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## RESEARCH ARTICLE

# Quantifying energy intake in Pacific bluefin tuna (*Thunnus orientalis*) using the heat increment of feeding

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## SUMMARY

**Using implanted archival tags, we examined the effects of meal caloric value, food type (sardine or squid) and ambient temperature on the magnitude and duration of the heat increment of feeding in three captive juvenile Pacific bluefin tuna. The objective of our study was to develop a model that can be used to estimate energy intake in wild fish of similar body mass. Both the magnitude and duration of the heat increment of feeding (measured by visceral warming) showed a strong positive correlation with the caloric value of the ingested meal. Controlling for meal caloric value, the extent of visceral warming was significantly greater at lower ambient temperature. The extent of visceral warming was also significantly higher for squid meals compared with sardine meals. By using a hierarchical Bayesian model to analyze our data and treating individuals as random effects, we demonstrate how increases in visceral temperature can be used to estimate the energy intake of wild Pacific bluefin tuna of similar body mass to the individuals used in our study.**

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Key words: heat increment of feeding, HIF, archival tag, caloric intake, hierarchical Bayesian model.

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## INTRODUCTION

Pacific bluefin tuna (*Thunnus orientalis*) is a highly migratory species distributed throughout the temperate waters of the Pacific Ocean (Bayliff, 1994). This species has the largest home range of any species of *Thunnus*. Electronic tagging indicates that juveniles utilize the whole North Pacific while adults make trans-Pacific migrations for spawning and range as far south as eastern Australian and New Zealand waters. Bluefin tuna (*Thunnus maccoyii*, *Thunnus orientalis* and *Thunnus thynnus*) attain a large body size and are long lived; *T. orientalis* may reach a mass of ~275 kg and age of 30–35 years.

Bluefin tuna display the highest level of endothermy among teleost fishes, using metabolic heat to maintain their body temperature above the ambient water temperature (Carey and Gibson, 1983; Block et al., 2001; Walli et al., 2009). They also exhibit a high metabolic capacity and rates of digestion relative to other fishes (Brill, 1996; Blank et al., 2007; Clark et al., 2010). Bluefin tuna experience a wide range of ambient temperatures in the ocean and are often exposed to large changes in ambient temperature (~10°C) through their horizontal and vertical movements (Gunn and Block, 2001; Walli et al., 2009; Kitagawa et al., 2007b; Boustany et al., 2010; Lawson et al., 2010). Heat conservation improves muscular performance (Altringham and Block, 1997); it also contributes to the bluefin tuna's capacity to exploit a broad thermal niche, with an expanded latitudinal and vertical range (Block et al., 1993; Graham and Dickson, 2004). In bluefin tuna, the difference between body and ambient water temperature (thermal excess) is greater in cooler water (Carey et al., 1984; Kitagawa et al., 2007b; Lawson et al., 2010), potentially enhancing swimming performance and the speed of digestion in cooler waters.

Specific dynamic action (SDA) refers to the energy used for ingestion, digestion, absorption and assimilation of a meal (Jobling, 1983; Secor, 2009). The well-observed postprandial increase in metabolism is also termed the heat increment of feeding (HIF) or diet-induced thermogenesis (DIT). In bluefin tuna, metabolic rate and visceral temperature have both been found to increase on ingestion of a meal (Carey et al., 1984; Gunn et al., 2001; Fitzgibbon and Seymour, 2009; Clark et al., 2010). Direct measurements of oxygen consumption rates in a flume have shown that postprandial changes in visceral temperature closely track changes in metabolic rate, with a small lag (Clark et al., 2010).

Visceral warming in bluefin tuna and other vertebrates results from the heat produced by digestion, absorption and lipid oxidation (Carey et al., 1984). In bluefin tuna, visceral heat is retained by extensive vascular counter-current heat-exchangers (hepatic retia mirabilia) in the circulatory pathway to key organs involved in digestion (Carey et al., 1984). The lag in the visceral temperature increase may be a result of thermal inertia, but could alternatively be caused by active regulation of the blood flow through the heat exchanger to minimize convective heat loss (Clark et al., 2010). Untangling this process is complicated by the placement of implanted tags, and the fact that multiple organs contribute to heat generation.

The advent of archival electronic tags that can be surgically implanted in the peritoneal cavity (Block et al., 1998; Gunn and

Block, 2001; Lawson et al., 2010) has allowed accurate measurement of visceral and ambient temperature with high temporal resolution. Although measuring changes in postprandial metabolism in a swimming tuna is difficult, the postprandial increase in visceral temperature has become relatively well-studied using implantable tags (Gunn et al., 2001; Walli, 2007; Clark et al., 2008). Captive populations of bluefin tuna have accelerated advances in our understanding of specific dynamic action in tuna. Experiments first conducted with Atlantic bluefin tuna in pens (Carey et al., 1984) and later with southern bluefin tuna (*Thunnus maccoyii*) and Pacific bluefin tuna held in captivity have shown that visceral warming measured with internally placed archival tags provides an accurate record of the occurrence of feeding events (Gunn et al., 2001; Itoh et al., 2003; Clark et al., 2010). Further work recording changes in metabolism and visceral temperature simultaneously has demonstrated that visceral warming is a proxy for metabolic activity (Clark et al., 2010).

The challenge in marine pen systems is controlling ambient temperature and the diving behavior of the tuna while recording feeding physiology. Using tanks with tightly controlled ambient temperature circumvents these problems. Here, we used a controlled tank setting to investigate further the capacity to model postprandial physiology in archival-tagged juvenile Pacific bluefin tuna.

We present results from an experiment with archival-tagged Pacific bluefin tuna held in captivity over a year, in which we measured the visceral temperature increment associated with meals consisting of fresh squid (*Loligo opalescens*) and sardine (*Sardinops sagax*) of known caloric value.

We investigated the effect of ambient temperature on HIF by conducting feedings at three tank temperatures (15, 20 and 22°C). We used hierarchical Bayesian regression (random slopes model) (Gelman et al., 1995) to model HIF area as a function of meal energy and ambient temperature, and capture differences between individual tuna. Hierarchical Bayesian models (HBMs) are appropriate when data from different units can be thought of as exchangeable (i.e. the units are similar but not identical, and there are no unaccounted for covariates that can be used to explain differences between units) (Gelman et al., 1995; Lunn et al., 2013).

Our overall objective was to develop a laboratory-calibrated HIF model that can be applied to estimate energy intake using visceral temperature data collected from archival-tagged wild fish of similar body size.

## MATERIALS AND METHODS

The juvenile Pacific bluefin tuna, *T. orientalis* (Temminck and Schlegel 1844), used in our study were collected off Baja California by hook and line on the fishing vessel *Shogun* in 2009. The fish were held in seawater-filled aerated live wells aboard the vessel for 1–3 days, before transportation to the Tuna Research and Conservation Center (TRCC), Stanford University, CA, USA. Tuna were held in a 109 $m^2$ circular seawater tank; prior to the SDA experiment they were fed a diet of squid, sardines and a vitamin supplement three times a week (see Farwell, 2001).

Each of the three fish in the experiment was tagged in April 2010 with a Lotek LTD 2310 archival tag (Lotek Wireless Inc., St John's, Newfoundland, Canada). Prior to surgical implantation, archival tags were soaked in alcohol and betadine. A stainless steel trochar (rinsed in alcohol and betadine) was inserted into the ventral musculature 3–4 cm in front of the vent, to make a path to the visceral cavity for the tag. The tag was inserted into the peritoneum and secured in place with two Ethilon monofilament sutures tied directly to a suture pin on the tag to limit movement (the same tagging protocol is used for tagging wild Pacific bluefin tuna). Archival tags were programmed to record ambient and visceral temperature, pressure and light every 8 s. The animals were also tagged with a uniquely colored external tag for visual identification (Hallprint tags, Victor Harbor, SA, Australia) during feedings. The curved fork lengths of the tuna at tagging were 84 cm (D1474), 85 cm (D1629) and 85 cm (D6052). The tank's oxygen concentration was measured by an oxyguard type 840 dissolved oxygen probe (Point Four Systems, Coquitlam, BC, Canada). In our study we use visceral ($T_b$) and ambient ($T_a$) temperatures measured by the implanted peritoneal tags.

Tuna were acclimated to 20°C for 90 days prior to feeding trials. Experimental observations were made at 19.9±0.2°C (mean ± s.d.) over a period of 42 days. In order to assess the effects of ambient temperature on visceral warming, the tanks were held for 32 days at 15.1±0.4°C, and for 30 days at 21.9±0.2°C. Data from the first feeding after the tank's temperature was changed were not included in analyses. A range of ration sizes was fed to each fish at each temperature level (Table 1). This was achieved by recording ration sizes as a percentage of body mass fed to each fish, and targeting individuals to a lesser or greater extent to obtain ration sizes that had not yet been fed. Tagged individuals were identified by dorsally placed Hallprint tags. Experimental feedings were conducted on Mondays, Wednesdays and Fridays; one feeding consisted of purely sardine, the second of purely squid and the third a mixture of the two.

The feeding protocol during each experimental feeding was as follows: the sardine and/or squid for each feeding was removed from the freezer at least 24 h prior to feeding to defrost. All sardines or squid were then weighed individually to the nearest gram and sorted into 10 g mass classes (wet mass), creating as many classes as were necessary to accommodate all the food items. After weighing and sorting all the food, each mass class was placed into a separate ziplock bag, and the number of food items in each mass class and their total mass were recorded. Bagged food items were then placed in a well at the side of the tank for 15–20 min. Once the food items were at a similar temperature to the tank's water temperature, food items were dropped into the tank from a platform above the tuna one by one, and the type and number of items in each mass class that were consumed by an archival-tagged tuna were recorded. Two observers fed the fish and one recorded the food items ingested by the externally tagged bluefin. The number of squid and sardine calories fed to each tagged fish in each feeding was then estimated using the recorded number of items consumed, their mass class and their caloric value. Fish were fed to an approximate ration level on each feeding, and rations were randomized from 1% to about 12% of wet mass.

The caloric content of sardines and squid was determined by bomb calorimetry and lipid and protein assay; two samples of sardine (total of 28 sardines) and two samples of squid (total of 16 squid) were sent to NP Analytic Laboratories (St Louis, MO, USA) over the course of the experiment (in May and June 2010). Energetic values as well as fat and protein content were determined on an individual basis and the individual masses and lengths of each sardine or squid in the sample were recorded. This was done to assess whether caloric value per gram might differ between small and large individuals (e.g. due to changes in the ratio of soft to hard body parts or condition with size). A non-linear (asymptotic exponential) model was fitted to the caloric value per gram (kcal $g^{-1}$) and sardine mass data using the nls function in the stats package in R (version 2.14.1, R Core Team, 2012).

Archival tag data for D1474 and D1629 were retrieved in September 2010 (body masses 14.4 and 13.8 kg, respectively); data for D6052 were retrieved in September 2011 (body mass 16.7 kg).

