The development of blood oxygen stores in bottlenose dolphins (*Tursiops truncatus*): implications for diving capacity

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Abstract

Enhanced oxygen reserves in the blood facilitate diving in marine mammals. For pinnipeds (seals and sea lions), a developmental period of 4 to 24 months is required for blood oxygen stores to reach adult capacities. We investigated whether a similar developmental period for the blood occurs in cetaceans (dolphins and whales), a group of mammals that are exposed to diving immediately after birth. Blood samples were collected from wild and zoological park bottlenose dolphins *Tursiops truncatus* aged 0–12 years. Red blood cell number (RBC), haemoglobin content (Hb), haematocrit (Hct), mean corpuscular volume (MCV), mean cell haemoglobin (MCH), and mean corpuscular haemoglobin concentration (MCHC) were determined for each sample. We found that during postnatal development, RBC, Hb and Hct decreased from 0 to 1.5 months and then increased from 1.5 to 6 months, reaching adult levels by 3 years. MCV and MCH both increased from birth. MCHC decreased from 0 to 3.2 months and then increased. Adult levels for MCV were attained as early as 2 months of age while adult levels for MCH and MCHC were attained by 6 months of age. These results indicate that, for bottlenose dolphins, the development of the blood and its capacity to store oxygen is not complete with weaning, which generally occurs at 1.5 years old. The lower oxygen storage capacity of immature dolphins is likely to limit dive capabilities. Calculated aerobic dive limits (cADLs) for 0- to 2-year-old dolphin calves are 1.9–3.6 min, compared to 4.8–5.4 min for 3- to 12-year-old dolphins. Increases in cADLs from 0 to 3 years are attributed to increases in both body mass and mass specific oxygen stores while body mass alone explains the increases in cADLs from 3 to 9 years. The limited diving capacity of young dolphins may influence the foraging behaviours of newly weaned juveniles and females accompanied by calves.

Key words: dolphins, blood, development, aerobic dive limit, oxygen stores, *Tursiops truncatus*

INTRODUCTION

Foraging and locomotion by marine mammals often requires long periods of apnoea, which are supported by the use of oxygen stores in the lung, blood and muscle. When compared to terrestrial mammals, diving mammals have higher total body oxygen stores, mainly resulting from increased blood and muscle oxygen stores (Kooyman, 1989). In small cetaceans, blood oxygen stores can be up to threefold greater than those in similar-sized terrestrial mammals (Snyder, 1983).

Although it is well known that adult marine mammals have comparatively high body oxygen stores (Snyder, 1983; Kooyman, 1989), only a few studies have examined the development of these stores in immature animals. Studies on the development of the blood have focused primarily on pinnipeds (Bryden & Lim, 1969; Geraci, 1971; Lane, Morris & Sheedy, 1972; Kodama, Elsner & Pace, 1977; Thorson, 1993; Horning & Trill-mich, 1997*a*; Costa, Gales & Crocker, 1998; Sepúlveda, 1999). These studies have demonstrated that the primary period of development for blood oxygen stores in seals and sea lions occurs while the animals are on land. The final stage of development seems to be associated with an overall increase in dive duration during the first foraging trips of the pinnipeds at sea (Thorson, 1993; Burns & Castellini, 1996; Burns, Schreer & Castellini, 1997; Horning & Trillmich, 1997*b*; Costa *et al.*, 1998; Burns, 1999).

In contrast to pinnipeds, cetaceans are born and develop in water, and must be able to dive immediately. In addition, cetaceans generally have a longer period of dependency in comparison to that of most pinnipeds. If

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dolphins and whales require similar developmental changes for enhancing oxygen stores during growth, then these young animals may be especially challenged during their first dives.

Several reports have demonstrated that cetaceans require a developmental period to obtain the muscle oxygen stores of adults (Dolar et al., 1999; Noren, Williams, Pabst et al., 2001). For example, the primary locomotor muscles of bottlenose dolphins Tursiops truncatus require up to 3 years after birth to reach adult myoglobin levels (Noren, Williams, Pabst et al., 2001). With the exception of a study on foetal haemoglobin levels in sperm whales (Tawara, 1950), little is known regarding the development of blood in cetaceans. In view of this, the development of the blood oxygen store in the bottlenose dolphin was investigated. This species was chosen because both immature and adult animals were accessible for blood sampling in the wild and in a zoological park. Measurements were made of red blood cell number (RBC), haemoglobin (Hb), haematocrit (Hct), mean corpuscular volume (MCV), mean cell haemoglobin (MCH), and mean corpuscular haemoglobin concentration (MCHC) for bottlenose dolphins aged 0–12 years. This study investigated the time taken for dolphin calves to develop blood oxygen stores equivalent to those in adults and the effect that reduced blood oxygen store has on the diving capacity of young dolphins.

