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Editorial

Climate Change and Human Impacts On Our Oceans and Seas- It's a Sad Time to be a Marine Species.

The earth's global climate is under going dramatic changes and these changes are happening at an exceptional rate mostly due to human influences (5,10). Research indicates that few animal species seem to be free from the potentially disastrous effects of climate change when looking at individual species or the latitude of their habitat (9). As a result, numerous species are now facing extinction. In fact it has been recently published that the extinction rate from climate change is accelerating at an alarming rate (11). The present risk of extinction is at 2.8% which rises to 5.2% with the present international policy of a targeted 2^0 C rise in global temperatures. However, as most experts do not believe that this 2^0 C is achievable, a 3^0 C rise in global temperatures would raise the extinction risk to 8.5% (11). Some scientists, researchers, individuals, and environmental groups are calling this the 6th great extinction as 5 previous times our planet has seen the extinction of up to 90 % of its species (12). In a recent issue of Science (2014), it was reported that in the past 500 years huge numbers of animal species have gone extinct or are now threatened, mostly due to "human biodiversity loss" (or losses due to human impacts) which has resulted in the loss of approximately 11,000 to 58,000 species per year (4).

When looking at the marine ecosystems and the species living within them, it would seem that they will also be dramatically affected by climate change. Increasing thermal stratification, or the temperature differences that separate layers of water, would reduce nutrient upwellings, melt sea ice, or decrease pH. These changes would alter the blooming of phytoplankton which would affect the food chain, thereby having an impact on numerous species from krill, seabirds, and marine mammals (10). Moffitt *et al* recently published a study examining the decreased amount of oceanic oxygen concentrations through climate change (6). They found that this oxygen loss in the oceans would dramatically change the distribution of marine life living on the margins of the continents, causing the ocean ecosystems to change and require several millennia to recover (6). However, climate change is not the only threat to marine species as other human impacts may further increase their rate of extinction.

Elasmobranchs (sharks, rays, skates) are one of the top apex predators of the marine environment and having a healthy elasmobranch population is critical for a well balanced ecology (7,13). However, it has been recently shown that numerous species of elasmobranchs are seriously endangered by over fishing or from habitat degradation, both of which are man made stressors (13). Multi- stressor research must now be instigated to uncover what impact a combination of climate change and other man made stressors such as over fishing or habitat loss may have on these important species that are already threatened. Logically it can be assumed that the combination of climate change and other stressors will further push these species to the brink of extinction and some preliminary research bears this out. A recent publication from Di Santo (2015) reveals that climate change in combination with ocean acidification has multiple effects on elasmobranch embryos such as increased metabolic costs with decreasing pH, lowered body condition, and decreased survival (3). It is therefore obvious that more than ever, research is required to study and evaluate the effects of climate change in combination with other environmental stressors on the marine ecology and the various species found within it.

With the need for further climate change research, it is critical that government agencies, who have legislative control over these areas of concern

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and that offer research opportunities/funding, take a leading role in furthering research into climate change to protect the marine environment. Instead it seems they continue to withdraw research support in an area that is quickly becoming critical and also has implications for human health. A leading Canadian scientist in the area of elasmobranch research has recently retired and claimed that government scientists were “muzzled”: "We have very strict directives of what we can say and the approval steps we have to go through, and very often that approval seems to be withheld for totally arbitrary reasons." He also said that “...government scientists often have to find their own funding, travel is often turned down and they are rarely allowed to talk to the media, even about their own groundbreaking research.” (1). As well, critical government programs that were looking into “research and stock assessment of shark species...” have been “temporarily closed” (2).

Climate change, human made stressors, and the threatened existence of top apex predators can only lead to the marine ecology and our environment being pushed further into a state of ever growing instability. In 2006, the Chinese river dolphin went extinct and within 4 more years the vaquita (a small porpoise) will also disappear from the earth’s waters. NOAA Fisheries has reported that there are 125 endangered or threatened marine species under their jurisdiction with another 2 shark species presently being petitioned for protection (8). Despite the efforts of many non-governmental groups, scientists/researchers, and private citizens, and regardless of any amount of public outcry, government agencies seem to ignore the scientific data, all pointing to the gravity of climate change. They continue to procrastinate having international meetings to discuss what needs to be done and issue reports. Meanwhile our environment continues to degrade further and more species are being threatened with extinction. It is truly a sad time to be a marine animal.

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ANNOUNCEMENT

JMATE launches new section featuring student manuscripts

JMATE is pleased to launch a new section under 'Original Manuscripts' specifically dedicated to encourage **current students** in the field of marine animal research to publish their work in a peer review journal. Though the manuscripts will undergo the same rigorous review afforded all submissions, consideration will be given that the first author is a student at the time of submission of the manuscript, and certain expectations will be adjusted. It is imperative that the work was done by a student under the supervision or mentorship of an active scientist in the field, who should be the senior author on the paper. Whenever possible, we hope to include at least one paper by a student with each issue, assuming the submission meets the appropriate criteria and standards of the journal.

We would like to encourage students at every level from undergraduate, masters or PhD training to consider submitting their work for review. It is our hope that supervisors/mentors of these future leaders in the marine animal field will support and promote this initiative; which will give students at all levels the opportunity to gain experience in publishing their research work.

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Body Condition Scoring System for Delphinids Based on Short-beaked Common Dolphins (Delphinus delphis)

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Abstract

Assessment of body condition is critical for examination of live and dead dolphins. Using live and dead stranded and dead bycaught short-beaked common dolphins (*Delphinus delphis*) from New England waters, a simple, practical body condition scoring (BCS) system was developed that has utility for all delphinid species. Using photographs, a non-invasive, 4-point visual scale was created based on anatomical landmarks which are indicative of body condition and emaciation. The consistency of using this BCS system was tested via a blind study with five trained and experienced stranding responders independently scoring a subset of *D. delphis* cases (n=30) using photo documentation only, and results showed a significant level of agreement among observers. Specific morphometric data relating to body condition were analyzed to determine parameters which, in association with the clinical evaluation of the animal, may be indicative of potential success after release during a live stranding event. Results showed a significant difference in length-to-girth ratios in both the axilla and anterior dorsal fin regions between animals which were released (mean for axilla: single stranded 1.75, mass stranded 1.76; mean for dorsal fin: single stranded 1.79, mass stranded 1.76) and those that died or were deemed unreleasable and euthanized (mean for axilla: single stranded 2.03, mass stranded 1.99; mean for dorsal fin: single stranded 1.99, mass stranded 1.87). Future studies are needed to validate the BCS system and its ability to predict such morphometric parameters and relative health. Use of this BCS system will allow for consistency in determining body condition in delphinid species, thus enabling stranding response agencies to better compare data relating to health and nutritional status in these animals. [JMATE 2014;7(2): 5-13]

Keywords: Body Condition, Stranding, Triage, Nutrition

Introduction

Cape Cod, Massachusetts consistently experiences one of the highest rates of common dolphin strandings worldwide, and it is essential for responders to be able to rapidly and efficiently make informed decisions regarding an animal's nutritional status to maximize triage efficiency (11). Currently, no standardized system has been implemented to assist in determining individual nutritional status and there is a lack of consistency of how delphinid body condition is determined in the field. For example, at the International Fund for Animal Welfare (IFAW) alone, delphinid stranding datasheets have changed throughout the years with body condition classified simply as either emaciated versus not emaciated versus robust, with no specific descriptors on how to classify an animal into one of these groups.

Body condition scoring (BCS) is an important subjective and semi-quantitative tool used to assess and make recommendations relating to nutritional status and overall health in a wide range of species (3, 12). Typically, a scale of 1-to-5 or 1-to-9 is used to assess body fat and muscle, with a lower score indicating emaciation and a higher score indicating obesity. The score of an individual animal is determined based on visual assessment of specific anatomical landmarks and analyses of morphometric parameters which indicate nutritional adequacy (3). BCS systems have been successfully developed to determine nutritional status and survivability in both right and grey whales using photographs providing evidence that a similar system may be useful in evaluating nutritive status and predicted releasability of stranded delphinids during field triage (2, 10).

Studies have shown a significant relationship between body condition and survivability in marine mammals, with failed animals showing a poorer body condition than those that survived (10, 11). A lower

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body condition score may indicate poor nutrition and underlying chronic illness, which may be correlated with decreased delphinid survival rates post-release (11). Currently, body condition of cetaceans can be assessed by analyzing morphometric data such as the relationship between girth, length and weight, as well as by using Body Mass Index (BMI) or ultrasound measurements of blubber thickness as a determinant of body fat condition (5-8, 11). Although useful in determining body condition, these assessments require specialized tools, equipment, and calculations that are not always feasible for use in the field.

The objective of this study is to create a simple, reliable tool to assess body condition of dephinids in the field without the need for specialized equipment such as ultrasound and weight scales. This BCS system will serve as a supplemental tool that will create a standardized method of assessing body condition and allow responders worldwide to compare data relating to nutritional status of stranded delphinids. This standardized BCS system may also be helpful in predicting post-release success in the context of the overall clinical evaluation of the animal.