Table 1. Ration sizes in the SDA experiment

| $T_a$ (°C) | D1474 | | | D1629 | | | D6052 | | |
|---|---|---|---|---|---|---|---|---|---|
| | Squid ration | Sardine ration | Total | Squid ration | Sardine ration | Total | Squid ration | Sardine ration | Total |
| | 2.20 | 0.61 | 2.81 | 0.00 | 3.31 | 3.31 | 0.00 | 2.17 | 2.17 |
| | 0.00 | 3.01 | 3.01 | 0.00 | 4.07 | 4.07 | 0.00 | 2.38 | 2.38 |
| | 0.00 | 4.34 | 4.34 | 0.00 | 4.36 | 4.36 | 0.00 | 3.90 | 3.90 |
| | 0.00 | 5.81 | 5.81 | 0.00 | 4.37 | 4.37 | 0.00 | 5.03 | 5.03 |
| | 6.15 | 0.00 | 6.15 | 0.00 | 5.67 | 5.67 | 4.20 | 1.04 | 5.24 |
| | 0.00 | 6.86 | 6.86 | 6.31 | 0.84 | 7.15 | 0.00 | 6.19 | 6.19 |
| 15 | 4.58 | 2.36 | 6.94 | 8.20 | 0.00 | 8.20 | 4.25 | 1.96 | 6.21 |
| | 7.00 | 0.31 | 7.31 | 7.33 | 1.15 | 8.48 | 3.72 | 2.75 | 6.47 |
| | 9.26 | 0.00 | 9.26 | 8.60 | 0.00 | 8.60 | 8.37 | 0.00 | 8.37 |
| | 0.00 | 9.34 | 9.34 | 7.23 | 1.50 | 8.72 | 8.64 | 0.00 | 8.64 |
| | 6.86 | 2.53 | 9.39 | 9.24 | 0.00 | 9.24 | 5.85 | 3.26 | 9.12 |
| | 10.62 | 0.00 | 10.62 | 9.98 | 0.00 | 9.98 | 9.83 | 0.00 | 9.83 |
| | 11.61 | 0.00 | 11.61 | 10.51 | 0.00 | 10.51 | 10.04 | 0.00 | 10.04 |
| | 12.66 | 0.00 | 12.66 | 9.77 | 1.77 | 11.54 | 10.58 | 0.00 | 10.58 |
| | 0.00 | 0.90 | 0.90 | 0.00 | 1.64 | 1.64 | 0.00 | 0.00 | 0.00 |
| | 0.00 | 1.20 | 1.20 | 0.00 | 2.08 | 2.08 | 0.00 | 2.14 | 2.14 |
| | 2.81 | 0.00 | 2.81 | 1.80 | 0.47 | 2.27 | 0.00 | 3.48 | 3.48 |
| | 0.00 | 3.51 | 3.51 | 0.00 | 2.47 | 2.47 | 5.42 | 0.78 | 6.20 |
| | 3.87 | 0.00 | 3.87 | 2.97 | 0.00 | 2.97 | 6.25 | 0.00 | 6.25 |
| | 2.52 | 1.41 | 3.92 | 3.30 | 0.00 | 3.30 | 6.79 | 0.00 | 6.79 |
| | 0.00 | 4.22 | 4.22 | 0.00 | 3.37 | 3.37 | 9.32 | 0.00 | 9.32 |
| | 0.00 | 4.65 | 4.65 | 1.31 | 2.37 | 3.68 | 6.60 | 3.31 | 9.91 |
| | 0.00 | 4.80 | 4.80 | 0.00 | 4.41 | 4.41 | | | |
| | 3.37 | 1.95 | 5.32 | 0.00 | 4.72 | 4.72 | | | |
| 20 | 0.00 | 6.77 | 6.77 | 5.15 | 0.00 | 5.15 | | | |
| | 7.15 | 0.00 | 7.15 | 0.00 | 5.25 | 5.25 | | | |
| | 0.00 | 8.03 | 8.03 | 2.73 | 4.17 | 6.90 | | | |
| | 5.11 | 3.17 | 8.28 | 8.03 | 0.00 | 8.03 | | | |
| | 2.90 | 5.86 | 8.76 | 3.80 | 4.33 | 8.13 | | | |
| | 9.47 | 0.00 | 9.47 | 0.00 | 8.69 | 8.69 | | | |
| | 5.79 | 3.83 | 9.62 | 9.11 | 0.00 | 9.11 | | | |
| | 10.01 | 0.00 | 10.01 | 9.30 | 0.00 | 9.30 | | | |
| | 10.12 | 0.00 | 10.12 | 5.84 | 3.52 | 9.36 | | | |
| | 10.85 | 0.00 | 10.85 | 4.41 | 4.96 | 9.37 | | | |
| | 8.19 | 3.65 | 11.84 | 9.89 | 0.00 | 9.89 | | | |
| | 9.79 | 2.22 | 12.01 | 6.79 | 4.16 | 10.95 | | | |
| | 0.00 | 1.88 | 1.88 | 0.00 | 2.55 | 2.55 | 1.55 | 0.96 | 2.51 |
| | 0.00 | 2.67 | 2.67 | 0.00 | 3.20 | 3.20 | 0.00 | 4.07 | 4.07 |
| | 4.63 | 0.65 | 5.28 | 0.00 | 4.01 | 4.01 | 5.52 | 0.00 | 5.52 |
| | 5.25 | 0.90 | 6.14 | 4.02 | 1.41 | 5.43 | 0.00 | 6.47 | 6.47 |
| | 6.34 | 0.00 | 6.34 | 5.97 | 0.00 | 5.97 | 6.49 | 0.00 | 6.49 |
| | 0.00 | 6.77 | 6.77 | 7.52 | 0.00 | 7.52 | 5.15 | 1.82 | 6.97 |
| 22 | 5.69 | 1.27 | 6.96 | 7.71 | 0.37 | 8.08 | 3.15 | 4.21 | 7.36 |
| | 7.42 | 0.00 | 7.42 | 6.21 | 2.17 | 8.38 | 7.61 | 0.00 | 7.61 |
| | 4.79 | 2.89 | 7.69 | 7.85 | 0.61 | 8.45 | 8.62 | 0.00 | 8.62 |
| | 0.00 | 8.33 | 8.33 | 0.00 | 9.83 | 9.83 | 0.00 | 8.86 | 8.86 |
| | 10.99 | 0.00 | 10.99 | 10.51 | 0.00 | 10.51 | 0.00 | 9.19 | 9.19 |
| | 0.00 | 10.99 | 10.99 | 8.73 | 2.75 | 11.48 | 6.83 | 2.39 | 9.23 |
| | 8.12 | 3.42 | 11.54 | 11.65 | 0.00 | 11.65 | 5.98 | 3.41 | 9.39 |
| | 12.22 | 0.00 | 12.22 | 0.00 | 11.69 | 11.69 | 0.00 | 11.73 | 11.73 |

$T_a$, ambient tank temperature. SDA, specific dynamic action.
Data are wet mass of squid or sardine divided by tuna body mass (%).

We analyzed data from experimental feedings between the 18 April and 4 August 2010.

### Measuring HIF

We automated identification and measurement of HIF events using the experimental visceral and ambient temperature data. Ambient and visceral temperature data were first smoothed using a moving average to remove high frequency fluctuations in ambient and body temperature caused by thermostat-controlled heating of the tank in regular pulses.

In order to measure HIF area, it was necessary to account for baseline thermal excess ($T_e$), which is defined as the difference between body temperature and ambient temperature when there is no visceral warming due to feeding. We assume that the baseline thermal excess reflects the heat generated by routine metabolism, while warming above $T_e$ can be attributed to HIF. Resting body temperature ($T_b$) was defined as follows: $T_b$ measurements for which the absolute derivative of $T_b$ was less than $0.001\,^{\circ}\mathrm{C}\,8\,\mathrm{s}^{-1}$ and $T_b$ was within $0.1\,^{\circ}\mathrm{C}$ of its 4 day minimum were regressed on the synchronous ambient temperature measurements. The resulting

regression coefficients were then used to interpolate resting visceral temperature ($\hat{T}_b$) for the whole time series (including during feedings). Subtracting $T_a$ from $\hat{T}_b$ yielded predicted baseline thermal excess, $\hat{T}_e$ indicated in Fig. 1. $\hat{T}_b$ was subtracted from $T_b$ to yield the thermal increment by which body temperature exceeds its predicted baseline during digestion (i.e. this increment is equal to 0 when body temperature is at baseline thermal excess).

Warming (positive derivative of body temperature, °C 8 $s^{-1}$) above the resting body temperature was attributed to HIF when the absolute value of the derivative of body temperature was higher than the hourly modal derivative of ambient temperature. This latter condition was needed to avoid erroneous classification of changes in body temperature caused by oscillations in the tank's temperature as part of HIF events. The first time point in the warming part of the curve was taken to be the start of the HIF ($t_s$, Fig. 1). Visceral cooling that occurred during the HIF event was defined analogously for points in the $T_b$ time series with a negative derivative. The last time point in the cooling part of the curve was taken to be the end of the HIF ($t_f$, Fig. 1). $t_s$, $t_f$ pairs less than 4 h apart were removed to avoid attributing anticipatory warming (pre-ingestion) to HIF. HIF area was measured by integrating the difference between $T_b$ and $\hat{T}_b$ from the HIF start point to the HIF end point:

$$\varphi = \int_{t_s}^{t_f} (T_b - \hat{T}_b)\,dt\,, \quad (1)$$

where φ denotes HIF area. In addition, HIF duration ($t_{HIF}$) was measured for each HIF event as the time elapsed in hours between $t_s$ and $t_f$. Measurement of HIF area and HIF duration was implemented in Matlab R2011a (The MathWorks, Inc., Natick, MA, USA).

### Regression analyses

HIF area (φ) and HIF duration ($t_{HIF}$) were regressed on the estimated meal energy (in kcal) for each ambient temperature and food type. For mixed feedings (sardine and squid), φ and $t_{HIF}$ resulting from squid and sardine were obtained by multiplying the measured φ and $t_{HIF}$ by the proportion of the estimated total kcal from each food type. Regression analyses were performed in a Bayesian setting, with uninformative normal priors [N(0,$1000^2$)] for the slope parameters. We fixed the intercepts at 0 for all regressions. Linear and quadratic regressions were fitted for each ambient temperature and food type. The model with the lowest deviance information criterion (DIC) (Spiegelhalter et al., 2002) was selected as the final model. DIC can be seen as a generalization of Akaike's information criterion; the minimum DIC identifies the model that makes the best short-term predictions (Lunn et al., 2013). The coefficient of determination ($R^2$) was computed for each regression as 1 minus the observation error variance divided by the total regression sum of squares.