MATERIAL AND METHODS

Animals

Data were collected from immature and adult dolphins of known age from both a wild and zoological park population. The data from each population were analysed independently. For the wild population, 79 dolphins aged 1–12 years were sampled from 1987 to 2000, resulting in 191 samples for analysis. For the zoological park population, 7 dolphins aged 7 days to 10.2 years were sampled from 1991 to 2000, resulting in 106 samples for analysis. Data were collected from animals up to the age of 12 years because this age corresponds to the age in which both male and female bottlenose dolphins have typically achieved sexual maturity (Baird, Walters & Stacey, 1993).

Wild dolphins (Sarasota Bay, Florida)

This resident community of c. 120 individuals was sampled as part of a long-term capture–release programme that has routinely measured dolphins since June 1984. This population is under continual observation. Individuals are identifiable from dorsal fin markings and the ages of the younger animals are known from monitoring their mothers through time and then following the calves through time. For older, unidentified animals, a tooth was examined for age

determination following the methods of Hohn et al. (1989). Details of this programme and the sampling regime are described in Wells (1991).

Zoological park dolphins (Dolphinarium, Brugge, Belgium)

Analysis of blood from the zoological park population allowed us to obtain data for the early developmental stages that could not be obtained in the wild dolphins. The dolphins sampled were housed in a large 2 800 000-l artificial seawater tank. The water was treated by sand filters and chlorine. All animals used in the analysis were born at the facility from parents acquired from the wild. The dolphins were fed a daily diet of mackerel, herring, sprat, capelin and squid.

Blood sampling and analysis

Measurements of body mass and blood samples of wild dolphins were taken while the animals were temporarily restrained during capture–release efforts in June, July, December and January from 1987 to 2000. Blood samples were analysed at Smith Kline Beecham during 1987–97 (using Technicon H-1 and H-2 analysers), at University of Miami Department of Comparative Pathology during 1997–99 (using a CDC Hemavet Mascot analyser), and at Cornell University during 2000 (using a Advia 120 analyser). Dolphins at the zoological park were trained for voluntary blood sampling from the ventral part of the fluke using 21 g needles during routine veterinary evaluations. Younger calves, not yet trained, were herded by net and either held by a trainer on a deck or in the water. All calves were given a period of recovery following initial restraint to allow heart rate to slow to normal levels before blood sampling. Samples were analysed at Laboratorium voor Medicsche Biologie, Brugge, Belgium (using a Sysmex SF 3000 Analyzer). RBC, Hb concentration, Hct, MCV, MCH and MCHC were determined for all blood samples.

