Thermal tolerance in bottlenose dolphins (*Tursiops truncatus*)

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SUMMARY

Water and air temperature are potentially limiting factors to the pole-ward distributions of coastal bottlenose dolphins. This study assessed the lower critical temperature of captive bottlenose dolphins to air temperature (LCT_a) and water temperature (LCT_w) through the use of open flow respirometry. Five dolphins, ranging from 14 to 33 years of age and acclimated to the waters of the southern California coast (14.2–22.5°C), were subjected to water temperatures ranging from 0.2 to 18.0°C. Two of the animals were additionally subjected to air temperatures ranging from –2.4 to 17.8°C while maintaining water temperature approximately 3°C above their individual LCT_w. The LCT_w ranged from 5.5 to 10.6°C and generally decreased with increasing animal mass; for dolphins in excess of 187 kg, the LCT_w ranged from 5.5 to 5.7°C. No LCT_a could be determined across the range of air temperatures tested. Core body temperature remained within the limits of normal body temperatures reported for dolphins but demonstrated a direct relationship to water temperature in three subjects and varied across a range of 1.5°C. Air and water temperature had a minimal synergistic effect on dolphin thermoregulation, i.e. water temperature exerted the predominant impact on thermoregulation. For dolphins in excess of 187 kg, water temperature alone would appear to be insufficient to limit the use of habitat north of current bottlenose dolphin ranges along the coastal United States. However, thermal impacts to smaller dolphins, in particular adolescents, neonates and accompanying females, may work in concert with other factors (e.g. prey distribution, predator avoidance, social interactions) to influence coastal residency patterns and population structure.

Key words: bottlenose dolphin, *Tursiops truncatus*, thermoregulation, energetics, lower critical temperature.

INTRODUCTION

Bottlenose dolphins (*Tursiops truncatus*, Montagu 1821) inhabit almost all tropical and temperate oceans between 45°N and 45°S and are found in both coastal and pelagic environments (Wells and Scott, 1999). Bottlenose dolphins range from southern Nova Scotia southward to Patagonia and the tip of South Africa in the Atlantic Ocean, and along both sides of the Pacific Ocean from northern Japan and central California southward to Australia, Chile and New Zealand (Wells and Scott, 1999; Wells et al., 1990). Bottlenose dolphins have been observed at more extreme pole-ward latitudes, as far as 76°N (McBrearty et al., 1986), but such sightings are typically considered extralimital. Notable exceptions are populations that live along the UK coasts (57°N) (Bristow, 2004; Hammond and Thompson, 1991; Wilson et al., 1997). Dolphins reside in the waters around the UK year round but with seasonal fluctuations in the number of animals present; during the winter and spring, when the water temperatures are at their lowest, the number of animals decreases (Wilson et al., 1997; Wood, 1998).

Variations in water temperature and prey availability have been postulated as two factors that influence the distribution and seasonal movements of dolphins (Shane et al., 1986; Gaskin, 1968). Distinguishing the relative importance of each of these factors is a difficult task. Although water temperature and habitat selection are related in some dolphin species (Bräger et al., 2003; Maze and Wursig, 1999; Barco et al., 1999; Zolman, 2002), dolphin distributions and water temperature appear to be co-related to seasonal redistributions of prey species (Bräger, 1993; Bräger et al., 2003; Maze and Wursig, 1999; Wells et al., 1990). For example, bottlenose dolphin distribution extended northward along the Californian coast during some El Niño years (Wells et al., 1990). During these ocean-warming periods, the range of the dolphins' prey species also extended northward, making it difficult to determine whether the dolphins primarily responded to the redistribution of prey, or whether both they and the prey were primarily responding to the warmer water conditions.

Differentiating the relative influence of prey distribution and water temperature on dolphin habitat utilization requires an understanding of dolphin thermal limits. The lower critical temperature (LCT) defines the temperature at which an increase in the metabolic rate is required to compensate for an increasing amount of heat lost to the environment, i.e. it defines the lower limit of the range of thermoneutrality (Schmidt-Nielsen, 1997). Determining the LCT of the bottlenose dolphin provides a physiological context within which to evaluate dolphin habitat utilization as a function of water temperature. Empirical measurements of the LCT in bottlenose dolphins are few (Williams et al., 2001). By measuring the basal metabolic rate of three adult male bottlenose dolphins, Williams and colleagues were able to determine a mean LCT of 5.9°C. This average value provides the only published LCT for bottlenose dolphins to date. Because the data from all of the animals were pooled, the impact of individual animal size on the LCT is unknown. Further quantification of LCT as a function of body mass is warranted since mass has been positively related to cold water habitation by bottlenose dolphins (Ross and Cockcroft, 1990).

Air temperature may also influence dolphin thermal limits. Dolphins, unlike most terrestrial mammals and pinnipeds (seals and sea lions), lack nasal turbinates (Hillenius, 1992). In most terrestrial mammals, the bony nasal turbinates help to warm and humidify

Table 1. Animal sex, age, mass, mean resting metabolic rate (RMR) and lower critical water temperature (LCT$_w$)

Animal	Sex	Age (years)	Length (cm)	Mass (kg)	RMR (ml O$_2$ min^{-1} kg^{-1})	Acclimation temperature (°C)	LCT$_w$ (°C)
Tt1	M	27	252	177.3	3.50±0.28 (10)	22.5±0.7	7.8
Tt2	M	24	250	191.4	4.06±0.57 (10)	22.8±1.4	5.7
Tt3	M	26	262.5	219.7±1.6	3.05±0.45 (10)	15.2±0.4 (12.9–17.9)	5.6
Tt4	M	14	241	187.0±4.1	4.4±0.6 (11)	15.4±1.2 (13.8–17.9)	5.5
Tt5	F	33	239	178.2±1.9	4.0±0.2 (4)	14.2±0.2	10.6

Numbers in parentheses for RMR indicate the number of trials within the thermoneutral zone and therefore used to calculate the mean RMR (±s.d.). Mass was measured weekly during the study period, but only a single mass was measured for the dolphins that spent the entire measurement period in the environmental control facility (Tt1 and Tt2). Acclimation temperature range is given in parentheses.

inhaled air prior to reaching the lungs, diminishing the loss of heat from the core that is required to warm the lung air (Jackson and Schmidt-Neilsen, 1964). Lacking nasal turbinates, dolphins exposed to cold air would be required to warm air within the lung *via* heat transfer from associated tissues and may need to rely on regional increases in blood flow to combat the drying of the pulmonary mucosa (Baile et al., 1987). The exposure of lung epithelia to cold air could potentially affect epithelial performance and integrity, and the loss of heat from the core of the body could increasingly challenge thermal balance if coupled with cold water temperatures.

In this study we assessed the lower critical water temperature and the effects of cold air inhalation for five Atlantic bottlenose dolphins (*Tursiops truncatus*) acclimated to a mid-latitude climate. The dolphins, housed in open water pens within San Diego Bay, are acclimated to the coastal water temperatures of southern California and provide the opportunity to obtain information on thermal tolerance applicable to conspecifics within the same region. Wild Pacific bottlenose dolphins (also *Tursiops truncatus*) along the coast of California are generally found from the central coast (~38°N) southward; bottlenose dolphins have been observed as far north as Washington State (~46°N), although these sightings are considered extralimital (Ferrero and Tsunoda, 1989). The results of this study provide insight on the tolerance of bottlenose dolphins to cold water and air, thus permitting the relative importance of environmental temperatures to be related to habitat utilization.

MATERIALS AND METHODS
Animals
Five adult Atlantic bottlenose dolphins ranging from 14 to 33 years of age (Table 1) participated in the study. All subjects were operational animals within the US Navy Marine Mammal Program (MMP) and subject availability was dictated by the operational requirements of the animals. All procedures were approved by the Institutional Animal Care and Use Committee of the Biosciences Division, Space and Naval Warfare Systems Center (SSC) San Diego, CA, USA, and followed all applicable USA Department of Defense guidelines for the care of laboratory animals.