A HBM was used to regress meal energy (kcal) on HIF area for each of the three tank temperatures and two food types [note that the slopes in this model (kcal/HIF area) are the inverse of the slopes in the non-hierarchical regressions described above]. The purposes of this model were to account for between-individual differences in the relationship between meal energy and HIF and to obtain a posterior predictive distribution for expected caloric intake for wild Pacific bluefin tuna. Prior distributions are specified at two levels in the HBM; the individual (or unit) level and the population level. In this application, regression coefficients for tagged tuna (the individual level) were assumed to be independently distributed from a common population distribution whose parameters are unknown and assigned priors (i.e. hyperpriors). The population level distribution in our study describes the distribution of regression coefficients among Pacific bluefin tuna in a similar size range to the experimental fish. $R^2$ was calculated for each regression (combination of food type, ambient temperature and individual) in the HBM. Equations for the HBM can be found in the Appendix. All regression analyses were implemented in JAGS (Just Another Gibbs Sampler) version 3.2.0. JAGS was run from R version 2.14.1 using the rjags package.

To illustrate estimation of daily caloric intake in archival-tagged wild juvenile Pacific bluefin tuna, we applied our model to extracts from the visceral and ambient temperature traces from two

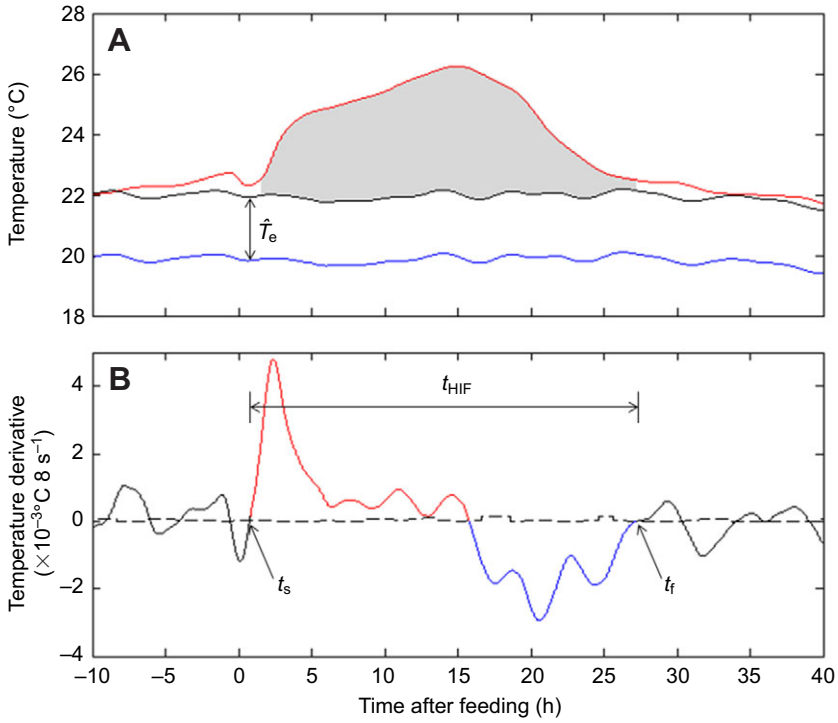


Fig. 1. (A). Smoothed ambient temperature ($T_a$, blue line) and visceral temperature ($T_b$, red line) from a captive archival-tagged Pacific bluefin tuna. The black line is the predicted visceral temperature at rest, $\hat{T}_b$ (i.e. without feeding). Thermal excess (the difference between predicted visceral temperature at rest and ambient temperature) is also indicated ($\hat{T}_e$). (B) The derivatives of ambient (dashed black line) and visceral (solid line) temperature, with the heat increment of feeding (HIF) start and end points ($t_s$ and $t_f$) and HIF duration ($t_{HIF}$) marked. The warming part of the HIF event is colored red, while the cooling part of the HIF event is colored blue.

Table 2. Mean and s.d. of visceral temperature and baseline/resting thermal excess at three ambient temperatures for the three archival-tagged Pacific bluefin tuna in the experiment

| | $T_b$ (°C) | | | $T_e$ (°C) | | |
|---|---|---|---|---|---|---|
| $T_a$ (°C) | D1474 | D1629 | D6052 | D1474 | D1629 | D6052 |
| 15.1±0.4 | 19.0±1.4 | 18.0±0.8 | 17.8±0.9 | 2.2±0.2 | 1.8±0.2 | 1.5±0.2 |
| 19.9±0.2 | 23.2±1.4 | 22.1±0.9 | 22.2±0.8 | 1.7±0.4 | 1.3±0.2 | 1.4±0.2 |
| 21.9±0.2 | 25.1±1.4 | 24.4±1.0 | 24.8±1.2 | 1.9±0.2 | 1.7±0.2 | 1.7±0.2 |

$T_b$, visceral temperature; $T_e$, baseline thermal excess; $T_a$, ambient temperature.

individuals. The applicability of the laboratory calibrated model to data from wild fish is discussed in the Appendix, with a description of how the algorithm used to define resting visceral temperature ($\hat{T}_b$) was modified for wild tuna.

The total magnitude of the visceral temperature increment per 24 h period was obtained by integrating $T_b$–$\hat{T}_b$ from midnight to midnight of the following day. Daily sea surface temperature (SST) was computed as the mean of the ambient temperature recordings within a 24 h period in the top meter of the water column. The daily visceral temperature increment and mean SST were then used as inputs to the HBM to obtain a probability density function for daily gross energy intake (kcal) for a new individual (see Appendix). For this example, we assumed a diet of 80% sardine-type food and 20% squid-type food.

## RESULTS

Experimental feedings in a controlled tank setting resulted in a data set suitable for analysis of HIF at three ambient temperatures; 15°C (14 feeding events), 20°C (22 feeding events) and 22°C (14 feeding events). Means and standard deviations for ambient temperature, visceral temperature and thermal excess are shown in Table 2. The HIF events, modeled baseline body temperature ($\hat{T}_b$) and ambient tank temperature for one archival-tagged bluefin tuna are shown in Fig. 2. Individual HIF events can be recognized from the increase in body temperature above $\hat{T}_b$ and subsequent decrease back to the baseline over 1–3 days (Fig. 2). HIF events were sometimes preceded by anticipatory warming as the captive tuna were fed at regular 2 day intervals; this anticipatory warming was most pronounced for feedings at 15°C (Fig. 2A). Ingestion of food was discernable for some feedings as a sudden drop in visceral temperature before the onset of HIF (e.g. feeding on 01/05/10, Fig. 2).

The mass-specific caloric value ($y$) of sardines increased with body mass ($x$) (Fig. 3). The best fitting exponential function was:

$$y = 206\,(\pm 16.5)(1 - e^{-0.031(\pm 0.0061)x}), \quad (2)$$

where numbers in parentheses are standard errors for the regression parameters ($n$=28). The mass-specific caloric value of squid did not vary with body mass so the mean value (92.30 kcal 100 g$^{-1}$) was used.

Ambient (tank) temperature had a significant effect on the magnitude and duration of visceral warming. Warmer ambient

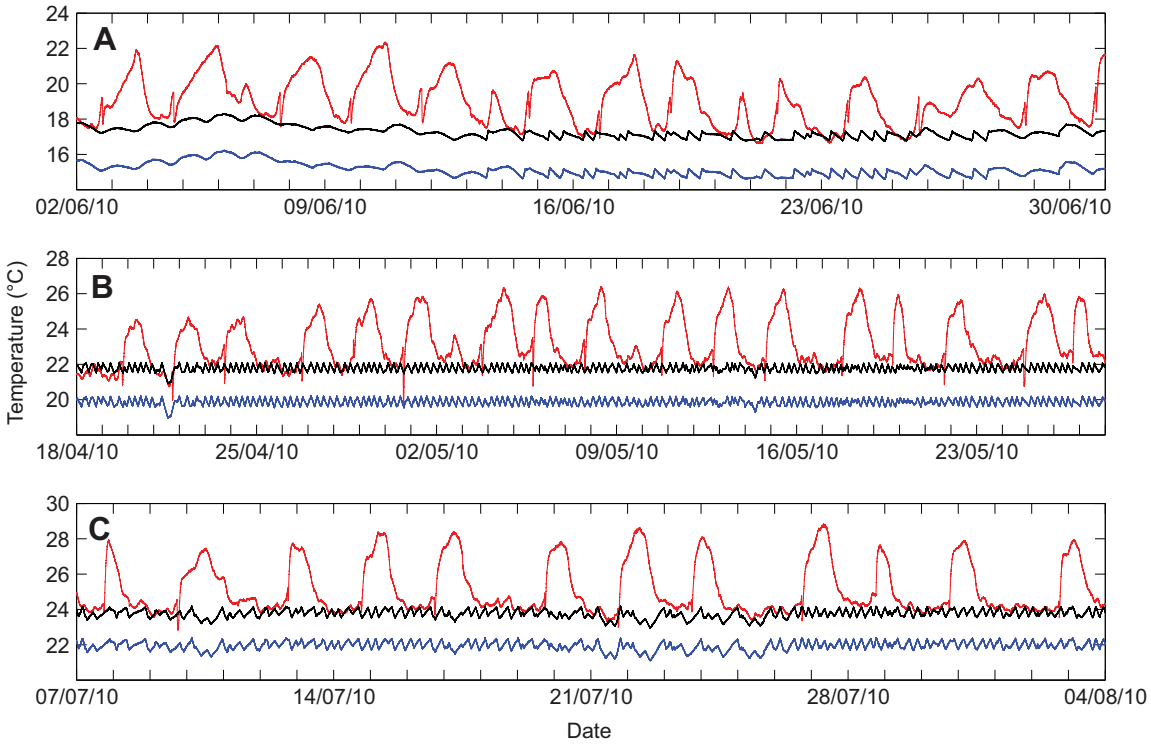


Fig. 2. An example data set for one archival-tagged tuna for feedings between April and August 2010. A–C correspond to feedings at three ambient temperature levels (A, 15°C; B, 20°C; and C, 22°C). Blue line, ambient temperature ($T_a$); red line, visceral temperature ($T_b$); and black line, predicted visceral temperature at rest, i.e. fasted ($\hat{T}_b$). The cycling in the ambient water temperature is due to the thermo-cycling in the tank to maintain water temperature.

