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Bioenergetics of captive Pacific bluefin tuna (*Thunnus orientalis*)

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ABSTRACT

Tuna bioenergetics can be described by the following relationship: the energy available for growth is equal to the food energy minus all metabolic costs. These costs include routine metabolic rate, specific dynamic action, increased activity level, eliminated waste, and gonadal development. Captive populations of Pacific bluefin tuna (*Thunnus orientalis*) were held at ~20 °C in fiberglass tanks and fed on a regular schedule with a diet formulated to achieve an energetic content of 176 ± 36 kJ · kg^{-1} of biomass · day^{-1} (mean ± s.d.). To conduct a bioenergetic study, growth rates during the captive period and tissue energy values post-mortem were empirically determined. Daily growth rates were obtained from a von Bertalanffy growth function based on curved fork length (CFL) measurements of live fish and post-mortem morphometrics. The parameters obtained for the captive bluefin growth function were 225.13 cm straight fork length (SFL), 0.173, and −0.497 years for L_∞, k, and t_o, respectively. The growth equation, $SFL = 225.13 \cdot (1 - e^{(-0.173(t-(-0.497)))})$ in conjunction with the length-mass regression (where body mass $M = 4.98 \times 10^{-6} \times SFL^{3.3186}$) gave a daily growth increase of 32.60 ± 2.40 g · day^{-1} for Pacific bluefin tuna of 2.2 years of age and 11.4 ± 1.0 kg (the average age and mass of a fish in the study). The average tissue energy value from four sampled tuna was 7.66 ± 0.40 kJ · g^{-1}, and applying the daily growth increase estimate provides a daily energy gain of 249.7 kJ, which is 12.4% of an ingested meal's total energy content. A food conversion ratio of 17.8:1 is estimated for a meal consisting solely of sardines and 22.6:1 for a mixed diet consisting of sardines, squid, and a gelatin-vitamin mixture at the stated feeding regimen. This paper presents the first data on actual food conversion ratios and bioenergetic utilization for Pacific bluefin tuna.

1. Introduction

Studying the bioenergetics of tunas is a challenge due to the difficulties inherent in studying large pelagic fish. Tunas are ram ventilators, highly mobile, and must swim continuously to ventilate and thus are difficult to keep in captive environs. To overcome these challenges, investigators first utilized shipboard measurements to study tuna metabolism (Gooding et al., 1980; Graham and Laurs, 1982). These first respiration measurements were made on skipjack (*Katsuwonus pelamis*) and albacore (*Thunnus alalunga*) tunas, which were considered too difficult to transport back to the laboratory. Shipboard research provided data from specimens soon after capture, thus eliminating the transport stress. However, fish were excited post-capture and while these studies were exemplary in their approach, they may have recorded the stress of the capture.

Land-based research on tunas was initiated with skipjack tunas, and later with yellowfin tuna (*Thunnus albacares*) at the Kewalo Research Facility, Southwest Fisheries Science Center, Honolulu, HI, which opened the research opportunity for studying tuna energetics (Brill, 1979, 1987, 1992). Additional land-based facilities at Achotines Laboratory in Panama, Kewalo Basin in Hawaii, Scripps Institution of Oceanography in La Jolla, California, and the Tuna Research and Conservation Center (TRCC), jointly operated by Stanford University's Hopkins Marine Station and the Monterey Bay Aquarium in Monterey, California began a twenty-year period of extensive study of tuna energetics. Scripps Institution of Oceanography also developed the capacity for utilizing wild tunas in experimental flumes for advanced measurements of metabolism (Dewar and Graham, 1994). The Achotines lab's studies initially focused on growth in relation to diet in larval and early-stage juvenile black skipjack (*Euthynnus lineatus*; Wexler, 1993) and later shifted

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to extensive study of yellowfin (Wexler et al., 2003). Other researchers concurrently examined metabolic rates (Boggs and Kitchell, 1990) and energy storage and utilization in migration (Dotson, 1998; Sharp and Dotson, 1977).

At the TRCC, early energetic studies focused on determining the routine metabolic rates of yellowfin tuna, bonito (*Sarda chiliensis*), skipjack, and Pacific mackerel (*Scomber japonicus*) in an effort to understand the evolution of metabolism in scombrid fish. In the early 1990s, a closed experimental chamber (tank) with periodic water changes was used for early metabolic measurements (Freund, 1999). Blank et al. (2007a, b), made the first measurements of Pacific bluefin tuna (*Thunnus orientalis*) and yellowfin tuna routine metabolic rates in a large-volume flume. At the TRCC, the emphasis was on studying tuna respiration under controlled laboratory conditions of temperature, water quality, and swimming speed for extended periods of time (Blank et al., 2007a,b). More recently, TRCC researchers focused on quantifying and modeling the specific dynamic action (SDA) of Pacific bluefin tuna (Clark et al., 2010; Walli et al. 2007; Whitlock et al., 2013).

Several aspects of bluefin tuna bioenergetics have been studied across the globe as the use of open ocean pens and land-based tanks has increased due to growing interest in tuna aquaculture. The processes of ingestion, digestion, absorption, and assimilation are now partially quantified in two species of bluefin tunas. The energy expended following a feeding event, also known as specific dynamic action, has been examined in two studies, one on Southern bluefin (*Thunnus maccoyii*; Fitzgibbon and Seymour, 2009) and one on Pacific bluefin tuna (Clark et al., 2010; Walli et al., 2007; Whitlock et al., 2013). Other aspects of fish bioenergetics including the energy available for growth, or retained energy (RE), ingested energy (IE), routine metabolic rate (RMR), active metabolic rate (AMR), and excretion and egestion (EE) have also been investigated (Beamish et al., 1975; Brett and Groves, 1979; Halver and Hardy, 2002; Kitchell et al., 1978).