The dolphins were housed at SSC San Diego in floating net pens within San Diego Bay. Water temperatures in San Diego Bay were obtained from a buoy moored inside San Diego Bay (Station ID: 9410170, latitude 32°42.8′N, longitude 117°10.4′W) that is operated by the National Oceanographic Data Center. All dolphins had been previously trained for short transports between the net enclosure and pools. Animals were fed a mixed diet of herring (*Clupea harengus*), mackerel (*Scomber scombrus*) and capelin (*Mallotus villosus*) and were supplemented with vitamins (Mazuri® Tabs, PMI Nutrition International, Richmond, IN, USA). Animals were weighed weekly to the nearest 0.5 kg during the period of the study using a hanging crane scale (Challenger 2, Model 3360,

Measurement Systems International, Seattle, WA, USA; Table 1). For each animal, blubber depth was measured weekly at two dorsal, two lateral and two ventral body sites (Fig. 1). Blubber depth measurements were made from the skin surface to the blubber muscle interface using a SonoSite Titan portable imaging ultrasound (SonoSite, Bothell, WA, USA) with a C60 (5–2 MHz), 60 mm broadband transducer and at 0.01 cm resolution. Measurements were made with the animal in the water while the animal voluntarily presented itself for the ultrasound measurements. Natural skin markings on the dolphins were used to consistently identify measurement sites for repeated ultrasound measurements. Dorsal (D1, D2) measurements were taken approximately 1 cm to the right of midline, whereas lateral (L1, L2) measurements were taken along the corresponding lateral midline (i.e. halfway between the ventral and dorsal surfaces). Ventral sites (V1, V2) were taken midline, approximately 10 cm cranial and caudal of the umbilicus.

Environmental control facility
An environmental control facility (ECF) was constructed at the SSC San Diego. The facility was created to control the air and salt water temperatures to which subject animals were exposed. The facility consisted of a spherical pool (4.9 m diameter×1.2 m deep; Vogue Pool Products, LaSalle, QC, Canada) within a 6.4 m×6.4 m×3.4 m insulated containment room (Ace Coolers, San Diego, CA, USA). The pool was filled with sea water from San Diego Bay. The water was continuously recirculated through a closed loop system with an ozone disinfection system and a course sand filter to remove particulate matter from the pool. Pool water temperature (T_w) was controlled by an Odyssey in-line water chiller (Trane, Cullen, LA, USA), which was capable of maintaining temperatures within ±1°C and permitted a minimum T_w of approximately 0.2°C. Air temperature (T_a) was controlled by a refrigeration unit (model KEZA030M6-HT3A-B, Keep Right Refrigeration, Ontario, Canada). The refrigeration system was capable of lowering air temperature to below 0°C and demonstrated a temperature variability of ±0.7°C after reaching the temperature set point. Both water and air temperature were monitored using digital thermometers (Aquathermo, XTE-5320, Aube Technologies, Québec, Canada).

Oxygen consumption
Oxygen consumption ($\dot{V}_{O_2}$) measurements were conducted while the dolphins were housed in the ECF. Of the five dolphins, two (Tt1, Tt2) were transported to the ECF and remained there for a period of 10 days. Two (Tt4, Tt5) were transported to the ECF for 3–4 day periods with 3–4 days between pool sessions until testing across all T_w was completed. Testing was completed on these dolphins within 30 days of the first measurement. Water temperature was decreased gradually overnight while the dolphins resided in the pool. On return trips to the pool (before a 3–4 day residency) water
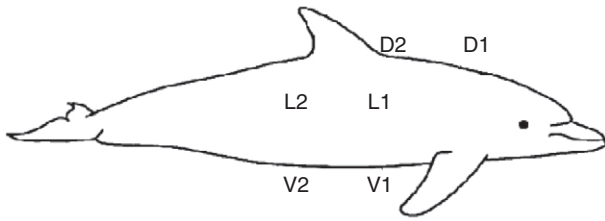
Fig. 1. Measurement sites for blubber depth on the bottlenose dolphins. The letters designate the six sites where the probe from the portable ultrasound machine was placed on the dorsal (D1, D2), lateral (L1, L2) and ventral (V1, V2) surfaces. Dorsal and lateral measurements were made on or just off the midline of the dolphin.
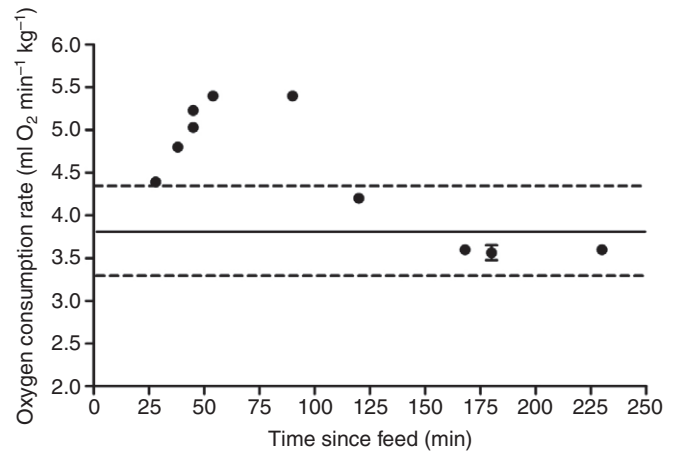

Fig. 2. Rate of oxygen consumption in relation to time since last feed (1.4 kg capelin). All points are single measurements from one dolphin (Tt3) with the exception of 180 min postfeed where the point represents the mean (±1 s.d.) of three measurements taken on different days. The solid line represents the mean rate of oxygen consumption for all dolphins at thermoneutrality. Dashed lines indicate ±1 s.d. of that mean.

temperature was preset to the test temperature. The largest difference between the ambient ocean temperature and the experimental pool temperature experienced by these animals was 9°C. Dolphin Tt3 was transported from the net pens (water temperature range, 12.9–17.9°C) to the ECF on a daily basis. Pool water was cooled to testing temperature prior to the arrival of the dolphin at the ECF. The maximum temperature difference between the net pens and pool temperature this dolphin experienced occurred in February and was 15.2°C.

The dolphins were trained to rest quietly under a clear acrylic metabolic dome (112 cm×76 cm×44.5 cm) for 10–20 min to permit the collection of respiratory gases. The metabolic dome floated on top of the water and was sealed against the water surface through the suction created by the flow of air through the dome (see below). A rope restraint connected the dome to the side of the pool and prevented the dome from being pulled underwater by the air flow. A neoprene-covered plate was mounted on the interior of the dome for the animal to bite onto and to help maintain its position while resting under the dome.

The respirometry system was calibrated daily with dry, ambient air (20.95% O_2) and weekly using dry nitrogen gas (100% dry N_2). The flow of calibration gases into the dome was controlled by a ball flow meter (Cole Palmer Instrument Company, Vernon Hills, IL, USA) that was accurate to within 2% of flow. The theoretical fraction of O_2 leaving the dome was calculated (Davis et al., 1985; Fedak et al., 1981) and compared with measured values from the oxygen analyzer.