Materials and Methods

Overview: For this study, data from 802 common dolphins, *Delphinus delphis,* stranded on Cape Cod between November 1999 and June 2014 was provided by the IFAW Marine Mammal Rescue and Research Division database. Twenty bycaught common dolphins from February 2006 to November 2012 provided by the National Oceanic and Atmospheric Administration (NOAA) Northeast Fisheries Observer Program were also examined. These records were screened to include only animals which live-stranded or were bycaught and contained photographs and morphometric parameters of interest: Standard straight lengths were measured in centimeters from the tip of the rostrum to the tail fluke. Girths were measured in centimeters at the level of the axilla and at the anterior dorsal fin from dorsal midline to ventral midline and then doubled (half girth), or as a circumferential measurement (full girth). In some cases blubber thickness from the dorsal and lateral axilla region was measured to the nearest millimeter via ultrasound. Overall weight was obtained by placing the animal onto IFAW's custom designed dolphin cart (Edson International, New Bedford, MA,USA) and then rolling the cart onto four industrial grade postal scales (Rubbermaid, Huntersville, NC). The weight from each scale was summed and the weight of the cart, padding and stretcher was subtracted. For animals that died or were euthanized on site, weight was measured during necropsy using a hanging dynamometer scale (TCI Scales Inc., Snohomish, WA, USA) or digital veterinary platform scale (A and A Scales LLC, Prospect Park, NJ, USA).

Creation of the BCS Chart: Photographs were analyzed in conjunction with the morphometric data and stranding reports to determine specific anatomical landmarks which serve as indicators of body condition or emaciation. The animals in the photographs were in ventral recumbency and were out of the water on either a firm surface, such as a trailer floor or a necropsy table, or on a sandy beach. Areas that were analyzed and were concluded to serve as markers of nutritive condition included: the epaxial section, determined by the degree of concavity or convexity ventrolateral to the dorsal fin; the nuchal crest or degree of depression posterior to the blowhole; the thoracic wall, determined by the visibility of the ribs; and the overall shape and symmetry of the trunk. These areas were categorized according to the degree of loss of body mass, both blubber and muscle, and similar to BCS systems in other species, a visual chart consisting of a 4-point scale was created . (7). During this analysis, a protocol describing the most useful angles to assess delphinid body condition via photographs was also developed.

Test for Consistency: A blind study was then conducted to determine the consistency of scoring animals using the BCS system between different observers. A set of 30 cases consisting of various photographs of stranded live and dead common dolphins were sent out to each of the five trained IFAW stranding responders who independently used the BCS system to score each animal. The non-parametric Kendall's W test was used to analyze the inter-rater consistency of scoring common dolphins via photographs.

Analysis of Morphometric Parameters: In an attempt to further quantify the scale and decrease subjectivity, we analyzed morphometric parameters to look for correlations between these values and the score given to an animal. Animals were grouped according to the type of stranding event; either mass stranded, single stranded, or bycaught. Mass stranded animals were part of an event involving two or more cetaceans stranding at the same time and place, excluding cow and calf pairs (4). The assumption is that this group includes both animals that are healthy and others that are diseased (4). Single stranded animals are likely to have some sort of pathology and a poorer body condition (1). Bycaught animals are those that died due to entanglement in fishing gear and were used as presumptive positive controls. Mass or single stranded animals were further grouped according to final disposition; either released or failed. Released animals were those that passed a field health assessment, were successfully released after stranding, and were not documented to have re-stranded (11). Failed animals are those that stranded alive and either died during the response effort, were initially released and later re-stranded, or were euthanized by IFAW responders due to poor health status. These animals are assumed to have a poorer body condition.

Differences in girth, length to girth ratio (L:G), weight, length to weight ratio, and blubber thickness between the groups were calculated in an attempt to correlate these parameters to the BCS scale and to develop morphometric "predictors of releasability". All statistical analyses were performed using SPSS software. The Kruskal Wallis H test, a non-parametric ANOVA, was used to look for statistically significant differences in morphometric data between groups. Confounding factors such as sex, age, and season were corrected for.

Results

A Body Condition Scoring chart illustrating the parameters for classification in each of the four conditions is shown in Figure 1. Representative photographs of common dolphins in each BCS category are shown in a lateral view of the head in Figure 2, cranio-caudal view in Figure 3, caudo-cranial view in Figure 4, and dorso-ventral view in Figure 5. A significant level of agreement among the raters was observed for the blind study via the inter-rater reliability test, Kendall's W= .664 (χ^2 (28) = 93.0, p<.001). This is considered a moderate-strong agreement considering the small sample size and lends evidence that the BCS chart may be useful in consistent scoring of live-stranded animals in the field.

Data for 121 live stranded common dolphins with length and axilla girth measurements, 94 of which had weight measurements, was available. Two animals, IFAW12-119Dd and IFAW12-340Dd, were excluded due to classification as calf. A calf was defined as having a length of less than 150 cm (4). Three animals, IFAW11-023Dd, IFAW12-118Dd, and IFAW13-124Dd, were excluded due to confirmed pregnancy status either via ultrasound exam or during necropsy. Data from 20 bycaught *D. delphis* were available, two of which, DO-6187 and HO-0009, were excluded due to classification as calf in the necropsy reports. In the mass stranded group, 63 were released (20 females and 43 males) and 33 failed (10 females and 23 males). Of the single stranded group, 7 were released (2 females and 5 males) and 14 failed (6 females and 8 males).

Post-hoc analysis (Figure 6) indicates a significantly higher L:G ratio for both the axilla region ($\chi^2(4) = 53.73$, $p < 0.001$) and anterior dorsal fin (ADF) region ($\chi^2(4) = 36.19$, $p < 0.001$) for the single stranded failed group (Axilla: mean= 2.03, n=14; ADF: mean=1.99, n=14) and the mass stranded failed groups (Axilla: mean=1.99, n=33; ADF: mean=1.87, n=33) compared to the bycatch (Axilla: mean=1.86, n=18; ADF: mean=1.76, n=18), single stranded released (Axilla: mean=1.75, n=7; ADF: mean=1.79, n=7) and mass stranded released (Axilla: mean=1.76, n=63; ADF: mean=1.76, n=57) groups. Other dependent variables were significant, but not at a level that would be appropriate to report given that multiple, independent tests (e.g. too high of a risk of an inflated family-wise type I error rate) were run.

Discussion

To increase consistency and decrease subjectivity, stranding responders should receive proper training prior to using this BCS system in the field. Although some animals may appear to fall into two categories and half points may be useful, (e.g. have a depression posterior to the blowhole but no concavity ventro-lateral to the dorsal fin) a strict 4-point scale was chosen due to the ability to visually discern 4 levels of condition at both the nuchal crest and epaxial area in certain angles of photographs and to help decrease subjectivity and increase the usefulness of future data-sets (2). We follow recommendations of Bradford *et al* to round-up to the higher score if an animal appears to fall within two scores (2). During assessment in the field, animals should be assigned into 1 of the 4

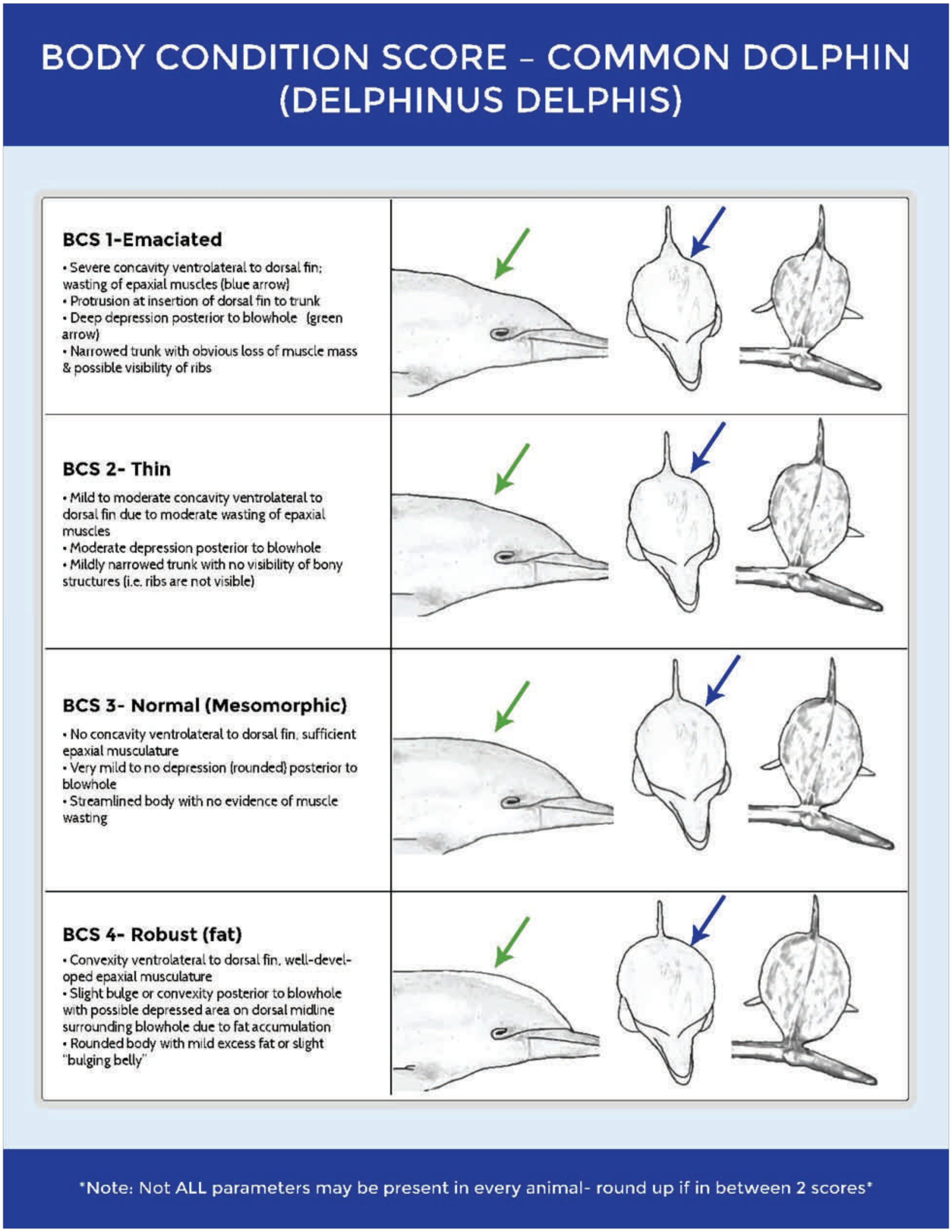

Figure 1: Delphinid Body Condition Scoring (BCS) chart using common dolphins (Delphinus delphis) as an example. Sketches highlight the primary areas of interest. This chart is meant to serve as a field guide for determining body condition during a stranding triage.

