known quantities of food consisting primarily of squid and sardine (meal energy range 1680–8749 kJ, ~4–13% of tuna body mass). Oxygen consumption rates ($\dot{M}_{O_2}$) were measured in a swim tunnel respirometer during the postprandial period at a swimming speed of 1 body length (*BL*) s^{-1} and a water temperature of 20°C. $\dot{M}_{O_2}$ was markedly elevated above routine levels in all fish following meal consumption [routine metabolic rate (RMR)=174±9 mg kg^{-1} h^{-1}]. The peak $\dot{M}_{O_2}$ during the SDA process ranged from 250 to 440 mg kg^{-1} h^{-1} (1.5–2.3 times RMR) and was linearly related to meal energy content. The duration of the postprandial increment in $\dot{M}_{O_2}$ ranged from 21 h to 33 h depending upon meal energy content. Consequently, the total energy used in SDA increased linearly with meal energy and ranged from 170 kJ to 688 kJ, such that the SDA process accounted for 9.2±0.7% of ingested energy across all experiments. These values suggest rapid and efficient food conversion in *T. orientalis* in comparison with most other fishes. Implanted archival temperature tags recorded the increment in visceral temperature (T_V) in association with SDA. $\dot{M}_{O_2}$ returned to routine levels at the end of the digestive period 2–3 h earlier than T_V. The qualitative patterns in $\dot{M}_{O_2}$ and T_V during digestion were similar, strengthening the possibility that archival measurements of T_V can provide new insight into the energetics and habitat utilization of free-swimming bluefin in the natural environment. Despite efficient food conversion, SDA is likely to represent a significant component of the daily energy budget of wild bluefin tunas due to a regular and high ingestion of forage.

Key words: specific dynamic action, SDA, heat increment of feeding, HIF, oxygen consumption rates, teleost fish, digestion, caloric intake, feeding, respirometry, metabolic rate, energy expenditure.

INTRODUCTION

Bluefin tunas (*Thunnus maccoyii*, *Thunnus orientalis* and *Thunnus thynnus*) are among the most highly evolved teleosts in the marine environment (Block and Finnerty, 1994; Graham and Dickson, 2004). They are endothermic, powerful predators that can traverse entire oceans in search of food (Block et al., 2005; Walli et al., 2009). An exceptional metabolic capacity and rapid growth rate in comparison with other fishes requires a high ingestion of forage (Korsmeyer and Dewar, 2001; Hearn and Polacheck, 2003; Polacheck et al., 2004). Indeed, specific dynamic action [SDA; or heat increment of feeding (HIF)], defined as the energy expended (or heat produced) during ingestion, digestion, absorption and assimilation of a meal, is likely to elevate metabolism for many hours following meal consumption and is therefore likely to represent a significant component of the daily energy budget of bluefin tunas (Carey et al., 1984; Korsmeyer and Dewar, 2001; Bestley et al., 2008; Clark et al., 2008c).

Despite its ecological importance, SDA of tunas has received scant attention due to the inherent difficulties of measuring energy expenditure of these animals under controlled conditions. Existing data on the postprandial metabolism of tunas come from a single species, the southern bluefin tuna (*T. maccoyii*). Fitzgibbon et al. introduced groups of three ~10 kg *T. maccoyii* into a large, sea-based polypropylene enclosure containing 350,000–400,000 liters of water, where they were fed rations of sardines ranging from 2.1% to 8.5% of tuna body mass at a water temperature of 19°C (Fitzgibbon et al., 2007). It was not possible to identify feeding events of individual tunas when food was introduced into the enclosure. Using the polypropylene enclosure as a respirometer, the study concluded that SDA accounts for 35% of gross ingested energy in *T. maccoyii*, while measurements for other species of fish typically range from 6% to 20% with a mean of 16% (Secor, 2009). The study on *T. maccoyii* suggests that tunas expend an exceptional amount of energy during SDA, perhaps as a mechanism to augment rates of digestion and protein turnover in preparation for the next meal (Jobling, 1983; Brill, 1996). Such a high energetic cost of digestion in comparison with other fishes has clear ecological implications, as it suggests that SDA may actually dominate the daily energy budget of bluefin tunas in the natural environment. However, it is challenging to interpret these findings in the absence of measurements of SDA from individual bluefin tunas under more controlled conditions where, for example, food intake of individual tunas can be accurately quantified and postprandial swimming speed can be kept constant.

While there exists few postprandial metabolic measurements from bluefin tunas, an associated postprandial increment in visceral temperature (T_V) has been extensively documented (Carey et al., 1984; Gunn et al., 2001; Kitagawa et al., 2007; Walli, 2007; Bestley et al., 2008; Clark et al., 2008c). Archival tags implanted within the visceral cavity of bluefin tunas have recorded T_V and revealed characteristic increments associated with daily feeding events, and the magnitude of the thermal increment has been correlated with meal size and energy content (Gunn et al., 2001; Walli, 2007; Bestley et al., 2008; Clark et al., 2008c). This phenomenon provides a unique

opportunity to utilize T_V profiles measured in wild bluefins to estimate energy intake and SDA, although no previous study has simultaneously measured T_V, energy intake and SDA in order to quantify the interrelations between these parameters.