Metabolic rates were estimated from $\dot{V}_{O_2}$ as measured by the open flow respirometry methods of Williams and colleagues (Williams et al., 2001). Air was pulled through the metabolic dome at a rate of 190–300 l min^{-1} (depending on animal mass) by a mass flow controller (Flow Kit 500, Sable Systems International, Henderson, NV, USA). A sub-sample of dome exhaust was dried (Drierite, WA Hammond Drierite Company, Xenia, OH, USA) and scrubbed of CO_2 (Sodasorb, WR Grace and Company, Cambridge, MA, USA) and dried again prior to entering the oxygen analyzer (FOXBOX, Sable Systems International). Oxygen content of the subsample was logged on a laptop computer every 1 s. Flow rates were corrected to STPD (standard temperature and pressure, dry) and gas contents were converted to oxygen consumption rates using equation 4b of Withers (Withers, 1977) with fasting respiratory quotient (RQ) assumed to be 0.85 (Schmidt-Nielsen, 1997). Steady-state resting $\dot{V}_{O_2}$ was estimated by selecting the most level 5 min section of continuous data points (Expedata, Sable Systems International).

Since fish were utilized as positive reinforcement for the completion of research behaviors, the potential impact of the heat increment of feeding on the timing of $\dot{V}_{O_2}$ measurements was

assessed. One dolphin (Tt3) was trained for daily transports to and from the ECF and tested once daily over a 3 week period. Oxygen consumption measurements were conducted at thermoneutrality (T_w=15°C, T_a=20°C) after feeding with 1.4 kg of capelin. Measurements were taken on separate days at 30, 40, 45, 55, 120, 170, 180 and 230 min following the feed. These data were then compared with resting metabolic rates (RMR) obtained after an overnight fast and used to determine the minimum time required for an animal to return to a fasting RMR following a maximum fish reward of 1.4 kg of capelin. The $\dot{V}_{O_2}$ reached a peak value at ~50 min following the consumption of 1.4 kg of capelin, when it was 57.1% over the mean resting oxygen consumption rate (Fig. 2). Thereafter, $\dot{V}_{O_2}$ declined steadily towards the resting level and returned to fasting oxygen consumption rates within 180 min of the feeding. This time was subsequently used as the minimum time between a $\dot{V}_{O_2}$ measurement and any feeding prior to the measurement. Feedings prior to a $\dot{V}_{O_2}$ measurement were always limited to 1.4 kg of capelin or less.

Animals that remained in the ECF continuously were tested twice daily; animals were fasted overnight prior to morning testing and for a minimum of 3 h between morning and afternoon sessions. Fish rewards for morning tests were limited to 1.4 kg of capelin or less. The dolphin that was transported daily was provided with a fish reward (≤1.4 kg capelin) for the transport to the ECF. Testing of this animal occurred at a minimum of 3 h following the consumption of the last fish provided as a reward. Dolphins were exposed to T_w varying from 0.2 to 18°C. Water temperature was gradually decreased 1–2°C overnight (12–14 h) prior to testing the following morning. Elevations in the RMR as a function of T_w were used to define the lower critical water temperature (LCT$_w$) for each dolphin (see 'Analyses' below). Additional testing was conducted on two of the dolphins (Tt3 and Tt4) to determine the influence of cold inspired air on the LCT. For these animals, the T_w was raised ~3°C above their individual LCT$_w$ and the T_a dropped. Air temperature varied from –2.4 to 17.8°C and was reduced by 2–3°C between sessions. Air temperature was returned to ambient between sessions, but each subject was exposed to the test T_a for a minimum of 3 h prior to the measurement of $\dot{V}_{O_2}$. Given the stable T_w, a LCT for
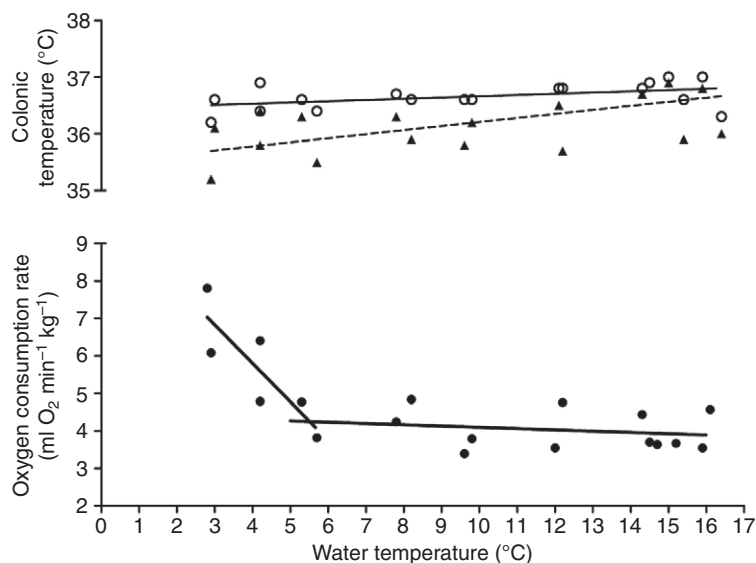
Fig. 3. Example of colonic body temperatures and corresponding linear regressions at 25 cm depth (open circles, solid line) and 15 cm depth (filled triangles, dotted line) and corresponding oxygen consumption (bottom; filled circles) in relation to water temperature in one adult male bottlenose dolphin (Tt2). The solid lines indicate the component linear regressions resulting from a segmented linear regression analysis. The intersection of the solid lines is taken as the lower critical temperature (5.7°C).

air LCT_a^t was determined, where t is the temperature of the water at the time of testing.

Body temperature and respiration rate

Respiration rate was monitored for 5 min at 30 min intervals once the animal entered the ECF. Additionally, animal behavior was classified as active (swimming) or inactive (floating at the surface of the water or resting on the bottom). To remove the effect of varying levels of activity on respiration rate, only respirations rates during periods of rest were used in the analyses.

As a proxy for core body temperature, deep colonic temperature was taken in the pens on non-$\dot{V}_{O_2}$ trial days as well as in the ECF immediately following oxygen consumption trials. Rectal temperature was taken at 25 cm depth (T_R25) for all dolphins and for a sub-set of dolphins (Tt2, Tt3) at both 25 and 15 cm depth (T_R15). These positions in the colon place the points of measurement within the countercurrent heat exchanger (25 cm) (Rommel et al., 1994) associated with the testes and posterior to the countercurrent heat exchanger (15 cm) (Rommel et al., 1994). Measurements were taken using a pliable rectal probe with a thermocouple (Traceable® digital thermometer accuracy ±0.05°C, Control Company, Friendswood, TX, USA). Rectal temperature was recorded once the temperature reading was stable for a period of 30 s.

Blood chemistry

Prior work has suggested that some hematological values may vary as a function of cold exposure and may be related to either the pathophysiology of cold stress or adaptation to cold exposure (e.g. Chun and Harris, 1978). In order to investigate whether there were any systematic variations in standard blood chemistries related to cold exposure, animals were trained to present their flukes voluntarily for blood sampling at the end of an exposure period. Mixed arterial–venous blood samples were drawn from vessels on the ventral surface of the dolphin's fluke. When possible, sample collection was performed every 2–3 days for dolphins that remained in the ECF for 10 days (Tt1 and Tt2), and was performed at each temperature exposure for the dolphin that was transported to and from the ECF daily (Tt3). Sample collection was irregular for the remaining two animals (Tt4 and Tt5) due to a more staggered pool residency schedule; only one blood sample was collected from Tt5 during the study. Samples were drawn into chilled 7.5 ml serum and

4 ml sodium heparin blood collection tubes (Becton Dickinson, Franklin Lakes, NJ, USA) and immediately centrifuged for 10 min at 1509 g. Serum samples were sent to Quest Diagnostics (San Diego, CA, USA) and replicate samples were analyzed on a Stat Profile© Critical Care Xpress system (Nova Biomedical, Waltham, MA, USA). Each sample was processed to determine pH, hematocrit (Hct), hemoglobin content (Hb), electrolyte levels, glucose, lactate and blood urea nitrogen (BUN).