Concerns about using automated cell counters to analyse blood samples, as done in the present study, have been raised by Castellini, Meiselman & Castellini (1996). Castellini et al. (1996) showed that for pinnipeds, the use of automated counters could result in up to 15% higher Hct values than those obtained by microcentrifugation. However, we did not find the same degree of variability between the 2 methods when measuring Hct from bottlenose dolphin blood samples. For comparison, at 1 of the laboratories, manual counts were run simultaneously with automatic cell counts to determine values for Hct; little difference was found between the 2 methods. Furthermore, S. Ridgway (pers. comm.), using blood samples taken from 76 bottlenose dolphins, found only a 2.15% (SD = 0.90) difference between the Hct values obtained from microcentrifugation (mean ± SD = 39.53 ± 2.65) and
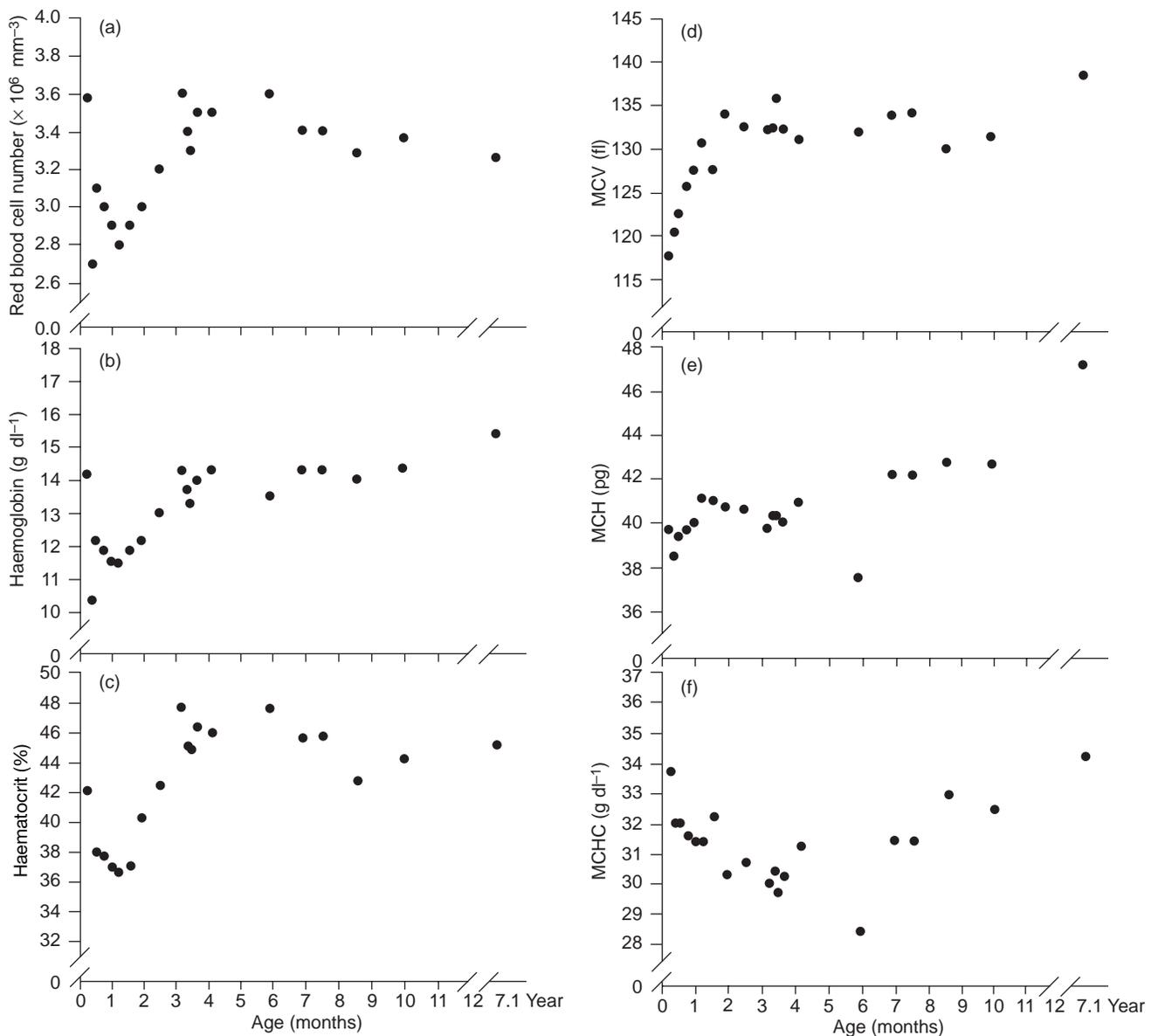
Fig. 1. Red blood cell count (a), haemoglobin content (b), haematocrit (c), mean corpuscular volume (MCV) (d), mean cell haemoglobin (MCH) (e), and mean corpuscular haemoglobin concentration (MCHC) (f) in one individual dolphin from the zoological park population as a representative of early development in bottlenose dolphins *Tursiops truncatus*. ●, a single blood sample from the dolphin.

from an electronic cell counter (mean ± SD = 41.68 ± 2.66). The method used in the present study (the use of automatic cell counters) is similar to methods used previously to analyse the blood of cetaceans (Koopman, Westgate & Read, 1999).

Statistics

Statistical analyses were performed using Sigma Stat (Jandel Scientific). Age class values for the wild population are reported as means ± 1 SEM. Inter-age class differences for RBC, Hb, Hct, MCV, MCH and MCHC were determined by a 1-way analysis of variance. When

statistical differences were found, an all pairwise multiple comparison procedure (Student–Newman–Keuls method) was used to determine which age classes were different. Regression analyses were used to determine the relationship between age and body mass, and age and mass specific blood oxygen stores. Results were considered significant at $P \leqslant 0.05$.

