

# The Effects of Reproductive Status and Water Temperature on the Caloric Intake of *Tursiops truncatus*

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## Abstract

Several studies have examined diurnal consumption patterns and energy requirements of Atlantic bottlenose dolphins (*Tursiops truncatus*), yet little is known about how these values change with respect to reproductive status and small-scale changes in water temperature. This study describes a comparative assessment of the caloric intake of three populations of resident Atlantic bottlenose dolphins, all housed in ambient seawater facilities, with respect to sex, reproductive state, and water temperature. Weekly caloric intake, based on catch-specific caloric values of each dietary component, was calculated for five adult males and eight adult females with repeated measurements on animals in the following reproductive states over the course of 12 to 24 months: males ( $n = 5$ ), pregnant/lactating ( $n = 2$ ), pregnant/non-lactating ( $n = 7$ ), non-pregnant/lactating ( $n = 6$ ), and non-pregnant/non-lactating ( $n = 7$ ). Although food was provisioned, rate and magnitude of provisioning was driven by appetitive responses exhibited by each individual dolphin. Males exhibited a significantly higher caloric load than non-pregnant/non-lactating females. Among females, lactating females (pregnant and non-pregnant) exhibited significantly higher consumption values when compared to females in all other reproductive states, highlighting the energetic cost of lactation. Additionally, there was an inverse relationship between mean monthly caloric intake and mean monthly water temperature, capturing the influence of temperature on the energetic demands of small odontocetes. These data can be utilized to estimate the carrying capacity of wild habitats, to improve management, and to serve as a baseline for the strategic development of provisioning protocols in managed care.

**Key Words:** caloric consumption, energetics, metabolic demands, reproductive state, temperature,

pregnant, lactating, bottlenose dolphins, *Tursiops truncatus*

## Introduction

The energetic requirements of Atlantic bottlenose dolphins (*Tursiops truncatus*) and other cetaceans have been broadly defined and quantified, yet little is known about how caloric intake changes in regards to reproductive state and small-scale changes in water temperature. Most efforts to quantify intake in odontocetes involve direct assessments of total and estimated kilograms or pounds ingested (Kastelein et al., 2002; Piercey et al., 2013), analyses of stomach contents post-mortem (Bernard & Hohn, 1989; Gómez-Campos et al., 2011), or estimated consumption as a proportion of total body mass (Kastelein et al., 2000a). Due to regional, seasonal, and species variations in kilocalorie (kcal) values of prey fish species and the limited opportunity for direct observations of feeding in marine mammals, caloric intake is rarely calculated (Kastelein et al., 2003a). However, studies of odontocetes in managed care have provided some insight regarding the influence of reproduction (Reddy et al., 1994; West et al., 2007) and season (Piercey et al., 2013) on appetitive response and consumption measured directly.

Biological, physical, ecological, and social factors influence the energetic requirements of odontocete species (e.g., Kastelein et al., 1993, 1999, 2000a, 2000b, 2001, 2002, 2003a; Reddy et al., 1993). Metabolic rate and relevant prey requirements are known to vary by size, activity, and life history stage (Kleiber, 1975; Kriete, 1995; Costa, 2002; Noren, 2002; Costa & Sinervo, 2004; Williams et al., 2006; Maniscalco et al., 2007; Williams & Noren, 2009), and lactation alters the caloric needs of females. For example, while energetic requirements in free-ranging female spotted

dolphins (*Stenella attenuata*) and bottlenose dolphins in human care during non-reproductive years only marginally changed during gestation (Kastelein et al., 2002) and were similar to those of adult males (Bernard & Hohn, 1989; Reddy et al., 1994; Kastelein et al., 2003a), lactating females consumed more and selectively nutrient rich fish (Bernard & Hohn, 1989; Kastelein et al., 2003a). Maternal strategy varies geographically among bottlenose dolphin populations, but the nursing period ranges from 2 to 7 y (Wells & Scott, 2009), which may require increased caloric intake for prolonged periods of time.

Similarly, changes in water temperature alter energetic expenditure as marine mammals work to mitigate heat loss in the highly conductive aquatic environment. For example, a decrease in water temperature initiates the thickening of the lipid-rich insulating blubber layer of cetaceans, which is supported by increased food intake (Williams & Friedl, 1990; Kastelein et al., 2000b; McEwen & Wingfield, 2003; Wells, 2009; Piercey et al., 2013). Kastelein et al. (2000b) noted a negative correlation between water temperature and food consumption among killer whales (*Orcinus orca*) at three zoological parks and an increased consumption with decreased water temperatures in a harbor porpoise (*Phocoena phocoena*; Kastelein et al., 2018). There was also a negative relationship between blubber layer thickness and water temperature noted among wild bottlenose dolphins (Williams et al., 1992; Meagher et al., 2008). However, the relationship between reproductive and thermoregulatory demands and food intake is not well defined (Kastelein et al., 1993, 1997, 2000b). In this study, a population of captive bottlenose dolphins was evaluated for changes in appetitive response and food intake with respect to reproductive status and small-scale changes in water temperature. Intake was reported using kilocalories calculated relevant to specific prey harvests, as well as a description of intake among various reproductive states, including females that were both pregnant and lactating. These data may contribute to a better understanding of odontocete energetics and models to predict the potential carrying capacity of various ecosystems, as well as improved energetic need estimates in managed care settings.