The key to understanding tuna bioenergetics is to quantify growth. Measurements of bluefin tuna growth have been collected from tagging studies, fisheries efforts, and tuna aquaculture operations. Growth results from energy being devoted to building somatic tissues rather than routine and active metabolism, reproduction, egestion, and excretion. Typically, growth is dependent on the nutritional value and abundance of food, age, water temperature, and level of sexual maturation.

In this study, the growth of Pacific bluefin tuna kept under a controlled ambient temperature and known diet was examined. This species has high importance to both commercial fisheries and aquaculture operations, and this study provided the opportunity to investigate aspects of bluefin tuna bioenergetics that have yet to be described in the literature. This area of research is important in mariculture operations because in coastal areas where bluefin tuna are raised, they are subjected to daily fluctuations in temperature, salinity, and dissolved oxygen. This natural variability can affect metabolic costs, food conversion efficiencies, and daily growth (Dizon, 1975), all of which can affect the economics of raising bluefin tuna. A sound understanding of growth and energetics will enable a better understanding of the economic viability of bluefin tuna mariculture operations.

2. Materials and methods

2.1. Collection of tuna

All of the Pacific bluefin tuna in this study were collected using rod and reel, primarily with live sardines (*Sardinops sagax*) as bait. Tunas were collected in accordance with Stanford University IACUC procedures. Captured tunas were placed into seawater-filled holds and kept alive aboard the fishing vessel *Shogun* for one to four days prior to transport back to the port of San Diego, California. Once in port, the bluefin were off-loaded to a specially designed transport container and trucked to Monterey, California. After arrival in Monterey, tuna were individually marked with a passive integrated transponder (PIT) tag (Avid Identification Systems, Inc., Norco, CA) and transferred in water-filled slings to holding tanks located in the Tuna Research and Conservation Center. The TRCC facility has two 110,000-L and one 340,000-L fiberglass tanks with a recirculating seawater system. Some tunas were transferred to the 3,800,000-L Outer Sea Exhibit at the Monterey Bay Aquarium (MBA). Although tunas were moved between different tanks throughout the study period, water temperatures were maintained at 19.9 ± 0.9 °C (mean ± s.d.) in all tanks. The TRCC life support, holding facility, and collecting techniques are described in greater detail in Farwell (2001).

2.2. Diet

Captive tuna in all of the holding tanks (TRCC and MBA) were fed a mixed diet of squid (*Loligo opalescens*) and sardines, plus a vitamin-gel mix (Mazuri Aquatic Gel Diet, Progress Drive, Richmond, IN). Food items were sent to a nutrition laboratory (N-P Analytical Laboratories, Checkerboard Square, St. Louis, MO) quarterly each year for proximate analysis and results were reported in percent composition of each food item. Protein values were determined using the Kjeldahl method (protein factor = 6.25), which measures the total nitrogen content of a sample, and fat values were determined using a Mojonnier fat acid hydrolysis technique to measure the crude fat content of a sample. The caloric values for each item were determined using the caloric equivalents of 23.87 kJ · g^{-1} and 36.43 kJ · g^{-1} for protein and fat, respectively (Brett and Groves, 1979; Dale et al., 2013; Halver and Hardy, 2002). The diet was fed three times per week with a diet formulated to achieve the calculated value of 176 ± 36 kJ · kg^{-1} of biomass · day^{-1}, and dietary fat was maintained at a level between 8% and 10% of the total food fed (Farwell, 2001). The variability in the diet fed is represented by the standard deviation of the caloric content of the sardine and squid measured across the archived proximate analysis data set. The standard practice for this dietary regimen was to estimate total biomass of captive tuna on a monthly basis, based on length measurements (described later), and adjust the composition and amount of food to adhere to the feeding objectives. All food input into the tank was typically eaten, and tunas that did not feed in captivity were not included in this analysis. Early experiences rearing yellowfin tuna in the TRCC facility showed that feeding levels in excess of the stated regimen bring concerns over water quality, obesity, and overall fish health, as well as more pronounced declines in tank oxygen levels due to increased post-prandial respiratory rates (Specific Dynamic Action). The feeding regimen did not change throughout the course of the study period.

2.3. Length and mass measurements

The main technique for monitoring growth in captivity was to measure the curved fork lengths (CFL) of living fish. CFL measurements were taken upon arrival of fish to the facility and at any subsequent handling opportunity (tank-to-tank moving or respirometry experiments) that experimental conditions permitted measurement. Curved fork lengths were measured with a flexible 3-m-long measuring tape laterally along the body from the tip of the rostrum underneath the pectoral fin, when possible, to the fork of the caudal fin. Curved fork length, straight fork length (SFL), and mass were measured post-mortem. SFL measurements were made with calipers from the tip of the rostrum to the fork of the caudal fin. CFL-to-SFL conversion equations were obtained by performing regressions of CFL vs. SFL for different size classes of bluefin tunas utilizing all available records in which both CFL and SFL data were obtained for individual fish (n = 166; Table 1). Age at the time of collection was determined from straight fork lengths estimated from the initial curved fork length measurements obtained upon arrival to the TRCC facility. Initial age estimates were derived from published data relating western Pacific bluefin tuna straight fork lengths to age conversions (Foreman, 1996; Shimose et al., 2009). Age at death for

Table 1
Regression values for calculating straight fork length (SFL) from curved fork lengths (CFL) measured from Pacific bluefin tuna (*Thunnus orientalis*).