Analyses

Prior to determination of the LCT_w, $\dot{V}_{O_2}$ values measured in afternoon and morning trials were compared for each animal using Student's paired t-test to determine whether the timing of the trials affected the $\dot{V}_{O_2}$. The LCT_w for each dolphin was determined by performing a segmented linear regression with breakpoint (Yeager and Ultsch, 1989) using $\dot{V}_{O_2}$ as the dependent variable and T_w as the independent variable. Using this approach, the data were divided into two sets by a breakpoint within the range of T_w to which the animals were exposed. The breakpoint was calculated as the division that results in the minimization of the squared errors for the two linear models (example given in Fig. 3). The same approach was used for determining the LCT_a^t but with T_a used as the independent variable. Regression analysis was used to determine whether core body temperature or respiration rate was related to T_w and to look for relationships between blood chemistry values and either T_w or T_a. The latter analysis was completed for all of the animals except Tt5 since only one blood sample was obtained from this animal during the study.

RESULTS
Oxygen consumption

Mean individual dolphin mass ranged from 177.3 to 218.7 kg during the study period and mean RMR for dolphins at thermoneutral conditions ranged from 3.05 to 4.4 ml $O_2 min^{-1} kg^{-1}$ (Table 1). For the four individuals tested twice daily, $\dot{V}_{O_2}$ measurements taken in the morning and the afternoon trials were similar (paired t-tests; Tt1, $t=0.13$, $P=0.90$; Tt2, $t=-1.33$, $P=0.21$; Tt4, $t=1.19$, $P=0.14$; Tt5, $t=2.33$, $P=0.5$); therefore, morning and afternoon trials were combined in the calculation of LCT_w for each dolphin. For all dolphins, a near-linear increase in $\dot{V}_{O_2}$ occurred at decreasing temperatures below the LCT_w (for example, see Fig. 3). LCT_w
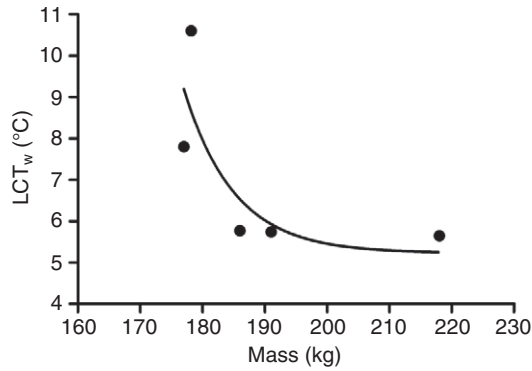
Fig. 4. Lower critical water temperature (LCT_w) in relation to individual body mass for dolphins residing in San Diego, CA. The line indicates the curvilinear relationship between body mass and lower critical water temperature according to the equation $LCT_w = 1.22e^{-0.12M}$.
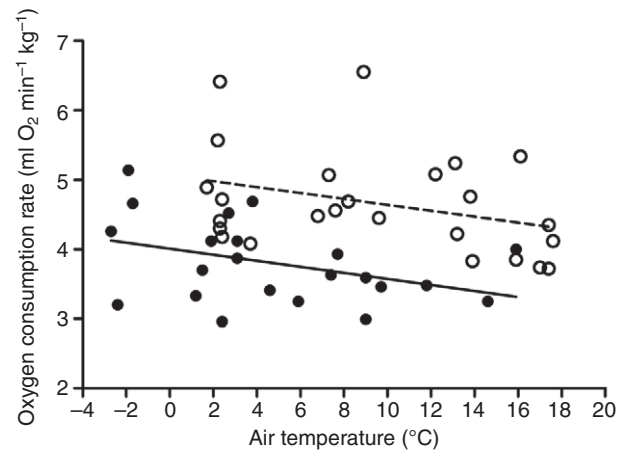

Fig. 5. The relationship between air temperature and rate of oxygen consumption for two adult male bottlenose dolphins (Tt3, filled circles; Tt4, open circles). For each $\dot{V}_{O_2}$ measurement, water temperature ranged from 7.0 to 8.3°C and was above the individual's LCT_w of 5.6 and 5.5°C.

demonstrated a curvilinear relationship with mass, with the LCT_w increasing rapidly below an animal mass of 180 kg (Fig. 4; $r^2=0.64$). LCT_w ranged from 5.5 to 10.6°C (Table 1) and was related to body mass following the equation:

$$LCT_w = 1.22e^{-0.12M}, \qquad (1)$$

where M is body mass in kg and LCT_w is the lower critical water temperature in °C. However, the LCT_w changed little for animals in excess of ~187 kg. The highest LCT_w did not belong to the smallest animal. It occurred in the only female dolphin tested (Tt5), which was also the oldest dolphin tested (33 years).

No LCT_a could be determined for the two dolphins exposed to decreasing T_a while being maintained at T_w slightly above the LCT_w, even though T_a values below –2°C were obtained within the ECF. Variability in $\dot{V}_{O_2}$ measurements was high across the range of T_a tested, and although no significant relationship between T_a and $\dot{V}_{O_2}$ was found, linear regression analysis suggested there was a slight trend of increasing metabolic rate with reductions in T_a (Fig. 5; Tt3, $r^2=0.15$, $P=0.07$; Tt4, $r^2=0.11$, $P=0.1$). The slopes of these relationships were not significantly different from one another ($F=0.002$, $P=0.98$, d.f.=43) nor were they significantly different from zero (Tt3, $P=0.07$; Tt4, $P=0.09$).

Rectal temperature

At 25 cm depth, colonic temperature ranged from 35.5 to 37.2°C. Mean T_R25 temperature for each dolphin at thermoneutrality was 36.8±0.2°C (Tt1, $N=5$), 36.7±0.2°C (Tt2, $N=26$), 36.5±0.2°C (Tt3, $N=29$), 36.8±0.2°C (Tt4, $N=12$) and 37.1±0.1°C (Tt5, $N=2$). There was a significant positive relationship between core body temperature and T_w (range, 0.3–18.0°C) for three dolphins (Tt1, $N=10$, $P=0.04$, $r^2=0.42$; Tt2, $N=24$, $P\ll0.001$, $r^2=0.52$; Tt3, $N=42$, $P=0.04$, $r^2=0.11$), an example of which is provided in Fig. 3. No relationship between core temperature and T_w was found in Tt4 ($N=11$, $r^2=0.0004$, $P=0.95$) or Tt5 ($N=6$, $r^2=0.003$, $P=0.9$).

For the two dolphins (Tt2, Tt3) sampled at both 25 and 15 cm depth, mean rectal temperature at 15 cm depth measured while each animal was in its respective thermoneutral zone was 36.6±0.25°C (Tt3, $N=29$) and 36.5±0.52°C (Tt2, $N=21$). The overall range at 15 cm was 35.2–37.5°C across water temperatures ranging from 0.2 to 22.5°C. There was a significant positive linear relationship between water temperature and T_R15 in both dolphins (Tt3, $r^2=0.24$, $P=0.0009$; Tt2, $r^2=0.56$, $P<0.0001$) and paired t-tests indicated there was a significant difference between 15 and 25 cm depth in both

dolphins (Tt3, $P<0.001$, $t=4.3$, d.f.=41, mean difference=–0.13; Tt2, $P<0.0001$, $t=6.19$, d.f.=22, mean difference=–0.43). Overall, T_R25 was warmer than T_R15 with the temperature difference between the two sites decreasing with increasing water temperature.

Blubber depth

Blubber depth demonstrated a non-uniform distribution with the lateral site, L2, being significantly thinner than the dorsal and ventral sites (ANOVA, all pair-wise comparisons $P<0.01$, *post-hoc* Tukey test) but not significantly different from L1 ($P=0.07$). Individual mean blubber depths for each site and the means for each site across all dolphins are presented in Table 2. There was no relationship between individual mean blubber depth and acclimation temperature or LCT_w.