## Methods

### Site Descriptions

This study was conducted at Dolphins Plus Marine Mammal Responder, Dolphins Plus, and Island Dolphin Care, three ambient seawater zoological facilities in Key Largo, Florida, with seasonal water temperatures that typically range from 23 to 30°C annually, with extremes noted

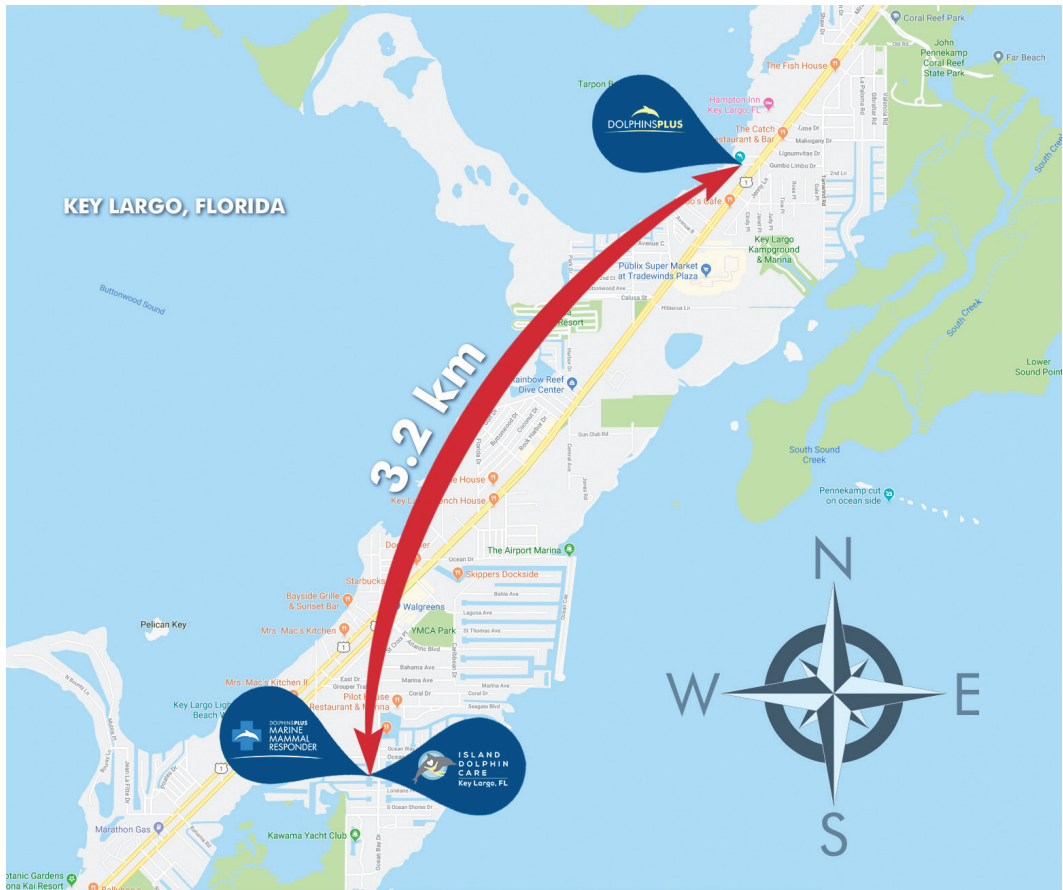
below 18°C and above 32°C (National Oceanic and Atmospheric Administration [NOAA], 2014). Dolphins Plus Marine Mammal Responder and Island Dolphin Care are located 3.2 km south of Dolphins Plus on a residential canal system open to the Atlantic Ocean with two rectangular lagoons, each encompassing an area of 1,672.30 m<sup>2</sup>, with a mean depth of 3.66 m ( $\pm 1.22$  m). Dolphin Plus is a single, 1,858.10 m<sup>2</sup> circular enclosure, with a mean depth of 5.50 m ( $\pm 1.22$  m), located on Florida Bay (Figure 1). Water temperatures for all sampled years and study sites ranged from 11.78 to 33.89°C ( $M = 26.02$ ,  $SD = 3.91$ ). Each study site was sampled independently *in situ* to ensure the relevant data accurately represented weekly caloric intake at each site.

### Subjects

Food intake, reflective of the appetitive response of each individual (see “Diet and Intake” for details) was assessed using diet records for 13 ( $n_{\text{males}} = 5$ ,  $n_{\text{females}} = 8$ ) sexually mature Atlantic bottlenose dolphins between 2002 and 2011. Daily diets were administered across various training sessions (e.g., husbandry, research, and interactions). Calves (dependent and nursing) and sub-adults (independent but not sexually mature), as defined by Eskelinen et al. (2015), were omitted from the study due to the potentially confounding influence of growth and maturation on metabolic demand. Annual caloric intake was compared among subjects and then grouped and compared relevant to specific life history stages or reproductive subpopulations as follows (number of years analyzed): adult males ( $n = 10$ ), adult females (not pregnant or lactating;  $n = 10$ ), pregnant females ( $n = 7$ ), non-pregnant and lactating females ( $n = 10$ ), and females that were pregnant and lactating simultaneously ( $n = 2$ ) (Table 1).

### Diet and Intake

Detailed dietary records were utilized to quantify intake (kcal) per calendar year among subjects and across reproductive subpopulations, analyzed as mean intake per week ( $n = 52$  for each sample year) to adjust for normal, small-scale fluctuations in caloric intake. The subjects’ diets were administered reactively, according to the appetitive response and under veterinary supervision of each individual, across a minimum of three feeds per day (e.g., training, husbandry, and research). A positive appetitive response was characterized by the subjects lifting their heads vertically above the water column with an open mouth, as well as increased proximity and physical following of animal handlers in possession of food or food containers. In contrast, a negative appetitive response was characterized by the subjects resting



**Figure 1.** Map of Key Largo, Florida, depicting distance between facilities and relevant watersheds

their heads horizontally on the water surface with a closed mouth in the presence of food, decreased proximity and attentiveness toward animal handlers, and/or spitting fish. Study subjects were not weighed routinely due largely to the lack of requisite instrumentation, and the basic assumption was that intake was balanced by demand, resulting in minimal to no change in overall body mass outside of pregnancy. For the duration of the study, animal care staff among the three facilities operated under the same training and husbandry practices and supervision.