Size ranges, CFL (cm)	Regression formula	R^2	N
65–74	SFL = 1.0381(CFL) − 5.7719	0.83	36
75–96	SFL = 0.9206(CFL) + 4.0604	0.95	73
103–140	SFL = 0.9738(CFL) − 0.256	0.98	25
146–209	SFL = 0.8942(CFL) + 5.442	0.97	13

each captive specimen was determined using the age at time of collection and adding the duration in captivity. If not measured directly, mass estimates were determined using a mass-length relationship derived from TRCC post-mortem measurement records for which both SFL and mass were taken ($n = 283$). Only healthy fish that fed regularly were included in the length-weight data set.

2.4. Growth rates

Growth over a twelve-year period was investigated by repeated length measurements of 166 individual fish held at 19.9 ± 0.9 °C. Individual fish were grouped by age classes for growth determination and the von Bertalanffy growth equation (VBGF; Fig. 1) was used to calculate length-specific daily growth rates. A non-linear least squares regression was used to fit the VBGF curve to the age and length data.

Changes in mass per year and per day, as well as percent change in mass were calculated using the derived formulas in Figs. 1 and 2. These growth estimates were then related to the energy content of an ingested meal using the equation: retained energy = ingested energy − [specific dynamic action + routine metabolic rate + active metabolic rate + excretion and egestion] (Table 2). Retained energy (RE) is considered to be the energy available for growth. Ingested energy (IE) was the daily food ration · kg^{-1} of tank biomass · day^{-1}. The values for routine metabolic rate (RMR) and specific dynamic action (SDA) for Pacific bluefin tuna were the mean values from Blank et al. (2007a) and Clark et al. (2010), respectively, which followed similar experimental protocols. The mean RMR value from Blank et al. (2007a) was scaled to the mean mass of the fish sampled in the proximate analysis component of this study (11.4 kg) using a metabolic scaling exponent of 0.698 (Killen et al., 2010). Killen et al. (2010) determined this scaling coefficient through an analysis of metabolic scaling in various species of pelagic fishes, including tunas. Estimates of waste energy (EE) values were obtained from Kitchell et al. (1978) and Brett and Groves (1979) and were the same values used by Dale et al. (2013).

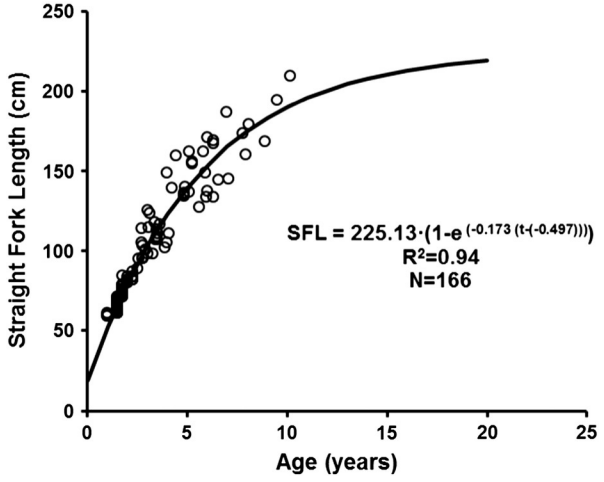

Fig. 1. Straight fork length (SFL, cm) at age (*t*, years) and the fitted von Bertalanffy growth curve for Pacific bluefin tuna (*Thunnus orientalis*).

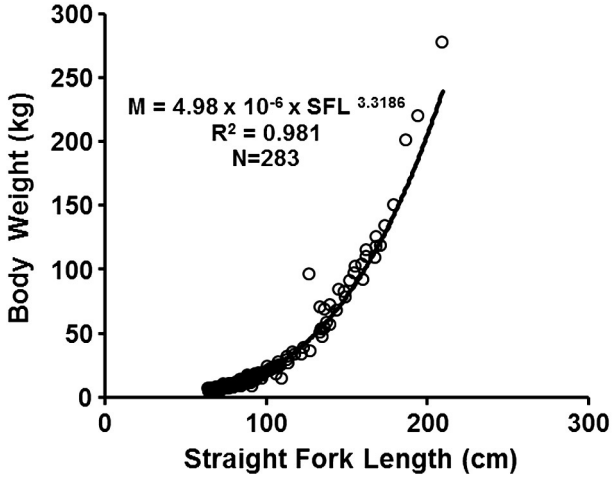

Fig. 2. Relationship between straight fork length (SFL, cm) and the whole body weight (M, kg) of Pacific bluefin tuna (*Thunnus orientalis*) in captivity.

Active metabolic rate (AMR) values were estimated using a similar method to Dale et al. (2013). Recent experiments with accelerometer tags indicate that the captive tunas in the TRCC facility exhibit higher tailbeat frequencies during sunrise hours and in anticipation of feeding events (Gleiss and Block, unpublished data). Using a regression of tailbeat frequency and swim speed from Blank et al. (2007a), swim speeds during these active periods were estimated to increase to an average of 1.1 body lengths · sec^{-1} relative to the average of 0.8 body lengths · sec^{-1}. Such periods of increased activity occurred for approximately 2–3 hours (10%) of the day. We estimated the metabolic rate of the fish during these periods of increased activity using previously published regressions of swim speed and metabolic rate (Blank et al., 2007a). Scaling this value to the body mass of the fish used in the proximate analysis component of this study (11.4 kg) provided a metabolic rate of 267 ± 28 $mgO^2 \cdot kg^{-1} \cdot h^{-1}$, which was converted to kilojoules and multiplied by 10% to provide an estimate of AMR for this energetic budget (Killen et al., 2010).