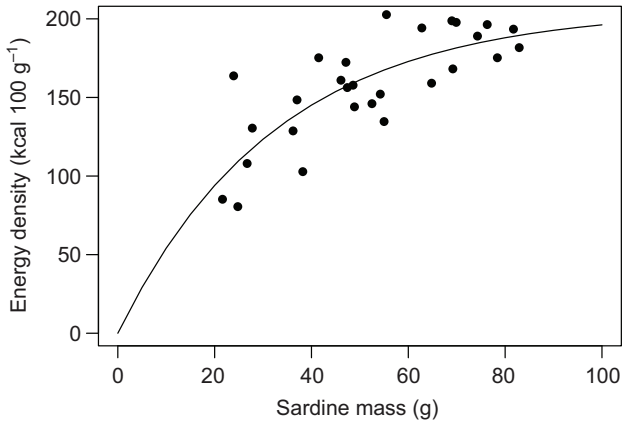


Fig. 3. Energy density from bomb calorimetry of Pacific sardines (*Sardinops sagax*) *versus* sardine body mass.

temperatures resulted in smaller HIF area for squid (Fig. 4A,C,E; Table 3) and sardine meals (Fig. 4B,D,F; Table 3). HIF area was significantly lower for squid meals at 22°C compared with 15 and 20°C (based on non-overlapping 95% posterior probability intervals for regression coefficients, $\beta_1$). HIF area was lower for sardine meals at 22°C compared with those at 15 and 20°C; the best fitting regression for sardine meals at 22°C was quadratic indicating that the rate of increase in HIF area decreases for very large meals (Table 3). Warmer ambient temperatures also resulted in significantly shorter HIF duration for both squid (Fig. 5A,C,E; Table 3) and sardine meals (Fig. 5B,D,F; Table 3). HIF duration was significantly lower for squid meals at 22°C compared with those at 15°C (Table 3).

Food type also had a significant effect on HIF magnitude and duration. Squid meals resulted in larger HIF than sardine meals (Fig. 4A,C,E *versus* 4B,D,F; Table 3). Differences in HIF area by food type were significant at 15 and 20°C (Table 3) with a smaller HIF area for sardine meals. Squid meals also resulted in longer HIF duration than sardine meals; the duration of HIFs was significantly longer for squid meals compared with sardine meals at 15°C. The best fitting regressions for the duration of HIFs from sardine meals at 20 and 22°C were quadratic, indicating that for very large sardine meals, HIF duration approaches an aymptote. There was also an interaction effect between ambient temperature and food type, where the decrease in HIF area with ambient temperature was larger for sardine meals (Fig. 4B,D,F) than for squid meals (Fig. 4A,C,E).

Results from the HBM also indicated a significant effect of ambient temperature on HIF magnitude, looking at individual level responses. There were significant differences in HIF area by temperature for sardine meals: for all three individuals, HIF area at 22°C was significantly lower than that at 20°C. HIF area was also significantly lower at 22°C than at 15°C for all three experimental fish (Table 4). For squid meals, one of the three individuals had a significantly lower HIF area at 20°C compared with that at 15°C. A different individual had a significantly lower HIF area at 20°C compared with that at 22°C, and all three had a significantly lower HIF area at 22°C compared with that at 15°C (Table 4). Fitted regression lines (averaged for the three individuals) for the three temperature levels and two food types are shown in Fig. 6.

There were no significant differences in mean HIF area by ambient temperature at the population level for squid or sardine meals (based on overlapping 95% posterior probability intervals for the population distribution mean parameters).

Results from the HBM also indicated a significant effect of food type on HIF magnitude, looking at individual level responses. Squid meals resulted in a significantly larger HIF area (slope of kcal/HIF area in Table 4 is lower for squid meals) for two out of three individuals at 15°C, two out of three individuals at 20°C and three out of three at 22°C (Table 4). This indicates an overall difference in the HIF generated per kcal of squid *versus* sardine as well as a potential interaction effect, where the magnitude of the difference

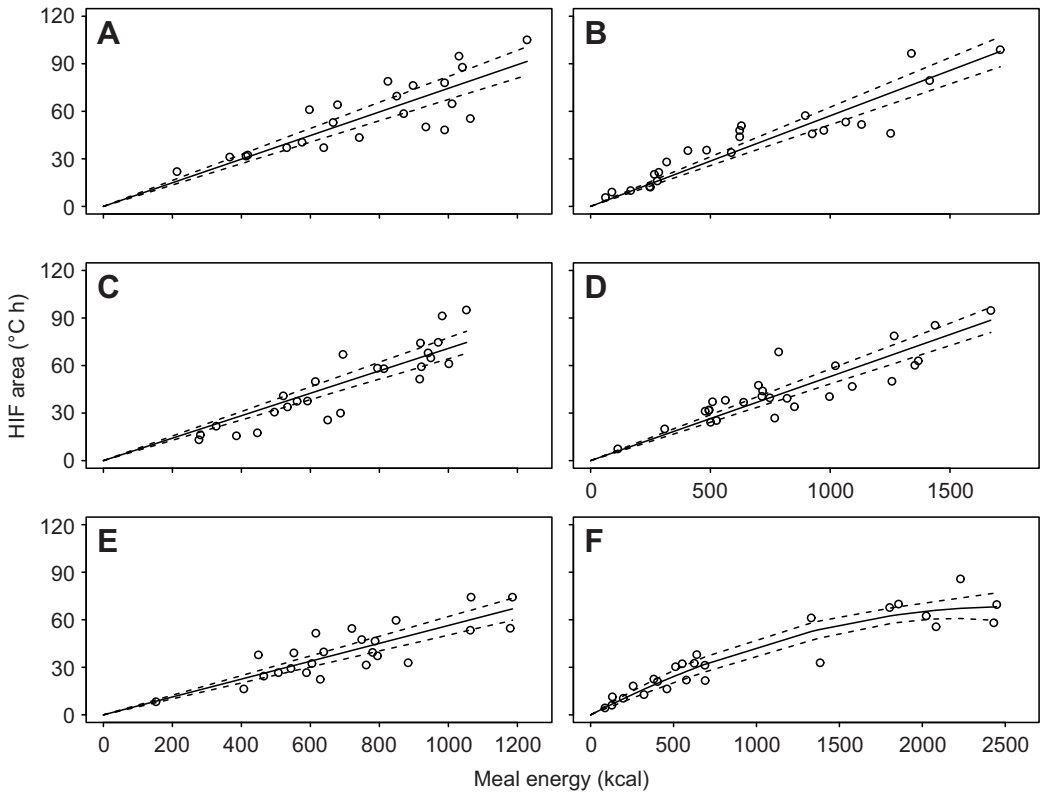


Fig. 4. HIF area and meal energy for three archival-tagged Pacific bluefin tuna. The solid black line is the posterior mean fitted regression, while the dashed lines denote the 95% posterior probability intervals. Each open circle represents one experimental feeding. (A) Squid and (B) sardine meals at 15°C; (C) squid and (D) sardine meals at 20°C; (E) squid and (F) sardine meals at 22°C.

Table 3. Posterior mean regression coefficients (s.d. in parentheses) for regressions of HIF magnitude and HIF duration on estimated meal energy (kcal)

| Response | Food type | $T_a$ (°C) | Model | $\beta_1$ | $\beta_2$ | $R^2$ | Fitted value† |
|---|---|---|---|---|---|---|---|
| HIF magnitude | Squid | 15 | Linear | $7.5\times10^{-2}$ ($3.3\times10^{-3}$)* | – | 0.65 | 75°C h |
| | Squid | 20 | Linear | $7.1\times10^{-2}$ ($3.1\times10^{-3}$)* | – | 0.73 | 71°C h |
| | Squid | 22 | Linear | $5.6\times10^{-2}$ ($2.7\times10^{-3}$)* | – | 0.65 | 56°C h |
| | Sardine | 15 | Linear | $5.7\times10^{-2}$ ($2.6\times10^{-3}$)* | – | 0.84 | 57°C h |
| | Sardine | 20 | Linear | $5.3\times10^{-2}$ ($2.2\times10^{-3}$)* | – | 0.75 | 53°C h |
| | Sardine | 22 | Quadratic | $5.7\times10^{-2}$ ($4.5\times10^{-3}$)* | $-1.2\times10^{-5}$ ($2.2\times10^{-6}$)* | 0.86 | 45°C h |
| HIF duration | Squid | 15 | Linear | $4.7\times10^{-2}$ ($2.8\times10^{-3}$)* | – | 0.63 | 47 h |
| | Squid | 20 | Linear | $3.7\times10^{-2}$ ($3.0\times10^{-3}$)* | – | 0.41 | 37 h |
| | Squid | 22 | Linear | $2.6\times10^{-2}$ ($1.8\times10^{-3}$)* | – | 0.45 | 26 h |
| | Sardine | 15 | Linear | $3.3\times10^{-2}$ ($2.5\times10^{-3}$)* | – | 0.64 | 33 h |
| | Sardine | 20 | Quadratic | $4.1\times10^{-2}$ ($5.4\times10^{-3}$)* | $-9.4\times10^{-6}$ ($4.6\times10^{-6}$)* | 0.60 | 32 h |
| | Sardine | 22 | Quadratic | $2.6\times10^{-2}$ ($3.5\times10^{-3}$)* | $-5.6\times10^{-6}$ ($1.7\times10^{-6}$)* | 0.65 | 20 h |

$T_a$, ambient temperature. HIF magnitude was measured by HIF area (in °C h).
The model chosen on the basis of the lowest deviance information criterion (DIC) is shown in the fourth column. The posterior mean proportion of the variance explained by the regression is shown in the final column.
*Significant regression (95% posterior probability interval for coefficient does not include 0).
†Fitted value for a 1000 kcal meal.

increases with ambient temperature. All population and individual level slopes were significantly different from 0, based on non-overlapping 95% posterior probability intervals.

There were no significant differences in mean HIF area by food type at the population level at any of the ambient temperatures (based on overlapping 95% posterior probability intervals for the population distribution mean parameters). More results from the HBM including plots of posterior probability distributions for individual level slope parameters for different food types and temperatures can be found in the Appendix and supplementary material Tables S1–3 and Figs S1–6.

The fit of the HBM (actual kcal fed *versus* estimated kcal given HIF area) is shown in Figs 7 and 8 for sardine and squid meals, respectively. Coefficients of determination ($R^2$) computed for the