The dietary records for each subject included the species and total weight (pounds) consumed per day. During the study period, nine species of fish were utilized (Table 2) in various combinations, with some species not available every year. To account for annual, intraspecies seasonal and geographic variation, samples of each species were sent to offsite laboratories (ABC Research Holding Company, Dairy One, and Eurofins Strasburger & Siegel, Inc.) each year for caloric analysis, and then

pounds were converted to kilocalories using the mean caloric value by species (Table 2).

#### *Water Temperature and Seasons*

Near surface water temperature was recorded one to three times a day (i.e., morning [~0800 h], midday [~1200 h], and afternoon [~1500 h]  $\pm 2$  h) at each facility for the duration of the study. Mean weekly temperature values were calculated per subject at their designated facility, respectively. Temperature was measured using a Laser Infrared Thermometer with a distance to spot ratio of 12:1 and an accuracy of  $\pm 2\%$  of reading or  $2^{\circ}\text{C}$ , whichever is greater. Missing data were supplemented by the National Data Buoy Center water station, located approximately 8 km from the facility at the Molasses Reef buoy  $25.010^{\circ}\text{N}$ ,  $80.380^{\circ}\text{W}$  ( $25^{\circ} 0' 36''\text{N}$ ,  $80^{\circ} 22' 48''\text{W}$ ). To ensure validity, water temperature data collected on-site ( $M_{\text{yearly}} = 25.85^{\circ}\text{C}$ ,  $\text{SD} = 3.46$ ) for a randomly selected year (i.e., 2007) were compared to the data buoy ( $M_{\text{yearly}} = 26.90^{\circ}\text{C}$ ,  $\text{SD} = 2.33$ ) water temperature data, revealing no significant

**Table 1.** Demographics of the study subjects, number of years analyzed, and reproductive status

Subject	Sex	Resident facility	DOB (d/mo/y)	Mean weight across study years (kg)	Sampled years	Data represented in reproductive state(s) (Number of years examined)
LB	M	Dolphins Plus Marine Mammal Responder	e1978	~312	2007-2009	Adult male (2)
AZ	M	Dolphins Plus	25/7/1993	216	2007-2009	Adult male (2)
KT	M	Dolphins Plus	27/8/1993	209	2007-2009	Adult male (2)
DE	M	Dolphins Plus	23/7/1994	248	2006-2008	Adult male (2)
BB	M	Dolphins Plus Marine Mammal Responder	28/8/1994	~204	2007-2009	Adult male (2)
DG	F	Dolphins Plus Marine Mammal Responder	e1977	~206*	2003-2011	Non-pregnant/lactating (2) Pregnant/non-lactating (1) Pregnant/lactating (1)
SY	F	Dolphins Plus	e1981	~184*	2002-2005	Non-pregnant/non-lactating (1) Non-pregnant/lactating (1) Pregnant/non-lactating (1)
ST	F	Island Dolphin Care	e1982	170*	2002-2010	Non-pregnant/non-lactating (1) Non-pregnant/lactating (2) Pregnant/non-lactating (1) Pregnant/lactating (1)
JA	F	Dolphins Plus Marine Mammal Responder	e1984	~189*	2003-2009	Non-pregnant/non-lactating (1) Non-pregnant/lactating (1) Pregnant/non-lactating (2)
SH	F	Island Dolphin Care	e1984	159*	2006-2010	Non-pregnant/non-lactating (1) Non-pregnant/lactating (2) Pregnant/non-lactating (1)
SA	F	Dolphins Plus Marine Mammal Responder and Dolphins Plus	e1984	~197*	2002-2011	Non-pregnant/non-lactating (2) Non-pregnant/lactating (1) Pregnant/non-lactating (1)
GE	F	Dolphins Plus	e1978	~225*	2006-2008	Non-pregnant/non-lactating (2)
NY	F	Dolphins Plus	e1983	~206*	2002-2007	Non-pregnant/non-lactating (1) Non-pregnant/lactating (1) Pregnant/non-lactating (1)

e = Estimated year of birth

~ = Approximate weight based on morphometrics as defined by Messinger et al. (2000): weight (in kg) =  $(0.08 \times \text{length}) + (0.0066 \times \text{girth}^2) - 140$  (measurement in cm) + 5.4 (only if male) - 9 (if age 2 to 10 y) + 9 (if girth is 1.5 m or greater)

\* = Mean weights not acquired during pregnancy

differences ( $t(102) = -1.83, p = 0.07$ ). Temperature was compared to caloric intake using 5°F intervals (converted to °C for analyses) to adjust for small-scale, diel fluctuations. Caloric intake was also compared among seasons in the Northern Hemisphere, using the relevant temperature data as follows: spring (March, April, and May), summer

(June, July, and August), fall (September, October, and November), and winter (December, January, and February).