2.5. Whole-body caloric values

The full body energy content was determined from proximate analyses of individual whole tunas. To do this, four regularly feeding, healthy Pacific bluefin tuna (average, 11.4 ± 1 kg) from the TRCC tanks were euthanized and weighed, and their internal organs and muscle mass were removed, along with the fins, skin, gills and head. All body components were weighed to determine relative percentage of total body mass for each component and samples were sent to N-P Analytical Laboratories for proximate composition analysis of protein and fat content. For the musculature, representative portions of slow twitch (red) muscle and fast-twitch (white) muscle (both dorsal and ventral samples) were taken from the 50% fork length area and sent for proximate analysis. The mean energy content (kJ · g^{-1}) for a pooled sample of four Pacific bluefin tuna was obtained from the percentage of fat and protein content of the test fish tissues as a percentage of the total body mass.

2.6. Energy and food conversion ratios

All proximate analyses and oxygen consumption values were converted to kilojoules using a conversion factor of 1 kcal = 4.1868 kJ (Schmidt-Nielsen, 1997). Food conversion ratios (FCR) were determined by comparing the daily calculated mass gain to the estimated food mass consumed daily for a mixed diet of sardines, squid, and gelatin mix and for a sardine-only diet. To estimate the range in FCR values, the food mass consumed daily was recalculated for a high and low

Table 2
Processes involved in converting the values and units used in ingested energy and the energetic costs into kilojoules, including references for each process. Calculations based on an 11.4-kg Pacific bluefin tuna (*Thunnus orientalis*).

	Process	Estimated energy	Reference
Ingested energy		**kJ**	
176 ± 36 kJ · kg^{-1} of biomass · day^{-1} at ~20 °C	1 kcal = 4.1868 kJ	2006.4	Brett and Groves 1979
Energetic costs			
RMR At 20 °C	20.8 ± 2.1 mgO^2 · h^{-1} 1 mgO^2 = 13.59 J	881.2	Blank et al., 2007a Jobling, 1994
SDA at 20 °C	9.2 ± 0.7% of ingested energy	184.6	Clark et al., 2010
AMR at ~20 °C	RMR at 1.1 body lengths · sec^{-1} = 23.4 ± 2.5 mgO^2 · h^{-1} for ~10% of day	99.3	Gleiss and Block (unpublished data) Blank et al., 2007a
EE at 20 °C	27% of ingested energy	541.7	Kitchell et al., 1978 Brett and Groves, 1979 Dale et al., 2013

scenario based on the range in the energy content measured in the selected feeds. The gross energy conversion ratio (GEC) was determined through comparison of the daily energy gained to the daily energy consumed. The energy available for growth, or retained energy (RE), was calculated as the gross ingested energy minus the metabolic costs associated with anabolic and catabolic activity, which is represented by the values for RMR, SDA, AMR, and EE. All variations around the reported means for these measurements are standard deviations.

3. Results

3.1. Diet analysis

Proximate analysis of sardines fed throughout this study period indicate on an average each sardine contains 17.8 ± 1.2% protein and 10.5 ± 5.0% fat (n = 50, sample mass = 86.8 ± 42.8 g (mean ± s.d.)), squid contain an average of 15.8 ± 2.2% protein and 1.9 ± 0.3% fat (n = 37, sample mass = 44.8 ± 15.7 g), and the gelatin mix contained an average of 27.8 ± 0.5% protein and 4.1 ± 0.1% fat (n = 8, sample mass = 70.7 ± 7.3 g). The caloric content of each diet constituent was then calculated to be 4.47 ± 0.6 kJ · g^{-1} for squid, 8.06 ± 2.1 kJ · g^{-1} for sardines and 8.13 ± 0.2 kJ · g^{-1} for the gelatin mix. To meet the target energetic input of 176 ± 36 kJ · kg^{-1} of biomass · day^{-1} for individual fish, where the standard deviation represents the range in caloric contents recorded in our archived proximate analysis data set, the diet consisted of 48% squid and sardines and 4% gelatin by mass.

3.2. Age and growth analyses

The Pacific bluefin tuna used in this study ranged in length from 63.5 to 108.8 cm straight fork length (SFL) at the time of placement into the captive facility, based on curved fork length (CFL) to SFL conversion equations determined from TRCC length records (Table 1). Based on previously published length-age regression estimates (Bayliff et al., 1991; Foreman, 1996; Shimose et al., 2009), these fish ranged from 1 to 3 years old at the time of capture. The fish remained in captivity for various periods (1 to 9 years). The tuna growth rates were based on measurements of fish ranging from 63.6 to 209 cm SFL (Figs. 1 and 2).

The fitted VBGF equation resulting from our analysis was SFL = $225.13 \cdot (1 - e^{(-0.173\ (t-(-0.497)))})$, ($R^2$ = 0.94; n = 166) where t = age, and the length-mass (M) regression was $M = 4.98 \times 10^{-6} \times \mathrm{SFL}^{3.3186}$ (R^2 =0.981; n = 283). Daily growth increments for mass and length were 32.60 ± 2.40 g · day^{-1} and 0.47 ± 0.24 mm · day^{-1} for an age of 2.2 years (the average age of the tuna utilized in the whole-body caloric analysis; Tables 3 and 4).

