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The development of diving bradycardia in bottlenose dolphins (*Tursiops truncatus*)

Abstract Bradycardia is an important component of the dive response, yet little is known about this response in immature marine mammals. To determine if diving bradycardia improves with age, cardiac patterns from trained immature and mature bottlenose dolphins (*Tursiops truncatus*) were recorded during three conditions (stationary respiration, voluntary breath-hold, and shallow diving). Maximum (mean: 117 ± 1 beats·min^{-1}) and resting (mean: 101 ± 5 beats·min^{-1}) heart rate (HR) at the water surface were similar regardless of age. All dolphins lowered HR in response to apnea; mean steady state breath-hold HR was not correlated with age. However, the ability to reduce HR while diving improved with age. Minimum and mean steady state HR during diving were highest for calves. For example, 1.5–3.5-year-old calves had significantly higher mean steady state diving HR (51 ± 1 beats·min^{-1}) than 3.5–5.5-year-old juveniles (44 ± 1 beats·min^{-1}). As a result, older dolphins demonstrated greater overall reductions in HR during diving. Longitudinal studies concur; the ability to reduce HR improved as individual calves matured. Thus, although newly weaned calves as young as 1.7 years exhibit elements of cardiac control, the capacity to reduce HR while diving improves with maturation up to 3.5 years postpartum. Limited ability for bradycardia may partially explain the short dive durations observed for immature marine mammals.

Keywords Heart rate · Bradycardia · Dive response · Development · Dolphins

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Abbreviations *ADL* aerobic dive limit · *cADL* calculated aerobic dive limit · *ECG* electrocardiogram · *HR* heart rate · *TDR* time–depth recorder

Introduction

The control of heart rate (HR) by marine mammals is a critical feature of the dive response that facilitates the prolonged breath-holds required for foraging and locomotion (Kooyman 1989). A hallmark of this control is bradycardia, a pronounced lowering of HR in response to submergence. Adult bottlenose dolphins (Irving et al. 1941) and killer whales (*Orcinus orca*; Spencer et al. 1967) exhibit a 50% reduction in HR upon submergence compared to resting on the water surface. Elsner et al. (1966) demonstrated an even more pronounced reduction in HR for an adult Pacific bottlenose dolphin (*Tursiops truncatus gilli*) trained to dive in a pool. In this animal, HR declined from 90–100 beats·min^{-1} while on the water surface to a minimum HR of 20 beats·min^{-1} within 60 s of diving. These studies on cetaceans and previous work on pinnipeds (Harrison and Tomlinson 1960; Irving et al. 1963; Van Critters et al. 1965; Ridgway et al. 1975; Andrews et al. 1997) suggest that bradycardia is a fundamental response to breath-hold by adult marine mammals.

Less is known about the cardiovascular responses of immature marine mammals or the relationship between diving capability and bradycardia during development. Postnatal development of cardiac control has been studied in terrestrial mammals, and to a lesser extent in marine mammals. Several changes in cardiorespiratory patterns characterize postnatal mammalian development. One of the most important is the link between respiratory and cardiac events. For example, cardiorespiratory patterns in human infants are irregular from birth to the sixth month of life (Patzak et al. 1996). The appearance of sinus arrhythmia, in which HR increases during inhalation and decreases during exhalation, is a normal pattern in terrestrial mammalian postnatal

development (Katona et al. 1980; Schubert et al. 1987; Leistner et al. 1990). As in terrestrial mammals, elephant seals (*Mirounga angustirostris* and *Mirounga leonina*) develop many features of cardiac control early in life (Castellini et al. 1994a, 1994b; Falabella et al. 1999). The changes in HR associated with normal sinus arrhythmia, sleep apnea, and diving apnea appear to be similar in seals, suggesting that regulation is by a common homeostatic control mechanism (Castellini et al. 1994b). Thus, attaining cardiac control during apneustic events prior to weaning likely facilitates the transition to sea, and the ability of seal pups to maintain bradycardia during diving. However, the development of diving bradycardia in marine mammals has only been examined in forced submergence studies on seals (Harrison and Tomlinson 1960; Irving et al. 1963; Hammond et al. 1969).

Although postnatal development of cardiac control has been studied in pinnipeds, little is known about changes in cardiac function with development in cetaceans. Unlike seals and sea lions, whales and dolphins are born directly into water and must breath-hold to swim and dive immediately postpartum. Consequently, cetaceans may exhibit evidence of cardiac control earlier in life than pinnipeds. Alternatively, because seals demonstrate a shorter period of maternal dependence and must independently forage sooner in life than dolphins, dolphins may be afforded a longer developmental period. For example, the developmental period required to attain mature oxygen stores that support diving is much shorter in seals than in dolphins (Noren et al. 2001); bottlenose dolphins do not attain mature oxygen stores in the muscle or blood until 3 years postpartum (Noren et al. 2001, 2002).

To determine the changes in cardiac response that occur with development in cetaceans, HR patterns of immature bottlenose dolphins (1.7–5.4 years old) were examined and compared to similar measurements collected from adult dolphins. We investigated instantaneous and average HR during stationary respiration on the water surface, voluntary breath-hold just below the surface, and shallow diving to depths of 4–5 m. Variability in HR throughout the respiratory cycle and the degree of bradycardia were quantified. The results show that although calves demonstrate elements of cardiac control by weaning, the level of bradycardia during diving improves with age until approximately 3.5 years postpartum.

