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<table>
<thead>
<tr>
<th>NAME OF RESPONSIBLE INDIVIDUAL</th>
<th>TELEPHONE (MAIN AND CALL)</th>
<th>CODE</th>
</tr>
</thead>
<tbody>
<tr>
<td>S. F. Shippee</td>
<td>(808) 257-5489</td>
<td>511</td>
</tr>
</tbody>
</table>

UNCLASSIFIED
CONDITIONING BOTTLENOSE DOLPHINS

(TURSIOPS TRUNCATUS GILLI)

FOR VOLUNTARY DIVING STUDIES.


Naval Research and Development Division of NCCOSC,
Hawaii Laboratory.
P. O. Box 997, Kailua, HI 96734.

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The International Marine Animal Trainers Association (IMATA).
Held in Freeport, Bahamas, November, 1992.
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Abstract

The behavior and physiology of diving has been extensively studied in several species of pinnipeds and marine birds. Less is known about the diving adaptations of the most completely aquatic mammals, the cetaceans. Early investigations of the diving abilities of dolphins were conducted using forced dive conditions in the laboratory, resulting in extreme physiological responses. The investigative technique improved during the late 1960's and 70's when dolphins were trained for diving experiments in the open sea, providing initial data on the natural diving physiology of these animals. In the present study, two Pacific bottlenose dolphins (*Tursiops truncatus gilli*) were trained to dive to specific depths and then return to station for fluke presentation allowing immediate post-dive blood sampling. The samples were analyzed for lactic acid content, providing an indication of anaerobic effort during the graded dives. A Time-Depth Recorder worn by the animals recorded dive profiles which allowed calculation of swimming speed, bottom time, and ascent/descent rates. Additional experiments conducted in the home pen involved training the animals to allow serial blood samples to be drawn during voluntary breathholds, thus providing information on blood gas changes. Simultaneous heart rate measurements were made to verify that forced dive bradycardia did not occur. These studies demonstrate the value of conditioning voluntary responses for scientific investigation, and additional uses for "medical" behaviors.

Introduction

Over 70 species of cetaceans play an important role in marine ecosystems, having evolved a high-energy predatory life-style to survive in the challenging environment of the sea (Kanwisher and Ridgway, 1983). To thrive, these animals must be adapted to the thermal stress of the marine environment, and be able to withstand the rigors of pressure and nitrogen accumulation which they encounter while diving to acquire food. Many cetaceans are recognized for utilizing food resources unavailable even to modern man, as is the case with sperm whales feeding on deep sea squid (Clarke, 1980). Other cetaceans are important constituents of multi-species communities sharing common resources, for example the association between pelagic tuna and dolphins in the eastern tropical Pacific. Owing to their entirely aquatic existence and the difficulty of observing their movements while submerged, we know relatively little about the physiological ecology of free-ranging cetaceans, especially dolphins. Studies on the foraging patterns and movements of cetaceans in the pelagic environment have depended largely on radio tracking techniques and direct surface observations from shorelines and boats (Pryor and Norris, 1989; Scott *et al.*, 1990). Yet information about the natural diving behavior of these animals remains very sketchy (Kooyman, 1989). Much of what is known about the ecology of pelagic whales and dolphins has been derived from whaling data and from
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specimens attained from incidental mortalities associated with human activities (Pryor, 1990). For many species, the data is entirely anecdotal.

To learn more about the diving capabilities of marine animals, laboratory studies began in earnest earlier this century (Scholander, 1940). By experimenting on a variety of different species ranging from ducks to seals, much was learned about the physiological responses to immersion and asphyxiation (Elsner et al., 1966a). Studies on pinnipeds using forced dive techniques led to important discoveries about these animal's diving capabilities and behaviors. However, attempts to forcibly dive a restrained porpoise (Phoceaena phoceaena) resulted in extreme cardiovascular responses and then death after a fairly short duration dive (Scholander, 1940).