The length increase rate of captive Pacific bluefin tuna in this study was slightly less, but not significantly different (unpaired Student's t-test, P = 0.22) than that of wild bluefin tuna for ages 1–4 (Shimose et al., 2009; Fig. 3a). However, the weight increase rates were significantly greater for captive fish relative to wild fish for ages 6–10 (unpaired Student's t-test, P = 0.02). The rate of weight change began to decrease at age 6 for wild bluefin and at age 7 for the captive bluefin in this study (Fig. 3b).

3.3. Whole-body caloric analyses

Proximate analysis of the captive bluefin (n = 4, average mass = 11.4 ± 1.0 kg) determined that the musculature (red and white muscle with tendons included), head, and skeleton represent the highest percentage of the total body mass at 55.7%, 14.3%, and 11.9%, respectively, and in total, contain 81% of the caloric energy of the whole tuna mass (Table 5). The proximate analysis accounted for 94.8% of the body mass, which yielded a total of 82,707.64 kJ, and when normalized for 100% of the body mass, a corrected average tissue energy value of 7.66 ± 0.40 kJ · g^{-1} was obtained. On average, these whole tuna were composed of 20.5% protein and 7.7% fat.

3.4. Energy and food conversion efficiency

The food conversion ratio (FCR) estimated for the mixed diet of squid, sardines, and gel fed throughout the study period was 22.6:1 (high scenario = 28.5:1, low scenario = 18.8:1), where the high and low scenarios incorporate the standard deviation in caloric content of feed items across our proximate analysis data set. If the tuna were fed a diet consisting solely of sardines amounting to the total caloric content of the target feeding level, this would return a food conversion ratio of 17.8:1 (high scenario = 23.9:1, low scenario = 14.1:1) to achieve the same growth rates measured in this study (Table 6). We calculated a

Table 3
Straight fork length (SFL) at age, t, taken from Fig. 1, the von Bertalanffy growth formula, SFL = $225.13^*(1-e^{(-0.173^*(t-(-0.497)))})$ and mass from Fig. 2 for Pacific bluefin tuna (*Thunnus orientalis*) where body mass, M, is solved by the regression $M = 4.98 \times 10^{-6} \times \mathrm{FL}^{3.3186}$.

Age	SFL	Growth	Mass at age	Mass increase	Daily change	Yearly percent change
Years	cm	mm · day^{-1}	kg	kg · $year^{-1}$	g · day^{-1}	%
1	52.3	0.9	2.5	2.4	6.7	96.6
2	80.3	0.8	10.4	7.9	21.7	75.9
3	103.7	0.6	24.4	14.0	38.3	57.3
4	123.4	0.5	43.4	19.0	52.0	43.8
5	139.9	0.5	65.8	22.4	61.3	34.0
6	153.7	0.4	89.9	24.1	66.1	26.8
7	165.2	0.3	114.4	24.5	67.1	21.4
8	174.9	0.3	138.2	23.8	65.3	17.2
9	183.1	0.2	160.7	22.5	61.6	14.0
10	189.9	0.2	181.4	20.7	56.7	11.4

Table 4
Comparative growth rates between captive and wild western Pacific bluefin tuna (*Thunnus orientalis*), from Table 3 and Shimose et al. (2009). A Student's *t*-test for paired means gives a significant difference ($P = 0.02$) at the 95% confidence level between the first 6 years of growth in mm · day^{-1} (SFL).

Age	Captive mm · day^{-1}	WPO, wild mm · day^{-1}	Captive g · day^{-1}	WPO, wild g · day^{-1}
1	0.91	1.04	6.7	6.8
2	0.77	0.87	21.7	22.5
3	0.64	0.74	38.3	38.1
4	0.54	0.62	52.0	49.6
5	0.45	0.52	61.3	56.5
6	0.38	0.44	66.1	59.4
7	0.32	0.37	67.1	59.2
8	0.27	0.31	65.3	56.8
9	0.22	0.26	61.6	53.0
10	0.19	0.22	56.7	48.4

gross energy conversion (GEC) value of 12.4% by comparing the daily energy gain to the energy in the daily consumed meal (Table 7).

4. Discussion

Data on the bioenergetics of tunas are difficult to obtain; however, the opportunity to study captive populations of Pacific bluefin at the TRCC facility has provided first estimates of energetics in this species. The results indicate a gross energy conversion of 12.4%, and thus 87.6% of the energetic content of ingested food is not available for direct growth, which is most likely attributable to physiological maintenance of an endothermic species with an elevated metabolic rate (Blank et al., 2007a, 2007b; Korsmeyer and Dewar, 2001). This GEC is higher than the reported estimate of 8% for yellowfin tuna (Olson and Boggs, 1986). This difference could be explained by having conducted the current study in captive tunas rather than wild tunas, or it may be related to the fact that bluefin tuna have a higher metabolic rate than yellowfin tuna (Blank et al., 2007a). The metabolic parameters used in this energy budget accounted for over 83% of the ingested energy. The remaining energy was either not accounted for by the selected parameters or was the result of error or uncertainty in these parameters.