Materials and methods

Animals

Nine dolphins (seven calves and juveniles, two adults) housed at the Dolphin Experience (Freeport, Grand Bahamas Island) were trained for the experiments. All dolphins were maintained in large (15 m×15 m×5 m deep) saltwater enclosures connected to the open ocean and fed a daily diet of capelin and herring supplemented with multi-vitamins (Sea Tabs). Studies were conducted over three field seasons (June–July 1999, May–June 2000, and June 2001). Mean temperatures for water and air were 29.0 ± 0.10 °C and 29.6 ± 0.29 °C, respectively. When possible, immature dolphins were studied longitudinally for all 3 years. The duration of the longitudinal studies for each dolphin depended on the age and level of training of the dolphin at the start of the study. All immature dolphins had been born at the facility and were of known age. Adults were sexually mature and had been maintained at the facility for at least 9 years; exact ages were unknown as they were originally obtained from the wild.

Body mass for each dolphin was calculated using Morphometric Calc pre-release Version 1.4 (Outernet Technologies International 1999, 2000) accessible at: http://www.outernet-tech.com/research/download/ (Messinger and Weissensel 1999). This equation utilizes gender, total body length, maximum girth, and age of the dolphin to estimate body mass. The equation was designed specifically for bottlenose dolphins; data used to formulate the equation were acquired from dolphins at four facilities including The Dolphin Experience. To validate the equation, both an adult and immature dolphin from the present study were weighed on a beaching scale. The measured and calculated body mass differed by only 4% and 9% for the adult and immature dolphin, respectively.

Heart rate

Data collection for HR was limited to calves that were old enough to be trained to wear the experimental equipment and to follow the experimental protocols (see below). Methods and equipment were similar to those used previously for assessing resting and active HRs in adult bottlenose dolphins (Williams et al. 1993b).

Instantaneous HR

To determine the relationship between respiratory events and instantaneous changes in HR, electrocardiogram (ECG) traces were recorded on grid paper for seven dolphins, one adult (age >9 years) and six immature animals (ages: 1.7–5.4 years). Two suction cups (8 cm diameter with a 2.5-cm diameter silver plate electrode) were attached to the dolphin. One cup was placed along the ventral midline directly below the pectoral fin insertions, and the other was placed above the right scapula. The two electrodes were attached by shielded wires to an electrocardiograph (Birtcher Model 365). Beat to beat measurements were recorded on an ECG trace while the dolphins performed two behaviors: (1) stationary respiration, and (2) voluntary breath-hold. For the stationary respiration trials, HR was recorded from breath to breath while the dolphin calmly rested on the water surface. One stationary respiration trial was conducted for each dolphin. The voluntary breath-hold trials were conducted to supplement shallow diving tests because equipment limitations prevented ECG recordings during diving. For these trials, each dolphin was trained to float upside-down on the water surface while ECG was continuously recorded from breath through submergence to the following voluntary breath (which ended the trial) according to Williams et al. (1993b). One voluntary breath-hold trial was conducted for five dolphins (age range: 2.0 years to adult). Throughout the trials, respiratory events of the dolphins were marked onto the ECG traces by an observer. HRs and breath-hold durations were determined from the scored traces (described in Instantaneous HR analysis below).

Average HR

To determine the effects of age on diving bradycardia, average HR was recorded for dolphins trained to dive to 4–5 m in depth. Suction cup electrodes were placed on the dolphins as described above, and attached to an HR monitor that was housed in a strap secured around the dolphin's pectoral fin. Signals from the HR monitor

were received continuously by a time–depth recorder (TDR; Mke 3 Wildlife Computers, Redmond, Wash.) also housed in the pectoral fin strap; average values for HR were recorded for each 10-s interval. HR was monitored while the dolphins performed two behaviors: (1) voluntary breath-hold as described above, and (2) shallow diving to 4–5 m. For the latter trials, the dolphins were trained to dive to a trainer on SCUBA stationed at 4–5 m sub-surface. The dolphins were rewarded with fish throughout the dive. Dive trials ended when the dolphin voluntarily swam to the surface and took a breath. Two adults and seven immature dolphins (studied over 1–3 years) were used in these tests, affording an age range for immature dolphins of 1.7–5.4 years. During each session, several voluntary breath-hold or dive trials were performed while HR was continuously measured. An observer recorded the behavior (voluntary breath-hold or dive) and respiratory events of the dolphins throughout each session. The observer's watch was synchronized with the TDR clock prior to each session to correlate cardiac, respiratory, and behavioral events.

Analysis and statistics

Data were used to assess differences throughout maturation in: (1) the rate of onset of bradycardia upon breath-hold, (2) the ability to maintain a stable steady state HR during breath-hold, (3) mean HR during stationary respiration, breath-hold, and diving, (4) maximum HR at the surface, (5) minimum HR while diving, and (6) the percentage change in HR from surface breathing to diving. Statistical analyses were performed using Sigma Stat (Jandel Scientific 1999). Means ± 1SEM are presented and results deemed significant at $P < 0.05$ unless otherwise noted.

Instantaneous HR

Interbeat intervals were derived from the ECG trace (Fig. 1) after visually identifying each QRS complex. HR was calculated from each R-to-R interval visually, as the paper trace consisted of 1-mm square grids and the paper speed was set at 25 mm·s^{-1}. Apnea duration during stationary respiration and voluntary breath-hold was determined from the time interval between two adjacent breaths using the observer marks on the ECG strip.