RESULTS

As a result of sampling limitations for wild dolphins, the zoological park data were used to assess early post-partum development for bottlenose dolphins from 0 to
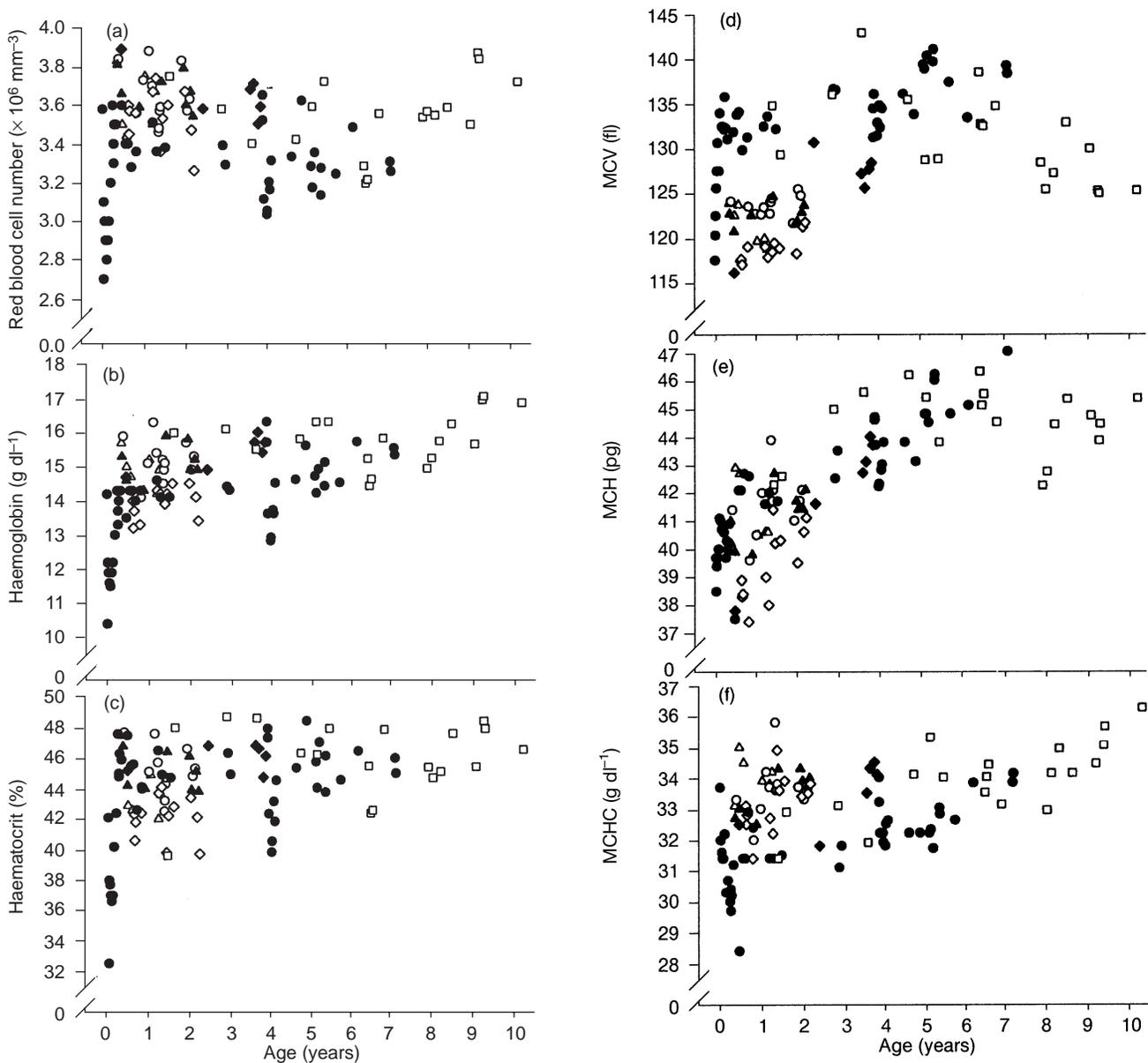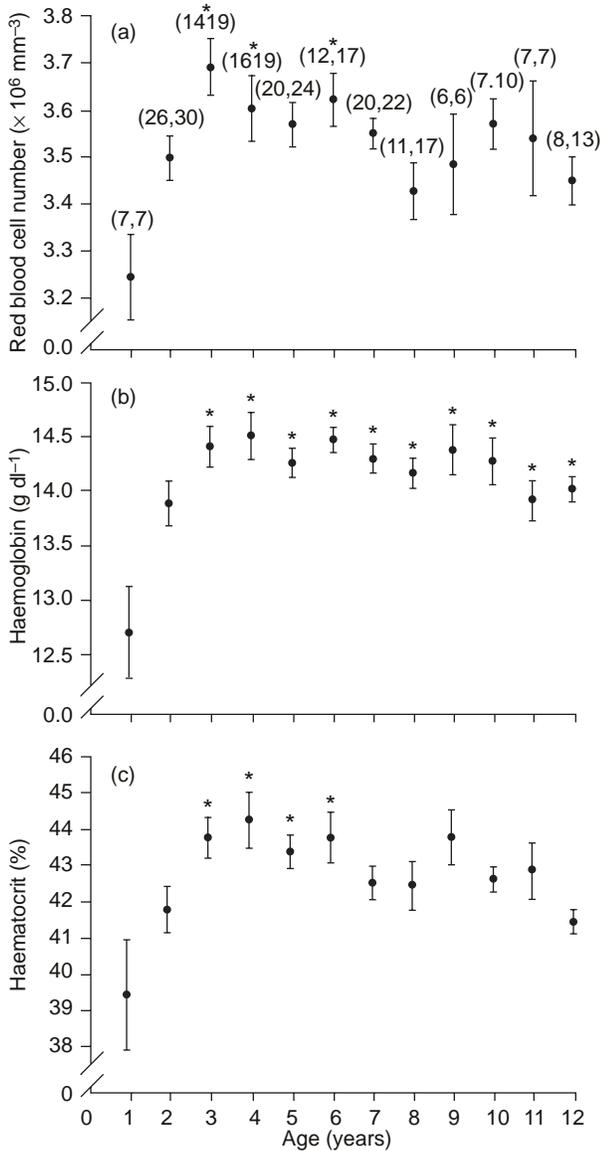
Fig. 2. Red blood cell count (a), haemoglobin content (b), haematocrit (c), mean corpuscular volume (MCV) (d), mean cell haemoglobin (MCH) (e), and mean corpuscular haemoglobin concentration (MCHC) (f) in the bottlenose dolphins *Tursiops truncatus* from the zoological park population. Each point, a single blood sample from an individual dolphin. Seven individuals are represented, and a different symbol denotes each individual (○, ●, △, ▲, ◇, ◆, □). ●, the individual dolphin represented in Fig. 1.