Proximate analyses of bluefin tuna tissues showed similar patterns in protein and fat composition to those observed in related studies. The percentage of total muscle tissue (red and white muscle) reported in Table 5 was comparable to the reported value of 60% (Graham et al., 1983), and the percentage of fat found in the head is comparable to previously published data (Nguyen et al., 2012; Selmi et al., 2008)

Table 5
Pacific bluefin tuna organ, muscle, and skeleton masses are shown. Total kilojoules were derived from percentage of protein and fat values from proximate analysis of individual tissues. Total kilojoules per gram and average percentage of protein and fat values are shown for the whole tuna. Values shown represent the means for four Pacific bluefin tuna (*Thunnus orientalis*) with masses of 10.15, 11.1, 11.6, and 12.6 kg with an average mass of 11.4 kg and calculated age of 2.2 years. Skeletal section 1 is comprised of the collar and pectoral fins. Skeletal section 2 covers the skeletal system running posterior of the head to the end of the first dorsal fin, including the pelvic fins. Skeletal section 3 consists of the area from the second dorsal fin down to the anal fin. *Atrium values were not available for all tunas sampled.

Sample	Weight (g)	Weight (% of total mass)	Protein (%)	Protein (kJ)	Fat (%)	Fat (kJ)	Total (kJ)
Liver	124.1	1.1	18.6	550.7	4.8	216.0	766.7
Atrium*	1.2	0.0	14.6	4.1	1.5	0.7	4.7
Ventricle	14.9	0.1	15.5	55.1	2.2	12.0	67.1
Bulbous arteriosis	7.0	0.1	17.1	28.6	2.2	5.7	34.3
Caecum	148.8	1.3	16.6	590.6	2.3	125.0	715.5
Spleen	45.8	0.4	23.0	251.2	1.3	21.8	273.1
Stomach	122.8	1.1	18.7	548.0	1.8	78.5	626.5
Intestine	27.3	0.2	16.8	109.1	1.9	18.9	128.0
Gonad	2.1	0.0	15.6	8.0	20.4	15.9	23.8
Gallbladder	10.4	0.1	9.1	22.6	3.1	11.8	34.4
Gills	563.4	5.0	16.1	2158.1	4.4	912.3	3070.4
Skin	566.0	5.0	18.2	2461.7	25.3	5216.0	7677.7
Red muscle	1888.0	16.7	22.4	10,070.2	2.6	1772.6	11,842.7
White dorsal muscle	1981.0	17.3	24.5	11,559.0	1.4	1029.0	12,588.0
White ventral muscle	2137.0	18.7	23.5	11,972.0	3.2	2475.3	14,447.3
Head	1539.0	13.6	13.7	5031.7	21.3	11,926.4	16,958.1
Skeletal section 1	642.5	5.7	18.4	2813.6	15.8	3691.8	6505.5
Skeletal section 2	407.0	3.6	17.6	1707.3	12.0	1775.5	3482.8
Skeletal section 3	228.5	2.0	20.4	1111.1	3.0	249.9	1361.0
Tail	321.0	2.8	20.5	1570.4	4.5	529.7	2100.1
Total	10,777.0	94.8		52,622.9		30,084.7	82,707.6

with values ranging from 12% to 14%. However, Nakamura et al. (2007) performed proximate analysis of wild and cultured Pacific bluefin (average mass = 33 kg) and reported lipid contents in the musculature that were substantially higher than those found in this study. We attribute this observed difference in fat content to the fact that the cultured bluefin in Nakamura et al. (2007) were fed daily to satiation, and wild Pacific bluefin typically feed at similar levels

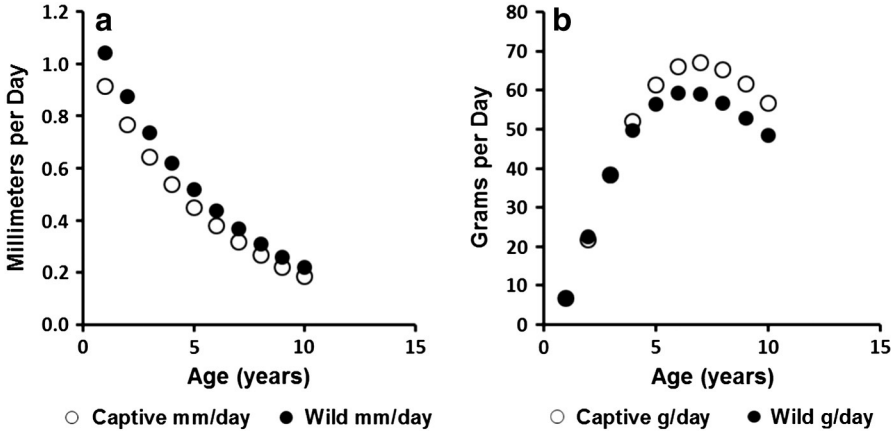

Fig. 3. (a and b) Comparison of captive and wild growth rates expressed in millimeters and grams per day for Pacific bluefin tuna (*Thunnus orientalis*) from calculated fork lengths using the von Bertalanffy growth function (VBGF) and the relationship between straight fork length and mass. Data represented in Table 4, including wild data from Shimose et al. (2009).

Table 6
Food conversion ratio (FCR) is the estimated mass of food ingested daily ($g \cdot day^{-1}$) to supply the target feed level divided by the daily growth rate ($g \cdot day^{-1}$), estimated here for an all-sardine and mixed diet. Values calculated for bluefin tuna with a body mass of 11.4 kg.