ECG records from stationary respiration trials were used to calculate mean resting HR. ECG records from voluntary breath-hold trials were used to assess mean steady state HR during breath-hold. In order to calculate mean steady state HR during breath-hold, the data were first smoothed by transforming the heartbeats into running means (based on the average of three consecutive heartbeats). The transformed data were then plotted against time into the trial and visually inspected to determine the inflection points that defined the beginning and end of the steady state period during breath-hold (where HR remained at a consistently low rate). The incline sections prior to and after this segment represented transitional HRs associated with breathing. The transformed HR data representing the steady state segment were averaged, and to ensure that all heartbeats associated with the steady state segment were included in the final analyses, the steady state segment was extended on each end by accepting all transformed HRs equivalent to the mean + 2 SD. The non-transformed HR data corresponding to this extended steady state segment were averaged to determine the mean steady state HR, and the variance around the mean was calculated. All heartbeats in the transition period prior to the steady state segment were used to determine the rate of change for HR at the onset of breath-hold; the slope was determined by least squares regression analysis.

Pearson product moment correlation tests were used to assess the influence of age on resting HR, steady state HR during breath-hold, the variance in steady state HR during breath-hold, and the rate of change in HR during the transition from breathing to steady state bradycardia. Adults were excluded from the correlation analyses because their exact ages were unknown. The resting HR and steady state HR from all dolphins combined were compared by a t-test.

Average HR

The accuracy of determining HR from 10-s averages was assessed by comparing average HR recorded by the TDR to instantaneous HR from ECG recordings during voluntary breath-holds of similar duration performed by the same dolphins. HRs recorded for the entire breath-hold cycle were similar for each method ($t = 1.870$, $df = 4$, $P = 0.135$).

Bradycardia varies with dive duration (Ridgway et al. 1975), thus longer dives for each dolphin resulted in a more pronounced cardiac response. To ensure that the most pronounced cardiovascular response was analyzed and each dolphin contributed equally, the five longest dives performed by each dolphin were used for the analyses. The following were determined for each dive: maximum HR at the surface, mean steady state HR while diving (described above), minimum HR during submergence, and the percentage reduction in HR while diving, where percent change in HR is equivalent to:

$$[(\text{maximum surface HR} - \text{minimum diving HR})/$$
$$(\text{maximum surface HR})] \times 100 \qquad (1)$$

Data for HR were grouped into five age classes, 1.5–2.5 years, 2.5–3.5 years, 3.5–4.5 years, 4.5–5.5 years, and adult. The two youngest age classes represent calves that are still developing oxygen stores (Noren et al. 2001, 2002). The two older immature age classes represent juveniles that have mature muscle and blood oxygen stores but have not yet attained adult body size. Maximum HR at the surface, mean steady state HR while diving, minimum HR during diving, and the percentage reduction in HR were compared across age classes using a one way ANOVA in combination with Tukey all pairwise comparisons.

Fig. 1 Representative electrocardiogram trace recorded from a 1.7-year-old bottlenose dolphin calf during stationary respiration on the water surface. Time and a representative QRS complex and R-to-R interval, which were used to determine heart rate, are indicated
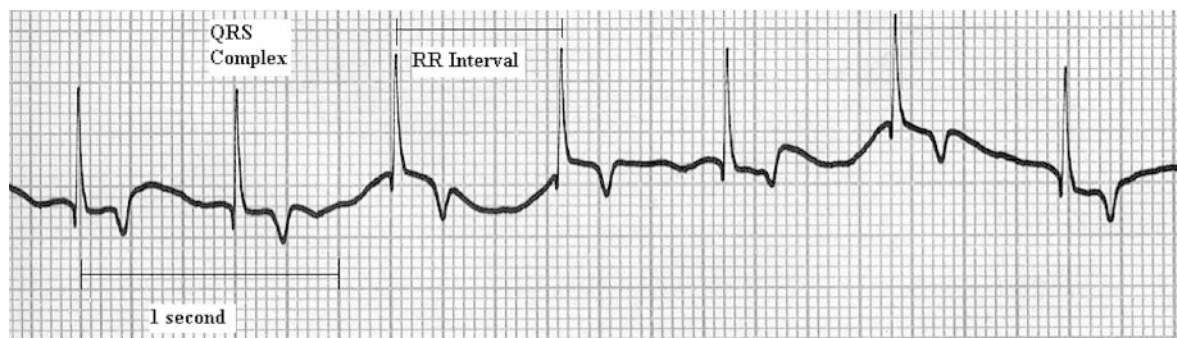
Developmental trends in cardiac control were also assessed for calves that were studied longitudinally over 2–3 consecutive years. Data obtained from individual calves were compared across years and differences in the level of bradycardia during diving throughout maturation were either determined by a *t*-test or one-way ANOVA in combination with Tukey all pairwise comparisons.

Dive performance

Similar to the HR analyses, differences in observed dive durations between age classes were quantified by a one way ANOVA in combination with a Tukey all pairwise comparison. Longitudinal analyses of dive capacity for individual calves were either determined by a *t*-test or a one-way ANOVA in combination with Tukey all pairwise comparisons.

To determine whether voluntary dives and their associated HRs were representative of maximal aerobic efforts, aerobic dive limits (ADLs) were determined for each dolphin in the study. ADLs were calculated by dividing total body oxygen stores by metabolic rate according to Kooyman (Kooyman 1989). Methods for calculating ADLs of immature and mature bottlenose dolphins are described in detail in Noren et al. (2002). Briefly, ADLs for all dolphins were calculated assuming a metabolic rate of two times basal metabolic rate (Kleiber 1975) as measured for adult bottlenose dolphins (Williams et al. 1993b). Values for the calculated aerobic dive limit (cADL) and the five longest dive durations for 1.7–5.4-year-old dolphins were plotted against age. Slopes for these relationships were determined by least squares regression analyses, and compared using one-way ANOVA.

Variables influencing HR

Because the older dolphins were larger and exhibited longer dive durations, it was necessary to differentiate between the effects of dive duration, body mass, and age on HR. Data from the longest dive performed by each dolphin were used in a forward stepwise regression to determine which variable (age, body mass, or dive duration) best predicts maximum HR at the surface, mean steady state HR during diving, minimum HR during diving, and percentage reduction in HR. Adults were excluded from these analyses, as their exact ages were unknown.