Blood chemistry

Most of the blood chemistry parameters showed no relationship to variations in T_w or T_a and there was no indication of pathological consequences of the cold exposures. However, in one animal that remained in the pool for all of the cold exposures and measurements (Tt1), a significant inverse relationship between Hct and T_w existed (Tt1: $P=0.04$, $r^2=0.81$). For Tt1, the Hct was 9% higher at the coldest T_w than at the warmest T_w. A similar trend was observed for the other animal that resided in the pool for the duration of the experiment (Tt2: $P=0.14$, $r^2=0.72$), but an insufficient number of samples were collected at the coldest exposure temperatures to fully explore this relationship. Still, the Hct at the coldest T_w for which a blood sample was collected was 13% higher than that at the warmest T_w.

Respiration rate

Across all dolphins, respiration rate ranged from 4 to 45 breaths over a 5 min period and depended on T_w and T_a. Respiration rate increased with decreasing T_w in four of the five dolphins (Tt2, $N=177$, T_w range=3.0–19.2°C, $P<0.001$, $r^2=0.20$; Tt3, $N=103$, T_w range=0.2–8.3°C, $P<0.001$, $r^2=0.14$; Tt4, $N=192$, T_w range= 3.9–17.9°C, $P<0.001$, $r^2=0.19$; Tt5, $N=155$, T_w range=4.6–16.8, $P=0.04$, $r^2=0.30$). For the two dolphins exposed to cold air, respiration rate also increased with decreasing T_a (Tt3, $N=78$, T_a

Table 2. Mean blubber depth (±1 s.d.) by site for individual dolphins in the period during which daily oxygen consumption measurements were made

	D1	D2	L1	L2	V1	V2
Summer						
Tt1 (2)	2.5±0.1 (2.4–2.6)	2.4±0.1 (2.3–2.4)	1.9±0.1 (1.9–2.0)	1.8±0.1 (1.7–1.9)	2.4±0.3 (2.2–2.6)	2.5±0.1 (2.5–2.6)
Tt2 (2)	3.1±0.1 (3.0–3.2)	2.5±0.1 (2.4–2.5)	2.2±0.1 (2.1–2.3)	1.8±0.1 (1.7–1.9)	2.7±0.3 (2.2–3.0)	2.5±0.2 (2.3–2.7)
Winter/spring						
Tt3 (5)	3.4±0.3 (3.0–3.9)	3.1±0.2 (3.1–3.8)	3.2±0.4 (2.6–3.9)	2.6±0.3 (2.3–3.3)	3.2±0.2 (3.2–3.9)	3.8±0.2 (3.3–4.1)
Tt4 (2)	2.7±0.1 (2.6–2.7)	2.5±0.3 (2.1–2.9)	1.9±0.2 (1.7–2.3)	1.6±0.1 (1.5–1.7)	2.5±0.2 (2.3–2.8)	2.8±0.2 (2.4–3.1)
Tt5 (2)	3.2±0.01 (3.16–3.17)	2.7±0.04 (2.62–2.68)	2.5±0.3 (2.2–2.7)	2.3±0.1 (2.2–2.3)	3.1±0.1 (3.0–3.1)	2.9±0.1 (2.9–3.0)
Mean ± s.d.	3.1±0.4	2.9±0.6	2.6±0.6	2.2±0.5	3.2±0.6	3.2±0.6

Depths are given in cm and values in parentheses under each of the mean depths represent the range for that animal. Dorsal, lateral and ventral sites are indicated by D, L and V, respectively. Locations of each specific site are shown in Fig. 1. Values in parentheses following the subject ID reflect the number of times that the complete set of measurements was made for that animal.

range=–2.4–16.1°C, $P<0.001$, $r^2=0.17$; Tt4, $N=138$, T_a range= 1.7–17.5°C, $P<0.001$, $r^2=0.24$).

DISCUSSION

The LCT_w measurements for the bottlenose dolphins were generally inversely related to mass, as might be expected due to the direct relationship between thermal inertia and mass. However, the LCT_w measurements for the smallest animals (Tt1=7.8°C and Tt5=10.6°C) were 1.9 and 4.7°C higher than an average LCT_w of 5.9°C reported for three male bottlenose dolphins that were also acclimated to the water temperatures of San Diego Bay (Williams et al., 2001). The average mass of the dolphins involved in the study by Williams and colleagues was reported to be 148.6 kg, which is ~30 kg less than the smallest animal tested in this study. These results at first seem contradictory as it would be expected that smaller animals would have a higher LCT_w. However, the RMR of the dolphins in the study by Williams and colleagues was ~63% higher than that measured here. The differences are unlikely to be due to the acclimation temperatures, which were similar between studies, but may exist because of the different methodologies employed, different physical characteristics of the individual dolphins or the ages of the animals tested. The dolphins tested by Williams and colleagues were held in a 'metabolic box' for the measurements, which effectively constrains the animal and may have contributed to an acute stress response that elevated the metabolic measurements (Williams et al., 2001). Alternatively, the animals may not have been completely mature (ages are not provided in their study) and the blubber composition may have been different. Within odontocetes, the chemical composition and depth of blubber are known to vary with ontogeny, time of year and diet (Costa and Williams, 1999; Dunkin et al., 2005; Koopman, 2007; Montie et al., 2008; Samuel and Worthy, 2005; Struntz et al., 2004), and these factors can affect the thermal conductivity of the blubber layer (Dunkin et al., 2005; Meagher et al., 2008).

Complex nasal turbinates occur in nearly all mammals with the exception of cetaceans and some primates (for a review, see Hillenius, 1992). Nasal turbinates provide a surface for exchange of heat and water. They function to warm and humidify ambient air during inhalation prior to reaching the alveoli and to cool exhaled air and reduce respiratory water loss *via* condensation. This respiratory heat exchange has been postulated to help to retain body warmth and it is bypassed (i.e. panting) to facilitate cooling in some mammals (Baker, 1982). Although no LCT_a could be determined for two dolphins maintained at a constant water temperature slightly above the LCT_w, a trend of increasing $\dot{V}_{O_2}$ and variance in $\dot{V}_{O_2}$ as air temperature decreased was observed (Fig. 5). Anecdotally, along with this trend, a change in breath 'quality' was observed as the air

decreased in temperature; breaths became short and shallow compared with the deep breaths observed at ambient air temperatures. Variations in breathing patterns may be expected if the inhalation of smaller amounts of cold air serves to protect the pulmonary mucosa from cold-related trauma, drying and alterations in lung mechanics (Baile et al., 1987; Jammes et al., 1983). If so, then the contribution of inhaled cold air to the thermal budget of a bottlenose dolphin is probably relatively minor in comparison to the impact of water temperature for the temperature range measured in this study.

An increase in Hct was observed in the two dolphins that remained in the pools for the duration of the experiment. These animals had the longest uninterrupted exposures to cold water temperatures of the five dolphins studied. This pattern was not observed in the animal that was moved back and forth to the pool on a daily basis and insufficient samples were taken from the animals that remained in the pools for ~3 days at a time to determine whether the trend was observed in them. An increase in Hct with decreasing water temperature was previously observed in bottlenose dolphins under similar temperature exposure conditions (Chun and Harris, 1978). Over a 13 day period, three bottlenose dolphins were maintained in a temperature-controlled facility while the water temperature was reduced from approximately 17.2 to 3.3°C. During this period, the Hct of the dolphins increased from 5% to 12%, which is comparable to the 9% and 13% increase observed in this study. It is feasible that the increase in Hct reflects an increase in oxygen-carrying capacity through the proliferation of red blood cells, as the oxygen-carrying capacity and viscosity of blood have been observed to increase in other mammals under relatively short cold acclimation periods (Mercer et al., 1999; Viscor et al., 2003). Alternatively, the increase in Hct could reflect cold-induced dehydration, as has been observed in rats (Sun et al., 1999). However, no other potential indicators of dehydration were observed in the dolphins involved in this study.

