

THE DIVING PHYSIOLOGY OF BOTTLENOSE DOLPHINS (*TURSIOPS TRUNCATUS*)

I. BALANCING THE DEMANDS OF EXERCISE FOR ENERGY CONSERVATION AT DEPTH

TERRIE M. WILLIAMS^{1,*}, J. E. HAUN² AND W. A. FRIEDL³

¹Department of Biology, Earth and Marine Science Building, A-316, University of California, Santa Cruz, CA 95064, USA, ²SPAWAR Systems Center, D35, 53560 Hull Street, San Diego, CA 92152-6506, USA and ³CEROS, 73-4460 Queen Kaahumanu Highway, Kailua-Kona, HI 96740, USA

*e-mail: williams@darwin.ucsc.edu

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Summary

During diving, marine mammals must rely on the efficient utilization of a limited oxygen reserve sequestered in the lungs, blood and muscles. To determine the effects of exercise and apnea on the use of these reserves, we examined the physiological responses of adult bottlenose dolphins (*Tursiops truncatus*) trained to breath-hold on the water surface or to dive to submerged targets at depths between 60 and 210 m. Changes in blood lactate levels, in partial pressures of oxygen and carbon dioxide and in heart rate were assessed while the dolphins performed sedentary breath-holds. The effects of exercise on breath-hold capacity were examined by measuring heart rate and post-dive respiration rate and blood lactate concentration for dolphins diving in Kaneohe Bay, Oahu, Hawaii. Ascent and descent rates, stroke frequency and swimming patterns were monitored during the dives. The results showed that lactate concentration was $1.1 \pm 0.1 \text{ mmol l}^{-1}$ at rest and increased non-linearly with the duration of the sedentary breath-hold or dive. Lactate concentration was consistently

higher for the diving animals at all comparable periods of apnea. Breakpoints in plots of lactate concentration and blood gas levels against breath-hold duration (P_{O_2} , P_{CO_2}) for sedentary breath-holding dolphins occurred between 200 and 240 s. In comparison, the calculated aerobic dive limit for adult dolphins was 268 s. Descent and ascent rates ranged from 1.5 to 2.5 ms^{-1} during 210 m dives and were often outside the predicted range for swimming at low energetic cost. Rather than constant propulsion, diving dolphins used interrupted modes of swimming, with more than 75% of the final ascent spent gliding. Physiological and behavioral measurements from this study indicate that superimposing swimming exercise on apnea was energetically costly for the diving dolphin but was circumvented in part by modifying the mode of swimming.

Key words: dolphin, *Tursiops truncatus*, diving, heart rate, energetics.

Introduction

Previous studies have demonstrated that swimming dolphins modify their energetic costs by altering their mode of locomotion. Porpoising (Au and Weihs, 1980; Blake, 1983a), wave-riding (Williams et al., 1992) and travelling at energetically efficient routine speeds (Williams et al., 1993a,b) are behavioral options that significantly lower locomotor costs in these animals during transit swimming near the water surface. Diving poses a unique challenge to locomotion because of changes in pressure with depth. On especially long dives, small dolphins will take advantage of these pressure changes and switch from constant propulsion to interrupted patterns of swimming (Skrovan et al., 1999). Although the energetic benefits of this behavior have not been determined for marine mammals, alternating periods of accelerated motion and powerless gliding have been found to increase the swimming efficiency of other streamlined aquatic vertebrates (Weihs, 1974). For example, the predicted cost of swimming

may be reduced by as much as 50% in some species of fish that utilize interrupted swimming patterns in comparison with those using steady swimming at the same average velocity (Weihs, 1974; Videler and Weihs, 1982).

Energetically efficient modes of swimming by marine mammals are especially important during diving when access to oxygen is limited to reserves sequestered in the lungs, blood and muscles (Kooyman, 1989). Lower locomotor costs in diving marine mammals potentially provide the added advantage of parsimoniously utilizing oxygen reserves and, hence, prolonging aerobic metabolic support during submergence. For the foraging aquatic mammal, the result may be realized as an increase in the time available for locating and capturing prey (Dunstone and O'Connor, 1979; Kramer, 1988).

The demand for energy conservation will probably depend on the duration and depth of the dive. Diving cetaceans, like other marine mammals, must balance metabolic demands

associated with supporting exercise while conserving a limited oxygen store (Castellini et al., 1985; Hochachka, 1986). Short-duration dives, compared with longer dives, presumably permit greater flexibility in speed and swimming mode because the relative impact on oxygen stores is lower. Longer dives in which oxygen reserves become limiting should result in the expression of energy-conserving strategies by the animal (Williams et al., 1996). Consequently, dolphins may tailor their mode of propulsion, routine speed and coincident energetic costs to match specific tasks or when performing longer dives.

This study examines the physiological responses and locomotor strategies of adult, bottlenose dolphins *Tursiops truncatus* performing long- and short-duration dives. To differentiate between responses associated with apnea alone and with apnea during submerged exercise, the study was divided into two parts; sedentary breath-holding on the water surface, and open-ocean diving. Plasma lactate concentration, heart rate and partial pressure of oxygen and carbon dioxide in the blood were determined during apnea on the water surface. We also monitored swimming style, heart rate and post-dive lactate production and respiration rate in dolphins trained to dive to submerged buoys positioned at a depth of 60–210 m. These results were compared with the maximum exercise response of dolphins during stationary exercise pushing against a force transducer and transit swimming on the water surface (Williams et al., 1993a). The results show a graded diving response that correlated with swimming activity, dive depth and duration of submergence. Energy-conserving modes of locomotion appear to slow the rate of oxygen utilization and, consequently, extend dive duration. This strategy is more apparent for longer, deeper dives than for relatively short dives.