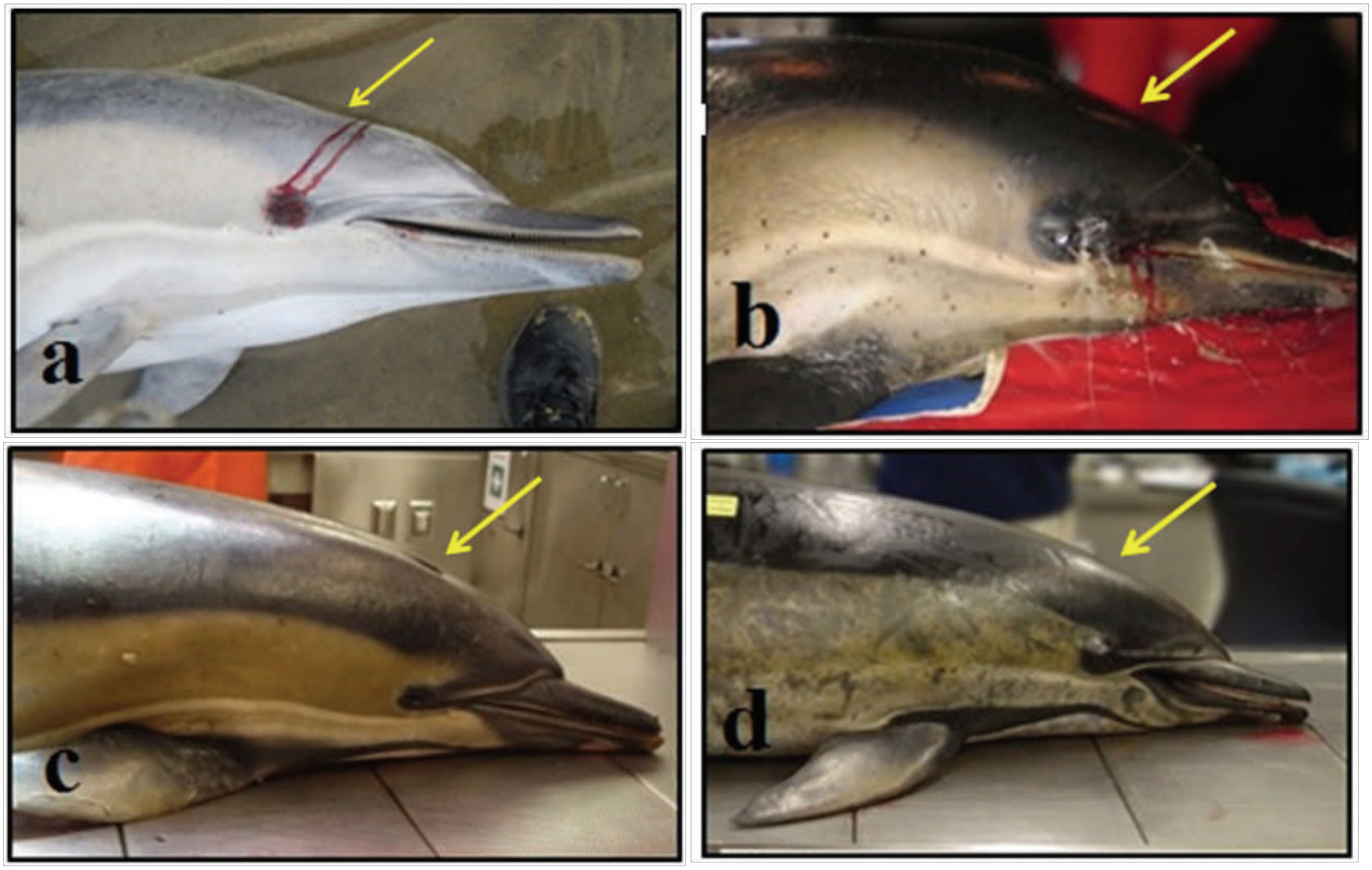

Figure 2: Representative lateral photographs of Common Dolphins (Delphinus delphis) to illustrate anatomical areas indicative of body condition: (a) Note the severity of the cranial dip or "peanut head" in the animal with a BCS of 1; (b) BCS of 2 has less of a cranial dip; (c) BCS of 3 has no cranial dip; (d) Fat accumulation creating a slight bulge in this area can be noted on the animal with a BCS of 4. All photographs are copyright of IFAW and reproduced with permission.

condition categories based on the severity of these characteristics: 1 - Emaciated, 2- Thin (Ectomorphic), 3- Normal (Mesomorphic), 4 - Robust (Endomorphic).

The most consistently observed marker of emaciation in common dolphins is the degree of concavity or depression in the area posterior to the blowhole, best visualized in photos with an eye level lateral view of the head. This area, which has been described as a post-cranial dip or "peanut-head", is currently the standard visual measure of cetacean body score and considered to be the most indicative of emaciation (2, 10). Mesomorphic common dolphins were shown to have a smooth rounded profile caudal to the nuchal crest, while robust animals present with fat accumulation or convexity in this region, a feature that has also been noted in right whales (10). The next most consistently seen marker of emaciation is the wasting or development of the epaxial musculature. This is determined by the degree of concavity or convexity ventrolateral to the dorsal fin. In emaciated animals the muscle atrophy in this region may be so severe that a protrusion at the lateral insertion of the dorsal fin and the trunk may be visible. This parameter is best visualized using photographs with cranio-caudal and caudo-cranial views just above eye level.

The overall shape of the trunk, either narrowed or rounded, was also determined to be indicative of nutritive status and body condition in common dolphins. This parameter can best be determined in aerial photographic views, but differences tend to be more subtle and more difficult to discern in animals with a higher BCS. The wasting of muscles of the thoracic

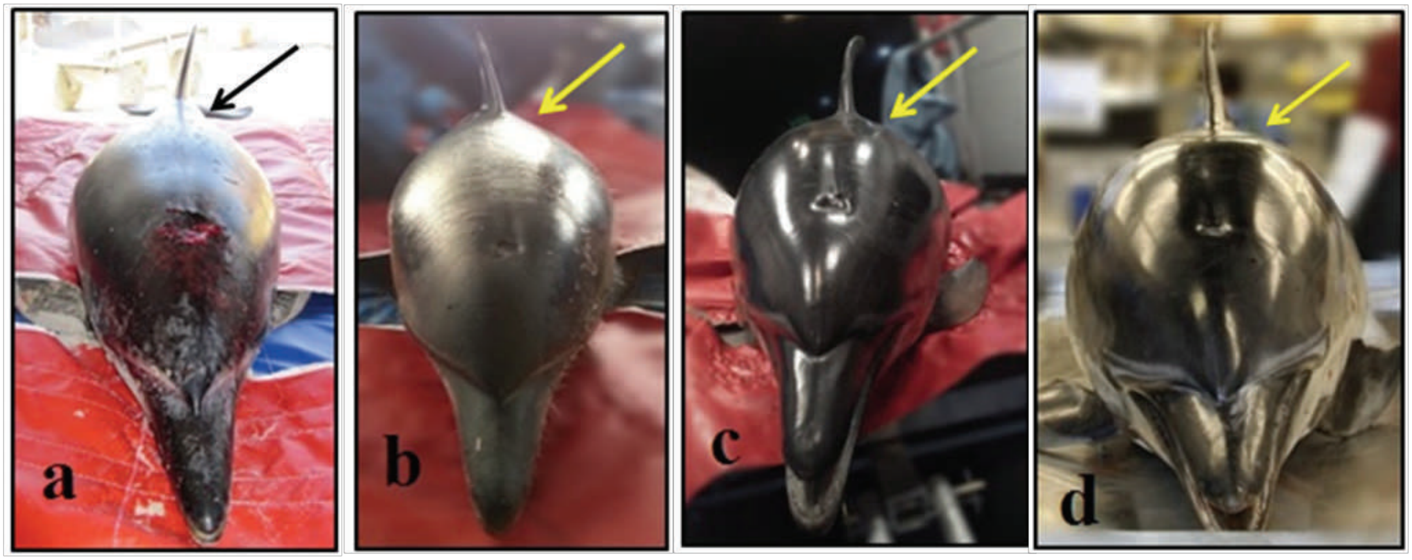

Figure 3: Representative cranio-caudal photographic view of Common Dolphins (Delphinus delphis) to illustrate anatomical areas indicative of body condition: (a) Note the severity of the wasting of the epaxial musculature in the animal with a BCS of 1; (b) BCS of 2 wasting not as severe; (c) BCS of 3 neutral appearance of epaxial muscles; (d) Visible development of the epaxial musculature can be noted on the animal with a BCS of 4. All photographs are copyright of IFAW and reproduced with permission.