This paper presents data obtained from experiments spanning three years. We utilized a swim tunnel respirometer to examine the postprandial metabolism of individual Pacific bluefin tunas (*Thunnus orientalis*) swimming at a controlled velocity [1 body length (BL) s^{-1}] and at 20°C. The same tunnel respirometer has been utilized successfully in the past to obtain high-resolution metabolic measurements from fasted and steadily swimming bluefin tunas for continuous periods of up to six days (Blank et al., 2007a; Blank et al., 2007b). In particular, this study sought to examine the time course of the SDA events, the energetic costs of bluefin SDA in comparison with current dogma, and the interrelations between SDA and the postprandial increment in T_V to determine if the latter may provide an accurate estimate of the former.

MATERIALS AND METHODS

Animal capture and handling

The seven juvenile Pacific bluefin tunas [*Thunnus orientalis* (Temminck & Schlegel 1844)] used in this study were from wild collections caught in January–March of 2006 (3 fish), 2007 (3 fish) and 2008 (1 fish). Experiments were conducted during the same year that fish were collected. Wild tunas were captured from the fishing vessel *Shogun* using barbless circle hooks off the coast of Mexico, where sea-surface temperatures ranged from 18.4°C to 19.8°C. Following capture, the tunas were held onboard the fishing vessel in seawater-filled, aerated live wells for 1–3 days before being transported to the Tuna Research and Conservation Center (TRCC), Stanford University, CA, USA, using a large transport tank. Bluefin tunas were held at the TRCC in a 109 m^3 circular tank containing 20±0.5°C seawater, and were fed a diet of squid, sardines and vitamin-enriched gelatin three times per week, as previously described (Farwell, 2001). Fish were acclimated for 1–2 months and then each individual was tagged externally with a colored identification tag (Hallprint Tags, Victor Harbor, Australia) in the dorsal musculature. Additionally, electronic archival tags (model 2310 C or D series, Lotek Wireless, Inc., Newmarket, Ontario, Canada) were surgically implanted into the visceral cavity (4 cm from the vent) of two fish to measure visceral and ambient temperature every 20 s [implantation methods described previously (Block et al., 1998)]. Fish were allowed to recover from tagging for at least four weeks to ensure that they were in excellent physical condition. The fish had to reach an appropriate size to be used in the swim tunnel respirometer, and so some fish were maintained for 3–6 months prior to experiments. Mean (±s.e.m.) body mass and BL of the seven fish used in this study were 10.4±0.2 kg and 81.2±0.4 cm, respectively.

All procedures and experiments were conducted with the permission of the Stanford University Animal Care and Use Committee.

Respirometry and fish training

The swim tunnel respirometer used in the present study has been described previously (Blank et al., 2007a; Blank et al., 2007b). Briefly, the respirometer had a water volume of 871 l and the fish was restricted to a clear Plexiglas working section that had the dimensions 135 cm×45 cm×45 cm (length×width×depth). A removable lid on the working section allowed for introduction and removal of the fish. The entire respirometer sat within a 1500-l rectangular water-filled external tank that provided thermal control. Water velocity through the respirometer was regulated by a propeller that was attached *via* a stainless steel shaft to a variable-speed motor. Swimming speed of the tuna within the respirometer was maintained at 1 $BL\,s^{-1}$ throughout all experiments, and water temperature was maintained at 20°C. The solid blocking effect and the BL of each individual fish were taken into consideration when calculating the desired speed of 1 $BL\,s^{-1}$. The respirometer was automatically flushed with oxygenated seawater for 10 min during a 20 min cycle, and measurements of oxygen consumption rates ($\dot{M}_{O_2}$) were determined from the decline in oxygen saturation inside the respirometer during the 10 min periods between flushes. The respirometer was regularly cleaned such that the background respiration was negligible. The body mass of the fish was subtracted from the volume of water in the respirometer during calculations of $\dot{M}_{O_2}$ to account for water displacement.

Bluefin tunas are inherently difficult animals on which to conduct research. Details of the protocol to 'train' fish to swim in the tunnel respirometer have been documented previously (Blank et al., 2007a; Blank et al., 2007b). Briefly, on the day of a training event, the water level of the 109 m^3 holding tank was dropped to 1 m of depth (taking about 30 min), and a team of 3–4 people entered the tank and caught the desired fish using a water-filled vinyl sling (see Farwell, 2001). The sling was passed to two people outside of the tank, and the fish was transported 15 m and placed in the working section of the respirometer in which the water speed was pre-set at 1 $BL\,s^{-1}$. For initial training attempts, an experimenter remained with the fish at all times, and the lid on the working section of the respirometer remained partially open to allow quick removal of the fish if a problem arose. On subsequent training attempts when the fish seemed comfortable, the lid was sealed completely after 20–40 min and the experimenter left the area that contained the respirometer. The respirometer was shielded from external stimuli at all times using dark plastic curtains that surrounded the respirometer and extended from the floor to the roof of the building. All subsequent monitoring of the fish was conducted using real-time video footage that was displayed on a television in a nearby office. An experimenter monitored the fish at all times during respirometry experiments and periodically measured tail beat frequencies using the real-time footage. Each fish was trained to swim in the respirometer on 3–5 occasions (>3 h on each occasion) prior to the commencement of experiments. The seven fish included in the present study are those that adjusted well to swimming inside the respirometer for long periods of time, and some of them had been used previously for respirometry trials (Blank et al., 2007a; Blank et al., 2007b). For every one fish that was successfully trained, there were two or three fish that did not swim steadily enough to be used in the experiments.

Experimental protocol

Each fish underwent both a 'fasted' and a 'digesting' respirometry protocol. For the 'fasted' protocol, a fish that had been fasted for 45–72 h was captured in a sling and transported to the respirometer as described above. The fish remained in the respirometer at 1 $BL\,s^{-1}$ and 20°C typically for 48 h to monitor routine $\dot{M}_{O_2}$ under these conditions (N=5 for 48 h, N=1 for each of 32 h and 18 h).