Materials and methods

Animals

Three adult, female Pacific bottlenose dolphins (*Tursiops truncatus gilli* Gervais, 1855) were used in sedentary breath-hold studies and open-water diving tests. An additional male, hybrid (*Tursiops truncatus gilli* × *Tursiops truncatus truncatus*) was also used in the open-water tests. The mean body mass of the dolphins was 197.5 ± 17.8 kg (mean ± S.D.). The animals were housed in floating pens (7 m × 7 m × 3 m deep) in a saltwater bay and were fed 2–3 times daily on mackerel, herring and smelt supplemented with vitamins. Mean water temperature in the pens during the experimental period was 24.6 ± 1.20 °C (mean ± S.D.).

Experimental design

The dolphins were studied under two experimental conditions: (1) sedentary breath-holding on the water surface, and (2) voluntary diving in open water. Surface breath-hold tests were conducted to determine the effect of apnea alone on physiological responses. Serial blood samples taken during these tests were used to assess the relationships between

voluntary breath-hold duration, blood gas levels, lactate concentration and blood pH. Open-water diving sessions assessed the combined effects of exercise and apnea on heart rate, muscle lactate production and post-dive respiration rates. Dives were conducted in Kaneohe Bay and along Mokapu Peninsula on Oahu, Hawaii. To avoid prolonged swimming exercise before the diving trials, the dolphins were transported in a 21 foot motorboat (Boston Whaler) to a predetermined dive site. The boat was stationed over a submerged buoy using a hand-held global positioning system (GPS) unit. The dolphins performed straight-line dives to a buoy anchored on the bottom and returned directly to the boat. Diving depths ranged from 60 to 210 m.

Sedentary breath-holding

The dolphins were trained using standard operant conditioning techniques to station next to a deck and to bite onto a padded mouthpiece mounted on a strut. The mouthpiece swiveled, enabling the resting dolphin to be inverted with its closed blowhole under water. This position ensured that the dolphin remained in breath-hold and provided access to blood vessels on the ventral fluke. Following a preparatory breath, the dolphin was inverted, and a butterfly catheter (21 gauge × 3/4 inch) was inserted into the fluke vein. Blood samples were drawn anaerobically into heparinized syringes (10 ml) at 1 min intervals for the duration of the breath-hold. If air bubbles were detected in the catheter line or syringe, the sample was discarded. Capped syringes were placed in an ice slurry and processed immediately. Each sample was analyzed for pH and for the partial pressures of oxygen and carbon dioxide. In addition, plasma lactate concentration was determined for the final sample of each session (see below).

Care was taken to avoid a forced dive response by training the animals for voluntary breath-holds (Ridgway et al., 1975). Behavioral conditioning for the breath-hold tests occurred over several months, as described by Shippee et al. (1994). The dolphins determined the duration of each breath-hold session by biting onto or releasing the mouthpiece. Voluntary breath-holds ranged from 1 to 6 min. The difference between a forced or voluntary breath-hold response was assessed from changes in heart rate (described below). A heart rate below $10 \text{ beats min}^{-1}$ was considered indicative of a forced dive response (Elsner et al., 1966; Kooyman, 1989). After training, heart rates for the dolphins during the breath-hold tests remained above $30 \text{ beats min}^{-1}$ and were similar to levels recorded during voluntary open-ocean diving.

Open-ocean diving

Dolphins were transported by boat to a dive site within 8 km offshore of Oahu, Hawaii. Once released into the water, the animals stationed next to the boat so that a harness could be attached. Each dolphin carried one of two instruments during the dive sessions. The first was a time/depth recorder (1.3 cm × 3.7 cm × 6.2 cm; Wildlife Computers, Inc., Woodenville, WA, USA) mounted onto a pectoral fin strap (1.9 cm nylon

ribbon padded with polyvelvet cloth). This instrument recorded changes in depth and water temperature at 1 s intervals throughout the dive. The recorder was pressure-tested to 450 m before and after the experimental period and was accurate to ± 2 m. During sessions in which heart rate was monitored, a second instrument that recorded heart rate as well as time and depth was attached to a mid-thorax harness (see below).

The dolphins were trained to dive in a straight-line path between the boat and a buoy. Respiratory rate was monitored for 1 min periods immediately following each dive. A blood sample was subsequently drawn from the ventral fluke vein for determination of lactate concentration. Training the animals to present their flukes voluntarily for sampling facilitated blood collection. Using this technique, we obtained the samples within 2–4 min after completion of the dive. Previous experiments in which serial blood samples were taken demonstrated that peak lactate concentrations were obtained during this period (Williams et al., 1993a). Test tubes containing whole blood samples were placed in an ice slush and delivered to the laboratory within 1 h. Data from the microprocessors were downloaded to a personal computer following each diving session and analyzed for average descent and ascent rates, dive duration and dive depth.

Blood gas levels, pH and lactate concentration

The pH and partial pressures of oxygen and carbon dioxide (P_{O_2} , P_{CO_2}) were determined from whole-blood samples of sedentary breath-holding dolphins. The blood gas analyzer (158 pH/blood gas analyzer, Ciba-Corning) was calibrated daily, and the electrode membranes were changed immediately before each experimental session. Buffer calibration standards ranged from pH 6.84 to pH 7.38. Gas standards ranged from 0 to 10 mol% for CO_2 and from 0 to 12 mol% for O_2 . All measurements were made at 37 °C.

Plasma lactate concentration was determined for blood samples taken from both the sedentary breath-holding and open-ocean diving dolphins. Chilled samples (10–12 ml in heparinized vacutainers; Becton Dickinson) were centrifuged (approximately 1000 g for 10 min), and the plasma was analyzed for total lactate concentration (YSI Industrial Analyzer, model 27, and Kodak Ektachem 700C). The analyzers were calibrated daily with lactate standards ranging from 0 to 133.6 mg dl⁻¹ (0–15.0 mmol l⁻¹).

Heart rate

An electrocardiograph (Birtcher Heart Rate Monitor, model 365) and a heart rate/dive depth microprocessor (2.5 cm diameter \times 15 cm long; Wildlife Computers, Inc.; Woodenville, WA, USA) were used to monitor the heart rate of sedentary and diving dolphins, respectively. Electrocardiograph (ECG) signals were monitored continuously during four breath-hold tests using two cross-thorax surface electrodes placed on the sternum between the pectoral fins and on the mid-lateral axillary area. Each electrode consisted of a 3.0 cm diameter silver plate mounted

in an 8.5 cm suction cup. Insulated wires from the electrodes were connected to the monitor and recorded ECG signals at 2.5 cm s⁻¹.