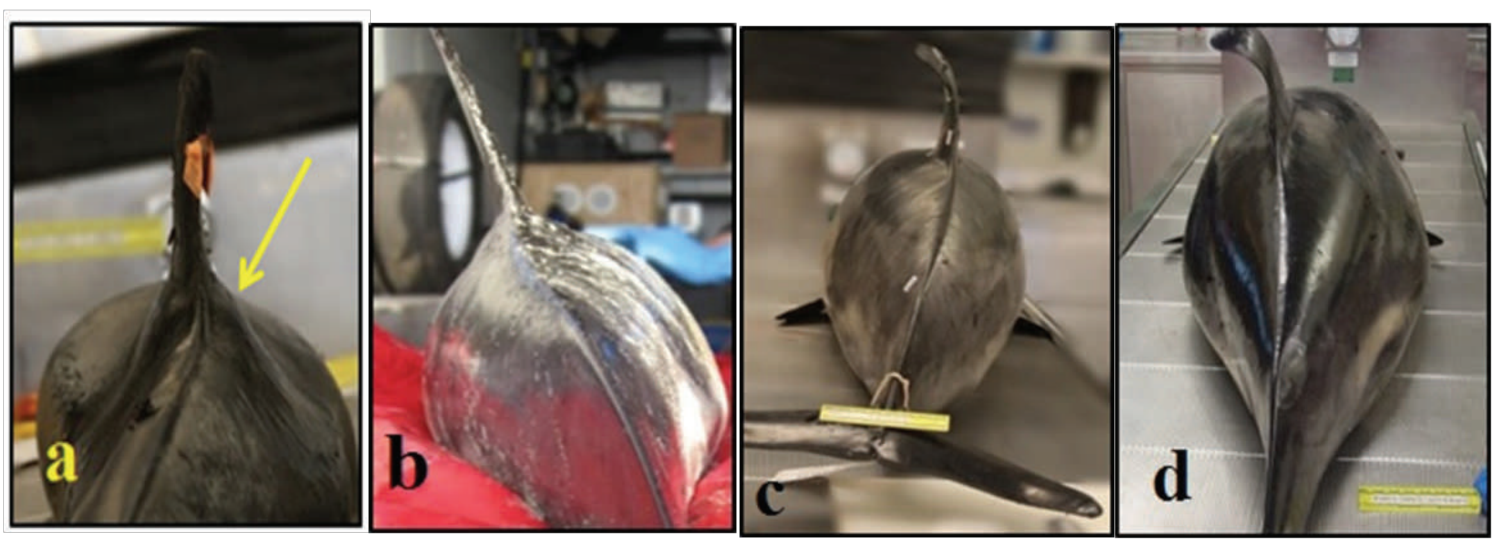

Figure 4: Representative caudo-cranial photographic view of Common Dolphins (Delphinus delphis) to illustrate anatomical areas indicative of body condition: (a) Note the severe concavity ventrolateral to the dorsal fin and the protrusion at the insertion of the dorsal fin to the trunk (arrow) in the animal with a BCS of 1; (b) BCS of 2 concavity not as severe; (c) BCS of 3 neutral appearance of epaxial muscles; (d) Visible development of the epaxial musculature can be noted on the animal with a BCS of 4. All photographs are copyright of IFAW and reproduced with permission.

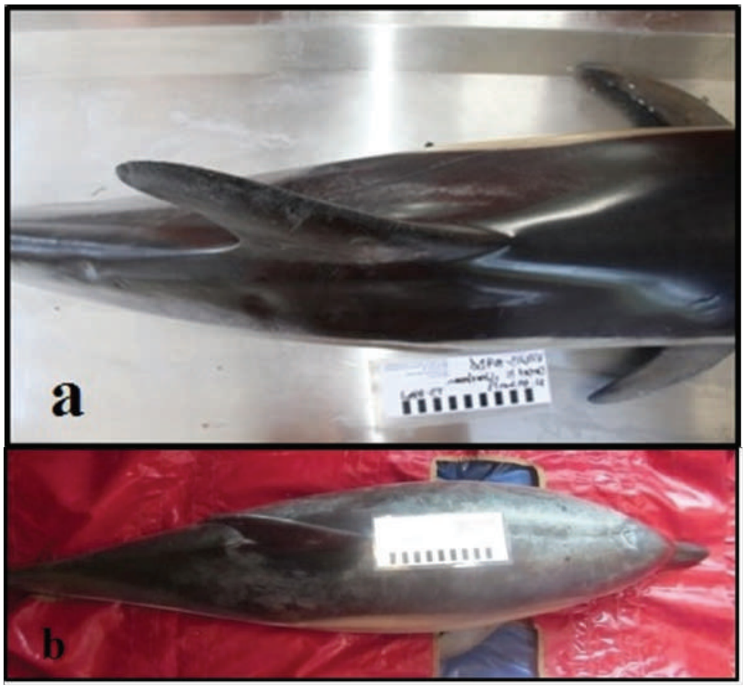

Figure 5: Representative dorso-ventral photographic view of Common Dolphins (Delphinus delphis) to illustrate the overall shape of the truck indicative of body condition: (a) Note the narrowing of the trunk and visibility of the ribs in the animal with a BCS of 1; (b) Rounded shape of the trunk in the animal with a BCS of 4. All photographs are copyright of IFAW and reproduced with permission.

wall, determined by the visibility of the ribs, was our final parameter in our description of the 4-point scale. Although rarely seen in the photographs of our animals, this parameter is indicative of a more advanced level of emaciation and helps to further define those animals with a BCS of 1.

A distinct correlation between the morphometric values and BCS that was assigned via photographs could not be determined. Also, lack of consistent, standardized categorization of body condition in the past made it impossible to realistically compare morphometric data to any sort of vague previous classifications that were given in the field and provided by the retrospective data. Although the L:G ratios correlated well with releasability, some animals with higher L:G ratios did not necessarily appear thin or emaciated in pictures as would be expected, while some animals with lower L:G ratios (assumed more robust) appear thin in pictures, presenting a major caveat for this study. This is likely due to lack of consistent, appropriate photographs to determine BCS and hence

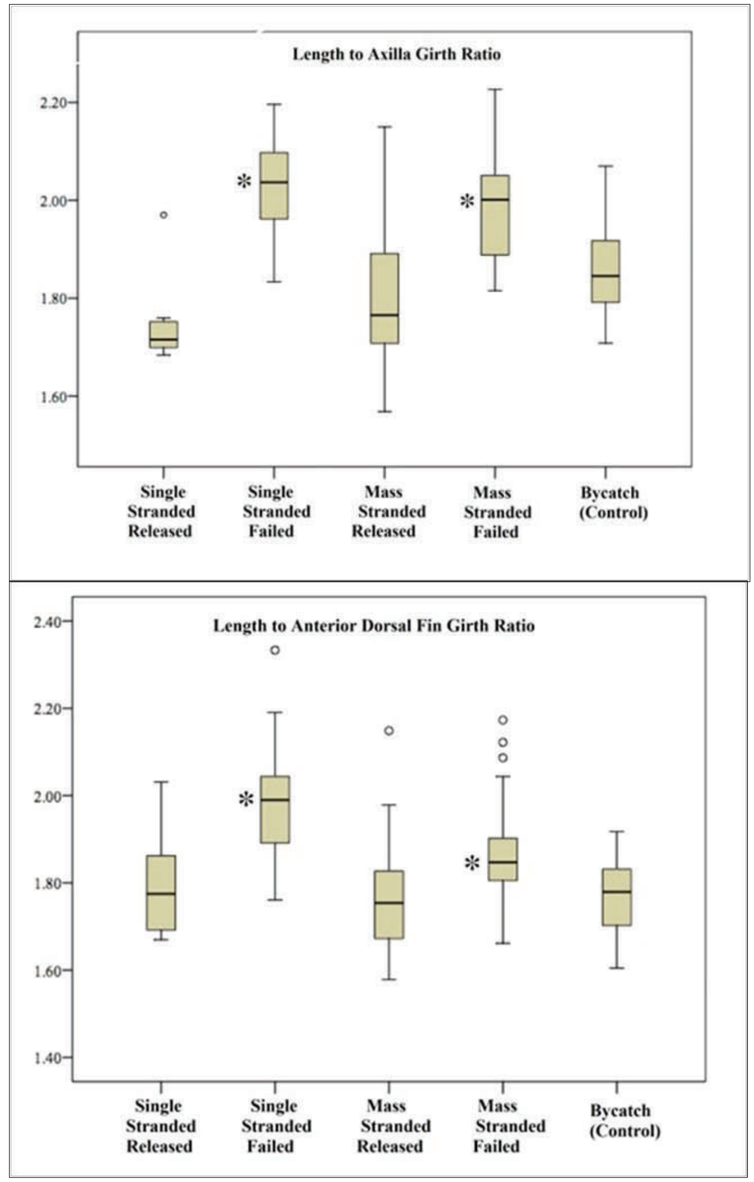

Figure 6 : Box and whisker plots summarizing the results from the Kruskal-Wallis H Test. (a) The mean Length to Axilla Girth Ratio between stranding groups; (b) The mean Length to Anterior Dorsal Fin (ADF) Girth ratio between stranding groups. Asterisk represents statistically significant differences between the stranding groups. Circles represent potential outliers.

led us to develop a photographic protocol. This could also be due to human error and difficulties of field data collection. Due to difficulties in manipulating live dolphins and to limit potential stress due to extended handling, half girths were measured on many of the released animals, rather than full girths as can be done at necropsy, thus calculated L:G ratio of the released animals may be skewed. In the future, full girth measurements should always be taken in the field whenever possible. Similar results were presented by Sharp *et al*. and according to Bradford *et al.*, girth measurements may be able to reflect nutritional status and is considered a better indicator of body condition than blubber thickness in cetaceans (2,11). This provides evidence that decreased girth or a “narrowed trunk” may be indicative of decreased nutritive status and decreased success of release from a stranding triage.