Results

### Cardiac control during respiration and breath-hold

The mean resting HR during stationary respiration on the water surface was not associated with age ($n = 11$, $r = -0.155$, $P = 0.649$). Evidence of cardiac control in response to prolonged apnea was demonstrated by dolphins of all ages. HR declined significantly with the onset of breath-hold and the rate of change did not correlate with age for either voluntary breath-hold ($n = 4$, $r = 0.837$, $P = 0.163$) or shallow diving ($n = 14$, $r = -0.174$, $P = 0.551$; Fig. 2). For all dolphins, mean resting HR was significantly greater than mean steady state HR during breath-hold ($t = 5.561$, $df = 15$, $P < 0.001$). At the end of breath-hold, all dolphins demonstrated an anticipatory tachycardia as HR abruptly increased within a few seconds before breathing (Fig. 2).
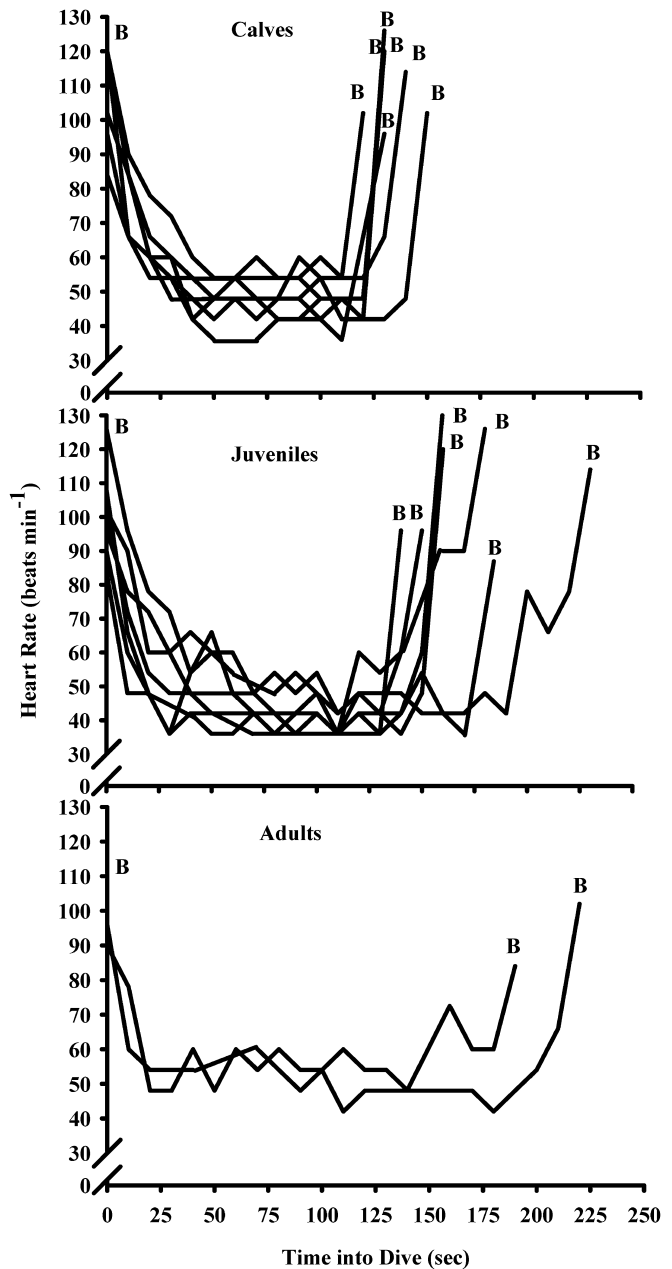
Fig. 2 Average heart rate (HR) throughout a shallow dive cycle for calves (1.5–3.5-year-olds), juveniles (3.5–5.5-year-olds), and adult bottlenose dolphins. HR was averaged over 10-s intervals using a time–depth recorder (TDR) microprocessor worn by the diving dolphins. The longest dive performed by each dolphin is shown. *B* indicates the point when the dolphins took a breath. All dolphins show a pattern of bradycardia during submergence and anticipatory tachycardia before surfacing

The effect of development on bradycardia

Mean steady state HR during voluntary breath-hold was not correlated with age ($n = 4$, $r = -0.533$, $P = 0.467$). The inability to detect a significant relationship between age and mean steady state HR during breath-hold may be a result of limited sample size; the 2-year-old calf (youngest animal in this portion of the study)
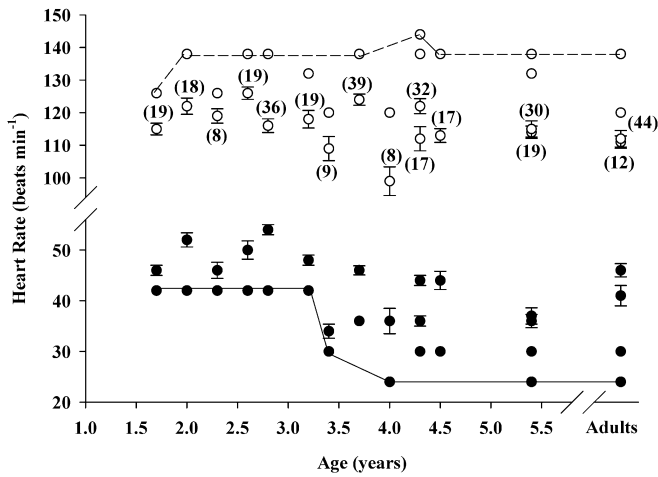
Fig. 3 Minimum and maximum HR recorded during shallow dive cycles >1 min in relation to age for bottlenose dolphins. *Points with error bars* represent minimum HR during diving ± 1 SEM (*filled circles*) and maximum HR at the water surface ± 1 SEM (*unfilled circles*) for individual dolphins. *Filled circles connected by a solid line* represent the absolute minimum HR recorded while submerged. *Unfilled circles connected by a dashed line* represent the absolute maximum HR recorded at the surface. The *numbers in parentheses* are the number of dives