For the 'digesting' protocol, food pieces were offered, one at a time, to the fish in the holding tank while observers counted the number of pieces consumed by each tuna from a lookout above the tank (individual fish were identified by dorsal colored tags). Meal sizes ranged from 4% of tuna body mass up to satiation feeds nearing 13% of tuna body mass. Approximately 1.5–2 h following meal consumption (this period allowed settling of the tunas post-feeding

Table 1. Nutritional content of the squid (*Loligo opalescens*), sardines (*Sardinops sagax*) and vitamin-enriched gelatin fed to Pacific bluefin tunas (*Thunnus orientalis*)

Constituent	Squid	Sardine	Gelatin
Lipid (%)	1.9	14.5	2.1
Protein (%)	12.2	16.9	15.8
Ash (%)	1.5	2.6	3.5
Moisture (%)	84.4	66.0	78.6
Energy (kJ kg^{-1})	3298	9595	4860
(kcal kg^{-1})	787	2290	1160

Energy content calculated assuming that protein and lipid have energy equivalents of 23.6 kJ g^{-1} and 39.5 kJ g^{-1}, respectively (Brett and Groves, 1979).

and was aimed at preventing regurgitation of the meal), the targeted, trained tuna was captured and placed in the respirometer to monitor the postprandial period (1 *BL* s^{-1} and 20°C). Fish handling and sealing of the respirometer took around 30 min, and so the first $\dot{M}_{O_2}$ measurement occurred at 2.5±0.3 h following meal consumption. None of the tunas regurgitated any portion of their meals using this protocol. The meal typically consisted of a mixed diet of market squid (*Loligo opalescens* Berry 1911) and Pacific sardines (*Sardinops sagax* Jenyns 1842), although vitamin-enriched gelatin was sometimes included, and on one occasion the meal consisted solely of sardines (Table 1; Table 2) (see Farwell, 2001). The mean mass of each food type was determined by weighing a subsample (30 pieces) prior to feeding, and the mean energy content was determined by proximate analyses of a subsample of each food type (Table 1; N.P. Analytical Laboratories, St Louis, MO, USA). These data were subsequently used to determine the meal intake of each tuna in percentage of tuna body mass and in kJ.

Data analyses and statistics

Upon placing the tunas in the respirometer during initial training sessions, $\dot{M}_{O_2}$ was usually elevated and was somewhat sporadic due to the activity of moving the fish. Training reduced this response such that $\dot{M}_{O_2}$ stabilized rapidly in the fish used in this study. The following variables were quantified for each fish during the respirometry experiments. Routine metabolic rate (RMR) was calculated for each fish during the fasted protocol using the lowest mean $\dot{M}_{O_2}$ measured during any 3 h period typically following at least 20 h residence in the respirometer. Peak metabolic rate during digestion ($\dot{M}_{O_{2peak}}$) was calculated as the highest mean $\dot{M}_{O_2}$ measured for a 3 h period once $\dot{M}_{O_2}$ had stabilized from transport to the respirometer ($\dot{M}_{O_2}$ typically stabilized ~30 min post-transport). The duration of the postprandial increment in $\dot{M}_{O_2}$ ($\dot{M}_{O_{2dur}}$) was calculated as the time between the end of the feeding event, as recorded by the observer, and the return of $\dot{M}_{O_2}$ to stable, baseline levels. $\dot{M}_{O_2}$ was considered to be at baseline levels, and thus digestion was considered to be complete, when (1) values for a particular individual were within one standard deviation of the RMR measured for the same fish during the fasted protocol, and (2) the slope of $\dot{M}_{O_2}$ *versus* time over a 3 h period was no longer significantly negative ($\dot{M}_{O_{2dur}}$ was calculated based on the starting point in this 3 h period). SDA was calculated in two steps. (1) Using the baseline levels of $\dot{M}_{O_2}$ at the end of the digestion protocol, partial specific dynamic action (SDA_{part}) was calculated as the excess energy expended above baseline levels throughout the digestion protocol. (2) To account for the period between when a particular fish finished feeding and when $\dot{M}_{O_2}$ measurements commenced (2.5±0.3 h), SDA_{part} was corrected assuming a linear increase in $\dot{M}_{O_2}$ to $\dot{M}_{O_{2peak}}$ during the first 2 h following feeding (shaded section, Fig. 1B). The area of this segment was determined and then added to the value of SDA_{part} to give SDA. The rationale behind this interpolation stemmed from the fact that (1) digestion and associated visceral heat production commenced shortly after meal ingestion (section bound by vertical broken lines in Fig. 1A), and (2) the heart rate of *T. maccoyii* increases at the time of feeding and does not return to baseline levels until digestion is complete (Clark et al., 2008c). Nevertheless, we present SDA_{part} as well as SDA in Table 2, and we include alternative calculations in the Results to highlight the effects of the interpolation on the calculated value of SDA. The SDA coefficient was calculated as 'SDA as a percentage of meal energy'.

Conversion of $\dot{M}_{O_2}$ to its energy equivalent was performed assuming 14.32 J of energy was expended per 1 mg of oxygen consumed (Beamish and Trippel, 1990). Values given are means ± s.e.m. unless otherwise indicated. Statistical tests were performed using SigmaStat 3.0.1 software (Systat Software, San Jose, CA, USA). Linear regression analyses and paired *t*-tests were used where appropriate to determine significant effects of meal size and to compare between fasted and digesting states. Significance was considered at $P<0.05$.