The nylon harness used to carry the heart rate/dive depth recorder had a streamlined, low profile (<5 % of the frontal area of the dolphin) designed to minimize drag (Culik and Wilson, 1991). Previous open-water swim tests with the dolphins demonstrated that there was no significant difference in physiological responses (respiration rate and blood lactate) with and without the harness at speeds up to 2.9 ms⁻¹ (Williams et al., 1993a). Therefore, we assumed that there was little additional energetic cost associated with the harness at relatively low speeds during diving.

Heart rate was averaged for 10 s intervals throughout the dive and for 2 min periods before and after each dive. Dive depth and duration were recorded simultaneously by the microprocessor at 1 s intervals. Following each dive session, the data were downloaded to a personal computer. Changes in heart rate in relation to dive depth and duration were determined using Dive-Analysis (Wildlife Computers, Inc.; Woodenville, WA, USA). To ensure the accuracy of the heart rate microprocessor and electrode sensitivity, we periodically tested average heart rates from the microprocessor against values determined from ECG waveforms (Birtcher heart rate monitor, model 365) for dolphins resting on the water surface.

Breath-hold duration and respiratory rate

Sedentary breath-hold duration, respiratory rate and dive duration of the dolphins were recorded by observers with stopwatches. Mean respiratory rate was determined by counting the total number of inspirations for 1 min periods immediately following each dive and during resting in saltwater pens.

Swimming mode during diving

During open-water dive sessions, a hand-held video camera (Canon model H680 in an Ewa Marine Aqua-Video housing) was used to record the first 25 m of the dolphins' descent and the final 25 m of ascent. Water clarity and SCUBA limits of the human divers dictated the depth range of recording. The video tapes were analyzed for swimming mode (continuous or interrupted dorso-ventral undulation), stroke frequency and percentage of time spent gliding. Stroke frequency was calculated as the total number of strokes divided by the recorded ascent or descent interval. Gliding was defined as forward movement of the animal while the flukes were positioned in line with the lateral axis of the body (Lang, 1966). The percentage of time spent gliding was calculated from the sum of the durations of the glide periods divided by the duration of the ascent or descent recorded.

Statistical analyses

Data for heart rate, respiratory rate, blood gas levels, plasma lactate concentration and stroke frequency are presented as

mean ± 1 standard error of the mean (S.E.M.). Differences between means were tested using Student's *t*-tests according to Zar (1974). Linear and non-linear relationships were calculated by least-squares procedures using statistical software (Sigma Stat 2.0, Jandel Scientific, Inc.). Best-fit correlations for individual relationships are presented. Critical breakpoints in the data were used to assess the relationship between the calculated aerobic dive limit and changes in post-dive plasma lactate accumulation and blood gas levels. Breakpoints for the relationships between breath-hold duration and blood gas levels and between dive duration and plasma lactate concentration were defined from the intersection of multiple regressions using Yeager and Ultsch (1989). Low sample number prevented the critical breakpoint for plasma lactate concentration in relation to the duration of sedentary breath-holding from being determined. Heart rate was calculated by dividing the total number of ECG waveforms or fractions thereof by the test interval according to Chabot et al. (1989).

Results

Sedentary breath-holding

Blood gas levels and pH

Partial pressures of oxygen (P_{O_2}) and carbon dioxide (P_{CO_2}) in mixed venous blood samples showed linear relationships with breath-hold durations up to 225 s (Fig. 1); longer durations were characterized by a decline in the rate of change in the partial pressure of the gases. P_{O_2} decreased with breath-hold duration (t_B) and is described by the equation:

$$P_{O_2} = 58.5 - 0.16t_B \quad (1)$$

($N=13$, $r^2=0.70$, $P<0.001$) for breath-holds less than 225 s, where P_{O_2} is in mmHg (1 mmHg=0.133 kPa) and breath-hold duration is in seconds. The relationship between P_{O_2} and breath-hold duration for the period exceeding 240 s is:

$$P_{O_2} = 32.8 - 0.03t_B \quad (2)$$

($N=9$, $r^2=0.08$, $P=0.45$), but was not statistically significant. This gas averaged 25 ± 2 mmHg ($N=9$) with breath-hold durations of 240 s or longer. In contrast, P_{CO_2} increased linearly with breath-hold durations up to 225 s. The least-squares linear regression for this relationship is:

$$P_{CO_2} = 47.7 + 0.06t_B \quad (3)$$

($N=14$, $r^2=0.64$, $P<0.001$), where P_{CO_2} is in mmHg. As observed for P_{O_2} , a breakpoint occurred in the relationship between P_{CO_2} and breath-hold duration for periods exceeding 240 s. The relationship for longer breath-hold durations is:

$$P_{CO_2} = 52.2 + 0.02t_B \quad (4)$$

($N=8$, $r^2=0.21$, $P=0.254$). Mean P_{CO_2} for breath-holds exceeding 240 s was 60 ± 1 mmHg ($N=8$). Blood pH was independent of breath-hold durations ranging from 60 to 405 s. Mean pH for all breath-hold tests was 7.34 ± 0.04 ($N=25$).

Plasma lactate concentration

Plasma lactate concentration of resting Pacific bottlenose

dolphins floating in water was 1.10 ± 0.1 mmol l^{-1} ($N=5$). The concentration of plasma lactate increased curvilinearly with the duration of sedentary breath-holding (Fig. 2) and was described by the equation:

$$[\text{Lactate}] = 1.16 + 0.0002t_B^{1.5} \quad (5)$$

($N=8$, $r^2=0.55$, $P=0.138$), where lactate concentration is in mmol l^{-1} . Plasma lactate concentration was 2–3 times higher than resting levels following breath-holds exceeding 300 s. However, the values were consistently lower than those measured for actively diving or exercising dolphins for comparable periods of apnea (see below).