Diet type, and amount fed	Squid	Sardine	Gelatin	Total fed	Daily growth rate	Food conversion ratio
–	$g \cdot day^{-1}$	$g \cdot day^{-1}$	$g \cdot day^{-1}$	$g \cdot day^{-1}$	$g \cdot day^{-1}$	–
Mixed diet	354 g	354 g	30 g	738 g	32.6	22.6:1 FCR
All-sardine diet	0	581 g	0	581 g	32.6	17.8:1 FCR

(between 46 and 201 $kJ^{-1} \cdot kg^{-1} \cdot day$) but at greater frequency (daily vs. 3 days $\cdot$ $week^{-1}$) than the captive tunas in this study (Whitlock et al., 2013).

Concise estimates of food conversion ratios (FCRs) in pen-raised bluefin tuna are difficult to obtain due to a lack of data on fish size, biomass, and amount of food actually consumed. Estimates of FCRs range from 10 to 25:1 for all bluefin tuna species, and from 7 to 20:1 for Pacific bluefin specifically (Ottolenghi, 2008; Zertuche-González et al., 2008). The FCRs estimated for this study fall within the estimated range for bluefin tunas with a calculated value of 22.6:1 for the mixed diet of sardine, squid, and gelatin, and 17.8:1 when the diet is calculated using only sardines. The lower FCR resulting from the all-sardine diet can be attributed to the higher lipid content of the sardine diet relative to the mixed diet because a lower quantity of food would be fed to achieve the target feeding level and result in the growth rate observed in this study. These authors chose not to analyze the moisture content of the different feed items in the present study. However, Clark et al. (2010) utilized the same feeds in their study of tuna digestion and metabolism and reported moisture contents of 84.4%, 66.0%, and 78.6% for squid, sardine, and gel, respectively. These moisture content values may be representative of the feed from the present study and it may be possible to compare the wet-weight FCRs calculated in this study to FCRs from tuna aquaculture operations that feed with dry formulated feeds.

There are several potential explanations for the differences in weight gain observed between the captive fish in this study and wild Pacific bluefin studies reported to date, as shown in Fig. 3b. Certainly, both diet and water temperature play major roles in determining growth rates of fish; however, sexual maturation and confinement in tanks may contribute to the observed differences in growth. It is difficult to disentangle the relative contribution of any one of these factors; however, it is interesting to compare this captive scenario to others in an effort to explore this topic more fully.

The TRCC tuna collection was maintained at a relatively constant 20 °C, and according to archival tagging records from Pacific bluefin tunas tagged in the eastern Pacific, this temperature is slightly warmer than the average temperature occupied by wild tunas (~18 °C; Kitagawa et al., 2007; Boustany et al., 2010; Block et al. 2011). Blank et al. (2007b) conducted respirometry experiments at the TRCC and found that Pacific bluefin exhibited minimum routine metabolic rates between 15 °C and 20 °C water temperatures and they suggested that temperatures warmer than optimal would result in slightly increased routine metabolic rates and potentially reduced growth rates. Lovern (1950) also noted that warmer than optimal temperatures may have a negative impact on fat deposition.

However, Pacific bluefin have been found to occupy much warmer waters (>28 °C) for short periods during the spawning season in the western Pacific, and Masuma (2009) demonstrates that other factors, namely diet and the conditions of captivity, may outweigh the negative effects of higher than preferred temperatures on growth rate. Masuma (2009) conducted a growth study on Pacific bluefin in a 14-hectare, 30-m-deep net pen in Japanese waters averaging 24 °C across seasons. He documented growth rates exceeding those of the tunas in this study and the wild tunas studied by Shimose et al. (2009) at this high temperature (Fig. 4; Masuma, 2009). However, two important distinctions from the present study are that Masuma (2009) fed tunas daily to satiation, and the concentration of tuna biomass in the net pens was likely kept low to reduce captive stress and promote spawning activity. In comparison, the dietary regimen in the present study was to feed only three times weekly, and the TRCC tanks typically contain between 0.5 and 2 kg of biomass $\cdot$ cm^{-3}. Masuma (2009) noted that increased water temperatures up to 24 °C were correlated with increased feeding rates, and this increased energetic intake may have outweighed any metabolic costs associated with increased water temperatures.

Another possibility is that the divergence of the wild and captive weight-age curves may be related to some aspect of maturation. There are reports that wild fish caught in the Sea of Japan can be sexually mature by age five (Tanaka, 2006; Yamada et al., 2009). The maximum gonadal somatic index (GSI) values for captive, immature bluefin tuna at TRCC/Monterey Bay Aquarium ($n = 137$) maintained at ~20 °C ranged between 1.0 and 1.2, ages 5–9 years, both sexes (male = 60, female = 38, unknown = 39; Farwell, 2011), compared to a maximum value of 3.2 to 3.6 for spawning bluefin tuna of similar age held at Tokyo Sea Life Park at 24 °C (Mimori et al., 2008). This suggests that the conditions of captivity, specifically, the constant 19.9 ± 0.9 °C tank temperature, could potentially inhibit or delay sexual maturation in this population of captive tuna. Wild bluefin may undergo a shift in energy utilization and require increased fat energy deposition for gonadal development, whereas the immature captive tunas in this study continued to devote energy towards somatic growth at later ages. More research is needed to understand the maturation schedules of Pacific bluefin.