#### Statistical Analysis

Statistical differences in caloric intake attributed to season (winter, spring, summer, and fall), water

**Table 2.** Species of fish fed to subjects and their individual nutritional value (2002 to 2011)

Fish species	Mean caloric value kcal/lb (kg)	Range of caloric value kcal/lb (kg)	Range of fat %/lb (kg)
Large fatty (Atlantic) herring <sup>1,2,3</sup> ( <i>Clupea pallasii</i> )	782.22 (1,724.50)	750.00-823.28 (1,653.47-1,815.02)	11.00-12.62 (4.99- 5.72)
Small fatty (Pacific) herring <sup>1,2,3</sup> ( <i>Clupea pallasii</i> )	703.31 (1,550.53)	575.61-831.00 (1,269.00-1,832.04)	8.10-13.00 (3.67-5.90)
Lean (Atlantic) herring <sup>1,2,3</sup> ( <i>Clupea harengus</i> )	504.66 (1,112.58)	503.48-545.00 (1,109.98-1,201.52)	3.06-6.00 (1.39-2.72)
Capelin <sup>1,2,3</sup> ( <i>Mallotus uillosus</i> )	422.93 (932.40)	381.02-449.96 (840.01-991.11)	3.20-4.25 (1.45-1.93)
Sardines <sup>1,2,3</sup> ( <i>Sardina pilchardus</i> )	635.11 (1,400.18)	479.90-810.00 (1,058.00-1,785.74)	2.60-5.40 (1.18-2.45)
Atlantic rainbow smelt <sup>1,2,3</sup> ( <i>Osmerus mordax</i> )	408.27 (900.08)	361.96-467.76 (797.99-1,031.23)	3.50-5.27 (1.59-2.39)
Peruvian smelt <sup>1,2,3</sup> ( <i>Odontesthes regia regia</i> )	525.97 (1,159.57)	484.00-585.43 (1,067.04-1,290.65)	4.30-6.47 (1.95-2.93)
Columbian river smelt <sup>1,2,3</sup> ( <i>Eulachon</i> )	748.69 (1,650.58)	597.38-900.00 (1,317.00-1,984.16)	9.30-15.00 (4.22-6.80)
Silversides <sup>1,2,3</sup> ( <i>Atheriniformes</i> )	608.98 (1,342.57)	560.00-666.77 (1,234.59-1,469.98)	6.46-8.00 (2.93-3.63)

Laboratories utilized for analyses:

<sup>1</sup>ABC Research Holding Company, 3437 SW 24th Avenue, Gainesville, FL 32607, USA

<sup>2</sup>Dairy One, Forage Laboratory, 730 Warren Road, Ithaca, NY 14850, USA

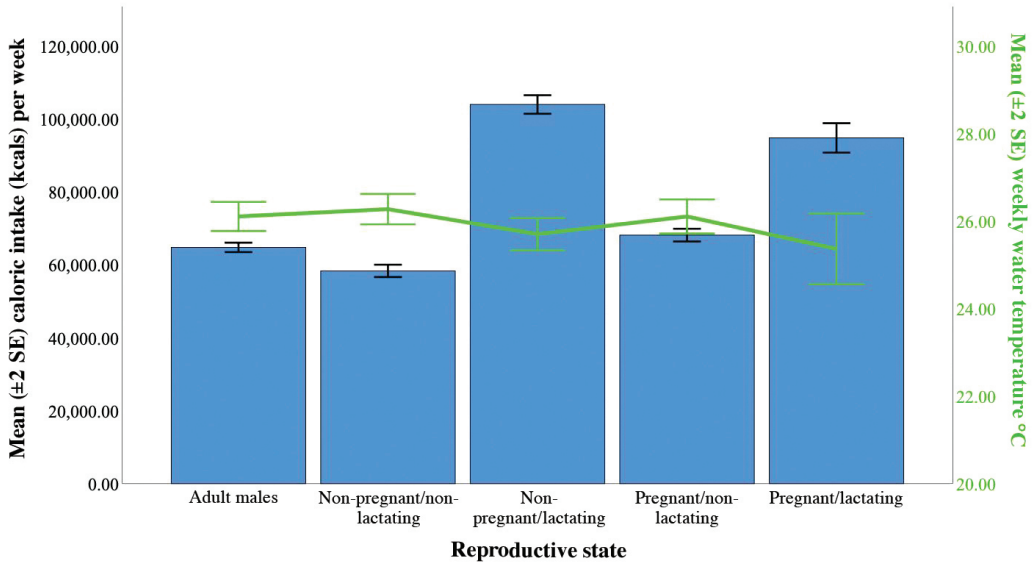
<sup>3</sup>Eurofins Strasburger & Siegel, Inc., 7249 National Drive, Hanover, MD 21076, USA

temperature, and reproductive state (i.e., categorical fixed factors) were assessed separately using Linear Mixed-Effects (LME) models with Restricted Maximum Likelihood (REML) test due to both fixed effects (season, water temperature, and reproductive state) and random effects (animal) within the model (Laird & Ware, 1982; Pinheiro & Bates, 2000). The model enables data from each subject within testing years and data from individual subjects across reproductive states to be examined as a repeated measure, taking into account correlations between and among repeated measures. Subjects were treated as random effects to account for repeated measures. Likelihood-ratio tests compared the likelihood of the models (Pinheiro & Bates, 2000). For significant differences, a post-hoc Tukey test was conducted to determine between group differences. Data were analyzed using R ('lme4' package) and SPSS, Version 24, data analysis software. Caloric consumption and other values are presented as mean  $\pm$  SE.