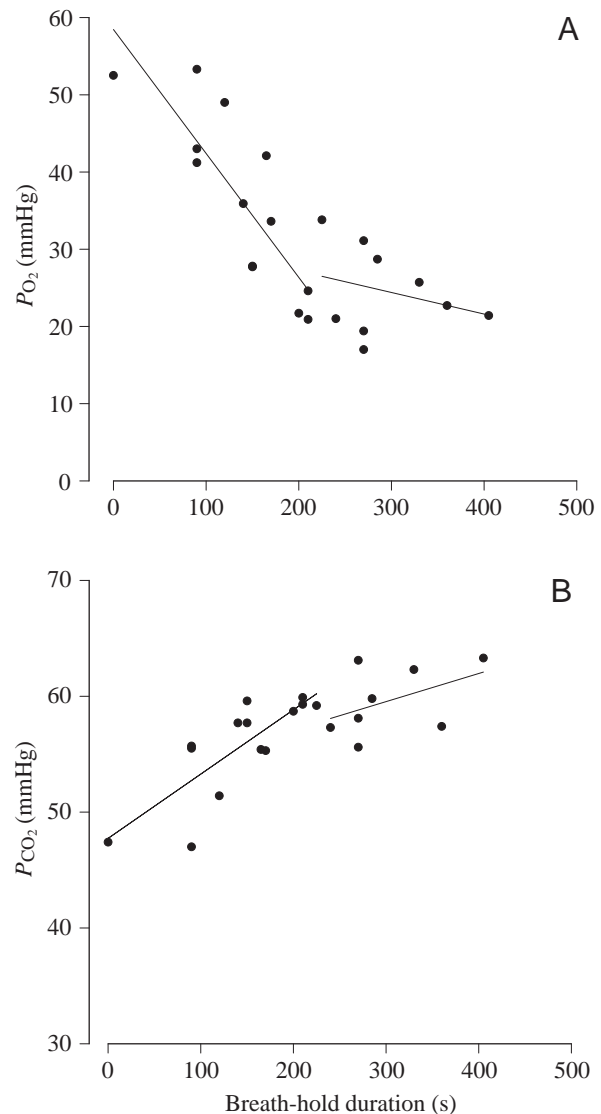


Fig. 1. Partial pressure of blood oxygen P_{O_2} (A) and carbon dioxide P_{CO_2} (B) in relation to breath-hold duration in sedentary bottlenose dolphins. Values for three adult, female Pacific bottlenose dolphins are presented. Each point represents a single blood sample. Solid lines are least-squares linear regressions for the data points. Breakpoints between regressions were determined statistically according to Yeager and Ultsch (1989). Equations and statistics are presented in the text. 1 mmHg=0.133 kPa.

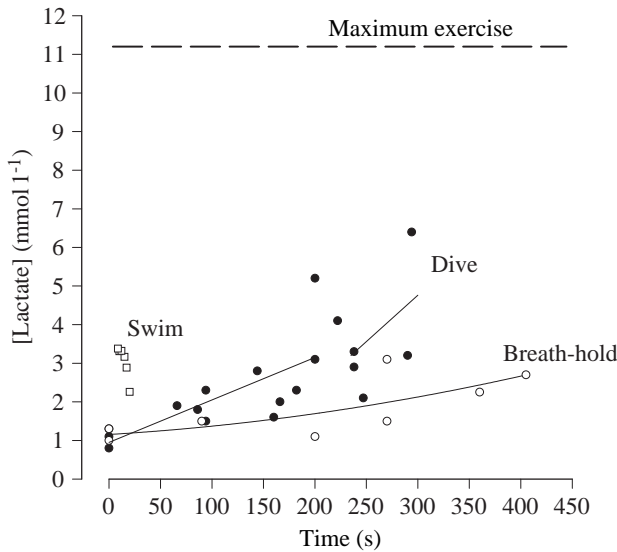


Fig. 2. Plasma lactate concentration in relation to the duration of apnea in sedentary (open circles), open-ocean diving (filled circles) and transit-swimming (open squares) dolphins. Values for four adult bottlenose dolphins are presented. Each point represents a single post-submergence blood sample. Solid lines are the least-squares linear or power regressions through the data points (see text). The upper dashed line shows the maximum plasma lactate level obtained for a dolphin during stationary exercise (Williams et al., 1993a).

Open-water diving

Heart rate

Changes in heart rate during 60 m and 210 m dives are shown in Fig. 3A and 3B, respectively. Average heart rate for the 2 min period before the shallower dives was 101.8 ± 0.7 beats min^{-1} ($N=6$); the rate before the deeper dives was 111.3 ± 2.2 beats min^{-1} ($N=6$). Upon submergence, the animals showed a rapid and marked bradycardia. Although the maximum response was achieved in approximately 30 s during the 60 m dives, maximum bradycardia did not occur until nearly 60 s during the 210 m dives. In addition, the final bradycardia response differed significantly (at $P < 0.05$) between the two dive depths. Average heart rate at the bottom depth was 37.0 ± 1.8 beats min^{-1} ($N=6$) for the 60 m dives and 30.0 ± 2.2 beats min^{-1} ($N=6$) for the 210 m dives.

Heart rate also showed considerable variability during the ascent phase of dives at both depths. This may have been due in part to an anticipatory tachycardia during the ascent, as described for free-ranging pinnipeds (Hill et al., 1987; Fedak et al., 1988; Thompson and Fedak, 1993; Andrews et al., 1997). Average heart rate during the 2 min immediately following the dives was 6–10% lower than pre-dive levels, although the results were not significantly different (at $P > 0.10$). Post-dive heart rate was 95.8 ± 5.3 beats min^{-1} ($N=6$) for 60 m dives and 101.1 ± 4.2 beats min^{-1} ($N=5$) for 210 m dives.