Table 2. Morphometrics, meal information and metabolic variables for fasted and digesting Pacific bluefin tunas (*Thunnus orientalis*)

Tuna ID	Body mass (kg)	Body length (cm)	RMR (mg kg^{-1} h^{-1})	Meal constituents (Sq./Sard./ Gel.) (%)	Meal size (% tuna mass)	Meal size (kJ)	$\dot{M}_{O_{2peak}}$ (mg kg^{-1} h^{-1})	Factorial scope	$\dot{M}_{O_{2dur}}$ (h)	SDA_{part} (kJ)	SDA (kJ)	SDA coefficient (%)
1	10.5	80.5	172	56/44/0	12.6	8036	388	2.3	32.5	556	641	8.0
2	10.6	80.3	156	56/44/0	10.5	6733	366	2.3	32.8	527	548	8.1
3	10.8	83.0	178	67/23/10	5.9	3109	323	1.8	28.2	326	360	11.6
4	10.5	80.5	164	85/11/4	9.7	4148	350	2.1	28.3	442	473	11.4
5	11.0	81.0	202	88/5/7	4.1	1680	300	1.5	21.1	157	170	10.1
6	10.1	80.8	139	60/35/5	6.4	3603	250	1.8	27.8	226	269	7.5
7	9.7	82.0	206	0/100/0	9.4	8749	440	2.1	31.8	645	688	7.9
Mean	10.4	81.2	174	–	8.4	5151	345	2.0	28.9	411	450	9.2
s.e.m.	0.2	0.4	9	–	1.1	1016	23	0.1	1.5	68	73	0.7

'Meal constituents' represents the percentage of squid (Sq.), sardine (Sard.) and gelatin (Gel.) in the meal based on meal mass rounded to the nearest percentage. Routine metabolic rate (RMR) was measured in fasted fish, while all other metabolic variables were measured in digesting fish. Factorial scope was calculated as $\dot{M}_{O_{2peak}}$/RMR. See text for description of abbreviations. 1 kJ=0.2388 kcal.

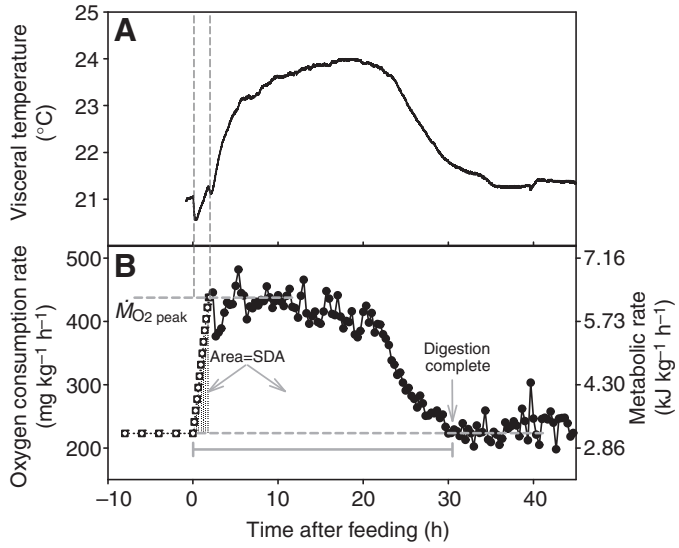

Fig. 1. Representative traces of (A) visceral temperature (T_V) and (B) oxygen consumption rate ($\dot{M}_{O_2}$) of a Pacific bluefin tuna (*Thunnus orientalis*) during digestion of a meal. The feeding bout (~15 min duration) ceased at 0 h, and the fish was placed into the swim tunnel respirometer (20°C, 1 *BL* s^{-1}) at 2.3 h. Filled circles are measured data from the fish in the respirometer whereas crossed square symbols are hypothetical data assuming the fish was at baseline $\dot{M}_{O_2}$ in the holding tank and linearly increased to $\dot{M}_{O_{2peak}}$ (peak metabolic rate attained during digestion) during the first 2 h following feeding. The methods used to calculate particular variables are illustrated (see Data analysis and statistics for descriptions of abbreviations and further details).