Due to the limited time frame and lack of strandings during this project, we were unable to validate the use of the BCS system and determine the consistency among responders scoring live animals in the field as we did with photographs. BCS systems in other species have been validated by techniques such as ultrasound measurement of blubber thickness in the anatomical areas of interest (9), comparing BCS scores to body condition index (BCI) formulas and dual X-Ray Absorptiometry (12, 13). In the future, responders should assign a body condition score to all animals in the field and collect relevant morphometric data including length, girth, and if possible, weight and blubber thickness measurements, as well as appropriate photographs. Future studies should be aimed at determining if the BCS score can actually serve as a proxy for morphometrics if equipment is unavailable, or as a potential predictor for success upon release.

The development of a metric in order to determine the degree of concavity or convexity, both ventrolateral to the dorsal fin (epaxial muscles) and for the area posterior to the blowhole ‘peanut head’, would be extremely useful to further define each point on the scale. This could be done using photogrammetric methods with a grid-board behind the animal or perhaps by using a set of 3D rulers to measure the height, which would be a determinant of concavity, between the body of the animal and a second ruler. By allowing observers to discover differences in body curvature and measure a proportion of the body above and below a reference line, such techniques could create a quantitative metric that would be helpful in decreasing the subjectivity of the BCS system. Such techniques, however, would not be feasible for use in the field and are more relevant to an academic study.

Conclusion

This BCS system was created to serve as a simple, non-invasive supplementary tool that can be used to assess nutritional status of stranded delphinids. The goal is that this system will be useful for stranding responders who do not have access to specialized equipment such as weight scales and ultrasound in the field. Although this BCS chart was developed based on characteristics of *Delphinus delphis*, we believe that this system will have utility for use in all delphinid species. Standardization in determining body condition of delphinids will provide consistency in stranding data and allow responders worldwide to be able to compare information regarding nutritional status in these animals.

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Southern California Bight marine mammal density and abundance from aerial surveys, 2008-2013

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Abstract

We conducted 18 aerial surveys for marine mammals in the Southern California Bight in the vicinity of San Clemente Island from October 2008 to July 2013. Data were collected to obtain density and abundance estimates, as well as focal behavioral observations of marine mammals. The primary platform used was a *Partenavia* P68-C or P68-OBS (glass-nosed) high-wing, twin-engine airplane. A total of 76,989 km were flown with 2,510 marine mammal groups sighted. Nineteen marine mammal species were identified. Density and abundance estimates were made using line-transect methods and DISTANCE 6.0 software. Due to limited sample sizes for some species, sightings were pooled to provide 4 detection function estimates for baleen whales, large delphinids, small delphinids, and California sea lions. Estimates were limited to species observed at least 20 times during line-transect effort. For the May-October warm-water season, the estimated average numbers of individuals present (and coefficient of variation) were as follows: short-beaked common dolphins (8,520, CV=54%), long-beaked common dolphins (3,314, CV=54%), Risso's dolphins (1,450, CV=66%), California sea lions (818, CV=40%), bottlenose dolphins (496, CV=87%), fin whales (137, CV=49%), and gray whales (6, CV=13%). During the November-April cold-water season, estimates were: short-beaked common dolphins (15,955, CV=51%), long-beaked common dolphins (6,440, CV=51%), California sea lions (1,454, CV=53%), Risso's dolphins (993, CV=51%), bottlenose dolphins (290, CV=61%), gray whales (221, CV=53%), and fin whales (140, CV=33%). Several other species were observed for which sightings were too few to estimate numbers present and/or were seen only off effort: blue, Bryde's, minke, humpback, sperm, Cuvier's beaked, and killer whales; Pacific white-sided and northern right whale dolphins; Dall's porpoise; and northern elephant and harbor seals. [JMATE 2014;7(2):14-30]

Keywords: dolphin, whale, sea lion, line-transect analysis, population biology

Introduction

The Southern California Bight (SCB) is extensively used by humans for shipping, military activities, recreation, and fishing, among other uses. These waters are also heavily used by a wide diversity and relatively high numbers of marine mammal species for feeding, reproduction, migration, and other important life functions. Thus, the potential for spatio-temporal conflict not only exists, but is high. Ship-based marine mammal surveys of the entire United States (U.S.) West Coast Exclusive Economic Zone have been conducted by the National Marine Fisheries Service (NMFS) in the eastern North Pacific Ocean since the early 1980s (with more extensive and consistent coverage since the early 1990s). These surveys have provided estimates of marine mammal abundance and density, and in some cases trends, for U.S. waters of California, Oregon, and Washington (2, 4- 6, 8-10, 15, 23-25). Results represent large-scale data and associated densities over a wide geographic region, as determined by following widely-spaced survey lines. Effort has focused on the late summer to autumn period (July-November) with relatively little coverage in the cold-water season (November-April), when weather conditions are generally unfavorable for marine mammal survey work. Recent (2004-2013) vessel-based surveys published by Douglas *et al*. and Campbell *et al*. are an exception, with relatively even coverage across the year (14, 22).

Waters off San Diego (SD) County are heavily used by the U.S. Navy (USN) for various training operations from several coastal naval bases, in particular the San Clemente Island (SCI) region. Operations include exercises involving low- and mid-frequency active sonars and underwater detonations implicated as causing disturbance, and in some cases even injury and mortality, to some marine mammal species (20). To assess and mitigate impacts, smaller-scale density estimates than those discussed above, specific to ocean

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areas associated with USN at-sea training ranges are needed, but such information is limited. Carretta *et al.* conducted extensive year-round aerial surveys of waters near SCI in 1998 and 1999 (19). This information has been very useful for USN marine mammal resource management; however, the estimates are now over 15 years old and are thus out-of-date. Furthermore, there is compelling evidence that the distribution and density of some marine mammal species have changed in the area during this time period (41).

To provide relevant information, aerial surveys were conducted across the seasons to monitor behavior relative to USN activities, and to provide the most recent and comprehensive up-to-date information currently available on year-round marine mammal density and abundance in portions of the SCB used by the USN for training operations (total study area of 17,556 km^2).

Methods

Data Collection: Three types of aircraft were used. Most (79 or 88%) of the 90 survey days were conducted from a small high-wing, twin-engine *Partenavia* P68-C or P68-OBS (glass-nosed) airplane equipped with bubble observer windows on the left and right sides of the middle seats; the remaining 11 survey days (12%) occurred from an *Aero Commander* airplane (9 days) or a helicopter (2 days), both of which had flat observer windows (Table 1). Survey protocol was similar to previous aerial surveys conducted to monitor for marine mammals and sea turtles in the SOCAL Range Complex, and elsewhere, as described below (39, 40, 42, 43).

The 18 surveys were conducted at least once during 11 of the 12 calendar months: October and

Survey Year	Survey Dates	# Cold-Water Survey Days*	# Warm-Water Survey Days**	Aircraft	Observer Window	SOCAL Sub-area Surveyed
2008	17–21 October	0	5	P	B	SCI, SCatB, S SCI
2008	15–18 November	4	0	P	B	SNB, SCI, S SCI
2009	5–11 June	0	6	P	B	SCatB, SNB
2009	20–29 July	0	8	P	B	SCatB, SNB
2009	18–23 November	6	0	P	B	SCI, SCatB, SNB
2010	13–18 May	0	5	P	B	SCatB, SNB
2010	27 July–3 August	0	5	P	B	SCatB, SNB
			2	H	F	
2010	23–29 September	0	6	P	B	SCatB, SNB
2011	14–19 February	4	0	P	B	SCatB, SNB, Silver Strand
2011	29 March –3 April	3	0	P	B	SCatB, SNB
2011	12–20 April	9	0	AC	F	SCatB, SNB, Silver Strand
2011	9–14 May	0	6	P	B	SCatB, SNB, Silver Strand
2012	30 January–5 February	7	0	P	B	SCatB, SNB
2012	13-15 March	3	0	P	B	SCatB
2012	28 March–1 April	5	0	P	B	SCatB
2013	25-30 March	6	0	P	B	SCatB, SNB
2013	22-26 May	0	5	P	B	SCatB, SNB
2013	24-29 July	0	6	P	B	SCatB, SNB

*Table 1: List of Southern California Bight aerial surveys from 2008 to 2013. P = Partenavia; H = Helicopter; AC = Aero Commander; B = Bubble; F = Flat; SCI = San Clemente Island; S SCI= Ocean area south of San Clemente Island; SCatB (Santa Catalina Basin: representing the area between SCI and the California mainland); SNB (San Nicolas Basin: area west of SCI); *cold-water (November-April); ** warm-water (May-October).*

November 2008; June, July, and November 2009; May, July, and September 2010; February, March, April, and May 2011; January, February, March, and April 2012; and March, May, and July 2013 (Table 1).