Respiratory rate

The mean respiratory rate (\dot{V}) of resting adult dolphins at the surface was 3.9 ± 0.2 breaths min^{-1} ($N=10$). This rate was higher during the first minute following diving and was

positively correlated with both dive depth (h) (Fig. 4A) and dive duration (t_D) (Fig. 4B). The resulting equations were:

$$\dot{V} = 4.1 + 0.03h \quad (6)$$

($N=25$, $r^2=0.71$, $P < 0.001$) and

$$\dot{V} = 4.0 + 0.02t_D \quad (7)$$

($N=25$, $r^2=0.78$, $P < 0.001$), where respiratory rate is in

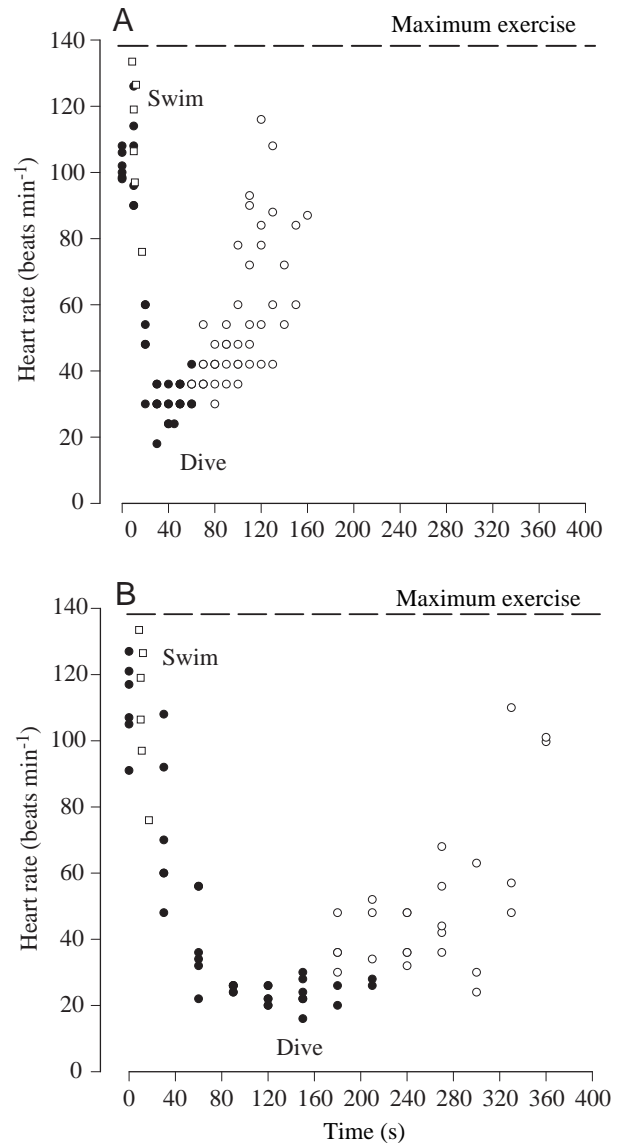


Fig. 3. Heart rate in relation to dive duration for two Pacific bottlenose dolphins. Data for six dives each at 60 m (A) and 210 m (B) are presented. Each point represents the average heart rate for a 10 s interval during the dive. Filled circles denote values for heart rate during the descent phase of the dives; open circles are for the ascent phase. Note the variability in heart rate during ascent. Average heart rate values for transit-swimming dolphins on the water surface (squares) are shown for comparison. Transit-swimming sessions were 10–25 min in duration at a constant speed ranging from 2.1 to 3.8 m s^{-1} . The upper dashed lines illustrate the maximum heart rates for bottlenose dolphins during stationary exercise (Williams et al., 1993a).

breaths min^{-1} , dive depth is in meters and dive duration is in seconds. Respiratory rate following the deepest dives examined in this study was 2.5 times the value for resting dolphins (Fig. 4A) and approached the maximum exercise rates for dolphins pushing against a load cell (Williams et al., 1993a).

Plasma lactate concentration

Plasma lactate concentration was $1.94 \pm 0.14 \text{ mmol l}^{-1}$ ($N=5$) following 60 m dives and was not significantly different (at $P < 0.005$) from resting values. Mean values for plasma lactate concentration increased with dive depth and were significantly

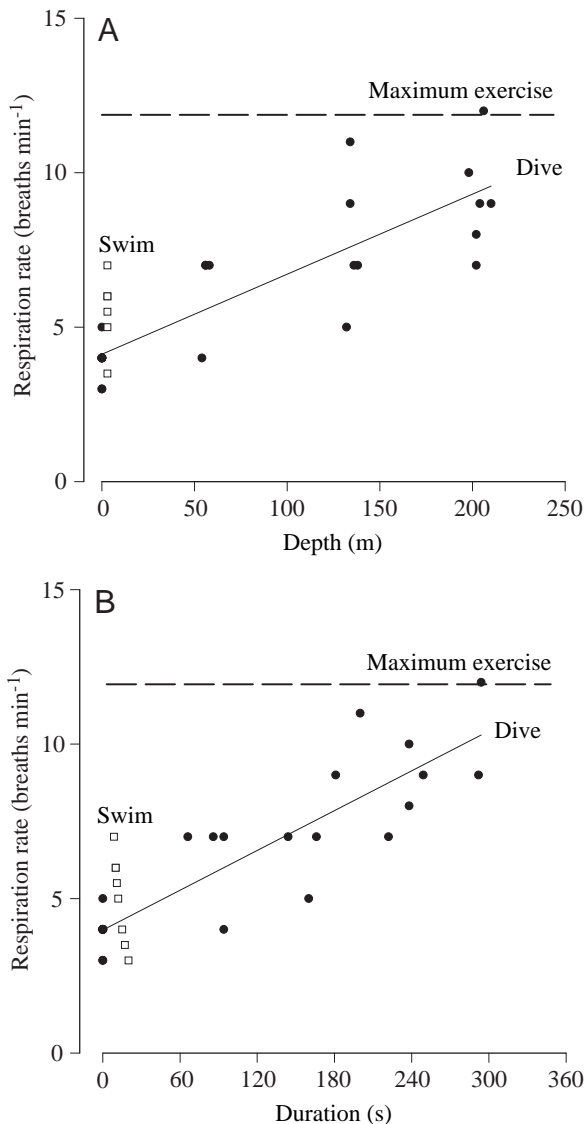


Fig. 4. Respiration rate of Pacific bottlenose dolphins in relation to dive depth (A) and dive duration (B). Each point represents a single measurement taken for 1 min at rest or immediately following a dive. Solid lines are the least-squares linear regressions through the data points. The upper dashed lines represent the maximum respiratory rate for dolphins during stationary exercise on a force transducer (Williams et al., 1993a). Statistics are presented in the text.

different (at $P=0.04$) once depth exceeded 140 m. Lactate concentration subsequently increased to $3.76 \pm 0.52 \text{ mmol l}^{-1}$ ($N=7$) following 210 m dives, representing a 3.4-fold increase over resting levels (Fig. 2).