Table 1. Previous Diving Studies with Trained Delphinids

<table>
<thead>
<tr>
<th>Principle Investigator(s)</th>
<th>Year</th>
<th>Species</th>
<th>Max. Depth Attained (m)</th>
<th>Type of Study</th>
</tr>
</thead>
<tbody>
<tr>
<td>Norris. et al.</td>
<td>1965</td>
<td><em>Steno bredanensis</em></td>
<td>30</td>
<td>Diving Ability</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(&quot;Pono&quot;)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Elsner</td>
<td>1966</td>
<td><em>Tursiops t. gilli</em></td>
<td>3</td>
<td>Heart Rate</td>
</tr>
<tr>
<td>Ridgway, Scronce &amp; Kanwisher</td>
<td>1969</td>
<td><em>Tursiops truncatus</em></td>
<td>300</td>
<td>Diving Physiology</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(&quot;Tuffy&quot;)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hall</td>
<td>1970</td>
<td><em>Lagenorhynchus obliquidens</em></td>
<td>215</td>
<td>Diving Ability</td>
</tr>
<tr>
<td>Bowers &amp; Henderson</td>
<td>1972</td>
<td><em>Globicephala scammioni</em></td>
<td>610</td>
<td>Diving Ability</td>
</tr>
<tr>
<td></td>
<td></td>
<td><em>Orcinus orca</em></td>
<td>265</td>
<td></td>
</tr>
<tr>
<td>McSheehy (unpublished)</td>
<td>1978</td>
<td><em>Tursiops t. gilli</em></td>
<td>535</td>
<td>Deep Diving Ability</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(&quot;Li')</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ridgway &amp; Howard</td>
<td>1979</td>
<td><em>Tursiops truncatus</em></td>
<td>100</td>
<td>N, Uptake and Washout</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ridgway, Bowers, et al.</td>
<td>1984</td>
<td><em>Delphinapterus leucas</em></td>
<td>647</td>
<td>Diving Ability &amp; Physiology</td>
</tr>
</tbody>
</table>

In the 1960's interest in the diving physiology of small odontocetes increased. With the advent of modern training techniques, it became possible to condition animals to allow handling for physiological experiments, and to induce voluntary behaviors including diving (Kooyman, 1985). This allowed measurements of telemetered heart rate from porpoises trained to wear instrumented harnesses in enclosed pools (Kanwisher, 1965; Elsner et al., 1966b; Baldwin, 1965; Ridgway, 1972). It also led to diving experiments with unrestrained animals in the open sea (Irwin, 1970; Wood, 1973). Various diving tests have been conducted with trained dolphins and whales up to the present (Table 1). Of note are the extensive physiology studies conducted by Ridgway et al. in 1969 and
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again in 1979. Deep dive studies conducted in 1978 demonstrated the capability of T. truncatus gilli. The animal completed a trained dive in excess of 500 meters, a feat requiring over 6 minutes (D. McSheehy, personal communication). A significant finding was made in 1984 when a beluga whale made the deepest trained dive on record to 647 meters, which took 16 minutes to complete (Ridgway et al., 1984).

Attachment of a radio tracking device in 1971 allowed measurements of dive depths to be made on a free swimming Delphinus, with a maximum recorded dive of 260m (Evans, 1971). Other investigators have since been successful in radio tracking dolphins and whales (e.g. harbor porpoise, spinner, dusky, white-sided, and bottlenose dolphins, pilot whales, and killer whales; Scott et al., 1990). Nonetheless, very little actual diving data has been collected on these free ranging odontocetes.

Scope and Training Requirements

Previous experiments conducted under Dr. Sam Ridgway with a trained dolphin, "Tuffy", measured O₂ and CO₂ content of expired air collected in a funnel as the animal surfaced (Ridgway et al., 1969; Ridgway and Harrison, 1986). A second experiment involved training two dolphins to perform serial dives in order to test the effects of nitrogen loading in the animal's tissues, commonly known as the bends (Ridgway and Howard, 1979; Kanwisher and Ridgway, 1983). However, measurements of lactic acid buildup and blood gas values were still lacking on diving dolphins. These types of physiological values are well documented in Weddell seals and marine birds (see Kooyman, 1989 for review). In light of this, we began a further investigation of diving physiology this past year. Our study attempts to establish baseline values for unrestrained quiescent animals, and to measure lactate levels in actively diving dolphins in the open sea. The scope of our study involves two components: in-pen breathhold experiments and open water diving experiments (Table 2).