One pilot (2008-2010) or two pilots (2011-2013), three professionally trained marine mammal biologists (at least two with over 10 years of related experience) or two such biologists and a computer scientist were aboard the aircraft. Two biologists served as observers in the middle window seats of the aircraft; the third biologist (or computer scientist) was the data recorder in the front right co-pilot seat (2008-2010) or in the rear left bench seat (2011-2013). Surveys were flown at speeds of approximately 100 knots and altitudes of approximately 227-357 meters (m) (800-1000 feet [ft]). In practice, altitude at the time of sightings averaged 261 ± 49 m, based on readings from a WAAS-enabled GPS. When the plane departed the survey trackline, the pilot usually returned to the transect line within 2 km of the departure point. Occasionally, the return point was several km from the departure point.

Established line-transect survey protocol was used (12, 19, 39). Parallel transect lines were positioned primarily along a WNW to ESE orientation generally perpendicular to the bathymetric contours/coastline to avoid biasing of surveys by following depth contours (Figure 1). The study area within the SOCAL Range Complex overlapped transect lines of previous aerial surveys conducted 1-2 times per month over approximately 1.5 year in 1998-99 by the NMFS/ Southwest Fisheries Science Center (SWFSC) on behalf of the USN (19) (see Figure 1 for comparison of the Carretta *et al.* study area with ours) (19). However, transect lines were different from and spaced closer together than the 22 km spacing used by Carretta *et al*. (19). Given the current goal to intensively survey in a prescribed area, we followed transect lines spaced approximately 14 km apart between the coast and SCI (the Santa Catalina Basin sub-area; 8,473 km^2). Our transect lines were spaced 7 km apart to the west (the San Nicolas Basin sub-area; 4,180 km^2), 19 km south of SCI (South of SCI sub-area; 4,903 km^2) (Figure 1).

We used the following hardware and software for data collection, including basic sighting and environmental data (observation effort, visibility, glare, etc.): (1) BioSpectator on a Palm Pilot TX (pull-down menus or screen keyboard) or an Apple iPhone or iTouch in 2008 and 2009; (2) a customized Excel spreadsheet on a Windows-based notebook computer (2010, 2011); or customized Mysticetus Observation Platform (Mysticetus™) software on a notebook computer (2011-2013). Each new entry was automatically assigned a time stamp, a sequential sighting number, and a GPS position. A Suunto handheld clinometer was used to measure declination angles to sightings when the sighting was perpendicular to the aircraft (2008-2010) and/or in 2011-2013 at the sighting location along with a horizontal bearing from the aircraft using Mysticetus. In 2008-2010, declinations were later converted to perpendicular sighting distance; in 2011-2013, declinations were instantly converted to perpendicular and radial sighting distances by Myticetus.

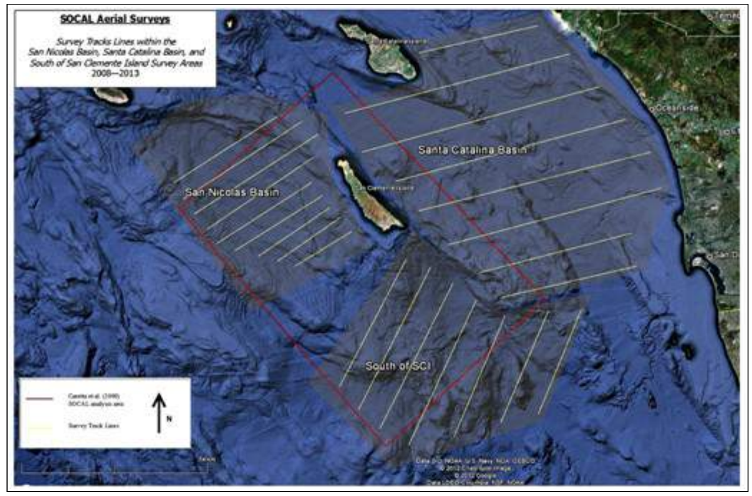

Figure 1: Systematic survey tracklines within the three survey sub-areas in the Southern California Bight, 2008–2013. Note that due to sample size considerations, estimates were only made for San Nicolas and Santa Catalina Basin areas.

Photographs and video were taken through a small opening porthole on either the co-pilot seat window (2008-2010) or the rear left bench-seat window (2011-2013). One of four Canon EOS or Nikon digital cameras with Image Stabilized zoom lenses was used to document and verify species for each sighting, as feasible/needed. A Sony Handycam HDR-XR550 or a Sony Handycam HDR-XR520 video camera was used to document behaviors when off effort. Observers used Steiner 7 X 25 or Swarovski 10 X 32 binoculars as needed to identify species, group size, behaviors, etc.

Environmental data including Beaufort sea state (Bf), glare and visibility conditions, were collected at the beginning of each leg and whenever conditions changed. Aircraft GPS locations were automatically recorded at 2 to 10-second intervals on WAAS-enabled GPSs. In 2008-2010, sighting and effort data were merged with the GPS data using Excel after the survey, based on the time-stamp information, to obtain aircraft positions and altitudes at recorded event times and to calculate distances to sighted animals. In 2011-2013, Mysticetus merged these data automatically in the field.

<u>Data analysis:</u> We used standard line-transect methods (conventional distance sampling) to analyze the aerial survey data (12). Estimates of density and abundance (and their associated coefficient of variation) were calculated using the following formulae:

$$\hat{D} = \frac{n\ \hat{f}(0)\ \hat{E}(s)}{2\ L\ \hat{g}(0)}$$

$$\hat{N} = \frac{n\ \hat{f}(0)\ \hat{E}(s)\ A}{2\ L\ \hat{g}(0)}$$

$$C\hat{V} = \sqrt{\frac{\hat{\text{var}}\ (n)}{n^2} + \frac{\hat{\text{var}}[\hat{f}(0)]}{[\hat{f}(0)]^2} + \frac{\hat{\text{var}}[\hat{E}(s)]}{[\hat{E}(s)]^2} + \frac{\hat{\text{var}}[\hat{g}(0)]}{[\hat{g}(0)]^2}}$$

Where: D = density (of individuals),
n = number of on-effort sightings,
f(0) = detection function evaluated at zero distance,
E(s) = expected average group size (using size-bias correction in DISTANCE),
L = length of transect lines surveyed on effort,
g(0) = trackline detection probability,
N = abundance,
A = size of the study area,
CV = coefficient of variation, and
var = variance.

Line-transect parameters were calculated using the software DISTANCE 6.0, Release 2 (44). Though previous estimates used both systematic and connector lines, those of Jefferson *et al.* and those herein did not (30-32). Due to concerns about possible bias, only survey lines flown during systematic (the main line-transect survey lines perpendicular to the coast) transects at a planned altitude of 213-305 m, with both observers on line-transect effort were used to estimate the detection function and other line-transect parameters (i.e. sighting rate, n/L, and group size). We used a strategy of selective pooling and stratification to minimize bias and maximize precision in making density and abundance estimates (12). Due to low sample sizes for most species, we pooled species with similar sighting characteristics to estimate the detection function. This was done to produce statistically robust values with sample sizes of at least 60-80 sightings for each of four groups: baleen whales, large delphinids, small delphinids, and California sea lions (see Table 2, Figure 2a-d).

Species Group	Species Included	n	f(0)	% CV
Baleen whales	*Balaenoptera musculus*, *B. physalus*, *Balaenoptera.* sp., *Megaptera novaeangliae*, *Eschrichtius robustus*, unidentified baleen whale	158 (113)	0.0018 Uniform/ Cosine	13
Large delphinids	*Grampus griseus*, *Tursiops truncatus*	194 (144)	0.0023 Hazard Rate/ Cosine	20
Small delphinids	*Delphinus delphis*, *D. capensis*, *Delphinus* sp., *Lagenorhynchus obliquidens*, *Lissodelphis borealis*, unidentified small dolphin	369 (270)	0.0016 Hazard Rate/ Cosine	16
California sea lions	*Zalophus californianus*, unidentified pinniped	229 (132)	0.0048 Uniform/ Cosine	8

Table 2: Estimates of the detection function (f[0]) for the four analyzed species groups. In the sample size column (n), two numbers are given: total sample size and the sample size after truncation (in parentheses). CV = coefficient of variation.