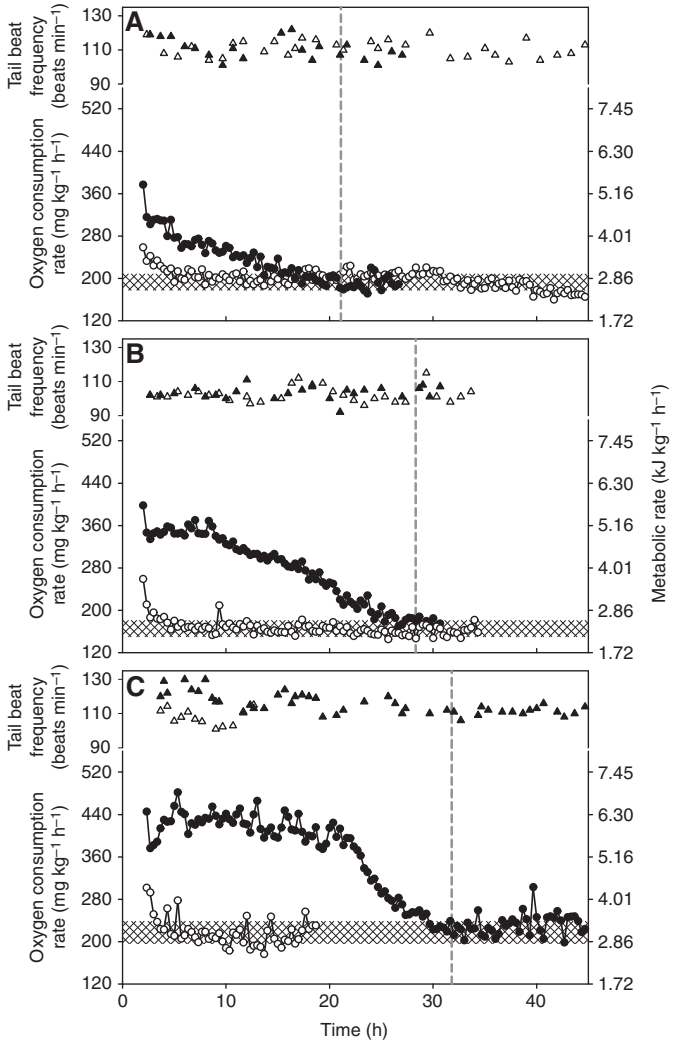

Fig. 2. Representative traces of fasted (open symbols) and digesting (closed symbols) oxygen consumption rate ($\dot{M}_{O_2}$; circles) and tail beat frequency (triangles) from three individual Pacific bluefin tunas (*Thunnus orientalis*). For digesting fish, the feeding bout (~15 min duration) ceased at 0 h, and $\dot{M}_{O_2}$ measurements commenced in the swim tunnel respirometer (20°C, 1 *BL* s^{-1}) at about (A) 1.8 h, (B) 2.1 h, and (C) 2.3 h. Meal energy contents were (A) 1680 kJ, (B) 4148 kJ, and (C) 8749 kJ. Traces for fasted fish have been shifted on the horizontal axis so that the time of the first $\dot{M}_{O_2}$ measurement lines up between fasted and digesting trials. Horizontal hatched line indicates the mean ± 1 s.d. value of routine metabolic rate of the fish during the fasted trial. Vertical broken line indicates the completion of digestion, as detailed in Data analysis and statistics.

RESULTS

Tuna training and routine metabolic rate

The seven tunas selected for this study had been well trained, and all fish remained calm throughout the experimental protocols. Indeed, the rapid drop in $\dot{M}_{O_2}$ to low levels during the RMR trials confirmed prompt recovery (~3 h) of the trained fish from the handling procedure (e.g. Fig. 2). Mean RMR for the seven tunas was 174±9 mg kg^{-1} h^{-1} (Table 2) and this was associated with a tail beat frequency of 109±2 beats min^{-1}.

Postprandial metabolic rate

The major experimental distinction and previously unexplored aspect of this set of experiments was whether a bluefin could be fed in the holding tank and then moved to the respirometer for measurements of postprandial metabolism. Confirmation for the success of this protocol stemmed from the fact that all seven tunas retained the entire experimental meal without regurgitation, and they rapidly acclimated to the handling and swimming protocols associated with postprandial metabolic measurements (e.g. Fig. 2).

All digesting fish had a significantly higher $\dot{M}_{O_2}$ upon entry to the respirometer than the same individual in a fasted state (Fig. 2), indicating that SDA had commenced shortly after the feeding event. The level of elevation in $\dot{M}_{O_2}$ of digesting fish, as characterized by $\dot{M}_{O_{2peak}}$, ranged from 250 to 440 mg kg^{-1} h^{-1} and was linearly related to meal energy (P=0.017; Fig. 2; Fig. 3A) and relative meal mass (i.e. percentage of tuna body mass; P=0.085). Consequently, the factorial scope between RMR and $\dot{M}_{O_{2peak}}$ increased linearly with meal energy (P=0.020) and relative meal mass (P=0.001) and ranged from 1.5 to 2.3 (Table 2). $\dot{M}_{O_{2peak}}$ was generally maintained for several hours before $\dot{M}_{O_2}$ began to slowly decrease towards baseline levels (Fig. 2). The duration between the feeding bout and the return of $\dot{M}_{O_2}$ to baseline levels, as characterized by $\dot{M}_{O_{2dur}}$, ranged from 21.1 h to 32.8 h and was also linearly related to meal energy (P=0.009; Fig. 2; Fig. 3B) and relative meal mass (P=0.013). The linear relationships for $\dot{M}_{O_{2peak}}$ and $\dot{M}_{O_{2dur}}$ with meal size were reflected in SDA, where values ranged from 170 kJ to 688 kJ and were linearly dependent on meal energy (P<0.001; Fig. 3C) and relative meal mass (P=0.007). Thus, larger meals elicited a greater SDA through increases in both $\dot{M}_{O_{2peak}}$ and $\dot{M}_{O_{2dur}}$ (Fig. 3). The SDA coefficient was independent of meal energy and averaged 9.2±0.7% across all experiments (range 7.5–11.6%; Table 2). Notably, if it is assumed that $\dot{M}_{O_2}$ increased to $\dot{M}_{O_{2peak}}$ immediately after feeding rather than gradually over 2 h (see Data analysis and statistics), the value of the SDA coefficient increases only marginally to 9.8±0.7%.