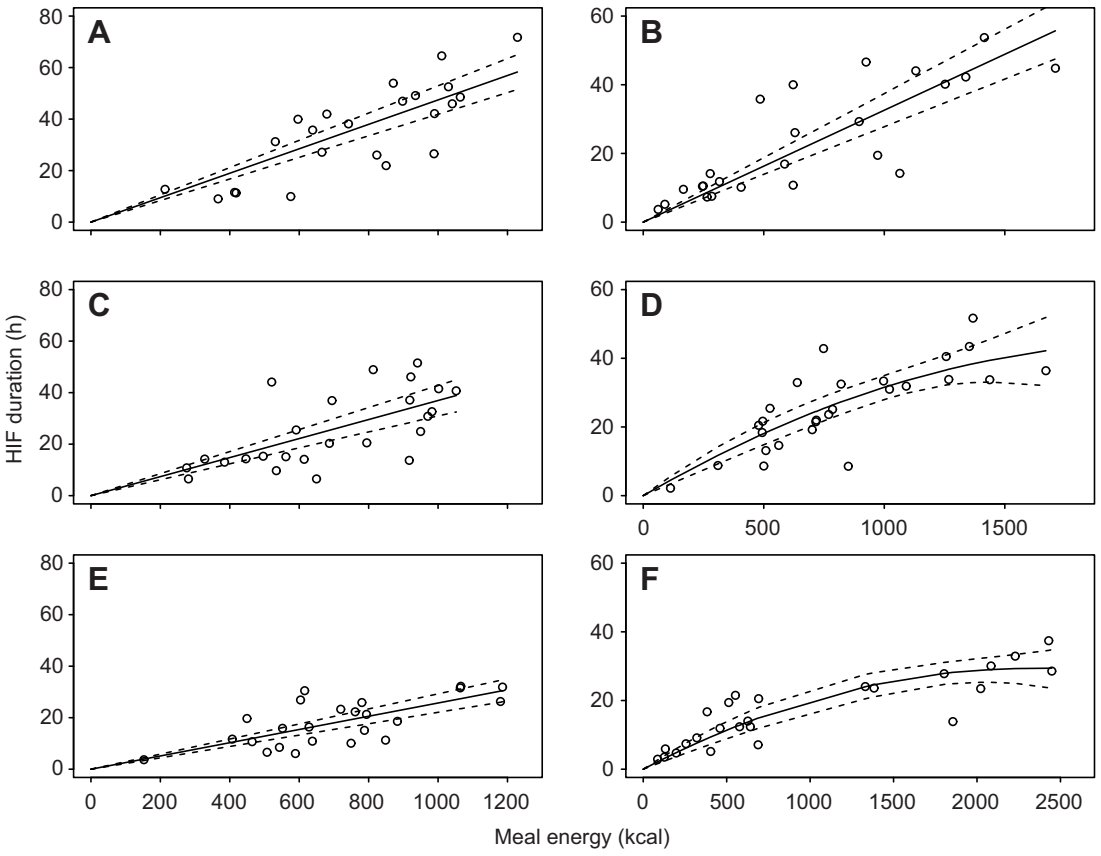


Fig. 5. HIF duration and meal energy for three archival-tagged Pacific bluefin tuna. The solid black line is the posterior mean fitted regression, while the dashed lines denote the 95% posterior probability intervals. Each open circle represents one experimental feeding. (A) Squid and (B) sardine meals at 15°C; (C) squid and (D) sardine meals at 20°C; (E) squid and (F) sardine meals at 22°C.

Table 4. Posterior means and s.d. for the individual-level regression coefficients (kcal/HIF area) from the HBM for the two food types and three ambient temperatures

| Food type | $T_a$ (°C) | Tag number | Posterior mean | Posterior s.d. |
|---|---|---|---|---|
| Squid | 15 | D1474 | 11.29* | 0.57 |
| | 20 | D1474 | 12.28* | 0.53 |
| | 22 | D1474 | 14.67* | 0.73 |
| | 15 | D1629 | 16.87 | 0.78 |
| | 20 | D1629 | 15.41*,† | 0.77 |
| | 22 | D1629 | 21.30* | 1.00 |
| | 15 | D6052 | 12.05*,† | 0.64 |
| | 20 | D6052 | 15.93 | 1.23 |
| | 22 | D6052 | 16.38* | 1.08 |
| Sardine | 15 | D1474 | 15.70 | 0.99 |
| | 20 | D1474 | 16.11† | 0.92 |
| | 22 | D1474 | 23.95 | 1.33 |
| | 15 | D1629 | 20.65 | 1.89 |
| | 20 | D1629 | 21.79† | 1.29 |
| | 22 | D1629 | 34.37 | 2.00 |
| | 15 | D6052 | 16.81 | 1.63 |
| | 20 | D6052 | 20.72† | 3.13 |
| | 22 | D6052 | 30.90 | 1.46 |

$T_a$, ambient temperature. HBM, hierarchical Bayesian model.
*Significant difference by food type (for the same individual and temperature); †significant difference with the next highest ambient temperature (for the same individual and food type).

HBM showed that HIF area and ambient temperature are good predictors of the energetic content of a meal, explaining a high proportion of the variation in the estimated caloric value of ingested food within an individual. High coefficients of determination ($R^2$) were obtained for all ambient temperature and individual combinations (Table 5), with most of the variation in meal energetic content being explained by HIF area (note that the coefficients in this model have units of kcal $\varphi^{-1}$).

Extracts from the visceral and ambient temperature traces from two archival-tagged Pacific bluefin tuna and the predicted baseline visceral temperature ($\hat{T}_b$) are shown in Fig. 9A, Fig. 10A and Fig. 11A. The corresponding distributions for expected daily caloric intake are summarized in Fig. 9B, Fig. 10B and Fig. 11B.

## DISCUSSION

Specific dynamic action (SDA) refers to the elevation in metabolic activity associated with the ingestion, digestion, absorption and assimilation of a meal (Secor, 2009). The thermal by-product of these metabolic processes (occurring in the visceral tissues) is detectable and is known as HIF. While SDA is evident as an increase in metabolic rate in most fishes and can be measured in a respirometer, measuring a thermal increment is problematic because of the heat losses associated with respiration and conduction. In bluefin tuna, counter-current visceral heat exchangers conserve the heat from digestion, leading to a measurable warming of the viscera that has been reported in all three bluefin species (Carey and Lawson, 1973; Gunn et al., 2001; Clark et al., 2010). Postprandial visceral warming in bluefin tuna is thought to be a result of increased heat production from the hydrolytic breakdown of food, the biochemical processes of digestion and assimilation, and heat conservation by the visceral retia mirabilia (Carey et al., 1984). Observations from captive Southern bluefin tuna that increased swimming speed (from 1.0 to 1.8 body lengths $s^{-1}$) does not lead to an appreciable increase in visceral temperature (Clark et al., 2008) support attribution of the postprandial thermal increment to digestive processes rather than enhanced activity. Direct measurements of the oxygen consumption rate in a flume indicate that the postprandial increase in visceral temperature in captive Pacific bluefin tuna closely tracks postprandial changes in metabolism, with a lag of 2–3 h (Clark et al., 2010). Given the similarity of the qualitative patterns in oxygen consumption and visceral temperature reported in that study, we suggest that the visceral temperature increment can be used as a proxy for SDA magnitude in Pacific bluefin tuna (at constant ambient temperature).

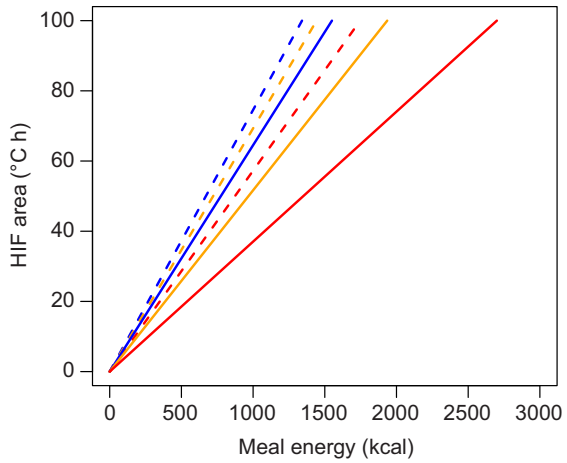


Fig. 6. Fitted regressions from the hierarchical Bayesian model (HBM). For clarity, each of the plotted curves is an average of three individual-level curves. Dashed lines, squid meals; solid lines, sardine meals. Blue lines, meals at 15°C; orange lines, meals at 20°C; red lines, meals at 22°C.

The use of implantable archival tags has led to extensive recordings of thermal physiology, both in wild and captive bluefin tuna (Gunn et al., 2001; Walli et al., 2009; Lawson et al., 2010; Kitagawa et al., 2007a; Bestley et al., 2008; Bestley et al., 2010). Direct quantification of energy intake in wild fish has so far been challenging because of a general lack of understanding of how HIF is correlated with prey type, changes in ambient temperature and body size. We conducted laboratory experiments with captive Pacific bluefin tuna in controlled tank conditions to quantify the effects of meal energy (for two representative food types, sardine and squid – see Appendix) and ambient temperature on HIF magnitude. Our

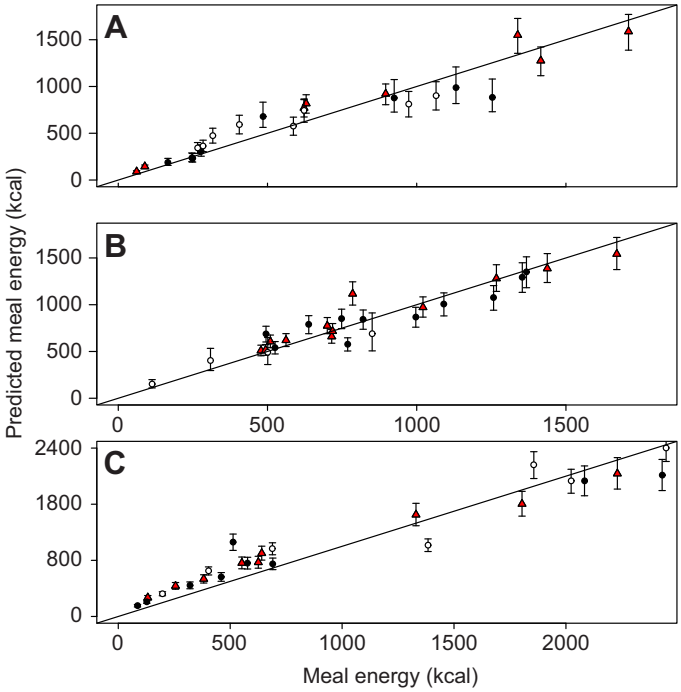


Fig. 7. Estimated and predicted meal energy from the HBM for 15°C (A), 20°C (B) and 22°C (C) for sardine meals. Different markers correspond to estimates for three archival-tagged bluefin tuna: red triangles, D1474; black circles, D1629; white circles, D6052.

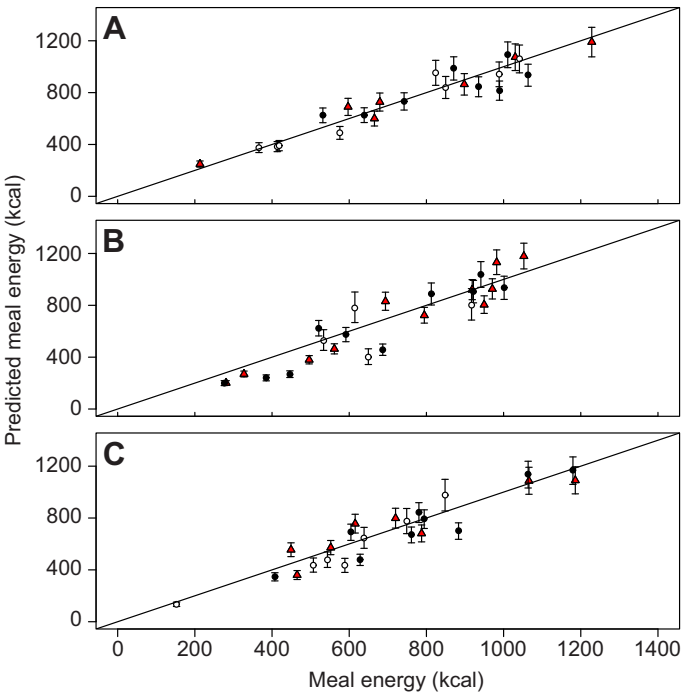


Fig. 8. Estimated and predicted meal energy from the HBM for 15°C (A), 20°C (B) and 22°C (C) for squid meals. Different markers correspond to estimates for three archival-tagged bluefin tuna: red triangles, D1474; black circles, D1629; white circles, D6052.

objective in correlating the thermal increment with gross energy intake in the laboratory was to estimate the energy content of food ingested by archival-tagged wild bluefin tuna of similar size to the experimental fish.