1 year of age (Fig. 1a–f). Broad developmental changes were assessed independently in both the zoological park dolphin population (Fig. 2a–f) and wild dolphin population (Fig. 3a–c).

In the captive bottlenose dolphins, early postpartum developmental trends were apparent in RBC, Hb, Hct, MCV, MCH and MCHC. The most pronounced changes in RBC, Hb and Hct occurred during the first 6 months of life (Fig. 1a–c). During the early developmental period these variables decreased with age from birth to *c.* 1.5 months. From 1.5 to 6 months, RBC Hb and Hct increased with age. RBC, Hb and Hct continued to increase with age for the first 3 years of life

(Fig. 2a–c). MCV increased with age from 0 to 2 months; values were similar to those of adults as early as 2 months postpartum (Fig. 1d). MCH increased after birth and approached adult values as early as 6 months after birth (Fig. 1e). MCHC decreased with age from 0 to 3.2 months and then increased, reaching values similar to those of adults as early as 6 months of age (Fig. 1f). For a few individuals, MCV and MCH values remained relatively low early in life, but for all immature dolphins, values were similar to adult levels by 3 years postpartum (Fig. 2d, e).

For the wild bottlenose dolphins aged 1–12 years, RBC, Hb and Hct increased with age from 1 to 3 years,

Fig. 3. Red blood cell count (a), haemoglobin content (b), and haematocrit (c) in the bottlenose dolphins *Tursiops truncatus* from the wild population. Each point and bar, the mean ± 1 SEM for each year age class; numbers in the parentheses, number of individual dolphins followed by the number of total blood samples for each age class; *, age classes that were significantly different from the 1-year-age class.

and RBC ($F = 2.59$, $P = 0.004$, d.f. = 190), Hb ($F = 3.52$, $P < 0.001$, d.f. = 190), and Hct ($F = 2.84$, $P = 0.002$, d.f. = 190) differed significantly between age classes (Fig. 3a–c). In contrast, MCV ($F = 1.09$, $P = 0.37$, d.f. = 190), MCH ($F = 1.41$, $P = 0.17$, d.f. = 190), and MCHC ($F = 1.58$, $P = 0.11$, d.f. = 190) did not differ between age classes for the wild dolphins aged 1–12 years.