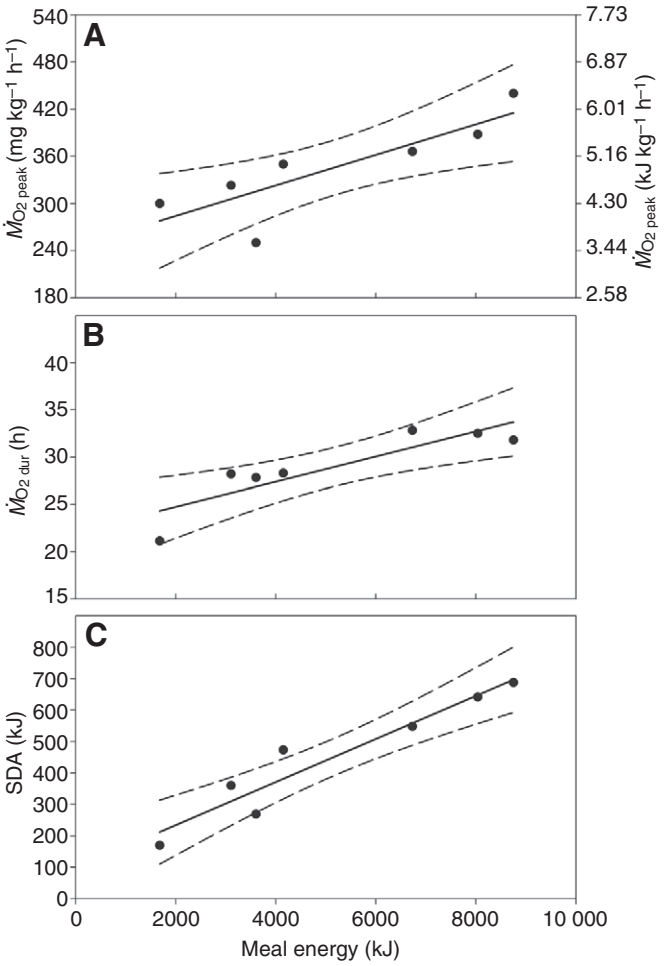

Fig. 3. Relationships for Pacific bluefin tunas (*Thunnus orientalis*) of meal energy and each of (A) peak metabolic rate during digestion ($\dot{M}_{O_2peak}$), (B) duration of the postprandial increment in $\dot{M}_{O_2}$ ($\dot{M}_{O_2dur}$), and (C) specific dynamic action (SDA) (N=7). The linear regressions (bold lines) are described by: (A) $\dot{M}_{O_2peak}$=0.019×meal energy+245.30 (R^2=0.71, P=0.017); (B) $\dot{M}_{O_2dur}$ =0.0013×meal energy+22.09 (R^2=0.77, P=0.009); and (C) SDA=0.069×meal energy+96.63 (R^2=0.92, P<0.001). Broken lines are 95% confidence intervals. Measurements were taken using a swim tunnel respirometer at a water temperature of 20°C and a swimming speed of 1 *BL* s^{-1}.

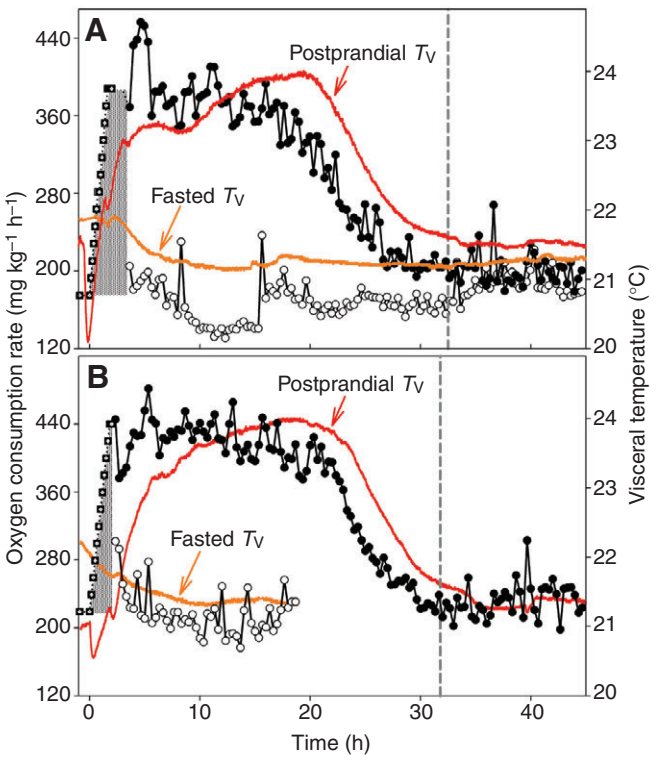

Fig. 4. Representative traces of fasted and digesting oxygen consumption rate ($\dot{M}_{O_2}$; open and closed circles, respectively) and visceral temperature (T_V; labeled on Figure) from two individual Pacific bluefin tunas (*Thunnus orientalis*) swimming at 1 *BL* s^{-1} and at 20°C. For digesting fish, the feeding bout (~15 min duration) ceased at 0 h, and $\dot{M}_{O_2}$ measurements commenced in the swim tunnel respirometer at about (A) 3.6 h and (B) 2.3 h. Crossed square symbols indicate hypothetical changes in $\dot{M}_{O_2}$ occurring shortly after feeding, and the associated shaded areas were included in calculations of specific dynamic action (SDA) (see Data analysis and statistics). Meal energy contents were (A) 8036 kJ and (B) 8749 kJ. Vertical broken line indicates the completion of digestion according to $\dot{M}_{O_2}$, as detailed in Data analysis and statistics. Traces for fasted fish have been shifted on the horizontal axis so that the time of the first $\dot{M}_{O_2}$ measurement lines up between fasted and digesting trials. Spikes in $\dot{M}_{O_2}$ for the fasted trace in A resulted from short bursts of unsteady swimming. Note the difference in vertical scale for $\dot{M}_{O_2}$ between panels.