Post-dive lactate levels were consistently higher than those of sedentary breath-holding dolphins at comparable durations of apnea. As observed for sedentary dolphins, plasma lactate concentration increased with the duration of apnea in the diving dolphins (Fig. 2). A linear increase in concentration occurred with dives up to 200 s in duration and is described by the relationship:

$$[\text{Lactate}] = 0.95 + 0.011t_D \quad (8)$$

($N=13$, $r^2=0.54$, $P=0.005$), where lactate concentration is in mmol l^{-1} and dive duration is in seconds. Longer dives resulted in a breakpoint in this relationship. The relationship between plasma lactate concentration and dive duration for periods exceeding 222 s is:

$$[\text{Lactate}] = 0.024t_D - 2.47 \quad (9)$$

($N=6$, $r^2=0.24$, $P=0.330$). The large variability in lactate concentration following longer duration dives contributed to the low significance level for this relationship.

Diving behavior

Descent and ascent rates for diving dolphins ranged from 1.4 to 2.5 m s^{-1} (Table 1). For the two depths examined, ascent rates were more than 51% faster than the corresponding descent rate. Assuming a straight path between the boat and submerged buoy, these rates demonstrate that the diving

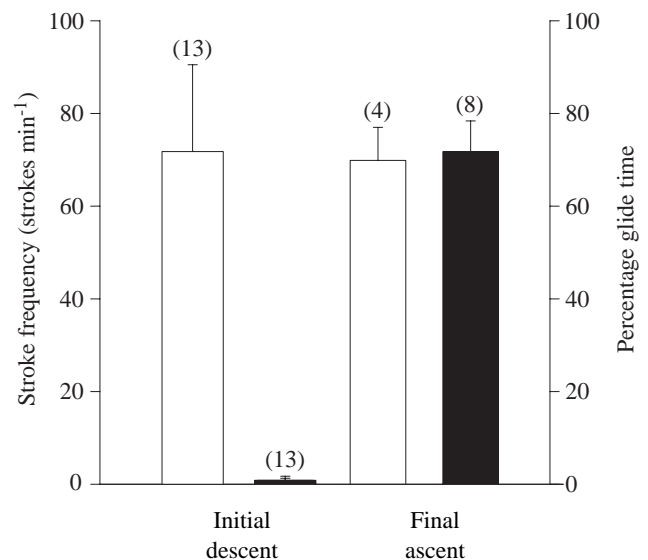


Fig. 5. Stroke frequency (open columns) and percentage glide time (filled columns) of bottlenose dolphins during the initial descent and final ascent from 210 m dives. The height of the columns and vertical lines represent the mean + 1 S.E.M. Numbers in parentheses denote the total number of dives. Note that, because of visibility, data from the top 25 m of the water column only are presented.

Table 1. Speed and duration of diving in adult Pacific bottlenose dolphins

	Depth 60 m			Depth 210 m		
	Descent	Bottom	Ascent	Descent	Bottom	Ascent
Speed (m s^{-1})	1.4±0.1	–	2.2±0.1	1.5±0.1	–	2.5±0.1
<i>N</i>	17		17	16		16
Duration (s)	44.3±3.6	10.1±1.9	25.8±0.9	131.8±4.4	29.5±5.0	82.4±2.1
<i>N</i>	20	20	20	16	16	16

The results for two females are presented.

Swimming speed, duration of travel and bottom time were determined from time/depth microprocessors carried by the diving animals.

Average speed of descent and ascent were calculated for each dive by dividing the straight-line transit distance between 5 m depth and a submerged target by the duration of performance.

N, the number of dives in each category. Values are means ± 1 S.E.M.

animals often moved at speeds outside the predicted range, 1.7–2.3 m s^{-1} , considered to be the most cost-efficient swimming speeds for bottlenose dolphins (Williams et al., 1993b). Average descent speed for both depths was slower than the predicted routine speed range. Ascent rate during the 210 m dives was higher than the predicted range. Both descent and ascent rates were consistently higher for 210 m dives than for shallower dives (Table 1) although the mean values were not statistically different ($P=0.141$ for descent, $P=0.113$ for ascent).

Videotaped and direct observations by SCUBA divers showed that the diving dolphins routinely used interrupted patterns of swimming. This was especially apparent for deeper dives (Fig. 5). The initial descent period was characterized by constant dorso-ventral stroking. For dives to 210 m, the dolphins swam steadily for more than 90 % of the time during this initial period. In contrast, the final ascent often included periods of active stroking followed by gliding phases; 75 % of the final ascent period of 210 m dives was spent gliding. Mean stroke frequency was 69.3 ± 1.8 strokes min^{-1} ($N=17$) during constant stroke periods for both the ascent and descent intervals.

Discussion

The effects of exercise on aerobic diving capacity in dolphins

The physiological responses of an actively diving mammal must account for the effects of apnea superimposed on swimming exercise (Castellini et al., 1985). While one response promotes the conservation of oxygen stores, the other simultaneously requires their utilization. Data from the present study in combination with previous work on swimming dolphins (Williams et al., 1993a) enable us to place the physiological responses attributable to apnea, exercise and the combination of both when diving within the physiological scope of the animals. On the basis of these findings, we can then examine the effect of these responses on the capacity of the animal to maintain aerobic metabolism during submergence.