DISCUSSION

Development of blood: marine and terrestrial mammals

The results of this study show that a developmental period is required for immature dolphins to obtain the elevated blood oxygen stores of adults. Both neonatal and juvenile bottlenose dolphins have lower red blood cell count (RBC), haemoglobin (Hb), and haematocrit (Hct) than adults (Figs 1a–c, 2a–c & 3a–c). These results are similar to those reported previously for pinnipeds (Bryden & Lim, 1969; Geraci, 1971; Lane *et al.*, 1972; Kodama *et al.*, 1977; Thorson, 1993; Horning & Trill-mich, 1997*a*; Sepúlveda, 1999). Therefore, marked changes in key blood variables occur from birth through weaning across many classes of marine mammals, regardless of whether development occurs on land or in water.

The developmental trends for RBC, Hb and Hct found in marine mammals are comparable to ontogenetic changes that occur in terrestrial mammals. For example, in humans (Rothstein, 1993) and sheep (Potocnik & Wintour, 1996), Hct decreases after birth and continues to decline for *c.* 2 months, followed by an increase to adult levels. Similar patterns were find for dolphins; Hct decreases from birth through the first month of life (Fig. 1c) and then increases until reaching adult levels by age 3 years (Figs 1c, 2c & 3c).

However, the developmental trends for mean corpuscular volume (MCV), mean cell haemoglobin (MCH = Hb/RBC), and mean corpuscular haemoglobin concentration (MCHC = Hb/HCT) found for marine mammals are different from those found for terrestrial mammals. For example, in the mountain gazelle *Gazella gazella* MCV and MCH values are high initially, decrease during the infant stage, and then increase to adult levels. MCHC levels remain relatively constant throughout development (Rietkerk, Delima & Mubarak, 1994). In contrast, in dolphins MCV and MCH values both increase during early development (Fig. 1d, e). Adult values for MCV are attained as early as 2 months (Figs 1d & 2d) and adult values for MCH are attained as early as 6 months (Figs 1e & 2e). While MCHC decreases during the first 3.2 months following birth (Fig. 1f), and then increases to near adult values by 6 months (Figs 1f & 2f). The overall increase in MCV and MCHC with age shown for bottlenose dolphin calves is similar to developmental patterns shown for southern elephant seal pups *Mirounga leonina* (Lane *et al.*, 1972).

It seems that dolphins and pinnipeds are born initially with blood characteristics similar to those of terrestrial neonates. Developmental trends for RBC, Hct and Hb in immature marine and terrestrial mammals are similar. Postpartum changes in RBC, Hct and Hb for both groups are probably associated with changes required for blood to extract oxygen from air rather than extracting oxygen from maternal blood while *in utero*. In contrast, the developmental trends for MCV, MCH and MCHC in immature marine and terrestrial

mammals differ. These differences may be associated with preparing for the demands of an aquatic lifestyle *vs* a terrestrial lifestyle. Adult marine mammals tend to have a lower number of red blood cells that are larger in size and contain larger amounts of haemoglobin than those of adult terrestrial mammals; therefore, MCV and MCH values for adult marine mammals are generally higher than those of adult terrestrial mammals (Lenfant, 1969; Ridgway *et al.*, 1970). Thus the developmental trends for MCV, MCH and MCHC found only in immature marine mammals are possibly associated with development of the red blood cell structure found in adults.

Impact of blood oxygen stores on dive capacity

Interestingly, for pinnipeds and dolphins the most rapid change in RBC, Hb and Hct occurs during the period when the young begin to practice diving. For pinnipeds, rapid blood development occurs while the pups live primarily on land and begin to dive in short bouts near shore (Thorson, 1993; Horning & Trillmich, 1997*a,b*). For dolphins, the most rapid change occurs from 1 to 6 months; at 6 months the calves begin to practice foraging (Perrin & Reilly, 1984). However, the time taken for blood to fully develop differs between pinnipeds and dolphins. The development of RBC, Hb and Hct is essentially complete for elephant seals *M. leonina* and *M. angustirostris*, 4 months postpartum (Bryden & Lim, 1969; Lane *et al.*, 1972; Thorson, 1993) and Galapagos fur seals *Arctocephalus galapagoensis*, 2 years postpartum (Horning & Trillmich, 1997*a*) before the pups begin to forage independently. In contrast, adult levels in dolphins are not obtained until 3 years postpartum (Fig. 3), which is well after weaning (weaning occurs at 1.5 years; Perrin & Reilly, 1984).