The mean tail beat frequency of digesting fish was not dependent on meal energy or digestive period and averaged 113±2 beats min^{-1}, a value that was not significantly different from the value of 109±2 beats min^{-1} obtained during RMR measurements (paired *t*-test, P=0.084; Fig. 2).

Links between postprandial metabolism and visceral temperature

Two fish that were equipped with archival temperature tags provided insight into the interrelations between the postprandial increase in $\dot{M}_{O_2}$ and the thermal increment in the viscera (Fig. 4). While fasted fish maintained T_V at 1–2°C above ambient water temperature during the RMR protocol, the feeding event caused a sharp drop in T_V followed by a large and rapid postprandial increase in T_V that peaked around 4°C above ambient water temperature in both fish (Fig. 4). The postprandial increment in T_V lagged behind the increment in $\dot{M}_{O_2}$, such that T_V continued to rise after $\dot{M}_{O_2peak}$ had been reached. Similarly, the return of $\dot{M}_{O_2}$ to baseline levels at the end of the digestive period occurred 2–3 h in advance of the return of T_V to baseline levels (Fig. 4). The peak postprandial T_V (24°C) and the duration of the postprandial T_V increment (34–35 h) were consistent with those measured in free-swimming *T. orientalis* of the same body mass after similar-sized meals (T.D.C. and B.A.B., unpublished data), providing good evidence that the postprandial metabolism of the tunas used in this study was not altered by their relocation to the swim tunnel respirometer.

DISCUSSION

Routine and postprandial metabolism of bluefin tunas

Tunas are obligate ram-ventilators and therefore must swim continuously to facilitate sufficient oxygen supply to the tissues of the body. Thus, while the ‘standard metabolic rate’ provides a measure of minimum energy requirements in most fish species, the minimum energy expenditure of tunas necessarily includes some level of locomotion and therefore is best described by RMR. The values of RMR presented here are consistent with previous reports

for the same species at the same water temperature and swimming speed (Blank et al., 2007a; Blank et al., 2007b). Considering that the minimum gross cost of transport of *T. orientalis* occurs around 1 $BL\,s^{-1}$ when swimming at a water temperature of 20°C (Blank et al., 2007a; Blank et al., 2007b), the reported values of RMR probably represent the minimum energy expenditure attainable at this temperature.

This study presents the first data on the postprandial metabolism of individual tunas of any species. As with other species of fish (Secor, 2009), a linear increase in SDA with meal energy content in *T. orientalis* was driven by increases in each of $\dot{M}_{O2peak}$ and $\dot{M}_{O2dur}$. A relatively constant SDA coefficient across the range of meal sizes indicated that a similar proportion of meal energy was utilized in the SDA process irrespective of meal energy content. The SDA coefficient of *T. orientalis* (9.2±0.7%) is lower than the mean value of 16% reported for fish in a recent review of SDA (Secor, 2009). As a more robust comparison with the present study, data from table 4 in Secor (Secor, 2009) were selected on the basis of meal type to include only those studies where the meal consisted of fish or squid. The resulting SDA coefficient across 18 species was 13.0±1.5% at a water temperature of 20.3±1.5°C and with a relative meal mass of 5.8±0.8% of body mass. The mean $\dot{M}_{O2dur}$ across the 18 species was 46.8±7.7 h, which is markedly greater than the $\dot{M}_{O2dur}$ of 25.9 h calculated for *T. orientalis* following an equivalent sized meal [$\dot{M}_{O2dur}$=1.17×relative meal mass+19.11 (R^2=0.74, P=0.013)]. These comparisons suggest that *T. orientalis* exhibits high food conversion efficiency, which may be linked with the observed postprandial elevation in T_V and augmentation of the activity levels of thermally dependent digestive enzymes including trypsin and chymotrypsin in the pyloric caecum (Carey et al., 1984; Stevens and McLeese, 1984). The capacity of bluefin tunas to acquire prey, rapidly digest a meal and efficiently process the energy may underpin the rapid growth rates documented for these species (Hearn and Polacheck, 2003; Polacheck et al., 2004). Patches of forage are often ephemeral in the open ocean, and so efficient and rapid processing of energy would assure bluefin tunas maximize the available resources.

The relative increase in $\dot{M}_{O2peak}$ for *T. orientalis* reached a maximum of 2.3-times the value of RMR during digestion of satiation meals (~13% of tuna body mass), which is consistent with other species of fish including *T. maccoyii* (Fitzgibbon et al., 2007; Secor, 2009). However, while $\dot{M}_{O2peak}$ reached a maximum of 440 $mg\,kg^{-1}\,h^{-1}$ for *T. orientalis* during digestion of a meal with an energy content of 8749 kJ, values of $\dot{M}_{O2peak}$ as high as 1290 $mg\,kg^{-1}\,h^{-1}$ have been reported for *T. maccoyii* during digestion of a meal with a lower energy content of 4760 kJ (Fitzgibbon et al., 2007). Consequently, the SDA coefficient of 9.2±0.7% from the present study is less than one-third the value of 35.0±2.2% reported for *T. maccoyii* (Fitzgibbon et al., 2007). The difference between species also exists in RMR estimates, where RMR for *T. maccoyii* has been reported as high as 460 $mg\,kg^{-1}\,h^{-1}$ (Fitzgibbon et al., 2008), which is 2.6-times higher than the RMR reported here for *T. orientalis* and higher than the $\dot{M}_{O2peak}$ measured during digestion of satiation meals (Table 2; Fig. 4). The discrepancies in RMR and SDA are surprising, given that swimming speed (~1 $BL\,s^{-1}$) and water temperature (19–20°C) were comparable between the studies, and hemoglobin concentration, relative heart mass and routine heart rate are similar between the two species [*T. maccoyii* (Clark et al., 2008b; Clark et al., 2008c); *T. orientalis* (Clark et al., 2008a; Galli et al., 2009) (T.D.C. and B.A.B., unpublished data)]. Rather than real differences between species, the disparity between studies may be an artifact resulting from differences in experimental equipment, including the difference in tuna:water volume ratios of the respirometers (1:86 *versus* 1:12,000) and the consequent effects on oxygen mixing characteristics [cf. Fig. 2 from present study, fig. 4 from Fitzgibbon et al. (Fitzgibbon et al., 2007)].