Although deep colonic temperature was significantly related to water temperature in three dolphins (Tt1, Tt2, Tt3) and in both dolphins in which rectal temperature was measured at 15 cm (Tt2, Tt3), the magnitude of the decrease in core body temperature with declining water temperature was less than 1.5°C and all body temperatures were within what is considered normal for the bottlenose dolphin (Hampton et al., 1971; Pabst et al., 2002; Pabst et al., 1995). None of the animals approached what would be considered hypothermic body temperature. The present study may be compared with a study in which a male and female dolphin were housed in the Thames River (New London, CT, USA) from November 1983 to January 1984 (Scronce and Bowers, 1985).

During the stay, water temperature ranged from 1 to 15°C and air temperature ranged from –18 to 16°C. Both animals exhibited a relationship between water temperature and core body temperature similar to that observed in some of the animals in this study. The larger male dolphin, whose mass varied from 220 to 241 kg during the residency, exhibited a mean core body temperature of 34.8°C during the coldest water temperatures. The female, whose mass varied from 207 to 214 kg, demonstrated a drop in core body temperature to 31.8°C during the coldest water periods. Because of the hypothermia experienced by this dolphin, she was moved to a heated enclosure where she regained normothermy (Scronce and Bowers, 1985). It is important to note that during these periods of coldest water, which lasted several weeks, the air temperature was also consistently between –18 and 0°C and provided a prolonged cold exposure of greater magnitude than was attempted in this study.

In dolphins, regional colonic temperatures are influenced by a countercurrent heat exchange system (CCHE) consisting of a network of vascular structures running from the dorsal fin and fluke to the region surrounding the intra-abdominal testes where it acts to regulate the temperature of the testes (Rommel et al., 1992; Rommel et al., 1994; Pabst et al., 1995). Regional temperatures within the colon may vary depending on total body length, activity state and thermal environment (Rommel et al., 1994; Pabst et al., 1995). Colonic temperatures in this study were measured in resting dolphins at two depths, 25 cm (within the CCHE) and 15 cm (posterior to the CCHE). In warmer water exposures (T_w>16.0°C;) the temperature at the two measurement sites was generally similar, being within 0.2°C. However, when the water temperature was cooled, the difference between the two sites increased, with the measurement site within the CCHE being warmer than the site posterior to the CCHE. Other researchers have used linear arrays of five to seven thermocouples placed 5 cm apart to measure colonic temperature in dolphins resting in water temperature ranging from 28.6 to 33.0°C (Rommel et al., 1994) and 25 to 26°C (Pabst et al., 1995). Under these conditions colonic temperature measured within the CCHE was 0.2–0.7°C and 0.2–0.9°C cooler than that measured outside the CCHE. By comparison, the water temperatures experienced by the dolphins in the present study were up to 32.8°C cooler than in previous studies. Under cooler environmental conditions, the vessels in the extremities, dorsal fin and fluke would shut down in order to conserve heat (Scholander and Sheville, 1955) and may explain the comparatively warmer temperatures within the CCHE than at the site 10 cm posterior. Thus, in resting dolphins, the differences in T_R25 and T_R15 measured in this study may be explained by an overall thermal shell effect, i.e. a thermal gradient that reflects an increase in heat storage from the skin surface to the core of the animal.

Relationship of LCT$_w$ to habitat utilization

Past work has demonstrated that the population size of dolphins residing at the high latitudes of the United Kingdom diminishes during the coldest times of the year (Wilson et al., 1997; Wood, 1998). Given the potential dependence of the LCT$_w$ on mass and that some animals remain resident during the coldest portions of the year, it would be worth determining which members of a population remain resident when the environmental temperatures are coldest. It may be that dolphins of small mass, particularly neonates and adolescents, are of insufficient size to tolerate extended periods of time in water temperatures below the LCT$_w$ and thus relocate to warmer water during the coldest times of the year. Logically, it would follow that attending adults, particularly nursing females, might relocate as well. Under such conditions it is predicted that dolphins remaining in high latitude regions during the time of

coldest water temperatures would be adults, possibly with a bias toward adult males. Future monitoring of high-latitude resident populations should strive to distinguish between the seasonal age and sex composition of the population to test this hypothesis.

Following the 1982–1983 El Niño, changes in residency patterns along the coast of western North America indicated that oceanographic events influenced the distribution of coastal bottlenose dolphins, resulting in a northward range extension into central California (Hansen and Defran, 1993; Wells et al., 1990). A small population of bottlenose dolphins continues to reside in this region even though the water temperature has returned to normal (Caretta et al., 2007). The range of bottlenose dolphins along the west coast of the United States appears northward limited at this point, just south of San Francisco (Carretta et al., 2007). If comparable to dolphins in this study, the average yearly water temperature along the west coast of the United States would appear to be tolerable throughout the year for wild coastal bottlenose dolphins with masses greater than 187 kg; the lower range of average water temperature reported for the west coast of the United States ranges from 5.6 to 21.1°C (United States National Oceanographic Data Center: http://www.nodc.noaa.gov). For dolphins exceeding this mass, the potential northward range along the Pacific coast of the United States would not appear to be limited by water temperature. Certainly, there are periods of time during which water temperature would briefly (i.e. of the order of days) drop below the LCT$_w$ of these larger dolphins. However, such drops would not preclude utilization of the environment, but may require an increase in energy acquisition to offset any increase in thermoregulatory costs.

One might expect that larger dolphins (with lower LCT$_w$) would range northward to reduce intraspecific competition for resources, while water temperature would restrict the movements of smaller dolphins (with higher LCT$_w$) to the southern California region. However, the current estimated population size for coastal bottlenose dolphins on the Pacific coast of the United States is approximately 323 animals and has remained relatively stable for the past 20 years (Dudzik et al., 2006). Given the small population size, it seems unlikely that intraspecific competition for food would be sufficient to promote a northward expansion. Similarly, if reproductive age females tend to remain in waters that are within the thermoneutral zone of attended offspring, it would not benefit males to expand their ranges northward at the cost of reproductive opportunity. It is interesting to note that the northernmost range use by Atlantic coastal bottlenose dolphins is seasonally varied. Dolphins are not typically observed north of Chesapeake Bay during the winter months, when water temperatures are below 9.5°C, and seasonally migrate southward as water and air temperature begin to cool (Barco et al., 1999; Read et al., 2003; Torres et al., 2005). If the LCT$_w$ of Atlantic coastal bottlenose dolphins is comparable to or higher than that measured for dolphins in this study, such water temperatures are probably outside the LCT$_w$ of smaller dolphins, particularly adolescents and neonates. This may, in part, explain the seasonal southward migration pattern observed in populations along the mid-Atlantic coast of the US (Barco et al., 1999; Kenney, 1990; Torres et al., 2005).