The duration of submergence rather than the level of exercise *per se* appears to dictate many of the cardiovascular

and respiratory responses of diving bottlenose dolphins. As reported for Weddell, harbor, grey and elephant seals (for a review, see Butler and Jones, 1997) and California sea lions (Ponganis et al., 1997), final bradycardia at depth is lower for longer dives or sedentary breath-holds by dolphins than for dives to shallower depths (Fig. 3). The bradycardia profile for actively diving dolphins was similar to that presented by Elsner et al. (1966) for trained submergence by bottlenose dolphins in an oceanarium pool. The previous study involved sedentary dolphins stationing at 2 m, while the present study used active dolphins performing dives up to 210 m (Fig. 3). Similarities in the level of bradycardia despite differences in activity for the study animals suggest an overriding influence of the dive response on exercise responses. This override feature appears to be most developed in highly adapted marine mammals. Other aquatic vertebrates, including many species of diving birds (Butler and Jones, 1997) and the hippopotamus (Elsner, 1966), show increased variability in heart rate when exercise is superimposed on apnea during submergence. Curiously, immature California sea lions maintain a response intermediate between that of dolphins and that of the hippopotamus. The level of bradycardia developed by immature sea lions was consistently less intense during trained dives than during submerged swimming at comparable periods of apnea (Ponganis et al., 1997). The young age of the sea lions and the nature of trained dives may have contributed to these results.

For diving marine mammals, the physiological effects of exercise and apnea on cardiovascular and respiratory systems may be manifest primarily during the post-dive recovery period. Several studies on pinnipeds (Thompson and Fedak, 1993; Andrews et al., 1997) have suggested that comparatively high physiological rates during the post-dive period benefit the marine mammal by reducing the requisite recovery time and shortening the inter-dive surface interval. Both the heart rate and respiratory rate of the dolphins during the initial post-dive recovery period were equivalent to or exceeded values reported for transit swimming (Figs 3, 4). The rates were positively correlated with dive depth and, hence, with the duration of submergence. Respiratory rate during recovery from the deepest dives reached the maximum level reported for dolphins

(Williams et al., 1993a). Similar patterns for respiratory rate have been reported for swimming and diving white whales. Like bottlenose dolphins, white whales demonstrate an increase in respiratory rate during recovery that correlates with the duration of trained dives (Shaffer et al., 1997).

Changes in blood parameters, most notably plasma lactate concentration (Fig. 2), of bottlenose dolphins also reflect the additive effects of exercise and apnea. As might be expected, sedentary breath-holding resulted in immediate changes in blood gas levels followed by a gradual change in lactate concentration. A linear decrease in blood P_{O_2} and increase in P_{CO_2} occurred during the first 225 s of apnea by sedentary dolphins (Fig. 1). The accompanying changes in lactate concentration during this period were insignificant (at $P < 0.05$), increasing by less than 40% over resting values (Fig. 2). Exercise, whether in the form of transit swimming on the water surface, stationary pushing against a force transducer or open-ocean diving, incurred higher lactate loads than sedentary breath-holding. The gradual rise in lactate level with the addition of exercise is comparable in scale with that of running terrestrial mammals (Seeherman et al., 1981) and is apparent in active dolphins regardless of continuous or interrupted access to air. Blood lactate level averaged $2.9 \pm 0.4 \text{ mmol l}^{-1}$ for dolphins swimming at 2.1 m s^{-1} ; this increased to $3.4 \pm 0.0 \text{ mmol l}^{-1}$ at 2.9 m s^{-1} (Williams et al., 1993a). Post-dive lactate concentration remained below these levels until the period of submergence exceeded 220 s (Fig. 2). Because diving dolphins tend to move over a slower range of speeds than transit-swimming animals (Table 1), the comparatively higher lactate concentrations undoubtedly result from the cumulative effects of apnea and exercise. Interestingly, the highest post-dive plasma lactate concentration, 6.4 mmol l^{-1} , was nearly six times resting values but only 57% of the maximum reported for bottlenose dolphins exercising against a force transducer. Dolphins in the exercise study were unable to sustain work at this level even though access to air was uninterrupted.

The onset of post-dive lactate accumulation may be predicted from the aerobic dive limit (ADL) of the animal (Kooyman and Ponganis, 1998). Defined as the maximum breath-hold that can be supported by available oxygen reserves without a post-dive rise in plasma lactate level (Kooyman, 1985), the ADL is calculated by dividing the on-board oxygen reserves by metabolic rate. Previous calculations for an adult bottlenose dolphin indicate a total oxygen reserve of $33\text{--}36 \text{ ml O}_2 \text{ kg}^{-1}$ body mass (Williams et al., 1993b; Kooyman and Ponganis, 1998). Using the calculations of Kooyman (1989), the predicted ADL based on total oxygen stores ($33 \text{ ml O}_2 \text{ kg}^{-1}$) and the resting metabolic rate ($7.39 \text{ ml O}_2 \text{ kg}^{-1} \text{ min}^{-1}$) of sedentary dolphins is 268 s (4.5 min). This decreases to 80 s (1.3 min) for a dolphin swimming continuously at 2.9 m s^{-1} .

The predicted values for ADL correspond well with the changes in blood gas levels and lactate concentration measured in sedentary breath-holding dolphins. Breakpoints in blood P_{O_2} and P_{CO_2} occurred following 225 s of sedentary breath-holding (Fig. 1). Lactate concentration remained within 27% of resting

values until the duration of apnea exceeded 270 s (Fig. 2). It is important to note that the ADL does not necessarily correlate with or imply the depletion of all oxygen reserves in a diving animal (Kooyman and Ponganis, 1998). The changes in blood gas levels in the mixed venous samples obtained in the present study indicate a breakpoint near the ADL but not a depletion in this oxygen reserve.