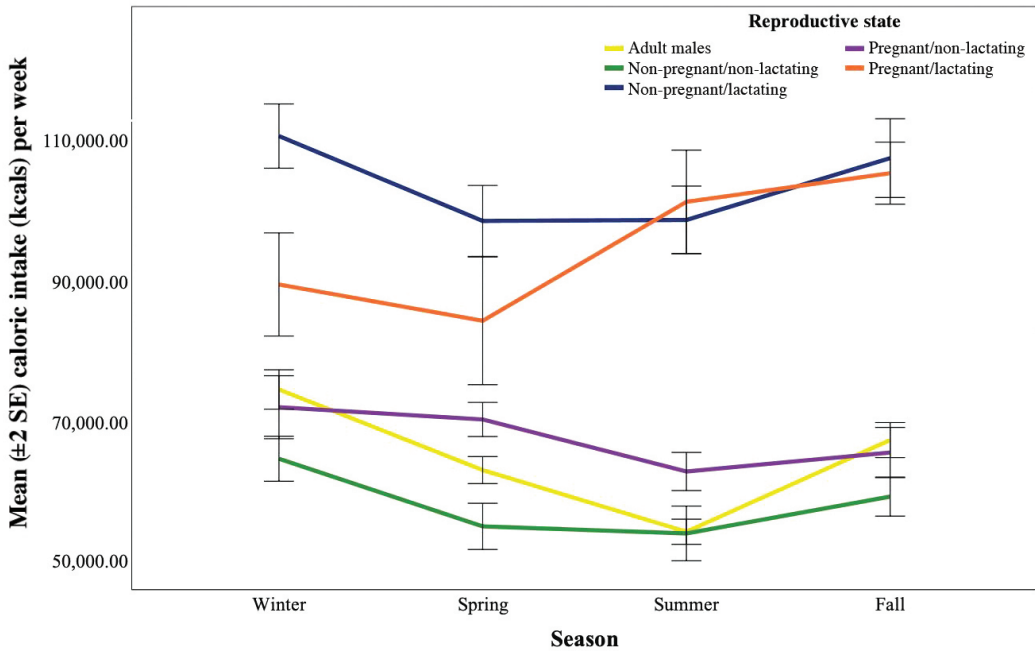
## Results

Mean weekly caloric intake (kcal) of adult males ( $M = 64,729.83$ ,  $SE = 643.99$ ), non-pregnant/non-lactating adult females ( $M = 58,292.81$ ,  $SE = 845.94$ ), non-pregnant/lactating females ( $M = 103,877.38$ ,  $SE = 1,270.65$ ), pregnant/non-lactating females ( $M = 67,911.60$ ,  $SE = 858.91$ ), and pregnant/lactating females ( $M = 94,719.22$ ,  $SE = 1,998.86$ ; Figure 2) was assessed using LME models with REML and revealed reproductive state as a significant predictor of caloric intake ( $p < 0.05$ ). Tukey post-hoc analyses revealed all reproductive categories differed significantly from one another except for adult males compared to non-pregnant/non-lactating as well as pregnant females ( $p < 0.05$ ), with lactating females (both pregnant and not pregnant) exhibiting the highest rates of intake and non-pregnant/non-lactating adult females exhibiting the lowest (Figure 2).

A log-likelihood ratio test revealed season as well as season and reproductive state combined significantly predicted caloric intake for all subjects combined ( $p < 0.05$ ). Tukey post-hoc analyses for all



**Figure 2.** Mean ( $\pm 2$  SE) caloric intake (kcal, bars) and mean ( $\pm 2$  SE) water temperature ( $^{\circ}\text{C}$ , line) per wk ( $n = 52$ ) for each reproductive subpopulation per sample year ( $N_{\text{years}} = 41$ )



**Figure 3.** Mean ( $\pm 2$  SE) caloric intake (kcal) per wk ( $n = 52$ ) for each reproductive subpopulation per sample year ( $N_{\text{years}} = 41$ ) by season: winter (December to February), spring (March to May), summer (June to August), and fall (September to November)

reproductive states combined revealed significantly higher intake in the winter ( $M_{\text{Water Temperature}} = 21.81^{\circ}\text{C}$ , range 11.78 to  $31.11^{\circ}\text{C}$ ) compared to spring ( $M_{\text{Water Temperature}} = 26.14^{\circ}\text{C}$ , range 16.94 to  $31.22^{\circ}\text{C}$ ) and summer ( $M_{\text{Water Temperature}} = 29.92^{\circ}\text{C}$ , range 18.66 to  $33.89^{\circ}\text{C}$ ) ( $p < 0.05$ ) but not when compared to fall ( $M_{\text{Water Temperature}} = 25.88^{\circ}\text{C}$ , range 14.72 to  $31.66^{\circ}\text{C}$ ); and fall was associated with a significantly higher intake than spring and summer (Figure 3).

Mean weekly caloric intake (kcal) of non-pregnant/non-lactating females and adult males for all seasons throughout the study years was  $63,167.91 \pm 1,185.88$  kcal. A LME with REML revealed season and water temperature ( $p < 0.05$ ), as well as the interaction between season and water temperature, as significant predictors of caloric intake ( $p < 0.05$ ). Mean caloric consumption was lowest during the summer ( $56,420.80 \pm 3,261.86$  kcal) and highest during the winter ( $68,747.87 \pm 2,106.16$  kcal). Tukey post-hoc analyses revealed a significantly higher intake in the winter ( $M_{\text{Water Temperature}} = 22.19^{\circ}\text{C}$ , range 14.44 to  $28.89^{\circ}\text{C}$ ) compared to spring ( $M_{\text{Water Temperature}} = 25.98^{\circ}\text{C}$ , range 16.94 to  $31.22^{\circ}\text{C}$ ), summer ( $M_{\text{Water Temperature}} = 30.08^{\circ}\text{C}$ , range 18.67 to  $33.88^{\circ}\text{C}$ ), and fall ( $M_{\text{Water Temperature}} = 26.17^{\circ}\text{C}$ , range 14.72 to  $31.67^{\circ}\text{C}$ ), as well as significantly lower intake in summer when compared to the fall ( $p < 0.05$ ) (Figure 4).