Potential sources of error in this study include its reliance on a previously published length-age data set, weighted heavily with Pacific bluefin sampled in the western Pacific, to assign age at the time of collection to tunas from the eastern Pacific. Polacheck et al. (2004) found that assigning age in this way can be susceptible to variability in length and mass of tunas of the same age stemming from environmental differences and fishing pressure. However, at this time, no other long-term,

Table 7
Retained energy (RE) = ingested energy (IE) − [specific dynamic action (SDA) + routine metabolic rate (RMR) + activity metabolic rate (AMR) + excretion and egestion (EE)]. All units for these components are given in $kJ \cdot day^{-1}$. Daily energy increase ($kJ \cdot day^{-1}$) of the tuna is the daily growth ($g \cdot day^{-1}$) multiplied by the tissue energy ($kJ \cdot g^{-1}$). The gross energy conversion (GEC) compares the daily energy gain to the estimate of daily energy intake. Values calculated for bluefin tuna with a body mass of 11.4 kg.

Ingested energy	RMR	SDA	AMR	EE	Retained energy	Daily growth	Tissue energy	Daily energy increase	Gross energy conversion
$kJ \cdot day^{-1}$	$kJ \cdot day^{-1}$	$kJ \cdot day^{-1}$	$kJ \cdot day^{-1}$	$kJ \cdot day^{-1}$	$kJ \cdot day^{-1}$	$g \cdot day^{-1}$	$kJ \cdot g^{-1}$	$kJ \cdot day^{-1}$	–
2006.4	881.2	184.6	99.3	541.7	299.6	32.6	7.66	249.7	12.4%

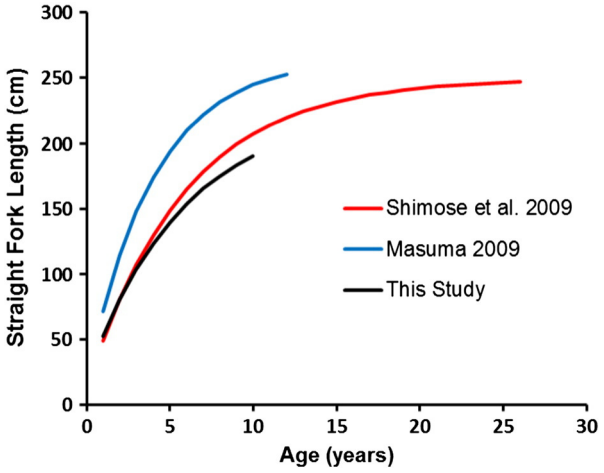

Fig. 4. A comparison of von Bertalanffy growth curves for Pacific bluefin tuna (*Thunnus orientalis*) under different environmental conditions, from Shimose et al. (2009) and Masuma (2009). (For interpretation of the references to colour in this figure, the reader is referred to the web version of this article.)

large sample size length-age data sets exist for Pacific bluefin tuna beyond those used by this study (Shimose et al., 2009).

A further issue encountered in the captive tanks is the broadcast feeding method used throughout this study period. Although common in tuna aquaculture and husbandry scenarios, this technique does not ensure that the distribution of food energy is exactly equal between individual tunas. Also, even though temperatures and feeding regimens were consistent between holding tanks, it is possible that tunas experienced slightly different growth rates resulting from the different tank sizes. It is assumed that the growth curve derived from this study is a composite that takes into account the variability in growth rates of individual tunas, and as such is as representative as possible of normal tuna growth in this unique captive scenario.

Additionally, all physiological and energetic values utilized in this study have some associated variability and uncertainty given the challenges of obtaining data on this difficult to study species. However, we have attempted to use the most representative data available to develop a first approximation of a Pacific bluefin tuna bioenergetic model unique to this captive scenario. For example, these authors estimated the active metabolic rate (AMR) of captive tunas based on preliminary findings on the tailbeat frequency of bluefin tunas in the research tanks (Gleiss and Block, unpublished findings). Other similar bioenergetic studies have combined estimates of swimming speed, daily energy budgets, and swim-tunnel respirometry measurements to estimate AMR (Dale et al., 2013). Future validation experiments are required to more rigorously develop an estimate of AMR for this captive scenario. In addition, more detailed studies are needed to accurately estimate the waste energy levels of tunas. In the absence of such studies, we relied on estimates of wasted energy (EE = 27% of IE) from a similar bioenergetic model for a species of carnivorous elasmobranch (Brett and Groves, 1979; Dale et al., 2013).

Last, these authors chose to utilize a metabolic scaling exponent of 0.698 from Killen et al. (2010); however, there are several other estimates of metabolic scaling exponents for tunas available in the literature. For this energy budget, applying a different metabolic scaling exponent from Fitzgibbon et al. (2008) results in a 1% change in the energy retained (RE) by bluefin tuna relative to using Killen et al.'s (2010) value. Such small levels of variation between scaling exponents used are of minor importance for the application of this model; however, future research is needed to improve our understanding of metabolic scaling in bluefin tunas.

This work provides important insights into the energy requirements of an endothermic tuna species. These data supply new information on growth, energy conversion, and food conversion ratios under controlled conditions, which may be useful in understanding Pacific bluefin tuna aquaculture operations and tuna ecology. However, many aquaculture operations are primarily focused on maximizing feed efficiency and animal growth whereas the dietary regimen fed throughout this study prioritized research needs and water quality maintenance in a recirculating seawater system. Furthermore, both wild and pen-raised bluefin tunas will likely encounter significantly greater environmental variability than the tunas utilized in this study. As such, it may not be acceptable to directly compare some of the results of this study to tuna aquaculture operations or to the study of wild tunas. However, this study provides a unique benchmark against which bioenergetic data on tunas from alternative scenarios, both captive and wild, may be compared and examined to further our understanding of this commercially and ecologically important species.

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