demonstrated the highest mean steady state HR during apnea (66 ± 0.8 beats·min^{-1}) while the adult demonstrated the lowest (45 ± 1.3 beats·min^{-1}). The variance associated with the mean steady state HR during breathhold (variance range: 8–20; SD range: 5–9) did not correlate with age ($r = 0.790$, $n = 4$, $P = 0.210$); the adult value for variance fell within the range demonstrated for immature dolphins. Thus, all dolphins showed similar abilities to maintain a stable HR during breath-hold.

Differences in minimum HR achieved during diving indicate that bradycardia is refined with maturation. The absolute minimum HR achieved during diving represents the most pronounced cardiovascular response; calves (<3.4 years old) demonstrated HRs as low as 42 beats·min^{-1}, but older calves, juveniles, and adults could attain lower HRs (24–30 beats·min^{-1}; Fig. 3). In contrast to minimum HR, there was no discernable pattern associated with age for maximum HR recorded at the water surface prior to or after a dive (Fig. 3).

When grouped according to age class, mean steady state diving HR, minimum HR during submergence, and percent HR reduction while diving also indicate developmental trends for dolphins (Fig. 4a). Minimum and mean steady state HR during diving differed significantly among age classes (minimum HR: $F = 6.297$, $df = 4$, 75, $P < 0.001$; mean steady state HR: $F = 5.794$, $df = 4$, 75, $P < 0.001$). Tukey all pairwise comparisons demonstrated that juveniles (3.5–4.5-year-olds and 4.5–5.5-year-olds) maintain significantly lower minimum and mean steady state HR during diving compared to calves (1.5–2.5-year-olds and 2.5–3.5-year-olds; minimum HR: $q = 4.587$, 5.721, 4.162, 5.382; $P < 0.05$; mean steady state HR: $q = 3.997$, 5.196, 4.090, 5.344; $P < 0.05$). Values for minimum and mean steady state HR during
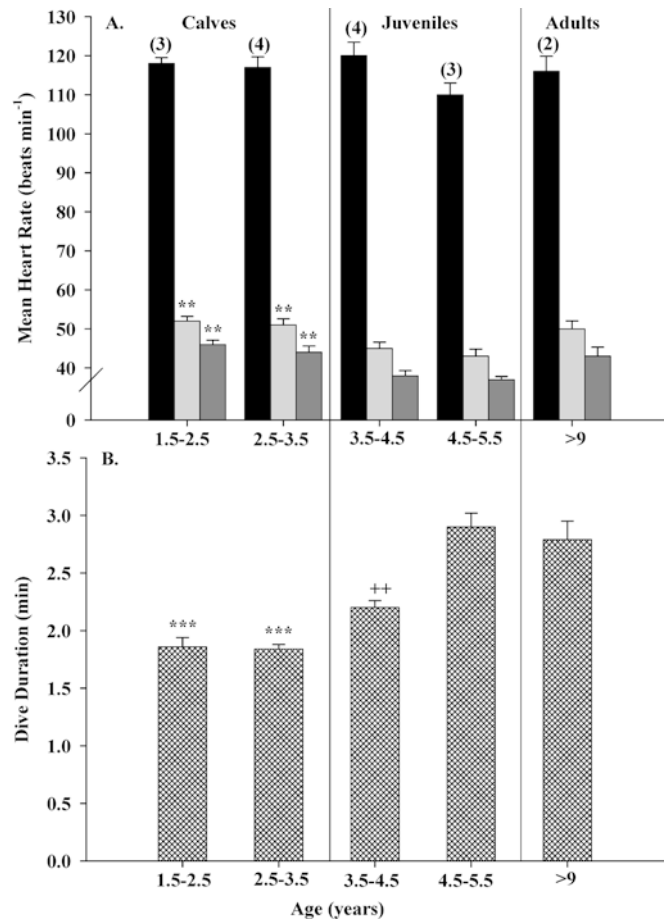
Fig. 4A–B HR (**A**) and dive duration (**B**) during shallow diving in relation to age class. *Bars with error lines* represent age class means + 1 SEM (includes data from the five longest dives performed by each dolphin). The number of animals in each age class is shown in *parenthesis*. **A** Maximum HR during surface breathing (*black bars*), mean steady state HR during diving (*light gray bars*), and minimum HR during diving (*dark gray bars*) are compared. Values for calves that are significantly greater then values for juveniles are denoted by *two asterisks*. Maximum HR at the surface was similar across age classes. See text for statistics. **B** *Crosshatched bars* represent mean voluntary dive durations. Values for calves that are significantly lower than values for juveniles and adults are denoted by *three asterisks*. Values for the 3.5–4.5-year-old juvenile age class that are significantly lower than values for the 4.5–5.5-year-old juvenile and adult age classes are denoted by *two plus signs*. See text for statistics

diving were similar for juveniles and adults. Although values for minimum and mean steady HR during diving appeared lower in adults compared to calves, these differences were not significant. In contrast, maximum HR recorded during surfacing were similar across all age classes ($F = 1.507$, $df = 4$, 75; $P = 0.209$). A consequence of the enhanced ability to lower HR during submergence in older dolphins is a significantly greater percent reduction in HR during diving compared to younger dolphins ($F = 5.820$, $df = 4$, 75; $P < 0.001$). Tukey all pairwise comparisons demonstrated that juveniles maintained significantly greater reductions in HR (67–68%) during diving compared to calves (61–62%;

$q = 5.321, 4.831, 4.733, 4.225; P < 0.05$). The ability for HR reduction during diving in juveniles was similar to that observed in adults (66%). Although adults appeared to demonstrate a greater capacity for HR reduction during diving than calves, these differences were not significant.