<table>
<thead>
<tr>
<th>ESTABLISH BASELINE VALUES</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>(Home-Pen Measurements)</strong></td>
</tr>
<tr>
<td>• blood-gas levels of oxygen, carbon dioxide, and pH</td>
</tr>
<tr>
<td>• lactic acid buildup in blood due to breathhold</td>
</tr>
<tr>
<td>• heartrate (EKG) to validate &quot;voluntary&quot; nature of dive</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>EXAMINE ACTIVE DIVING CONDITIONS</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>(Open Ocean Measurements)</strong></td>
</tr>
<tr>
<td>• dive profiles using TDR</td>
</tr>
<tr>
<td>• respiration rates after dives</td>
</tr>
<tr>
<td>• blood levels of lactic acid</td>
</tr>
</tbody>
</table>

Table 2: Scope of Study
Voluntary Dive Studies in Dolphins

In order to assess behavior during voluntary dives in the open sea, it was necessary to train our animals to carry instrument packages. Studies of the diving behavior of other marine animals such as seals and penguins (Kooyman, 1989; Gentry and Kooyman, 1986) have benefited from data collected on Time-Depth Recorders (TDR's). We hoped to use TDR's attached to our trained dolphins to ascertain features of their diving behavior during the open water sessions.

Consequently, the training requirements included the following (Table 3): conditioning each animal to perform voluntary breathholds, which involved the use of a stationing apparatus and depended on the animal's willingness to remain tranquil until signalled to release; to present tail flukes for blood sampling; and to accept attachment of heart rate electrodes. The animals also were required to carry instrument packages while working unrestrained in the open sea, exhibit reliable stimulus control of diving behavior, and allow a blood sample to be readily taken following a dive.

Two adult female Pacific bottlenose dolphins (*Tursiops truncatus gilli*) were chosen for the study, based on their prior open ocean conditioning, learned medical behaviors, and demonstrated diving ability. This species occurs commonly throughout the Pacific region, and is known to forage in the deep coastal waters of North and South America, Asia, and the Pacific Islands.

<table>
<thead>
<tr>
<th>Home-Pen Experiments</th>
</tr>
</thead>
<tbody>
<tr>
<td>• trained breathhold up to 6 minutes</td>
</tr>
<tr>
<td>• station for tail presentation during breathhold</td>
</tr>
<tr>
<td>• allow attachment of heart rate electrodes</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Open Ocean Experiments</th>
</tr>
</thead>
<tbody>
<tr>
<td>• wear instrument package (TDR)</td>
</tr>
<tr>
<td>• perform voluntary dive up to 200 meters</td>
</tr>
<tr>
<td>• readily present tail flukes for immediate post-dive blood sample</td>
</tr>
</tbody>
</table>

Table 3: Training Requirements
Methods and Equipment

In-pen sampling

To provide baseline data on the breathhold limits of dolphins, it was necessary to condition the dolphins for sampling procedures conducted in the home pen. Changes in blood lactate, pH levels, and blood O₂ and CO₂ concentrations were thus determined. To accomplish this, each animal was trained to position so that serial blood samples could be drawn during a surface breathhold. A padded biteplate mounted on a vertical board provided the animal with a means of staying motionless while in an inverted position, as well as being a focal point for attention. Its flukes were steadied by the trainer as venipuncture was accomplished on a ventral vessel using a butterfly catheter. Blood samples were drawn at timed intervals during the course of the breathhold for both lactate concentration and blood gas measurements. Blood gas samples were meticulously drawn to avoid introduction of environmental air into the heparinized syringe, requiring that the animal remain relatively still. Several repetitive sampling trails were conducted with each animal during breathholds ranging from 1 to 6 minutes. Both dolphins began to show signs of fidgeting after 3-5 minutes, but rarely released from the biteplate until signalled to do so.

To insure that the breathholds reflected voluntary dives and were not forced submersions, simultaneous heart rate measurements were taken on two occasions. This involved training both animals to accept placement of suction cup electrodes and wire leads along the sternum and side. Following a preparatory breath, the animal was inverted and positioned for tail presentation. After stationing on the biteplate, the suction cups were attached to the dolphin and adjusted for the best EKG signal. Heart rate was then monitored continuously during the breathhold. Once completed, the suction cups were removed and the animal was signalled to surface. Additional heart rate measurements were taken during normal respiration cycles with the dolphin in an upright position to give comparative values.