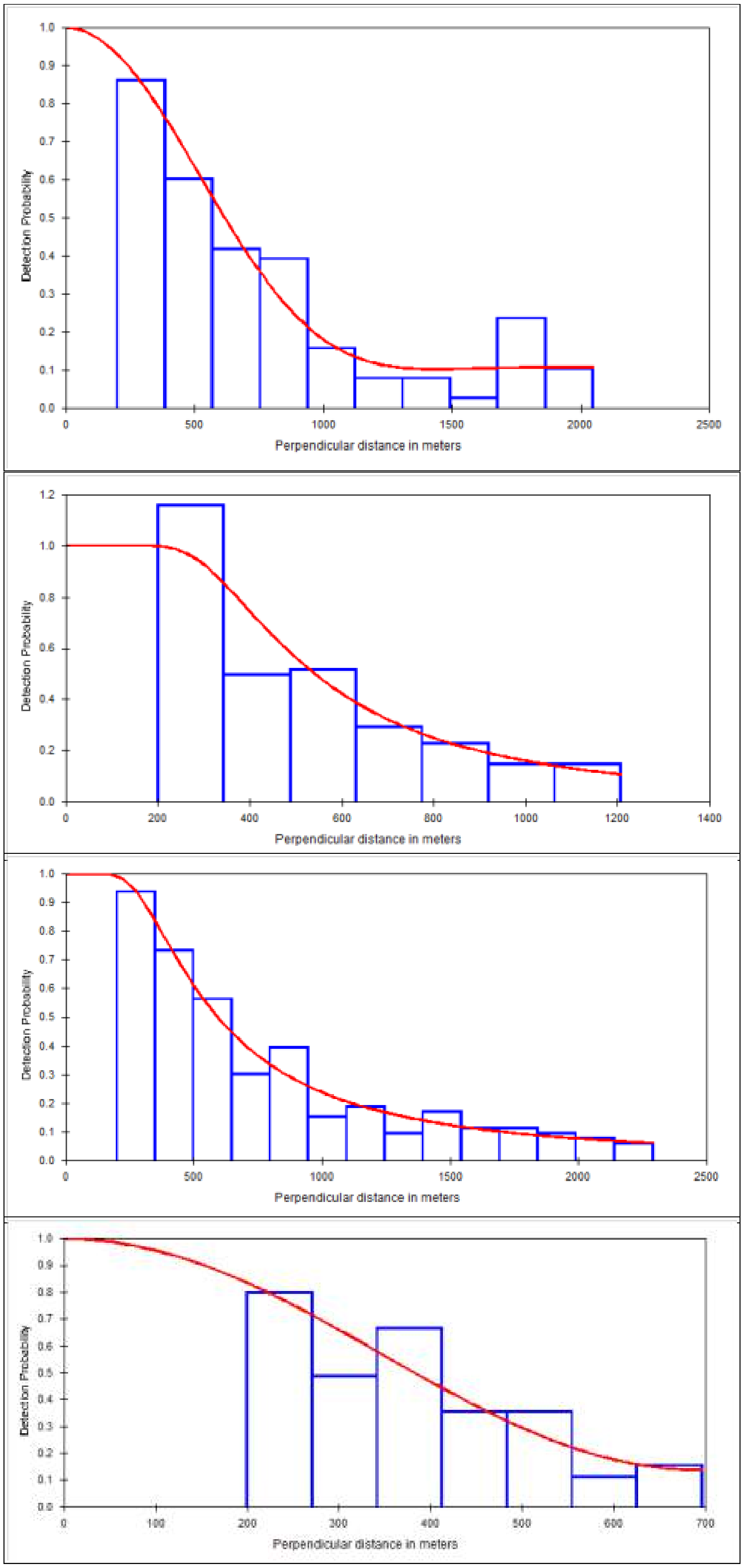

Figure 2: Perpendicular sighting distance plots and fitted detection functions for the four species groups: (a)baleen whales; (b)large delphinids; (c)small delphinids; (d) California sea lions.

We used all data collected in Bf conditions of 0-4 and did not stratify estimates by Bf or other environmental parameters. We produced stratified (in terms of sighting rate and group size) estimates of density and abundance for the two main survey sub-areas (Santa Catalina and San Nicholas Basins) and two seasons (warm and cold), using the pooled species-group f(0) values described above. We did not calculate density/abundance estimates for the South of SCI area, due to very small associated sample sizes. The seasons were defined as warm-water (May - October) and cold-water (November - April), after Carretta *et al.* (19).

Some sightings (19%) were unidentified as to species (although some of these were identified to a higher-level taxonomic grouping, e.g. unidentified baleen whale, unidentified small delphinid, unidentified pinniped, unidentified *Balaenoptera* sp., or unidentified *Delphinus* sp.). We thus pro-rated these sightings to species using the proportions of species in the identified sample, adjusted our sighting rates appropriately, and corrected the estimates with these factors. Because of the large proportion (81%) of sightings that were identified only to genus for *Delphinus*, we took a slightly different approach with this group. We calculated an overall estimate for *Delphinus* spp., then prorated the estimate to species (*D. delphis* and *D. capensis*), based on the proportion of each species represented in the known sample of sightings (0.72 for *D. delphis* and 0.28 for *D. capensis*).

To avoid potential overestimation of group size, we used the size-bias-adjusted estimate of average group size available in DISTANCE if it was less than the arithmetic mean group size. In most cases, group size for each estimate was calculated using a stratified approach (i.e. only groups from within a particular stratum were used to calculate average group size for that stratum).

Truncation involved the most-distant 5% of the sightings for each species group. We also used left truncation at 200 m, due to indications that poor visibility below the aircraft resulted in missed detections near the transect line (the 200 m cut-off was based on examination of the sightings by distance plots). This helped avoid potential underestimation of f(0) due to missed detection data immediately near the transect line.

We modeled the data with half-normal (with hermite polynomial and cosine series expansions), hazard rate (with cosine adjustment), and uniform (with cosine and simple polynomial adjustments) models, selecting the model with the lowest value for Akaike's Information Criterion.

We did not have data available to empirically estimate trackline detection probability [g(0)] for this study. However, since our surveys were very similar to those of Carretta *et al.*, values for g(0) from their study were used to adjust for uncertain trackline detection (19). This results in an underestimate of the variance for the final estimates of density and abundance. However, estimates of density and abundance were produced only for those species with at least 20 useable, on-effort sightings in the line-transect database (an arbitrary cut-off, based on past experience).

Results

Of the total 76,989 km flown, 25% (19,521 km) were flown during on-effort periods for line transect in good sea conditions (Bf 4 or less) on systematic lines, and thus available to estimate density and abundance. Of the total 2,510 marine mammal groups sighted during all survey states (on-effort, off-effort), 39.7% (n = 997) were used to estimate density and abundance herein (Table 3). We sighted at least 19 species of marine mammals, although not all sightings were identified to species level (Table 4). The most commonly sighted marine mammals meeting analysis criteria (with the number of sightings shown parenthetically) in descending order were common dolphins (n = 277, including both species), California sea lions (n = 212), Risso's dolphins (n = 158), fin whales (n = 69), gray whales (n = 47), and bottlenose dolphins (n = 36). Abundance was thus estimated for these seven species. The locations of the sightings identified to species and used in estimating density and abundance are shown in Figures 3-5. Line-transect estimates of density and abundance (and their associated coefficients of variation) are shown in Table 4.

Identification of common dolphins to species level was often not possible during flights, especially when weather conditions were less than ideal. For this reason, extensive photos were taken of common dolphin schools for later detailed examination. We examined a sample of these photos to see if we could identify the species, and we could in many cases. Short-beaked common dolphins predominated in these sightings. Based on the photo samples from which we were able to determine species, 72% of common dolphin sightings were *D. delphis* and only 28% were *D. capensis*. Photographs of representative groups of the two species are provided in Figure 6, showing the diagnostic characteristics we used to identify them to species.

SPECIES	nT	nD
California sea lion, *Zalophus californianus*	553	212*
Common dolphin, *Delphinus* sp.	521	196*
Risso's dolphin, *Grampus griseus*	328	158*
Unidentified delphinid	305	73
Fin whale, *B. physalus*	136	69*
Bottlenose dolphin, *Tursiops truncatus*	123	36*
Gray whale, *Eschrichtius robustus*	104	47*
Short-beaked common dolphin, *Delphinus delphis*	84	58*
Blue whale, *Balaenoptera musculus*	66	11
Unidentified. baleen whale	49	23
Unidentified. pinniped	47	17
Long-beaked common dolphin, *D. capensis*	44	23*
Unidentified. marine mammal	43	23
Pacific white-sided dolphin, *Lagenorhynchus obliquidens*	21	11
Minke whale, *B. acutorostrata*	19	9
Humpback whale, *Megaptera novaeangliae*	18	8
Northern right whale dolphin, *Lissodelphis borealis*	16	8
Harbor seal, *Phoca vitulina*	15	1
Northern elephant seal, *Mirounga angustirostris*	6	5
Dall's porpoise, *Phocoenoides dalli*	5	3
Bryde's whale, *B. brydeii/edeni*	2	1
Cuvier's beaked whale, *Ziphius cavirostris*	2	2
Killer whale, *Orcinus orca*	2	2
Sperm whale, *Physeter macrocephalus*	1	1
TOTAL	**2,510**	**997**

Table 3: Marine mammal species observed during the surveys listed in taxonomic order, with total sightings (nT) and sightings available for line transect analysis (nD). Density and abundance estimates were limited to those species denoted by an asterisk, based on nD ≥ 20.

Discussion

Potential Biases of the Estimates: As is true of any

	WARM SEASON				COLD SEASON			
SPECIES	**Di**	**N**	**N'**	**%CV**	**Di**	**N**	**N'**	**%CV**
Fin whale	**0.909**	**115**	**137**	**-**	**0.933**	**118**	**140**	**-**
SCatB	0.342	29	35	60	0.740	64	76	32
SNB	2.047	86	102	37	1.270	54	64	34
Gray whale	**0.059**	**5**	**6**	**-**	**1.162**	**197**	**221**	**-**
SCatB	0.058	5	6	13	1.791	152	171	29
SNB	0.000	0	0	n/a	1.066	45	50	76
Risso's dolphin	**11.459**	**1,450**	**1,450**	**-**	**7.848**	**993**	**993**	**-**
SCatB	16.428	1,392	1,392	36	11.041	936	936	32
SNB	1.407	58	58	96	1.378	57	57	70
Bottlenose dolphin	**2.584**	**327**	**496**	**-**	**1.510**	**191**	**290**	**-**
SCatB	3.564	302	459	72	2.263	191	290	61
SNB	0.577	25	37	102	0.000	0	0	n/a
Short-beaked common dolphin	**67.336**	**8,520**	**8,520**	**-**	**126.097**	**15,955**	**15,955**	**-**
SCatB	96.471	8,174	8,174	32	150.54	12,755	12,755	32
SNB	8.278	346	346	75	76.555	3,200	3,200	69
Long-beaked common dolphin	**26.191**	**3,314**	**3,314**	**-**	**50.897**	**6,440**	**6,440**	**-**
SCatB	37.519	3,179	3,179	32	61.322	5,196	5,196	32
SNB	3.229	135	135	75	29.761	1,244	1,244	69
California sea lion	**5.825**	**737**	**818**	**-**	**10.345**	**1,309**	**1,454**	**-**
SCatB	3.305	280	311	28	4.567	387	430	39
SNB	10.933	457	507	51	22.057	922	1,024	67