Longitudinal measurements on individual dolphins further demonstrate enhanced cardiac control during diving with maturation (Fig. 5). The ability to reduce HR while diving improved with age for each of the three youngest calves in this portion of the study. As a result, the level of bradycardia during diving increased throughout maturation for these individuals (Calf 1: One way ANOVA $F = 15.914$, $df = 2$, 12; $P < 0.001$, Tukey all pairwise $q = 4.405, 7.963$; $P < 0.05$; Calf 2: one way ANOVA $F = 3.794$, $df = 2$, 12; $P = 0.053$, Tukey all pairwise comparison $q = 3.061, 3.617$; $P < 0.10$; Calf 3: One way ANOVA $F = 4.406$, $df = 2$, 12; $P = 0.037$, Tukey all pairwise comparison $q = 3.945$; $P < 0.05$; Fig. 5). In contrast, the level of bradycardia during diving for the oldest calf, measured at 4.5 years and 5.4 years of age, did not change between years ($t = -2.150$, $df = 8$, $P = 0.064$). These results suggest that diving bradycardia

is refined by approximately 3.5 years postpartum (Fig. 5).

The effect of development on dive duration

Dive duration was significantly longer for older bottlenose dolphins (Figs. 4b, 5, 6). Age class comparisons demonstrated these differences ($F = 32.660$, $df = 4$, 75; $P < 0.001$; Fig. 4b), where calves had significantly shorter dive durations than 3.5–4.5-year-old juveniles ($q = 4.108, 4.713$; $P < 0.05$), 4.5–5.5-year-old-juveniles ($q = 11.884, 12.960$; $P < 0.05$) and adults ($q = 9.541, 10.282$; $P < 0.05$). Furthermore, 3.5–4.5-year-old juveniles had significantly shorter dive durations than the older juveniles ($q = 8.596$; $P < 0.05$) and adults ($q = 6.433$, $P < 0.05$).

A significant increase in dive duration with maturation was also evident for each of the three youngest calves studied longitudinally (Calf 1: one way ANOVA $F = 19.476$, $df = 2$, 12; $P < 0.001$, Tukey all pairwise comparison $q = 8.324, 6.699$; $P < 0.05$; Calf 2: one way ANOVA $F = 11.286$, $df = 2$, 12; $P = 0.002$, Tukey all pairwise comparison $q = 6.134, 5.442$; $P < 0.05$; Calf 3: one way ANOVA $F = 70.275$, $df = 2$, 12; $P < 0.001$, Tukey all pairwise comparison $q = 15.247, 13.662$; $P < 0.05$; Fig. 5). As found for diving HR, the oldest calf showed no difference in dive duration at the age of 4.5 years and 5.4 years ($t = 0.742$, $df = 8$, $P = 0.479$; Fig. 5).

Across all immature dolphins examined in this study, dive duration and cADL increased with age (Fig. 6). Although values for cADL are greater than the measured dive durations, the slopes of these relationships
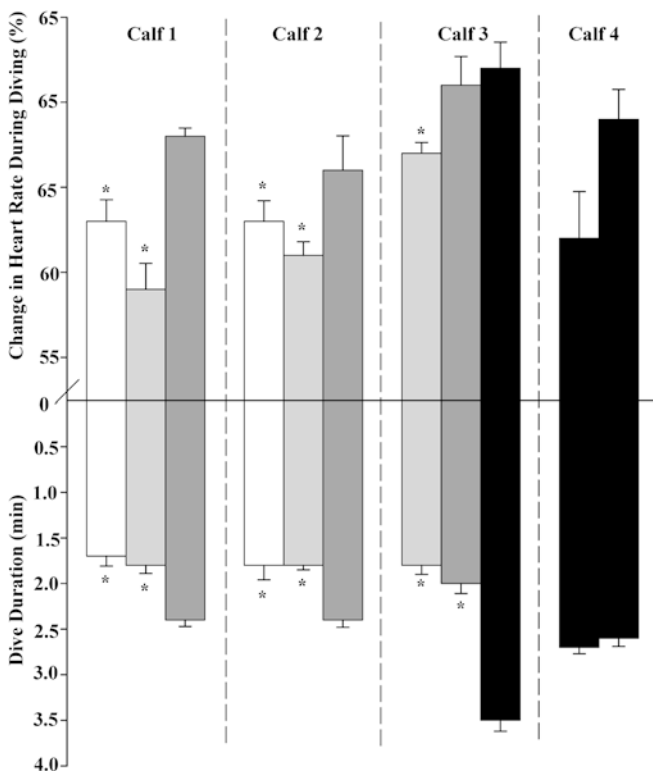
Fig. 5 The percentage reduction in HR during shallow diving and dive duration for four immature dolphins studied longitudinally over 2–3 consecutive years. Measurements for each calf are presented in longitudinal order. *Bars with error lines* represent the mean + 1 SEM for individuals and are colored according to age [1.5–2.5 years (*white*), 2.5–3.5 years (*light gray*), 3.5-4.5 years (*dark gray*), and 4.5–5.5 years (*black*)]. Intra-individual comparisons for Calf 1, 2, and 3 indicate differences between annual measurements and the *asterisk* denotes the measurements that were significantly different than the final (oldest) measurement. See text for statistics
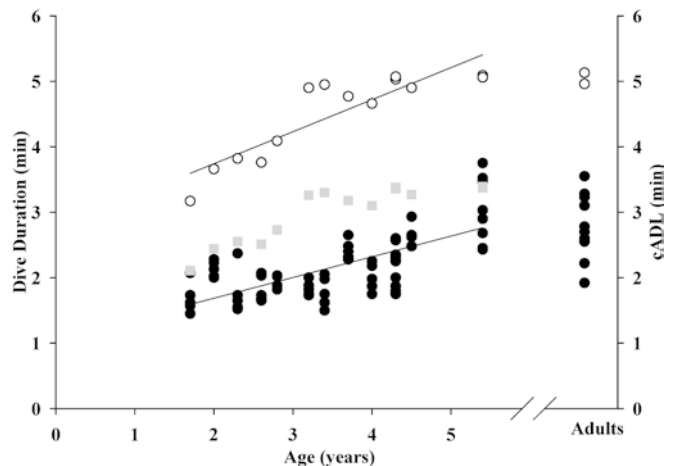
Fig. 6 Calculated aerobic dive limit and voluntary dive duration in relation to age. The calculated aerobic dive limit (*cADL; unfilled circles*) and the five longest dive durations (*filled circles*) for each dolphin are represented. *Solid lines* are the least squares linear regressions for the immature dolphins, where cADL = 0.5 age + 2.8 ($r^2 = 0.78$, $F = 43.298$, $df = 1$, 12; $P < 0.001$) and dive duration = 0.3 age + 1.1 ($r^2 = 0.51$, $F = 69.767$, $df = 1$, 68; $P < 0.001$). *Gray squares* represent cADLs that assume a metabolic rate of three times basal metabolic rate to account for additional metabolic costs for immature animals (Donohue et al. 2000)