Conclusions and future directions

The present study shows that it is possible to overcome the challenges of measuring postprandial metabolism in individual bluefin tunas, thus opening the door to more complex experimental protocols. The results suggest that *T. orientalis* is well adapted to satisfy postprandial oxygen demands while maintaining a broad scope for aerobic activity. Future experiments with digesting bluefin tunas at different water temperatures and swimming speeds will help to elucidate the relationships between $\dot{M}_{O2peak}$, maximum $\dot{M}_{O_2}$ and digestion efficiency. The observation that $\dot{M}_{O_2}$ returns to baseline levels prior to T_V following digestion is consistent with the situation for heart rate and T_V following digestion in *T. maccoyii* (Clark et al., 2008c), suggesting that $\dot{M}_{O_2}$ and heart rate remain tightly coupled during the digestive period. The lag in T_V may be a simple consequence of thermal inertia (linked with conductive heat loss) and the routine efficiency of the visceral heat exchanger reducing convective heat loss, although it is possible that blood flow through the heat exchanger is actively reduced following digestion to minimize convective heat loss and prolong the thermal increment. This possibility warrants further investigation in future studies.

There exist impressive data sets of T_V from bluefin tunas carrying archival temperature tags in the natural environment (Gunn and Young, 1999; Block et al., 2001; Kitagawa et al., 2007; Lawson et al., 2010), and some researchers have used the tag data sets to estimate ingested meal sizes (Walli, 2007; Bestley et al., 2008). With further controlled studies to quantify the interrelations between SDA and the postprandial thermal increment with different meal types (e.g. sardines, squid, etc.), different meal sizes (i.e. up to ~13% of tuna body mass), at different swimming speeds (i.e. interactions between T_V, SDA and swimming metabolism) and at different water temperatures, the present study suggests that T_V traces from wild bluefins could yield valuable information on ingested meal energy, SDA and daily energy budgets. Additionally, comparisons with other tuna species that are less endothermic, such as yellowfin tuna (*T. albacares*), might help to clarify the role of elevated T_V in the digestive process.

In addition to their ecological relevance, the findings of the present study are of interest from an aquaculture perspective. Aquaculture potentially offers a sustainable solution to the growing demand for bluefin tuna products. Farming of tunas is only now becoming a standard practice for *T. orientalis* (Sawada et al., 2005), and land-based aquaculture for *T. maccoyii* has been established in South Australia. A common aim of aquaculture research is to minimize SDA and therefore increase the amount of absorbed energy allocated to growth (LeGrow and Beamish, 1986; Fu and Xie, 2004). Measurements from this study indicate efficient food conversion in *T. orientalis* fed a standard diet at a water temperature of 20°C. Future studies of bluefin at different water temperatures and examining non-natural feed types with different levels of protein, carbohydrate and lipid, will be invaluable for advancing the bluefin aquaculture industry and reducing its impact on wild baitfish populations.

LIST OF ABBREVIATIONS

BL	body length
HIF	heat increment of feeding
$\dot{M}_{O_2}$	oxygen consumption rate

$\dot{M}_{O_2dur}$	duration of postprandial increment in $\dot{M}_{O_2}$
$\dot{M}_{O_2peak}$	peak metabolic rate attained during digestion
RMR	routine metabolic rate
SDA	specific dynamic action
SDA_{part}	partial specific dynamic action
TRCC	Tuna Research and Conservation Center
T_v	visceral temperature

ACKNOWLEDGEMENTS

The authors thank the owners, captains and crew of the *F/V Shogun*, particularly Captains Norm Kagawa and Bruce Smith for helping with the collection of wild Pacific bluefin tunas, and Mr Ted Dunn for his support of the TRCC program. We thank Dr Oscar Sosa-Nishizaki of CICESE for assisting with Pacific bluefin tuna research in Mexican waters, and the Mexican government for permitting access to bluefin tunas in their waters. Special thanks to Mr Alex Norton of the Monterey Bay Aquarium for assistance with maintaining the Pacific bluefin tuna in captivity and for help with conducting experimental protocols, the Husbandry Department of the Monterey Bay Aquarium for assisting with facility care in the TRCC, and Ms Julie Packard and Dr Chris Harrold for their enduring support of the TRCC mission. The research was funded by a National Oceanic and Atmospheric Administration (NOAA) aquaculture grant NA070AR4170447 and the Monterey Bay Aquarium Foundation. Partial support for T.D.C. was through a Killam Postdoctoral Fellowship at the University of British Columbia, Canada.

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