Two common hypotheses presented to explain the distributional limits of bottlenose dolphins are water temperature and prey redistribution. However, the distribution of dolphins is likely to be due to both biotic and abiotic factors and not to one factor alone. In addition to water temperature and prey redistribution, alternative factors include bathymetry, social structure and predator avoidance, each of which may itself be influenced by one of the other factors. On both the Pacific and Atlantic coasts, coastal bottlenose dolphins typically reside in areas where water depth is 25 m or less (Kenney,

1990; Torres et al., 2005). However, minimum population estimates of coastal bottlenose dolphins in the Atlantic exceed 10,000 individuals (Waring et al., 2007), which is substantially higher than the 323 animals estimated for the Pacific coastal stock. In contrast to the east coast, the bathymetry along the west coast of the US lacks a wide shallow continental shelf potentially limiting the habitat suitable to support large populations of coastal bottlenose dolphins. Whether or not this is a factor in determining coastal bottlenose dolphin distributions, and to what extent it affects distributions, requires further investigation. Additional factors, such as prey preference and predator avoidance, also exert some influence over coastal bottlenose dolphin distribution and are themselves likely to be related to coastal bathymetry and temperature (Hastie et al., 2004). For example, Heithaus and Dill demonstrated that water temperature indirectly affected dolphin habitat use by influencing the presence and absence of a predator (Heithaus and Dill, 2002). During cooler months when tiger shark presence was low, dolphins exploited areas of high prey biomass. During the warmer months, when tiger shark presence was high, dolphins avoided the area even though prey availability remained the same. The relationship of prey distribution to dolphin distribution is also not as clearly determined (Barco et al., 1999; Wells et al., 1990; although prey distribution must to some extent affect habitat selection (e.g. Barros and Wells, 1998), the observed plasticity in prey selection (Young and Phillips, 2002; Gannon and Waples, 2004) may minimize the impact resulting from temporal and spatial variations in specific prey availability in the bottlenose dolphin.

As in any wild population of mammals, the distribution and habitat utilization of the coastal bottlenose dolphin will be influenced by the movement of prey, water temperature, the minimization of predation pressure and the need for social interactions. With respect to water temperature, the LCT_w of bottlenose dolphins acclimated to the waters of coastal southern California seems sufficient to support the utilization of higher latitudes for larger animals (i.e. >187 kg). Cold air temperature common to the higher latitudes would appear to have a minor impact on thermoregulation in bottlenose dolphins and probably does not limit the range of these animals. However, the observed northward limits on coastal bottlenose dolphin ranges may result, in part, because of a need for neonates, adolescents and attending (presumably reproductive) females to remain in the warmer waters of the coastal USA. Similar propositions have been made with regard to the increase in abundance of animals in the inmost waters of the Moray Firth during the summer months when the water temperature is relatively high (Wilson et al., 1997). Future research into coastal bottlenose dolphin distribution should focus on whether habitats with seasonally cold water and with both resident and migratory individuals favor utilization by larger, male dolphins during the coldest months of the year.

REFERENCES

Baile, E. M., Dahlby, R. W., Wiggs, B. R., Parsons, G. H. and Pare, P. D. (1987). Effect of cold and warm dry air hyperventilation on canine airway blood flow. *J. Appl. Physiol.* **62**, 526-532.

Baker, N. A. (1982). Brain cooling in endotherms in heat and exercise. *Annu. Rev. Physiol.* **44**, 85-96.

Barco, S. G., Swingle, W. M., McLellan, W. A., Harris, R. N. and Pabst, D. A. (1999). Local abundance and distribution of bottlenose dolphins (*Tursiops truncatus*) in the near shore waters of Virginia Beach, VA. *Mar. Mamm. Sci.* **15**, 394-408.

Barros, N. B. and Wells, R. S. (1998). Prey and feeding patterns of resident bottlenose dolphins (*Tursiops truncatus*) in Sarasota Bay, FL. *J. Mammal.* **79**, 1045-1059.

Bräger, S. (1993). Diurnal and seasonal behavior patterns of bottlenose dolphins (*Tursiops truncatus*). *Mar. Mamm. Sci.* **9**, 434-438.

Bräger, S., Harraway, J. A. and Manly, B. F. J. (2003). Habitat selection in a coastal dolphin species (*Cephalorhynchus hectori*). *Mar. Biol.* **143**, 233-244.

Bristow, T. (2004). Changes in coastal site usage by bottlenose dolphins (*Tursiops truncatus*) in Cardigan Bay, Wales. *Aquat. Mamm.* **30**, 398-404.

Carretta, J. V., Forney, K. A., Lowry, M. S., Barlow, J., Baker, J., Hanson, B. and Muto, M. M. (2007). U.S. Pacific marine mammal stock assessments: 2007: National Oceanic and Atmospheric Administration. NOAA Technical Memorandum NOAA-TM-NMFS-SWFSC-414, p. 326.

Chun, N. K. and Harris, M. G. (1978). Cold water temperature tolerance test of three Atlantic bottlenose dolphins (*Tursiops truncatus*). San Diego: Naval Ocean Systems Center. NOSC TR 206, pp. 1-23

Costa, D. P. and Williams, T. M. (1999). Marine mammal energetics. In *Biology of Marine Mammals* (ed. J. E. Reynolds and S. A. Rommel), pp. 176-217. Washington: Smithsonian Institution Press.

Davis, R. W., Williams, T. M. and Kooyman, G. L. (1985). Swimming metabolism of yearling and adult harbor seals *Phoca vitulina*. *Physiol. Zool.* **58**, 590-596.

Dudzik, K. J., Baker, K. M. and Weller, D. W. (2006). Mark recapture abundance estimate of California coastal stock bottlenose dolphins: February 2004 to April 2005 National Oceanic and Atmospheric Administration. Administrative Report LJ-06-02C, p. 17

Dunkin, R. C., McLellan, W. A., Blum, J. E. and Pabst, D. A. (2005). The ontogenetic changes in the thermal properties of blubber from Atlantic bottlenose dolphin *Tursiops truncatus*. *J. Exp. Biol.* **208**, 1469-1480.

Fedak, M. A., Rome, L. and Seeherman, H. J. (1981). One step N_2 dilution technique for calibrating open-circuit O_2 measuring systems. *J. Appl. Physiol.* **51**, 772-776.

Ferrero, R. C. and Tsunoda, L. M. (1989). First record of a bottlenose dolphin (*Tursiops truncatus*) in Washington State. *Mar. Mamm. Sci.* **5**, 302-305.

Gannon, D. P. and Waples, D. M. (2004). Diets of coastal bottlenose dolphins from the U.S. mid-Atlantic coast differ by habitat. *Mar. Mamm. Sci.* **20**, 527-545.

Gaskin, D. E. (1968). Distribution of Delphinidae (Cetacea) in relation to sea surface temperatures off eastern and southern New Zealand. *N. Z. J. Mar. Freshwater Res.* **2**, 527-534.

Hammond, P. S. and Thompson, P. M. (1991). Minimum estimate of the number of Bottlenose dolphins *Tursiops truncatus* in the Moray Firth, NE Scotland. *Biol. Conserv.* **56**, 79-87.

Hampton, I. F. G., Whittow, G. C., Szekerczes, J. and Rutherford, S. (1971). Heat transfer and body temperature in the Atlantic bottlenose dolphin, *Tursiops truncatus*. *Int. J. Biometeorol.* **15**, 247-253.

Hanson, M. T. and R. H., Defran, R. H. (1993). The behavior and feeding ecology of the Pacific coast bottlenose dolphin, *Tursiops truncatus*. *Aquat. Mamm.* **19**, 127-142.

Hastie, G. D., Wilson, B., Wilson, L. J., Parsons, K. M. and Thompson, P. M. (2004). Functional mechanisms underlying cetacean distribution patterns: hotspots for bottlenose dolphins are linked to foraging. *Mar. Biol.* **144**, 397-403.

Heithaus, M. and Dill, L. M. (2002). Food availability and tiger shark predation risk influence bottlenose dolphin habitat use. *Ecology* **83**, 480-491.

Hillenius, W. J. (1992). The evolution of nasal turbinates and mammalian endothermy. *Paleobiology* **18**, 17-29.

Jackson, D. C. and Schmidt-Neilsen, K. (1964). Counter current heat exchange in the respiratory passages. *Proc. Natl. Acad. Sci. USA* **51**, 1192-1197.

Jammes, Y., Barthelemy, P. and Delpierre, S. (1983). Respiratory effects of cold air breathing in anesthetized cats. *Respir. Physiol.* **54**, 41-54.