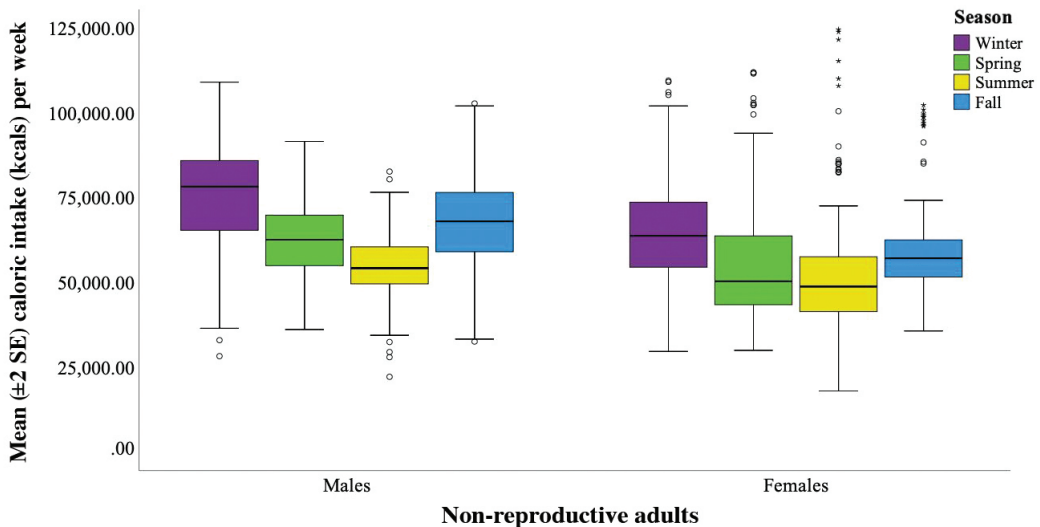
#### Reproductive Females

The mean caloric intake ( $92,508.23 \pm 3,363.73$  kcal) of reproductive females (i.e., pregnant/non-lactating, non-pregnant/lactating, and pregnant/

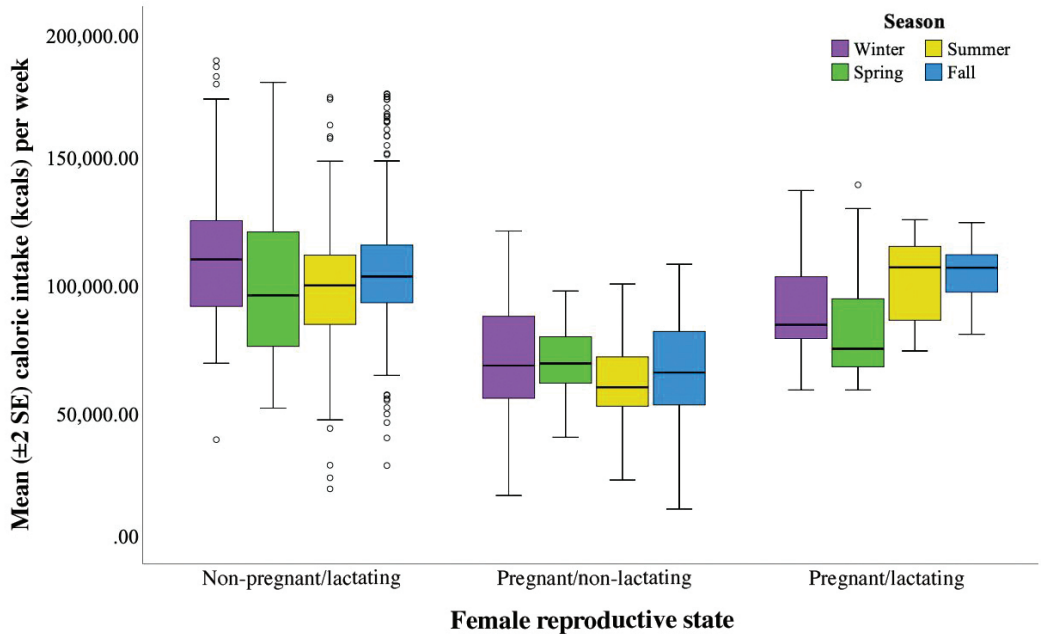
lactating) was analyzed using a LME with REML and revealed reproductive group and season as significant predictors of caloric intake ( $p < 0.05$ ). Tukey post-hoc analyses revealed a significantly lower intake in the spring ( $M_{\text{Water Temperature}} = 26.24^{\circ}\text{C}$ , range 16.94 to  $36.39^{\circ}\text{C}$ ) when compared to the fall ( $M_{\text{Water Temperature}} = 25.69^{\circ}\text{C}$ , range 14.72 to  $31.5^{\circ}\text{C}$ ) ( $p < 0.05$ ), and no significant differences for the summer ( $M_{\text{Water Temperature}} = 29.81^{\circ}\text{C}$ , range 24.78 to  $33.89^{\circ}\text{C}$ ) or winter ( $M_{\text{Water Temperature}} = 22.28^{\circ}\text{C}$ , range 11.77 to  $30.83^{\circ}\text{C}$ ) seasons. Among reproductive states, non-pregnant/lactating females consumed significantly more than pregnant/non-lactating and pregnant/lactating females ( $p < 0.05$ ), and pregnant/non-lactating females consumed significantly less than the other two reproductive states ( $p < 0.05$ ) (Figure 5).