The results of our experiment demonstrate that in Pacific bluefin tuna, HIF area (rather than duration alone) is the best metric for predicting the energetic value of an ingested meal in a captive laboratory population and controlled tank setting. A high proportion of the variance in estimated meal energetic value was explained by HIF area (the lowest $R^2$ value for any individual and ambient temperature treatment combination was 0.86). Most of the total regression variance (over 70%) was explained by measured HIF area. This finding is comparable with that of Fitzgibbon and colleagues (Fitzgibbon et al., 2007), who found that SDA magnitude showed a higher correlation with ration size ($R^2$=0.98) than SDA duration ($R^2$=0.38).

For food of a given type (sardine or squid), there were mainly linear relationships between meal caloric value and HIF area, and between meal caloric value and HIF duration. An exception to this was HIF area for sardine meals at 22°C, where a quadratic model provided a better fit to the combined data from three individuals. We chose to assume linearity in the HBM for individual-specific regressions; although a quadratic model provided a slightly better fit for two out of three fish at 22°C, the $R^2$ and DIC values were similar for linear and quadratic models. Our finding of a direct correlation between meal energetic content and HIF magnitude is similar to that of Gunn and colleagues (Gunn et al., 2001), who found that HIF area and duration in southern bluefin tuna increase linearly with ration size. In experiments with captive Pacific bluefin tuna at 20°C, Clark and colleagues (Clark et al., 2010) reported a linear increase in SDA with meal energy content. The linearity of the responses of SDA and the visceral temperature increment to meal energy is likely to result from extended stimulation of metabolic processes within the viscera for large meals, increasing oxygen consumption and heat production. Larger meals would require more ATP, lipid oxidation (prolonging adenylate demand) and protein metabolism. The end result is a longer duration of thermogenesis as a by-product of the sum of metabolic activity in the viscera.

A consistent observation across all three bluefin in our study was that at a given temperature, a larger heat increment of feeding is associated with digestion of squid compared with sardine. This indicates a higher cost of metabolism for squid compared with sardine. Adjusted for caloric value, meals of higher protein content have been found to be associated with larger SDA magnitudes (Jobling and Spencer Davies, 1980). Analyses of samples from the feeds used in our experiment indicated that they have a comparable protein content: sardine 18.6±0.9%, squid 16.7±0.9% (difference not significant). Thus protein content alone cannot account for the difference in our study. Furthermore, there were no significant differences in the protein content of squid or sardine samples sent for analysis at different times of the year. Other studies have found that the balance of protein with other energy sources in the diet (fat or digestible carbohydrates) may also affect SDA. Insufficient non-protein energy sources can lead to

Table 5. Coefficients of determination ($R^2$) for fits of predicted kcal to actual kcal for the archival tag data

| $T_a$ (°C) | D1629 | D1474 | D6052 |
|---|---|---|---|
| 15 | 0.87 | 0.95 | 0.87 |
| 20 | 0.86 | 0.94 | 0.95 |
| 22 | 0.94 | 0.96 | 0.96 |

$T_a$, ambient tank temperature.

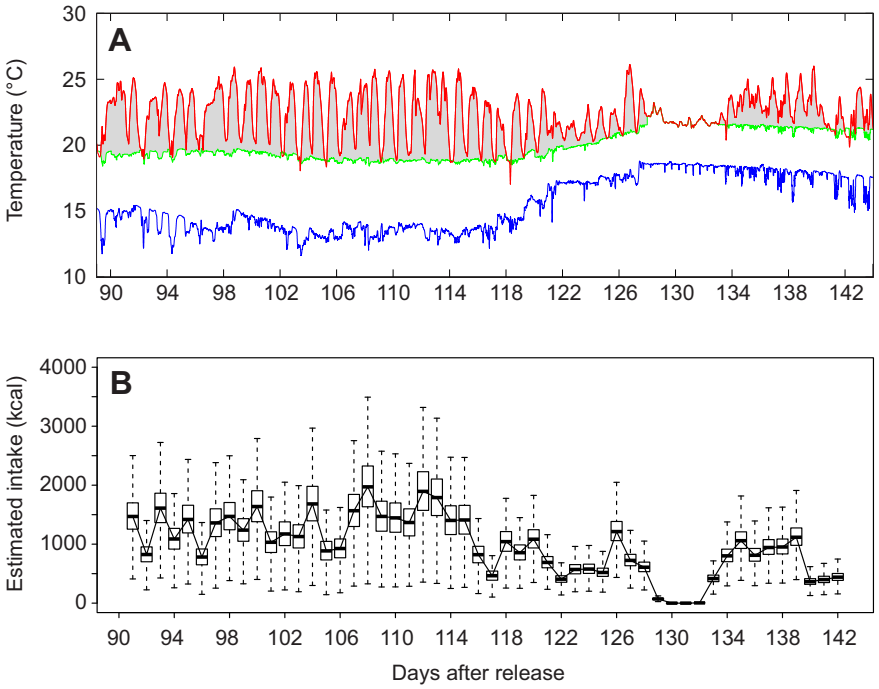


Fig. 9. (A) Extract from the visceral and ambient temperature trace from a tagged Pacific bluefin tuna released off Baja California on 25 August 2002 (length at release 94 cm). Red line, visceral temperature; blue line, ambient temperature; green line, predicted baseline visceral temperature ($\hat{T}_b$). (B) Posterior predictive distributions from the HBM for expected daily caloric intake for the archival tag data shown in A. Solid horizontal lines in each box indicate the posterior median; ends of the box are the 25th and 75th percentiles. Dashed vertical lines extend to the 2.5th and 97.5th percentiles of the posterior distribution for kilocalories ingested per day.

increased de-amination of amino acids and thus a greater energy cost of SDA (Beamish and Trippel, 1990; Peres and Oliva-Teles, 2001). The sardine used in our experiment had a lower protein to non-protein energy ratio than the squid, with a mean fat content of 10.4±3.6% *versus* 2.0±0.4% for the squid (smaller sardines tended to have a lower fat content, although still higher than the squid). Further explanations for this result could be that the amino acids found in squid require more catabolic steps for digestion than those present in sardine, or that the digestibility of sardine is lower (i.e. a higher proportion is egested).

Temperature had significant effects on visceral warming; a larger visceral heat increment and longer duration were found to be associated with meals consumed at cooler water temperatures. This is consistent with the finding of Gunn and colleagues (Gunn et al., 2001) that a given ration size (mass of pilchards consumed divided by body mass) produced less visceral warming at warmer ambient temperatures in juvenile southern bluefin tuna (*Thunnus maccoyii*). The summer and winter temperatures at which Gunn and colleagues made observations correspond to the experimental minimum and maximum temperatures in our study. The effect of temperature on the relationship between meal energetic value and HIF magnitude is also consistent with measurements of the temperature effect from another study (Walli, 2007) on Pacific bluefin tuna. Comparisons between the effect of ambient temperature on visceral warming and SDA may not be straightforward because of the possibility of changes in the rate of heat loss from the viscera with temperature.

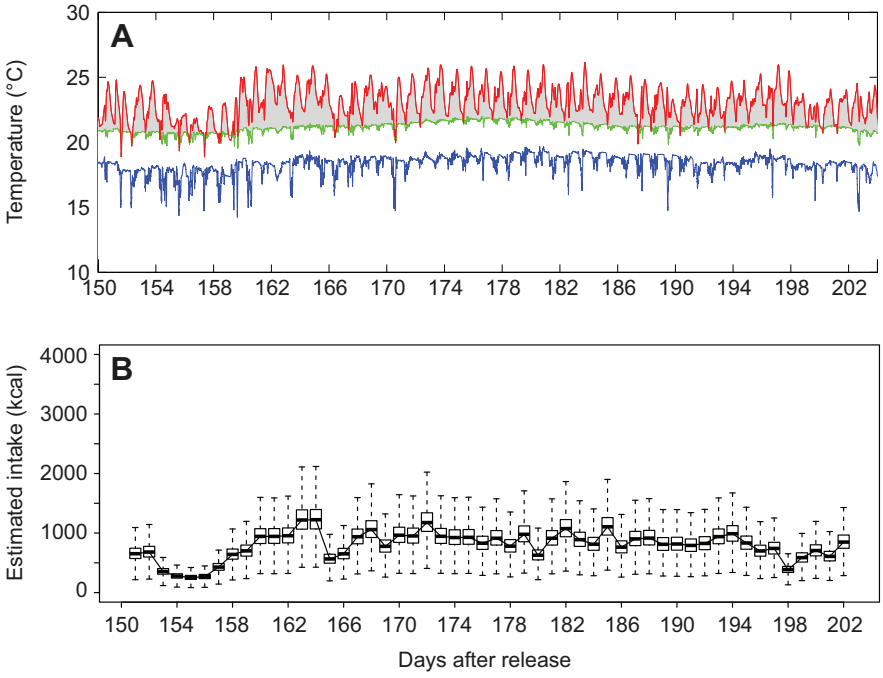


Fig. 10. (A) Extract from the visceral and ambient temperature trace from a tagged Pacific bluefin tuna released off Baja California on 25 August 2002 (length at release 94 cm). Red line, visceral temperature; blue line, ambient temperature; green line, predicted baseline visceral temperature ($\hat{T}_b$). (B) Posterior predictive distributions from the HBM for expected daily caloric intake for the archival tag data shown in A. Solid horizontal lines in each box indicate the posterior median; ends of the box are the 25th and 75th percentiles. Dashed vertical lines extend to the 2.5th and 97.5th percentiles of the posterior distribution for kilocalories ingested per day.