were not significantly different ($F = 3.645$, $df = 1$, 82, $P = 0.06$; Fig. 6); this suggests that the voluntary dives performed by the immature dolphins represented similar relative aerobic efforts.

Variables influencing HR

Although older dolphins had greater body mass and exhibited longer dive durations, results of the forward stepwise regression indicated that age, body mass, and dive duration were poor predictors of maximum HR at the surface. Age was the only variable that significantly predicted mean steady state HR during diving, minimum HR during diving, and the percentage reduction in HR during diving for 1.7–5.4-year-old dolphins according to:

$$\text{Mean steady state diving HR} = -3 \text{ age} + 56 \quad (2)$$

$(r^2 = 0.33$, $F = 5.810$, $df = 1$, 12; $P = 0.033)$

$$\text{Minimum HR during diving} = -4 \text{ age} + 54 \quad (3)$$

$(r^2 = 0.53$, $F = 13.46$, $df = 1$, 12; $P = 0.003)$

$$\text{HR reduction during diving} = 3 \text{ age} + 54 \quad (4)$$

$(r^2 = 0.60$, $F = 18.21$, $df = 1$, 12, $P = 0.001)$, where HR is measured in beats per minute, HR reduction is as a percentage, and age is in years. These results indicate that the refinement of diving bradycardia is not influenced by changes in body mass or dive duration throughout maturation. Rather, the development of physiological processes as young dolphins age appears to be the major factor leading to an increased ability to reduce HR and achieve a more pronounced bradycardia response during diving.

Discussion

Maturation of HR in mammals: developing cardiac control

Across mammals, HR patterns of mature individuals demonstrate similar features. This includes sinus arrhythmia, the capability to rapidly transition between elevated and reduced HRs during the respiratory cycle, and the ability to maintain a stable HR once a steady state has been reached. Rather than an inherent feature, HR adjustment in response to respiratory events requires a refinement in cardiac control and improves with maturation in mammals. For example, puppies (Haddad et al. 1987) and southern elephant seal neonates (Falabella et al. 1999) show smaller differences in HR during their respiratory cycle compared to adults, and the change in HR associated with respiration occurs more gradually in the immature animals compared to the adults. Furthermore, the HRs of young northern (Castellini et al. 1994b) and southern (Falabella et al. 1999)

elephant seal pups are more variable during sleep apnea than the HRs recorded during this period for older pups. After a period of postnatal development mature cardiac patterns appear, such that Northern elephant (Castellini et al. 1994a, 1994b) and harbor (*Phoca vitulina*; Lapierre et al. 2001; Greaves et al. 2001) seal pups demonstrate mature cardiac cycles associated with respiration before weaning.

Less detailed information concerning changes in cardiac control is available for young cetaceans due to the difficult logistics of attaining physiological data from this group. A mysticete calf (California gray whale, *Eschrichtius robustus*; Ponganis and Kooyman 1999) and a juvenile odontocete (harbor porpoise, *Phocoena phocoena*; Reed et al. 2000) demonstrated an ability to adjust HR during the respiratory cycle. Similarly, bottlenose dolphin calves as young as 1.7 years old demonstrate elements of cardiac control. Like mature bottlenose dolphins, calves lower HR in response to breath-hold and are capable of maintaining stable minimum HRs during breath-hold. Mean change in HR during the transition from breathing into breath-hold was 2.5 ± 0.9 beats $\text{min}^{-1} \cdot \text{s}^{-1}$, ($n = 5$). For bottlenose dolphins, this development has occurred by the age of weaning (weaning occurs at approximately 1.5–1.7 years postpartum; Perrin and Reilly 1984). Thus, newly weaned bottlenose dolphin calves have the necessary cardiac control that would be required to elicit bradycardia while diving.