## Discussion

Field metabolic rate (FMR) has been described as “the cost of living” (Speakman, 2000) or the energy required for all biological functions and activities. When animals increase energy expenditure above resting metabolic rate (RMR), whether for maintenance, activity, or heat production, the predicted response is to balance the energetic demand by increased food intake. To date, published studies of bottlenose dolphin prey requirements and dietary trends in managed care have been limited to measurements of intake as a proportion of body mass or standard units of mass (e.g., kilograms)



**Figure 4.** Mean ( $\pm 2$  SE) caloric intake (kcal) per wk ( $n = 52$ ) for adult females that were not pregnant or lactating per sample year ( $n_{\text{years}} = 9$ ) and adult males per sample year ( $n_{\text{years}} = 10$ ) across seasons: winter (December to February), spring (March to May), summer (June to August), and fall (September to November) ( $n = 5$  males,  $n = 7$  females)



**Figure 5.** Mean ( $\pm 2$  SE) caloric intake (kcal) per wk ( $n = 52$ ) for females that were non-pregnant/lactating per sample year ( $n_{\text{years}} = 11$ ), pregnant/non-lactating per sample year ( $n_{\text{years}} = 8$ ), and pregnant/lactating per sample year ( $n_{\text{years}} = 2$ ) across seasons: winter (December to February), spring (March to May), summer (June to August), and fall (September to November)

consumed (Kastelein et al., 2002, 2003a). This study quantified intake as kilocalories representative of specific fish harvests to expand upon these foundations with a more accurate measure of intake relevant to changes in water temperature and reproductive state. Results indicated that reproductive state, small-scale changes in water temperature, and season were significant predictors of caloric intake in bottlenose dolphins, suggesting that both thermoregulation and gestation/lactation significantly alter the energetic needs of bottlenose dolphins on an annual cycle.

When temperatures drop below the thermoneutral range, increased intake and utilization of metabolic substrates to produce heat is induced in an effort to maintain a constant internal body temperature, and water temperature exhibits an inverse relationship with food intake in a variety of odontocete species (e.g., killer whales: Kastelein, 2000a; Pacific white-sided dolphins [*Lagenorhynchus obliquidens*]: Piercey et al., 2013; and harbor porpoises: Kastelein et al., 2018). The results of this study further validate the central role thermoregulation plays on the energy budget of odontocetes, and significant changes in food intake were recorded relevant to changes in temperature of only 2.83°C. Although food intake is an indirect measure of cold-induced energy

expenditure, an increase in metabolism with temperature (see Cannon & Nedergaard, 2011) also reflects the endogenous activation of brown adipose tissue (BAT), which was recently identified, along with the mitochondrial uncoupling protein (UCP1) in bottlenose dolphin blubber (Hashimoto et al., 2015). Increased food intake also functions to adjust the lipid rich blubber layer for insulation (Williams & Friedl, 1990; Cheal & Gales, 1992; McEwen & Wingfield, 2003; Wells, 2009; Piercey et al., 2013), though this phenomenon occurs over comparatively longer time scales (Worthy & Edwards, 1990; Worthy, 1991).

Reproductive state and sex also had significant effects on the caloric intake of bottlenose dolphins. Previous studies of bottlenose dolphins in captivity revealed variations of caloric intake pre-, during, and postparturition (Reddy et al., 1994), as well as during lactation (West et al., 2007). The results of this study were similar but noted that the energetic requirements of non-reproductive females were less than adult males and lactating females (i.e., pregnant/lactating and non-pregnant/lactating). With little to no sexual dimorphism in the study population, this may reflect differences in energy expenditure between males and females. Adult males allocate a significant portion of their energy budget toward accessing and mating with



females, while adult females predominantly allocate resources toward caring for their offspring (Trivers, 1972; Krützen et al., 2004). As such, it is possible that a female without a dependent calf may experience lower energetic demands when compared to sexually mature adult males.

Caloric intake was also greater in pregnant/non-lactating females than non-reproductive adult females, which has also been documented in studies conducted by Reddy et al. (1991) (bottlenose dolphins) and Kriete (1995) (killer whales), though in both cases the increase occurred only during the last month of gestation. Lockyer (1981) estimated a 5 to 10% increase in food consumption among pregnant sperm whales (*Physeter macrocephalus*) starting during the 6th mo of gestation. Kastelein et al. (1994) identified only marginal changes in food intake during gestation in beluga whales, but this could reflect limitations associated with their use of kilograms as a measure of intake. Variations in mean caloric values of fish species ranged from  $\pm 408.27$  kcal in smelt to  $\pm 782.22$  kcal in herring, and Columbian river smelt exhibited a seasonal/yearly variation of  $\pm 302.62$  kcal per pound. Given these intraspecies variations, kcalories are a more accurate measure of energetic intake, making comparisons among studies difficult. Additionally, although lactation is known to be associated with an energetic demand significantly greater than gestation in marine mammals (Bernard & Hohn, 1989; Kastelein et al., 2003a), metabolic rate increases during pregnancy in most mammals (Brody, 1954; Gittleman & Thompson, 1988). In some exceptional cases, such as that of the northern elephant seal (*Mirounga angustirostris*), pregnant females have been noted to decrease their metabolic rate while engaging in energetically economic swim patterns, thereby stockpiling energy in preparation for the costly process of lactation (Maresh, 2014).