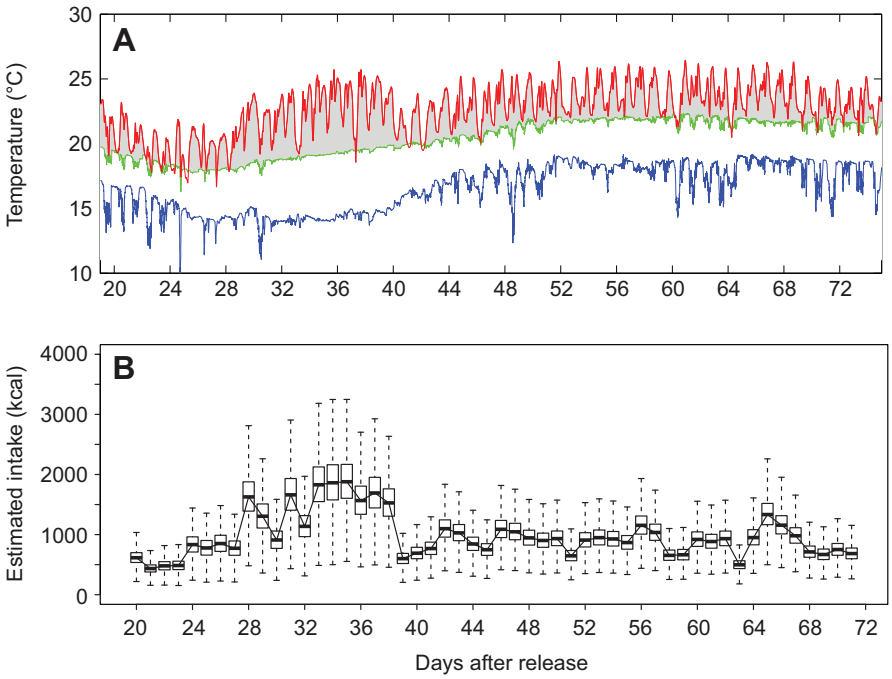


Fig. 11. (A) Extract from the visceral and ambient temperature trace from a tagged Pacific bluefin tuna released off Baja California on 25 August 2002 (length at release 89 cm). Red line, visceral temperature; blue line, ambient temperature; green line, predicted baseline visceral temperature ($\hat{T}_b$). (B) Posterior predictive distributions from the HBM for expected daily caloric intake for the archival tag data shown in A. Solid horizontal lines in each box indicate the posterior median; ends of the box are the 25th and 75th percentiles. Dashed vertical lines extend to the 2.5th and 97.5th percentiles of the posterior distribution for kilocalories ingested per day.

However, qualitatively similar findings may indicate a plausible mechanism for changes in visceral temperature. Jobling and Spencer Davies (Jobling and Spencer Davies, 1980) reported a shorter SDA duration at higher temperatures; thus, the shorter HIF duration we measured in warm water may reflect a shorter elevation of digestive metabolism. Baseline thermal excess was similar at the two ambient temperatures in our study (mean 1.8°C at 15 and 22°C), meaning that resting visceral temperature was higher in warmer water. Our results are therefore probably attributable to several mechanisms; changes in blood flow to the gut and in heat retention by the counter-current heat exchanger, and enhanced activity of thermally dependent digestive enzymes at higher visceral temperatures (Stevens and McLeese, 1984). Gunn and colleagues (Gunn et al., 2001) hypothesized that a change in heat conservation may be the primary mechanism accounting for differences in visceral warming in cold and warm water. A further mechanism could be a reduction in enzymatic efficiency at lower temperatures, leading to higher aerobic costs and increased thermogenesis. Cooler temperatures are also associated with bradycardia, which may result in lowered cardiac output and reduced rates of oxygen delivery to the digestive tissues, and consequently a lower rate of food metabolism (Blank et al., 2004). Our results also indicate that there may be an interaction between ambient temperature and diet; we found that temperature had a larger effect on HIF for sardine meals than for squid meals, although this interaction was not significant. Such an interaction might occur because of differences in the amino acid composition of squid and sardine, and the extent to which they contain amide bonds that are hydrolyzed by temperature-dependent enzymes (e.g. trypsin and chymotrypsin).

It has been hypothesized that SDA represents a large proportion of available metabolic scope in juvenile bluefin tuna because of the demands for protein synthesis and degradation related to their high growth rates (Fitzgibbon et al., 2007). Pacific bluefin tuna appear to exhibit higher food conversion efficiency than other fish species (evidenced by SDA accounting for a lower percentage of ingested energy) (Clark et al., 2010). This may be linked to elevated activity levels of thermally dependent enzymes as a result of visceral warming; thus, visceral warming may promote efficient digestion and assimilation of food, facilitating high rates of growth. The higher levels of visceral warming at lower ambient temperatures found for *T. orientalis* in our study and *T. maccoyii* in that of Gunn and colleagues (Gunn et al., 2001) might result from a compensatory acclimation mechanism to allow greater digestive efficiency at lower ambient temperatures. This could be an important adaptation to maintain optimal rates of food conversion over a wide range of ambient temperatures (Gunn et al., 2001; Fitzgibbon et al., 2007).

We used a HBM to analyze the data collected from three archival-tagged Pacific bluefin tuna. Hierarchical models are useful when some of the parameters can be regarded as related through the structure of the problem (Gelman et al., 1995). This structure is appropriate in our study as we expect the amount of visceral warming per kcal of ingested food (for a given ambient temperature level and food type) to be similar among individuals (they have similar body size and are subject to the same experimental protocol) but not identical (e.g. there may be differences in responses because of unknown variations in tag placement). The hierarchical Bayesian approach we used has a number of benefits. It can be used to obtain a posterior predictive distribution for the kcal consumed by an as yet unobserved Pacific bluefin tuna, given its measured HIF area (°C h). Adding observations from new experimental fish allows learning about other individuals (those that we have already made observations of and those that have not been observed yet), because of the assumption of relatedness inherent in the model structure.

Future laboratory studies to support this work may provide further information on the effect of body mass on HIF (we were not able to investigate the effect of body mass in our study as only juvenile Pacific bluefin tuna were available). Observations from other studies (Carey et al., 1984; Clark et al., 2008) indicate that the heating rate (standardized for meal size and tuna body mass) decreases with body mass. The mechanisms for the effect of ambient temperature on digestion and of diet composition on HIF and HIF magnitude also merit further investigation.

We have developed a probabilistic laboratory calibrated model to estimate daily caloric intake using records of visceral and ambient temperature from wild Pacific bluefin tuna. Although our model should be applied to bluefin tuna in a similar size range, it

is suitable for the analysis of a large dataset from archival-tagged juvenile Pacific bluefin tuna in the California current (over 100 individuals within 10 cm of the mean length of the experimental fish). The archival tag data we presented from two wild Pacific bluefin tuna show a sea surface temperature range of ~15–18°C. This range comprises the majority of observations for wild Pacific bluefin tuna in our data (see Appendix). The range of daily ration sizes observed for these wild fish was comparable to the ration sizes we fed in our experiment, although the weekly ration size of the wild tuna is higher owing to the fact that we fed the fish three times per week during the experiment. The 95% probability intervals for the estimated daily caloric intake of the wild tuna convey uncertainty resulting from the effects of meal size and ambient temperature on HIF magnitude, measurement uncertainty and the uncertainty in prediction that results from differences between individual tagged tuna (e.g. because of tag placement).

Future work will focus on applying the model developed here to a rich data set from archival-tagged juvenile Pacific bluefin in the wild, to quantify energy intake. By examining visceral temperature data alongside information on position and season we can illuminate foraging patterns and identify feeding 'hotspots' in the California current. Estimated energy intake can be analyzed in relation to oceanographic data, in an attempt to deepen our understanding of the physical and biological covariates underlying foraging success in the wild.

## APPENDIX

### HBM

Prior distributions (summarising knowledge about the parameter before making experimental observations) for parameters whose prior is not given below can be found in supplementary material Table S1.

Vague normal priors were placed on the population level priors for regression coefficients for squid and sardine feeds at 20°C:

$$\mu_{\beta_1,2} \sim \text{Normal}(0,1000^2), \quad \text{(A1)}$$

$$\mu_{\beta_2,2} \sim \text{Normal}(0,1000^2), \quad \text{(A2)}$$

where ~ means 'is distributed as', $\mu_{\beta 1,k}$ denotes the mean of the population level prior for squid feeds at temperature level $k$ and $\mu_{\beta 2,k}$ denotes the mean of the population level prior for sardine feeds at temperature level $k$. $k$=1 denotes feedings at 15°C; $k$=2 denotes feedings at 20°C; and $k$=3 denotes feedings at 22°C. Individual level regression coefficients at 20°C were assumed to arise from a common (population level) normal distribution with means given above and food type-specific precision parameters:

$$\beta_{1,i,2} \sim \text{Normal}(\mu_{\beta_1,2}, \tau_{\beta_1}), \quad \text{(A3)}$$

$$\beta_{2,i,2} \sim \text{Normal}(\mu_{\beta_2,2}, \tau_{\beta_2}), \quad \text{(A4)}$$

where $\beta_{1,i,k}$ is the squid feed regression coefficient for individual $i$ at temperature $k$ and $\beta_{2,i,k}$ is the sardine feed regression coefficient for individual $i$ at temperature $k$. $\tau_{\beta 1}$ and $\tau_{\beta 2}$ are the precisions of the population level priors for regression coefficients at temperature level $k$, for squid and sardine feeds, respectively.

A vague normal prior was also placed on the mean of the population level prior for the per degree change in the slope of kcal/HIF area for squid feeds, $\mu_{\delta 1,1}$, moving from 20°C to 15°C:

$$\mu_{\delta_{1,1}} \sim \text{Normal}(0,1000^2). \quad \text{(A5)}$$

Individual level priors for the per degree change in the slope of kcal/HIF area for squid feeds moving from 20°C to 15°C, $\delta_{i,1,1}$, were assumed to arise from a common (population level) normal distribution with mean given above:

$$\delta_{i,1,1} \sim \text{Normal}(\mu_{\delta_{1,1}}, \tau_\delta), \quad \text{(A6)}$$

where $\delta_{i,1,1}$ is the temperature effect parameter for individual $i$ for squid feeds, moving from 20°C to 15°C and $\tau_\delta$ is the precision of the population level prior for the per degree change in the slope of kcal/HIF area for squid feeds moving from 20°C to 15°C.

In order to maintain the ordering of the individual level temperature effect parameters, priors for individual level delta parameters for squid feeds moving from 20°C to 22°C were specified relative to those for 20°C to 15°C as:

$$\delta_{i,1,2} = \delta_{i,1,1}\eta, \quad \text{(A7)}$$

where $\delta_{i,1,2}$ is the temperature effect parameter for individual $i$ for squid feeds, moving from 20°C to 22°C and:

$$\eta \sim \text{Normal}(0,100^2). \quad \text{(A8)}$$

Similarly, priors for temperature effect parameters for sardine feeds were specified relative to the temperature effect parameters for squid feeds:

$$\delta_{i,2,1} = \delta_{i,1,1}\gamma_1, \quad \text{(A9)}$$

$$\delta_{i,2,2} = \delta_{i,1,2}\gamma_2, \quad \text{(A10)}$$

where $\delta_{i,2,1}$ is the temperature effect parameter for individual $i$ for sardine feeds, moving from 20°C to 15°C and $\delta_{i,2,2}$ is the temperature effect parameter for individual $i$ for sardine feeds, moving from 20°C to 22°C. $\gamma$ parameters were given vague normal priors (supplementary material Table S1).