The correlation between the predicted ADL and the onset of lactate accumulation is less clear for actively diving dolphins. When the metabolic costs of exercise are added to the calculations, the predicted aerobic limit is reduced. Using measured ascent and descent rates and bottom times (Table 1) and the corresponding metabolic rates of swimming and resting dolphins (Williams et al., 1992), the predicted aerobic dive limit is 181 s (3.0 min) for dolphins performing a 210 m dive. Changes in blood lactate concentration for open-ocean diving dolphins indicate a breakpoint at 222 s, approximately 23% longer than predicted (Fig. 2). Average duration for 210 m dives was 247 s for the dolphins in the present study; this was 36% longer than the predicted ADL. Differences between the predicted and measured ADL (based on changes in blood lactate concentration) reflect the lack of information regarding diving metabolic rates of free-ranging dolphins. Furthermore, it is difficult to estimate metabolic rates without knowing the behavior or activity level of the submerged dolphin. Forward velocity may be maintained by constant stroking or by interrupted patterns of swimming that incorporate prolonged glide sequences (Fig. 5). The energetic consequence of each on oxygen stores may be quite different.

Strategies for reducing locomotor costs during diving

Theoretically, the complex balance between exercise and diving responses will contribute to energetic savings during diving through the judicious use of oxygen stores. The strategy for many species of marine mammal appears to be adherence to a range of dive durations that is supported by aerobic metabolic pathways (Dolphin, 1987; Feldkamp et al., 1989; Kooyman, 1989; Kooyman et al., 1983; Ponganis et al., 1993; Martin et al., 1994; Shaffer et al., 1997). Indeed, the majority of dives (over 90% for some pinnipeds) fall within this 'aerobic dive limit'. Diving profiles of free-ranging, small cetaceans are well within the aerobic limits predicted in the present study. Coastal populations of wild *Tursiops* sp. in 7 m depths off Florida (Shane, 1990) and 39 m depths near Golfo San Jose, Argentina (Würsig, 1978; Würsig and Würsig, 1979), demonstrate mean dive times of less than 25 s. A male juvenile bottlenose dolphin off the coast of Pembrokeshire, Wales, had a mean dive duration of 55.3 s and a maximum dive duration of 150 s (Lockyer and Morris, 1987). Recent studies using satellite and dive recorder technologies on dolphins similar in size to *Tursiops* provide additional information on the range of diving behaviors of small cetaceans. A 127 kg Atlantic spotted dolphin (*Stenella frontalis*) diving in water 33 m deep along the Gulf coast of Texas maintained 93% of its dives to less than 120 s (Davis et al., 1996). The longest dives by this animal ranged from 300 to 360 s. In a study using time/depth

recorders, the diving behavior of spotted dolphins (*Stenella attenuata*) was recorded in the Eastern Pacific Ocean. Average dive duration for this species was 120 s, with 90% of dives lasting less than 210 s (M. Scott, personal communication; Scott et al., 1993).

To perform the 210 m dive in this study, bottlenose dolphins remained submerged for over 243 s (Table 1). Calculations based on swimming metabolic rates matched to ascent and descent rates indicate that the oxygen stores of the dolphin would have been exceeded by 28%. The apparent energetic shortfall was not compensated for by a large anaerobic response when compared with maximum exercise levels (Fig. 2). Rather, the diving animals changed their mode of swimming. Whereas transit-swimming dolphins moving near the water surface maintain constant stroking, diving bottlenose dolphins incorporated prolonged glide periods and interrupted patterns of propulsion (Fig. 5; see also Skrovan et al., 1999). Energetic savings associated with burst-and-glide activity (Weihs, 1974; Blake, 1983b) or simply the absence of muscular effort during extended glides probably contributed to the observed prolongation of dive duration.

Physical factors associated with diving at depth may also play a greater role in conserving oxygen stores of dolphins than previously appreciated. Ridgway et al. (1969) found that bottlenose dolphins swimming horizontally at a depth of only 20 m showed lower pulmonary oxygen reserves, and therefore greater metabolic demands, than the same animals diving vertically to 200 m. Lung compression (Ridgway et al., 1969) and modifications in swimming mode resulting from changes in buoyant forces (Skrovan et al., 1999) during deep vertical dives may have promoted the savings. Although we were unable to observe the dolphins in the present study throughout the entire dive, preliminary evidence suggests that changes in buoyancy and swimming mode during ascent and descent provide an important opportunity for saving energy at depth (Fig. 5). Similarly, it is becoming increasingly evident that changes in swimming mode, like changes in gait by running vertebrates, are an important characteristic for deep-diving mammals including elephant seals (Williams et al., 1996; Webb et al., 1998) and Weddell seals (T. M. Williams, unpublished data). This is discussed further for diving dolphins by Skrovan et al. (1999).

In summary, the results of this study indicate a complex relationship between the physiological responses to exercise and diving in bottlenose dolphins. Regardless of depth, apnea results in the initiation of bradycardia and a gradual transition to anaerobic pathways as oxygen stores are reduced. The addition of exercise results in a quicker transition, as shown by comparatively higher blood lactate concentrations. Similarly, Castellini (1988), using metabolic turnover techniques, concluded that even short periods of apnea in seals result in a metabolic transition that distinguishes diving from exercise responses. As oxygen stores theoretically become limited during prolonged dives, dolphins incorporate prolonged periods of gliding and interrupted patterns of swimming. These locomotor modes take advantage of changes in pressure and

buoyancy with depth and could provide unique opportunities for conserving limited oxygen stores during submergence in these cetaceans. Consequently, calculations based on the metabolic rate of dolphins actively swimming during ascents and descents will overestimate the actual energetic cost of the dive and underestimate the aerobic dive limit.

This series of papers on the diving physiology of dolphins was inspired by the work of Gerald L. Kooyman; they are dedicated to him in celebration of his remarkable research career and influence on all comparative physiologists. This study was supported by an ASSEE-ONT fellowship to T.M.W. and includes procedures and results from AMBS project SR02301. Additional research and analysis were supported by ONR grant N00014-95-1-1023 to T.M.W. The authors also thank S. Shippee and N. Chun for their invaluable assistance in sample collection at sea, and two anonymous reviewers and D. Noren, S. Noren, R. Davis and S. Kohin for helpful comments on drafts of the manuscript. All experimental procedures were evaluated and approved according to animal welfare regulations specified under NIH guidelines.

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