In addition to providing baseline diving values, this phase of our study also demonstrates an innovative application for trained medical behaviors beyond the scope of routine husbandry and health care.

Open ocean dives

Open water diving trials were conducted off the shores of Kaneohe Bay and the Mokapu Peninsula, on the island of Oahu, Hawaii. A variety of water depths are easily accessible within 8 Km of the NRaD laboratory. Work stations were established for this study in depths ranging to 250 meters. The dolphins had previously been trained to transport to the worksite in specially designed boats, thereby reducing transit times (e.g. Shinder,
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We developed two instrument packages that could be easily attached to the dolphin's pectoral fins. One housed a TDR (Wildlife Computers, Woodinville, WA) measuring 1x4x6 cm, or roughly the size of a metal cigarette lighter. This microprocessor stored information on changes in depth, water temperature, and light level encountered during each dive. The data was later downloaded to a computer and analyzed after every session. The second package held an analog depth gauge with maximum depth of dive indicator using a bourdon pressure coil (Sierra Precision Instruments, Cucamonga, CA), which provided the trainer with an immediate indication of the dolphin's dive effort. Each package was attached by a soft strap consisting of 3/4" nylon ribbon padded with polyvelvet cloth, a Fastex (R) quick-release buckle and a Velcro (R) adjustment tab. Fastening of the units to the animal's pectoral fins was made around the region of the axillary insertion of the fin where the circumference is the narrowest. The dolphin was trained to roll on its side and allow the watchband-like straps to be attached, adjusted, and the instruments to be inspected at any point during the work session. The compact nature of the instruments and the soft padding of the straps did not appear to cause discomfort or impede the movement of the animal during normal swimming, boat following, or diving.

In earlier dive training experiments in the 1960's and 70's, dolphins were trained to descend to a tethered pinger device which was lowered progressively deeper in the water column (Norris, 1966; Ridgway et al., 1969, 1979 & 1984; Hall, 1972). In our study, each animal was simply trained to conduct a routine dive to a buoy anchored on the bottom at a known depth and then return directly to the boat. The boat was accurately stationed above the buoy using navigational instruments. This method allowed the boat to be easily maneuvered and eliminated the time required for recovery of a pinger. Following the dive, the boat could be readily positioned to allow the animal to station for tail presentation and subsequent blood sampling.

Acoustic tones were used to signal the dolphin to dive and return to the boat. A 12 KHz pinger served as the standard recall device for instructing the animal to return to station and could be used at any point during a session. Initiation of a dive sequence needed to be controlled to prevent unwanted voluntary dives. To accomplish this, the dolphin was trained to station at the rear of the boat and await a signal from the trainer. A hand-held wand containing a hydrophone was then placed in the water, and a constant 5 KHz tone activated. The dolphin would take a final inhalation, touch the wand, and begin its descent at that location. Use of this technique allowed the trainer to control pre-dive movements, respiration, and point of dive origin. Also, the animal could anticipate the dive and make physiological and behavioral preparations beforehand.

Once the dolphin returned from a dive, an observer began to record elapsed surface
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time and respirations. The animal was given a one minute recovery period, and was then asked to station for a blood sample. The trainer steadied the dolphin's tail flukes while a third member of the team drew a 10cc sample from a ventral vessel. In this position, the animal was able to float comfortably and breathe normally while the blood was taken. Samples were easily obtained in this way in a variety of sea conditions.

Accurate records were made on the elapsed time of sampling and injection into the collecting tube (Vacutainer \( ^{R} \)). The tube was then placed on ice and stored for later laboratory analysis. The dolphin was always rewarded well for its efforts, yet there appeared to be little stress associated with this procedure.

To insure that previous diving exercise did not affect the sampled lactate levels, the animals were always given at least a 5 minute surface rest period prior to initiation of any sampled dives. Both dolphins were trained to make dives ranging from 60 to 200 meters. Several training dives might be made during each session prior to and after sampling. Data was continuously collected with the TDR on these training dives, providing additional information on the normal diving behavior of our free swimming dolphins.

Discussion of Preliminary Findings

Our findings will be extensively summarized and published elsewhere in the near future. This discussion is included to provide a brief synopsis of the type of data collected, and how it might be applied.