The effect of age on bradycardia: refining cardiac control

A similar pattern of accelerating and decelerating HR in response to breathing and breath-hold is a vital component of the dive response of marine mammals (Kooyman 1989), albeit in an exaggerated form. Presumably, the control mechanisms that govern cardiovascular responses to apnea on land may be used to modify cardiovascular responses during diving (Irving et al. 1935; Castellini et al. 1994a). Juvenile northern elephant seals show a similar cardiac response to both terrestrial and diving apneas, although the terrestrial response is less pronounced than the diving response (31% versus 64% reduction in HR; Andrews et al. 1997). By markedly reducing HR to a fraction of that exhibited when the animal is breathing, diving marine mammals are able to conserve limited on-board oxygen stores that support aerobic metabolism when submerged.

Studies of bradycardia during forced dives in young pinnipeds indicate that harbor (Harrison and Tomlinson 1960), fur (*Callorhinus ursinus*; Irving et al. 1963), and northern elephant (Hammond et al. 1969) seal pups are able to markedly lower HR in response to submergence. The level of bradycardia increases with age in harbor seals (Harrison and Tomlinson 1960) and fur seals (Irving et al. 1963). In contrast, freely diving juvenile elephant seals (Andrews et al. 1997), and forcibly

submerged neonatal (Hammond et al. 1969) and adult (Van Critters et al. 1965) elephant seals show similar levels of bradycardia.

As reported for harbor and fur seals, the present study demonstrates that bradycardia during diving changes with maturation in bottlenose dolphins. Absolute minimum HRs during shallow diving are lower in adults and juveniles than in calves (Fig. 3) with the result that mean steady state HR during diving is maintained at lower levels in older dolphins (Fig. 4a). Despite these absolute changes, differences between age classes for minimum and mean steady state HR during diving were only significant between calves and juveniles; calves were not significantly different from adults (Fig. 4a). One explanation for this discrepancy is that the full cardiac response in adults may not have been initiated because of their relatively short dive durations (Figs. 4b, 6). According to Williams et al. (1993a), adult bottlenose dolphins are capable of aerobically supported dive durations of 4.4 min. Without the "anticipation" of a long dive, which strongly influences the degree of bradycardia, the cardiac response can be highly variable (Kooyman 1989). Furthermore, Ridgway et al. (1975) demonstrated that the level of diving bradycardia varies with dive duration in adult bottlenose dolphins. If the adults in this study had prolonged dive duration, it is likely that the variability associated with the age class mean for mean steady state diving HR and minimum HR during diving would have been reduced and the actual values of these means may have been lowered. As a result, the ability to detect differences in diving HR between calves and adults would have been improved. Unlike the adult dolphins, the immature dolphins in this study were closer to their physiological dive limits during the diving trials (Fig. 6). Therefore, comparisons of diving HR between calves and juveniles were not encumbered by these factors. Regardless, the results of this study suggest that a mature bradycardia response during diving is established by approximately 3.5 years postpartum for bottlenose dolphins (Figs. 4a, 5).

Many factors undoubtedly influence the change in diving bradycardia that occurs during maturation. An evaluation of the relative effects of body mass, dive duration, and age demonstrates that throughout development, age is the primary predictor of minimum and mean steady state HR during diving for dolphins. This does not imply that this refinement in bradycardia is attributable simply to the lowering of HR with age as typically occurs in developing animals (Dittmer and Grebe 1959). For all dolphins in the present study, maximum HRs at the surface are identical while minimum HRs during diving decline with age (Figs. 3, 4a). These findings are similar to those reported in a preliminary study for diving harbor seal pups in which surface HRs did not change but HRs during submergence declined with age (Greaves et al. 2001). Therefore, the primary developmental change in cardiac function for a variety of marine mammals appears to occur on the physiological and/or psychogenic processes that control

the ability to reduce HR while diving; see Kooyman (1989) for a review of these processes.

Physiological development: implications for diving capacity

The interrelationships between HR, metabolic rate, oxygen stores and diving duration (Kooyman 1989) suggest that an inability to reduce HR limits apnea duration during intermittent breathing and breath-hold diving. This has been demonstrated for several species of seals in which maturation of cardiac control coincides with increasingly prolonged apneas (Castellini et al. 1994b; Falabella et al. 1999; Lapierre et al. 2001; Greaves et al. 2001) and extended periods of submergence (Harrison and Tomlinson 1960; Irving et al. 1963). Yet, many of the postpartum changes in HR observed for marine mammals are not associated with a requirement to dive. Developmental changes in HR patterns are complete within 11 weeks of birth for southern elephant seals (Falabella et al. 1999) and 15 weeks for northern elephant seals (Castellini et al. 1994b), before the pups leave the beach. The refinement in cardiac control during diving for harbor seal pups occurs before weaning and is therefore, temporally separated from the requirement to dive during independent foraging (Greaves et al. 2001).

Unlike pinnipeds, dolphins are subject to the immediate demands of swimming and diving at birth and demonstrate a comparatively protracted developmental period. Although newly weaned dolphin calves as young as 1.7 years old have already developed several elements of cardiac control, the ability for bradycardia during diving does not approach mature levels until 3.5 years postpartum (Figs. 4a, 5). Similarly, the development of the oxygen stores in the skeletal muscles (Noren et al. 2001) and blood (Noren et al. 2002) of bottlenose dolphins are not fully developed until 3 years postpartum. These developmental factors in combination with increased body size act synergistically to enable dolphins to increase breath-hold capacity as they mature. As a result, the diving capability of immature dolphins remains limited until development is complete; voluntary dive durations do not reach adult values until 4.5 years postpartum (Figs. 4b, 5, 6). This may explain in part the long associations (3–6 years) observed between bottlenose dolphin mom-calf pairs in the wild (Scott et al. 1990).

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