It seems counterintuitive that a long developmental period is required for dolphins, a group that must be prepared to dive immediately after birth. Sepúlveda (1999) suggested that increases in Hb and Hct with age in Juan Fernandez fur seal *Arctocephalus philippii* were associated with transitioning from a terrestrial life to a marine life. However, this cannot explain the developmental patterns found for dolphins because they live in the ocean immediately after birth. Previous studies on harbour seals *Phoca vitulina* (Kodama *et al.*, 1977) and tufted ducks *Aythya fuligula* (Stephenson, Turner & Butler, 1989) have demonstrated that dive conditioning does not change RBC, Hb, Hct, MCHC and MCH. Kodama *et al.* (1977) suggested that oxygen storage capacity is phylogenetically determined rather than the result of acclimatization to an increased frequency of apnoeic dives.

Studies on pinnipeds have shown that dive duration increases with age as a result of changes in oxygen storage capacity and increases in body size (Kooyman *et al.*, 1983; Lydersen & Hammill, 1993; Thorson, 1993; Lydersen, Hamill & Kovacs, 1994; Corpe *et al.*, 1995;

Burns & Castellini, 1996; Burns *et al.*, 1997; Horning & Trillmich, 1997*b*; Burns, Castellini & Testa, 1998; Costa *et al.*, 1998; McCafferty, Boyd & Taylor, 1998; Burns, 1999). Therefore, developmental changes in the blood oxygen stores of dolphin calves will theoretically result in changes in diving capacity. One-year-old and 2-year-old wild calves have 89% and 97% of the average mature haemoglobin content (14.3 g dl^{-1}), which will limit the ability of the blood to store oxygen. Total body oxygen stores are further limited in young dolphins because of limited oxygen stores in the muscles (Noren, Williams, Pabst *et al.*, 2001).

To assess developmental impacts on diving, we calculated the total body oxygen stores and aerobic dive limits for bottlenose dolphin age classes. Total body oxygen stores were calculated according to Shaffer *et al.* (1997) using age-specific values for bottlenose dolphins for muscle myoglobin content (Noren, Williams, Pabst *et al.*, 2001), percentage muscle mass (calculated from Dearolf *et al.,* 2000), and blood haemoglobin content (present study). Age-specific blood volumes were calculated using age-specific blood haemoglobin contents (present study) into the equation in the text such that blood volume = 813 haemoglobin – 38.6 ($r = 0.90$, $P < 0.00001$), from Snyder (1983). The equation of Snyder (1983) was derived from the relationship between haemoglobin concentration and blood volume in terrestrial and diving vertebrates, including bottlenose dolphins. Body mass for all age classes were determined from wild dolphins in the present study except for the zero age class, which was obtained from Dearolf *et al.* (2000). The calculated aerobic dive limit (cADL) was determined by dividing total body oxygen stores by metabolic rate according to Kooyman (1989). Because we were unable to measure metabolic rates in immature dolphins, two times basal metabolic rate was used (Kleiber, 1975) because this rate is associated with the minimum cost of transport for adult bottlenose dolphins (Williams, Friedl & Haun, 1993). This is a conservative estimate for metabolic rate across age classes. Admittedly, dolphin calves may have relatively higher metabolic rates than adults, as studies on pinnipeds have demonstrated that pups can have metabolic rates up to 5.2 times higher than that predicted by Kleiber (1975) (Donahue *et al.*, 2000). As a result, dolphin calves may have even lower aerobic dive limits than those presented in this study.