Priors for individual level regression coefficients at 15°C and 22°C were expressed relative to the individual level coefficient priors at 20°C (Eqns A11–14):

$$\beta_{1,i,1} = \beta_{1,i,2} + \delta_{i,1,1}(T_2 - T_1), \quad \text{(A11)}$$

$$\beta_{1,i,3} = \beta_{1,i,2} + \delta_{i,1,2}(T_3 - T_2), \quad \text{(A12)}$$

$$\beta_{2,i,1} = \beta_{2,i,2} + \delta_{i,2,1}(T_2 - T_1), \quad \text{(A13)}$$

$$\beta_{2,i,3} = \beta_{2,i,2} + \delta_{i,2,2}(T_3 - T_2), \quad \text{(A14)}$$

where $T_1$ denotes 15°C, $T_2$ denotes 20°C and $T_3$ denotes 22°C.

Predicted meal energy (kcal) for squid and sardine feeds is then given by:

$$\hat{y}_{1,i,j,k} = \beta_{1,i,k}\varphi_{1,i,j,k}, \quad \text{(A15)}$$

$$\hat{y}_{2,i,j,k} = \beta_{2,i,k}\varphi_{2,i,j,k}, \quad \text{(A16)}$$

where $\varphi_{1,i,j,k}$ is the TIF area for squid feed $j$ for individual $i$ at temperature level $k$, and $\hat{y}_{1,i,j,k}$ is the predicted meal energy in kcal for squid feed $j$ for individual $i$ at temperature level $k$ (Eqn A15) and analogously for sardine feeds (Eqn A16).

Measured meal energy values (kcal) for squid and sardine were assumed to be normally distributed around the predicted energetic value for a given individual, food type and tank temperature:

$$y_{1,i,j,k} \sim \text{Normal}(\hat{y}_{1,i,j,k}, \nu_1), \quad \text{(A17)}$$

$$y_{2,i,j,k} \sim \text{Normal}(\hat{y}_{2,i,j,k}, \nu_2), \quad \text{(A18)}$$

where $y_{1,i,j,k}$ is the measured caloric value of squid feed $j$ for fish $i$ at temperature level $k$, and $y_{2,i,j,k}$ is the measured caloric value of sardine feed $j$ for fish $i$ at temperature level $k$. The $\nu_1$ and $\nu_2$ parameters are observation error precisions for squid and sardine feeds, respectively.

To check that the structure of the hierarchical model was appropriate for the experimental data, DICs were computed for the model described above and a HBM with common regression parameters and temperature effect parameters for squid and sardine feeds. DIC was also computed for a HBM with intercept parameters.

### Extended results from the HBM

Values of the DIC computed for alternative HBM structures indicated that a model with separate regression coefficients and temperature effect parameters for squid and sardine provides a better fit to the experimental data compared with a model with common coefficients (a lower DIC was obtained for the HBM with food type-specific coefficients and temperature effect parameters) (supplementary material Table S2). A slightly lower DIC was obtained for a model without intercept parameters (supplementary material Table S2), although this difference was not sufficient to remove the model with intercepts from consideration. The results presented in this section are from a model with food type-specific coefficients and temperature effect parameters and no intercepts.

Estimated regression coefficients from the HBM indicated differences in the HIF magnitude generated per kcal among tagged individuals. Two individuals had significantly lower HIF magnitude for squid feeds at 15°C compared with the other two tagged tuna (supplementary material Fig. S1A). There were two significant differences out of three possible pair-wise comparisons between individual regression coefficients for squid feeds at 20 and 22°C (supplementary material Fig. S2A, Fig. S3A). There were no significant differences between estimated individual-level regression coefficients for sardine feeds at 15°C (supplementary material Fig. S1B), one significant difference between individual regression coefficients for sardine feeds at 20°C (supplementary material Fig. S2B) and two significant differences between individual regression coefficients for sardine feeds at 22°C (supplementary material Fig. S3B).

Estimated temperature effect parameters (per degree change in the slope of kcal/HIF area, $\delta$) for sardine feeds indicated higher slopes at higher temperatures (supplementary material Fig. S4). The temperature effect was stronger for warmer ambient temperatures, with a larger per degree change in visceral warming moving from 20 to 22°C, relative to 20 to 15°C (supplementary material Fig. S4). Estimated temperature effects were larger (in terms of absolute magnitude) for sardine than for squid, although not significantly so (supplementary material Fig. S4).

### Applying the HBM to archival tagging data from wild Pacific bluefin tuna

In this section we discuss application of our model to visceral temperature data from archival tags recovered from wild Pacific bluefin tuna. Juvenile Pacific bluefin tuna were tagged annually under the Tagging of Pacific Pelagics (TOPP) Program between 2002 and the present (see Marcinek et al., 2001; Kitagawa et al., 2007b; Boustany et al., 2010). Surgically implanted archival tags (Lotek, LTD 2310 series A-D, Lotek Wireless Inc.) were deployed during cruises at sea off the coast of California and Mexico (Kitagawa et al., 2007b; Boustany et al., 2010). Most of these juvenile tuna are in the same size range (supplementary material Fig. S5A) as the tagged experimental fish, so a large proportion of the recovered tags are suitable for our study.

The range of SST used by these individuals (as measured by the archival tags) is shown in supplementary material Fig. S5B; 86.6% of daily mean SST measurements fell between the range of temperatures (15–22°C) used for our experimental feedings. Tuna experience a wider range of temperatures because of changes in temperature experienced during dives; however, the archival tagging data show that tuna typically remain at the surface in warmer water after feeding/during a HIF event (an extract of the data from an archival tag recovered from a wild bluefin tuna showing this behavior is presented in supplementary material Fig. S6).

Inspection of the stomach contents of Pacific bluefin tuna in the Eastern Pacific Ocean (EPO) has revealed that oily fishes make up a large proportion of the diet. Pacific sardine (*Sardinops sagax*) and Northern anchovy (*Engraulis mordax*) are the dominant species of small pelagic fishes in the Pacific Ocean (Chavez et al., 2003; Zwolinski et al., 2012). It has been hypothesized that shifts in the dominance of either species in the EPO is driven by oscillations in the physical environment on a cycle that is approximately 25 years in length (Chavez et al., 2003). *Sardinops sagax* and *E. mordax* are comparable in terms of their nutritional value (supplementary material Table S3). Analysis of the stomach contents of 650 Pacific bluefin tuna caught off southern California and Baja Califonia between 1968 and 1969 showed that fish constitute 91.8% by number and 93.1% by volume of all food consumed (Pinkas et al., 1971). Northern anchovy was the most important food item, comprising 86.6% by number and 80.0% by volume of the bluefin diet (Pinkas et al., 1971), while cephalopods comprised 3.88% by number and 2.16% by volume. *Engraulis mordax* was the most important dietary item in all regions analyzed, making up 86% in Southern California, 56% in Northern Baja California, 98% in Central Baja California and 64% in Southern Baja California by volume. The lower percentages for Northern Baja California and Southern Baja California were accounted for mainly by unidentifiable fish species and pelagic red crab (*Pleuroncodes planipes*), respectively (Pinkas et al., 1971).

### Quantifying HIFs in wild Pacific bluefin tuna

A more complex algorithm was needed to define baseline thermal excess for wild tagged bluefin tuna, to accommodate changes in the ambient temperature associated with horizontal and vertical movements.

The mean of the lowest 5% of visceral temperature measurements at the surface (defined as the top 2 m of the water column) was taken over a 2 day window and a 5 day window. The lowest visceral temperature measurements when the tuna is at the surface should correspond to measurements when there is no warming due to HIF, i.e. the baseline or resting temperature. We assumed that resting body temperature was equal to the 5 day mean visceral temperature when the derivative of SST was below 0.5 times the derivative of the 2 day mean SST and equal to the 2 day mean visceral temperature when the derivative of SST was above this threshold. This was done to capture changes in the resting body temperature resulting from horizontal movement between water masses with a different ambient temperature (the 2 day mean visceral temperature captures these changes better). Defining baseline visceral temperature in wild fish is probably the most challenging aspect of applying our model outside the laboratory. Wild fish might consume a larger average daily ration than that used in our experiment, leading to a higher elevation of visceral temperature above ambient temperature at rest than what was observed in our experiment.

To account for changes in visceral temperature associated with vertical movements (dives into cooler water and subsequent return to warmer surface waters), deviations in ambient temperature from its 2 day mean (scaled to a smaller magnitude) were added to the predicted baseline visceral temperature. Finally, to account for times

when the tuna were not feeding, predicted baseline body temperature was set equal to measured body temperature when the standard deviation in daily mean body temperature was less than 0.5°C.

The total magnitude of the visceral temperature increment per 24 h period was obtained by integrating $T_b$–$\hat{T}_b$ from midnight to midnight of the following day. The distribution for the squid regression coefficient for a new individual, accounting for SST is then given by:

$$\beta_{1,i',\mathrm{SST}} = \beta_{1,i',2} + (1 - I_T)\delta_{i',1,1}(20 - \mathrm{SST}) + I_T\delta_{i',1,2}(\mathrm{SST} - 20), \quad \text{(A19)}$$

where:

$$\beta_{1,i',2} \sim \mathrm{Normal}(\mu_{\beta_1,2}, \tau_{\beta_1}), \quad \text{(A20)}$$

$$\delta_{i',1,1} \sim \mathrm{Normal}(\mu_{\delta_{1,1}}, \tau_{\delta}), \quad \text{(A21)}$$

$$\delta_{i',1,2} = \delta_{i',1,1}\eta, \quad \text{(A22)}$$

$i'$ denotes a new individual for which there are no experimental observations, SST is daily mean sea surface temperature (the average of ambient temperature measurements in the top metre of the water column) and $I_T$ is an indicator variable taking the value 0 when SST is less than 20°C and 1 when SST is above 20°C.

## LIST OF SYMBOLS AND ABBREVIATIONS

| | |
|---|---|
| DIC | deviance information criterion |
| DIT | diet-induced thermogenesis |
| HBM | hierarchical Bayesian model |
| HIF | heat increment of feeding |
| $R_2$ | coefficient of determination |
| SDA | specific dynamic action |
| SST | sea surface temperature |
| $T_a$ | ambient temperature |
| $T_b$ | visceral temperature |
| $\hat{T}_b$ | predicted visceral temperature |
| $T_e$ | thermal excess |
| $\hat{T}_e$ | predicted thermal excess |
| $t_f$ | HIF end point |
| $t_{HIF}$ | HIF duration |
| $t_s$ | HIF start point |
| φ | HIF area |

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## AUTHOR CONTRIBUTIONS

The authors of this study made the following contributions: R.E.W.: experimental design, conducting feedings, statistical analysis, drafting and revising the manuscript; A.W.: contribution to the analytical framework and Matlab code, revising the manuscript; P.C.: contribution to experimental protocol, conducting feedings and recording experimental data; L.E.R.: recording experimental data, assisting with feedings and providing technical support for the experiment; C.F.: advice and assistance with experimental design, oversight of experiments at the TRCC; B.A.B.: conception, experimental design, conducting surgeries, revising the manuscript and overall supervision.

## COMPETING INTERESTS

No competing interests declared.

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