Kenney, R. D. (1990). Bottlenose dolphins off the Northeastern United States. In *The Bottlenose Dolphin* (ed. S. Leatherwood and R. R. Reeves), pp. 369-386. San Diego: Academic Press.

Koopman, H. N. (2007). Phylogenetic, ecological, and ontogenetic factors influencing the biochemical structure of the blubber of odontocetes. *Mar. Biol.* **151**, 277-291.

McBrearty, D. A., Message, M. A. and King, G. A. (1986). Observations on small cetaceans in the north-east Atlantic Ocean and the Mediterranean Sea: 1978-1982. In *Research on Dolphins* (ed. M. M. Bryden and R. Harrison). Oxford: Clarendon Press.

Maze, K. S. and Wursig, B. (1999). Bottlenose dolphins of San Luis Pass, TX: occurrence patterns, site fidelity, and habitat use. *Aquat. Mamm.* **25**, 91-103.

Meagher, E. M., McLellan, W. A., Westgate, A. J., Wells, R. S., Blum, J. E. and Pabst, D. A. (2008). Seasonal patterns of heat loss in wild bottlenose dolphins (*Tursiops truncatus*). *J. Comp. Physiol. B* **178** 529-543.

Mercer, J. B., Osterud, B. and Tveita, T. (1999). The effect of short-term cold exposure on risk factors for cardiovascular disease. *Thromb. Res.* **95**, 93-104.

Montie, E. W., Garvin, S. R., Fair, P. A., Bossart, G. D., Mitchum, G. B., McFee, W. E., Speakman, T., Starczak, V. R. and Hahn, M. E. (2008). Blubber morphology in wild bottlenose dolphins (*Tursiops truncatus*) from the Southeastern United States: influence of geographic location, age class, and reproductive state. *J. Morphol.* **269** 496-511.

Pabst, D. A., Rommel, S. A., McLellan, W. A., Williams, T. M. and Rowles, T. K. (1995). Thermoregulation of the intra-abdominal testes of the Bottlenose dolphin (*Tursiops truncatus*) during exercise. *J. Exp. Biol.* **198**, 221-226.

Pabst, D. A., McLellan, W. A., Meagher, E. M. and Westgate, A. J. (2002). Measuring temperatures and heat flux from dolphins in the Eastern Tropical Pacific: is thermal stress associated with chase and capture in the ETP-tuna purse seine fishery? vol. report completed under contract 40ABNF108447, pp. 1-52: Southwest Fisheries Science Center, National Marine Fisheries Service, NOAA.

Read, A. J., Urian, K. W., Wilson, B. and Waples, D. M. (2003). Abundance of bottlenose dolphins in the bays, sounds and estruaries of North Carolina *Mar. Mamm. Sci.* **19**, 59-73.

Rommel, S. A., Pabst, D. A., McLellan, W. A., Mead, J. G. and Potter, C. W. (1992). Anatomical evidence for a counter current heat exchanger associated with dolphin testes. *Anat. Rec.* **232**, 150-156.

Rommel, S. A., Pabst, D. A., McLellan, W. A., Williams, T. M. and Friedl, W. A. (1994). Temperature regulation of the testes of the bottlenose dolphin (*Tursiops truncatus*): evidence from colonic temperatures. *J. Comp. Physiol. B* **164**, 130-134.

Ross, G. J. B. and Cockcroft, V. G. (1990). Comments on Australian Bottlenose dolphins and the taxonomic status of *Tursiops aduncus* (Ehrenberg, 1832). In *The Bottlenose Dolphin* (ed. S. Leatherwood and R. R. Reeves), pp. 101-128. San Diego: Academic Press.

Samuel, A. M. and Worthy, G. A. J. (2005). Variability in fatty acid composition of bottlenose dolphin (*Tursiops truncatus*) blubber as a function of body site, season, and reproductive state. *Can. J. Zool.* **82**, 1933-1942.

Schmidt-Nielsen, K. (1997). *Animal Physiology: Adaptation and Environment.* New York: Cambridge University Press.

Scholander, P. F. and Schevill, W. E. (1955). Countercurrent heat exchange in the fins of whales. *J. Appl. Physiol.* **8**, 279-282.

Scronce, B. L. and Bowers, C. A. (1985). Effects of a cold environment on Atlantic bottlenose dolphins (*Tursiops truncatus*), pp. 1-17. San Diego: Naval Ocean Systems Center.

Shane, S. H., Wells, R. S. and Wursig, B. (1986). Ecology, behavior and social organization of the bottlenose dolphin: a review. *Mar. Mamm. Sci.* **2**, 34-63.

Struntz, D. J., McLellan, W. A., Dillarman, R. M., Blum, J. E., Kucklick, J. R. and Pabst, D. A. (2004). Blubber development in bottlenose dolphins (*Tursiops truncatus*). *J. Morphol.* **259**, 7-20.

Sun, Z., Cade, R., Katovich, M. J. and Fregly, M. J. (1999). Body fluid distribution in rats with cold-induced hypertension. *Physiol. Behav.* **65**, 879-884.

Torres, L. G., McLellan, W. A., Meagher, E. M. and Pabst, D. A. (2005). Seasonal distribution and relative abundance of bottlenose dolphins, *Tursiops truncatus,* along the mid-Atlantic coast. *J. Cetacean Res. Manag.* **7**, 153-161.

Viscor, G., Torrella, J. R., Fouces, V. and Pagés, T. (2003). Hemorheology and oxygen transport in vertebrates: a role in thermoregulation? *J. Physiol. Biochem.* **59**, 277-286.

Waring, G. T., Josephson, E., Fairfield-Walsh, C. P. and Maze-Foley, K. (2007). U.S. Atlantic and Gulf of Mexico marine mammal stock assessments. NOAA Tech Memo NMFS NE 205, p. 415.

Wells, R. S. and Scott, M. D. (1999). Bottlenose dolphin *Tursiops truncatus* (Montagu 1821). In *Handbook of Marine Mammals: Dolphins and Porpoises* (ed. S. H. Ridgway and R. Harrison). Vol. 6, pp. 137-182. San Diego: Academic Press.

Wells, R. S., Hansen, L. J., Baldridge, A., Dohl, T. P., Kelly, D. L. and Defran, R. H. (1990). Northward extension of the range of Bottlenose Dolphins along the California Coast. In *The Bottlenose Dolphin* (ed. S. Leatherwood and R. R. Reeves), pp. 421-431. San Diego: Academic Press.

Williams, T. M., Haun, J., Davis, R. W., Fuiman, L. A. and Kohin, S. (2001). A killer appetite: metabolic consequences of carnivory in marine mammals. *Comp. Biochem. Physiol. A* **129**, 785-796.

Wilson, B., Thompson, P. M. and Hammond, P. S. (1997). Habitat use by bottlenose dolphins: seasonal distribution and stratified movement patterns in the Moray Firth Scotland. *J. Appl. Ecol.* **34**, 1365-1374.

Withers, P. C. (1977). Measurement of VO2, VCO2, and evaporative water loss with a flow-through mask. *J. Appl. Physiol.* **42**, 120-123.

Wood, C. J. (1998). Movement of bottlenose dolphins around the south-west coast of Britain. *J. Zool.* **246**, 155-163.

Yeager, D. P. and Ultsch, G. R. (1989). Physiological regulation and conformation: a BASIC program for the dertermination of critical points. *Physiol. Zool.* **62**, 888-907.

Young, R. F. and Phillips, H. D. (2002). Primary production required to support bottlenose dolphins in a salt marsh estuarine creek system. *Mar. Mamm. Sci.* **18**, 358-373.

Zolman, E. S. (2002). Residence patterns of bottlenose dolphins (*Tursiops truncatus*) in the Stono River estuary, Charleston County, SC, USA. *Mar. Mamm. Sci.* **18**, 879-892.