By measuring heart rate and respiration rate, we can validate data taken during breathholds and trained dives as being representative of voluntary behavior. Comparisons of heart rate on the surface breathholds fit the patterns measured previously on freely swimming dolphins (Kanwisher and Sundnes 1965, Elsner 1966b, Ridgway 1972) with normal tachycardia during breathing cycles and decreased heart rate following voluntary submersion.

Our measurements of blood-gas changes during breathholds will answer questions about oxygen partitioning, \( CO_2 \) tolerance, and the metabolic processes that occur during diving in dolphins. Measurement of blood lactate levels following deep dives will give us a further indication of the diving capacity of these animals. For example, our breathhold data on blood \( O_2 \) & \( CO_2 \) levels has allowed us to calculate that the aerobic dive limit for resting dolphins is about 4.5 minutes (Williams et al., in press). By comparison, human aerobic dive limits are calculated to be around 2 minutes.

By using Time-Depth Recorders on trained dolphins, we can calculate descent/ascent rates and bottom times, and make predictions about diving efficiency, possibly answering
Voluntary Dive Studies in Dolphins

Figure 1. Representative data collected on three distinct dives using Wildlife Computer's TDR and Strip Chart Program, allowing analysis of behavioral changes for each independent dive condition.

Figure 2. Swimming speed calculations and time allocation using Wildlife Computer's Dive Analysis program for data collected with the TDR on a sampled vertical dive. (AvgD & AvgA = average rate of descent and ascent; MaxD & MaxA = maximum rate of descent and ascent, based on a 15% or greater portion of total dive depth; Dur = total dive duration.)
questions about foraging strategies in diving animals (e.g. Dolphin 1987, Kramer 1988, Houston and Carbone 1992). To illustrate this, the three channels of the TDR measuring light, temperature and depth are shown in a representative printout in Figure 1. The lower axis indicates time, with hash marks at 30 second intervals. The first dive is a search dive to the bottom in an area where there was no buoy. Prior to the second dive, the boat was repositioned above a buoy. The animal dove, first searching for, and eventually finding the buoy, which it then circled before quickly returning to the boat. Following a brief surface interval, the animal was again asked to dive to the buoy, but this time made a more direct and deliberate dive in a shorter time period. Figure 2 displays a printout of the dive profile and computer calculations for rates of descent and ascent of a trained dive to 200 meters, with a duration of 4.3 minutes. This type of data will provide insights into the behavioral strategies employed by freely diving dolphins to balance energy expenditure against diving speed and duration (Williams et al., in press).

The combined information from these measurements may allow ecologists to interpret the observed diving behavior of wild dolphins and determine if prey aggregations at various depths are important food sources, perhaps explaining many mysteries about the niches dolphins occupy in the marine ecosystem. This knowledge will ultimately have benefits to fisheries management, and to conservation programs for marine mammals.

Conclusions

The goal of this paper is to demonstrate the capability of behavioral conditioning for studies into the natural history of marine mammals, in this case physiological ecology. Through operant techniques, we are able to direct dolphins to voluntarily perform natural behaviors in the open sea, and to allow physiological sampling procedures to be conducted without stress. In addition to improving our understanding about the basic physiology of these animals, we are also learning about the capabilities of trained dolphins working cooperatively with man in the open ocean. We also increase man's appreciation of the natural history of marine mammals and their importance in the global ecosystem. We hope to continue with this and similar studies in the future to further investigate the adaptations of marine mammals to life in the sea.

Acknowledgments

Many individuals provided assistance with the team effort that made this study possible. Innovative training techniques, hardware designs, and logistic support were contributed by Chip Fogg, Mike Nash, Tim Sullivan, Kelly Sullivan and Kipp Lawson. The Hawaii Lab veterinary staff, especially Dr. Pete Schroeder, Karl Keller, Eric Huber and Eileen Rawitz, provided valuable assistance with blood collection protocols and analysis. George Lingle and Joe Nolan assisted with visual documentation. And of course, very special acknowledgment is made of the contribution by our two highly cooperative dolphins, Popolo and Cookie. Funding for this project has been provided through the Naval Sea Systems Command and the American Society for Engineering Education (ASEE).
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### References


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