Mass specific blood oxygen stores of bottlenose dolphins were found to increase from 8 to 11 ml kg^{-1}, resulting in an overall increase in total mass-specific oxygen stores from 18 to 30 ml kg^{-1} during the first 3 years. The changes in body oxygen stores result in increases in cADL with maturation (Fig. 4). The cADL increases from 1.9 min at birth to 3.2, 3.6 and 4.8 min each year as dolphins grow from newborns to 3-year-old calves; these cADLs are further decreased to 0.8, 1.3, 1.4 and 1.9 min if a metabolic rate of five times basal is assumed. Although there are no further changes in mass-specific oxygen storage capacity in dolphins > 3 years old, the cADL continues to increase for
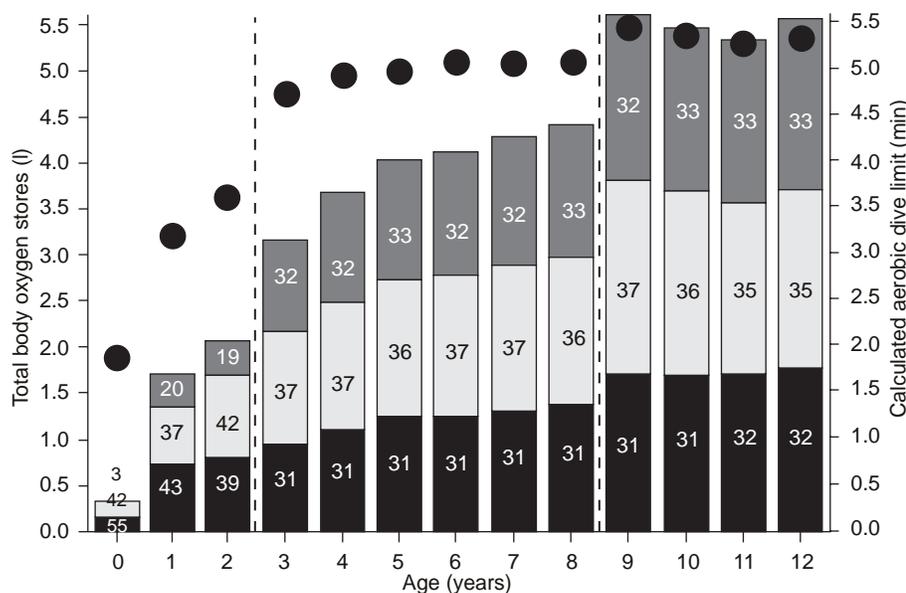
Fig. 4. Total body oxygen stores and calculated aerobic dive limits of bottlenose dolphins *Tursiops truncatus*. Total heights of the bars, the calculated total body oxygen stores; each bar is divided into three coloured bars that depict each oxygen storage compartment (black = lung oxygen store, light grey = blood oxygen store, dark grey = muscle oxygen store). Numbers within bars, percentage of the total body oxygen store represented by each respective storage compartment; closed circles, value for the calculated aerobic dive limit at each age; dashed lines separate dolphins < 3 years old who have not finished blood development and dolphins < 9 years old who have not grown to full body size (both factors influence dive capacity; see text). Mass specific blood oxygen stores increased from 8 to 10 ml kg^{-1} from 0 to 3 years according to the relationship: mass-specific blood oxygen store = 1.23 age + 7.25 ($r^2 = 0.95$, $F = 36.71$, $P = 0.03$, $n = 4$). Mass-specific blood oxygen stores plateaus at 11 ml kg^{-1} for dolphins $\geqslant$ 3 years old. Body mass increased from 19 to 151 kg from 0 to 9 years according to the relationship: body mass = 14.64 age + 51.27 ($r^2 = 0.89$, $F = 64.95$, $P = 0.00004$, $n = 10$). Body mass plateaus at 187 kg for dolphins $\geqslant$ 9 years old.

dolphins up to 9 years old. Larger body size facilitates larger total body oxygen stores and lower mass specific oxygen consumption rates in cetaceans (Noren, Williams, Ramirez *et al.*, 1999; Noren & Williams, 2000). Therefore, increases in cADLs for dolphins 0–3 years old are the result of the combined effects of increased mass-specific body oxygen stores and increased body mass. While for 4- to 9-year-old dolphins, increases in cADLs result primarily from increases in body mass (Fig. 4).

Unfortunately, we are unaware of any dive records from wild neonatal dolphins available for comparison to our cADL. However, detailed observations of a captive bottlenose dolphin calf showed that breath-hold duration increased with age and the most marked increase in breath-hold ability occurred at 6 months (Peddemors, 1990); at 6 months the first major increase in RBC, Hb and Hct is completed (Fig. 1a–c). Furthermore, the dive duration of a wild juvenile bottlenose dolphins (55.3 ± 28.9 s; Lockyer & Morris, 1987) was shorter than that of a wild adult bottlenose dolphin (112 ± 21 s; Lockyer & Morris, 1986).

In summary, the development of the blood oxygen store, like the muscle oxygen store, in bottlenose dolphins requires up to 3 years from parturition for completion. All marine mammals studied to date require postpartum development of the oxygen stores, yet this developmental period is longest in dolphins. The low mass-specific oxygen stores in neonates and juveniles contribute to short cADLs, which in turn, could influence the foraging behaviours of newly weaned juveniles and females accompanied by calves.

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