The energetic demand of lactation is associated with a marked increase in food intake in marine mammals (Bernard & Hohn, 1989; Recchia & Read, 1989; Kastelein et al., 1993, 1994, 2002, 2003a; Reddy et al., 1994; Kriete, 1995; Noren et al., 2012), ranging from 129 to 204% in captive bottlenose dolphins (Reddy et al., 1991). This may reflect specific needs of individual calves as well as the ability of calves to successfully obtain solid foods during weaning. In this study, the ages of dependent calves ranged from neonates through 2 y, and each calf began consuming fish during the annual sampling period. On average, lactating females consumed 52.04% more kcalories when compared to non-reproductive years and the intake of other non-reproductive females. To assess the energetic demand across multiple years of lactation and the development of a single calf, supplemental

analyses of two females (DG and JA) revealed a sustained, elevated caloric intake, with no significant differences across the first three consecutive years of lactation. Additionally, lactating females consistently consumed more calories than other reproductive subpopulations despite variations in water temperature. This indicates the high total cost of the lactation period over the course of a year, during which time females may experience periods of excess energy storage in warmer months, with those energy savings then utilized to support the thermoregulatory demands of colder months concurrently with milk production.

Pregnant/lactating females consumed less than non-pregnant/lactating females but sustained consistently high levels of food intake that did not vary across seasons. There is a paucity of published data regarding the combined energetic cost of a female marine mammal that is both pregnant and lactating. The discrepancy in energetic demand could reflect the thermoregulatory requirements of the fetus, which is kept cool despite being surrounded by muscle and blubber via a specialized arteriovenous plexus that transports cooled blood from the surface of the body (i.e., dorsal fin and flukes) to the uterus (Rommel et al., 1998). Pregnant and lactating females could strategically utilize the requisite exposure to cold water, resulting in a net reduction in the total heat conservation demand. This critical reproductive state may also result in improved energy economy via alterations in locomotion and periods of hypometabolism, which has been identified in seals during gestation (Renouf & Gales, 1994; Hedd et al., 1997; Sparling et al., 2006; Ochoa-Acuña et al., 2009; Maresh, 2014). This result may be due to the difference in body volume to surface area ratio despite the energetic requirements of a growing fetus (McBride & Kritzler, 1951; Tavolga & Essapian, 1957; Kastelein et al., 2003a). The discrepancy could also reflect the comparative milk yield of lactating vs pregnant/lactating females, with the former likely remaining high and consistent through late pregnancy and the latter reducing significantly in quantity and quality as the fetus develops (see Partidge et al., 1986).

It is highly plausible that pregnant and lactating females selectively allocate energy toward the fetus and fetal growth vs milk production for a weaning calf, potentially explaining why lactating females consumed more than pregnant/lactating females. Biochemical increases of milk fat and milk protein have been observed in *Tursiops* sp. during lactation with concurrent pregnancy, which may be influenced by parity and increased body stores, as well as calorically dense and stable diets (West et al., 2007). Pregnant/lactating females exhibited consistent and stable diets, with a higher relative proportion of fat rich fish species (e.g., Pacific and

Atlantic “fatty” herring [*Clupea pallasii*]), and routinely exhibited more robust body conditions (Turner et al., 2017) when compared to all other reproductive states, making them ideal candidates to balance the allostatic load of lactating with a concurrent pregnancy.

This study is limited by both the assumption of a state of energy equilibrium (e.g., lagoon size) and the use of appetitive response and food intake as a measure of energetic demand. Since all the study subjects were not weighed routinely, due largely to the lack of requisite instrumentation, the morphometric equation (Messinger et al., 2000) was conducted quarterly at minimum on all subjects. Thus, the basic assumption was that intake was balanced by demand, resulting in only seasonal changes in overall body mass outside of pregnancy. Although data regarding animal weights were not available, there were no noted increases or decreases in body mass among non-pregnant subjects, and all immature animals were excluded to avoid the implication of growth on energy budgets. Even so, organismal metabolism interacts with temperature and body mass in complex ways in terrestrial mammals (see Gillooly et al., 2001), and these phenomena are minimally understood in marine mammals (Kastelein et al., 2018). Given the difficulties associated with accessing marine mammals in the wild and the complex and invasive nature of standard metabolic methods, evaluating food intake is often the only realistic metric for assessing the energetic needs of marine mammals. However, although appetite and consumption work on a feedback loop regulated predominantly by physiological signals (e.g., hormones and levels of circulating glucose; see Lam & Ravussin, 2016), there are some cognitive and environmental influences such as sensory stimulation and the regular timing of meals (Sørensen et al., 2003; Mattes, 2005). These interactions are poorly understood in non-human animals and, as such, were not evaluated in this study.

This study contributed to the body of literature of appetitive response and food intake in odontocetes, specifically utilizing calories representative of select prey harvests as a measure of energy intake of various reproductive states, including females that were simultaneously pregnant and lactating. Though additional studies are needed to further elucidate metabolic demand and interactions between thermoregulation and reproduction, including evaluations of both energy intake and expenditure relative to body mass, these data add valuable baseline information to the understanding of marine mammal energetics. Conservative estimates of caloric need derived from studies of captive odontocetes may be

extrapolated to wild conspecifics to estimate the carrying capacity of specific habitats, the resilience of cetaceans in the face of increasing anthropogenic pressures and environmental change, and the impact cetaceans have on prey resources and trophic cascades (e.g., Breverton, 1985; Trites et al., 1997; Boyd, 2002). These findings may also prove valuable to aquarium curators and animal care staff in establishing balanced diets, particularly among dolphins housed in natural seawater facilities with seasonal changes in water temperature.

### Acknowledgments

We are grateful to all the animal care/training staff members at each facility for the precise record-keeping throughout the years. We are also grateful for the many hours Robin Bates, Brittany Jones, and the Dolphins Plus research interns (Alexandra Fine, Kelsie Murchy, and Trista Burch) dedicated to data entry and management. Additionally, thank you to Benjamin Young for his statistical guidance, Kelley Winship and Shaun Perisho for their comments on the manuscript, and Theater of the Sea for providing NY’s (adult female) records for use in this project.

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