Table 4: Estimates of individual density (Di, individuals/100 km^2), abundance (N), abundance incorporating proration of unidentified sightings (N'), and coefficient of variation (%CV) for marine mammals in the Southern California (SOCAL) Bight study area for the warm-water (May through October) and cold-water (November through April) seasons. The first line for each species is for the entire SOCAL Range Complex and the next two lines are stratified by the two survey sub-areas: Santa Catalina Basin (SCatB) and San Nicholas Basin (SNB). The species are listed in taxonomic order.

statistical technique, there are certain assumptions that must hold for line-transect estimates of density and abundance to be accurate. For instance, there are different ways to calculate correction factors for prorating unidentified sightings, and these differ in their statistical reliability. Therefore, we urge readers to view our prorated estimates with some caution, and we have presented the unprorated estimates alongside them for comparison. Below we go through the various assumptions of line transect and other issues that may cause bias in our estimates.

<u>Assumption 1: Certain Trackline Detection:</u> Animals on and very near the trackline must be detected to avoid estimates that are biased low (13). This is a central assumption of basic line-transect theory. However, in reality, it is often violated, especially by diving animals like marine mammals. This can be addressed by incorporating a factor into the line-transect equation that accounts for the proportion of missed animals (trackline detection probability, g(0)).

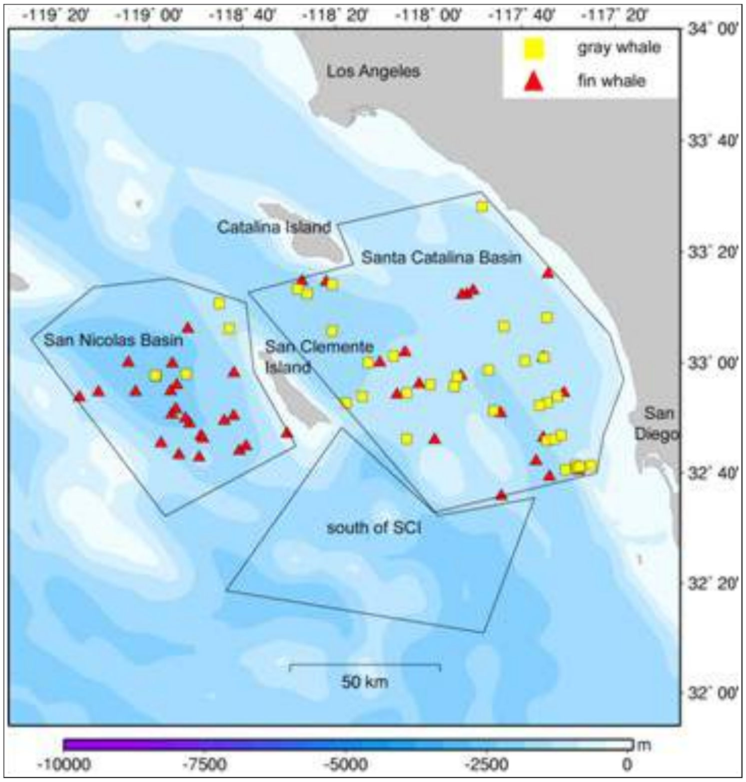

Figure 3: Sightings (identified to species) used for estimation of density and abundance of large whales in this study, 2008–2013.

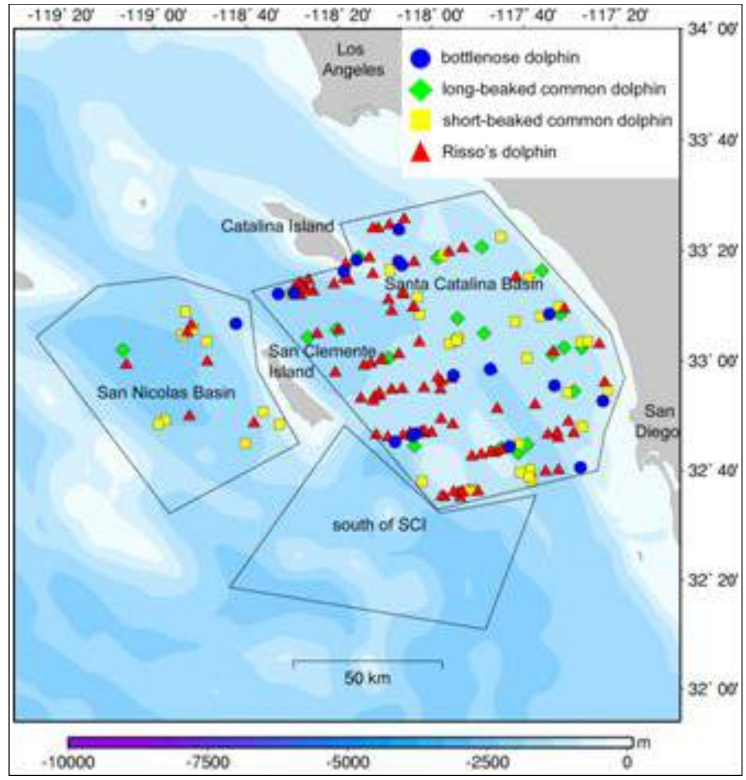

Figure 4: Sightings (identified to species) used for estimation of density and abundance of dolphins in this study, 2008–2013.

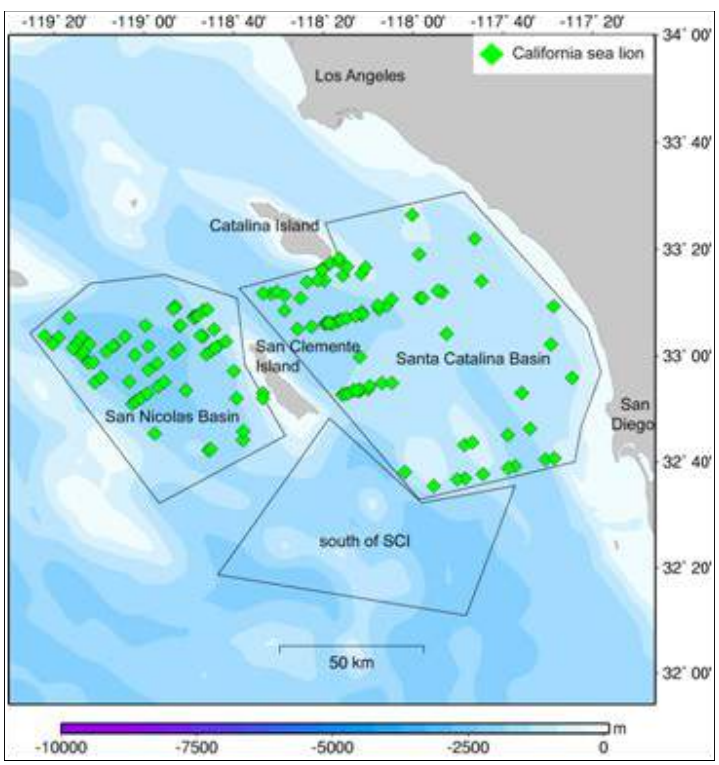

Figure 5: Sightings (identified to species) used for estimation of density and abundance of California sea lions in this study, 2008–2013.

Figure 6: Photographs of the aerial views showing the features used for species identification. (a) Delphinus delphis - *For* D. delphis*, the short beaks, robust bodies, white beak blazes, and frequent white patches on the dorsal fins and flippers can be seen. (b)* D. capensis - *For* D. capensis*, the long beaks, more-slender bodies, shallow foreheads, wide gape-to-flipper stripes, and infrequent light patches on fins can be seen. Used with permission.*

We did this in the present study, by using g(0) factors from studies by other researchers of the target species. However, these often only account for part of the potential bias.

Visibility bias in marine mammal surveys is generally divided into two categories. Availability bias is the proportion of animals on the trackline missed due to being on a dive and thus unavailable to be seen by the observers. It is usually modeled from information on dive times (3, 7, 19). Perception bias, on the other hand, is the proportion of animals on the trackline that was available to be seen, but was not detected by the observers due to operational factors (such as adverse conditions or observer fatigue). It is well known that certain species (e.g. blue whales and Risso's dolphins) are more easily seen, due to their large size, "showy" behavior, or highly visible coloration. Perception bias is usually modeled based on detection data collected from multiple-platform or independent/conditionally independent observer studies (17, 25, 26). Ideally, both should be accounted for in marine mammal surveys, but in practice suitable data are usually not available to correct for both types of bias. Since our estimates for some species do not account for both of these types of bias, this results in some residual underestimation.

The inability to see all animals directly under the aircraft also clearly affects the trackline detection. Due to aircraft and personnel limitations, we did not have the ability to use a belly observer. We have strived to minimize the potential effects of this limitation on the resulting density and abundance estimates by using a 200-m left-truncation approach. It is uncertain how much remaining bias from this factor may affect our estimates.

Assumption 2: No Responsive Movement : Although it is often stated that there must be no responsive movement to the survey platform, this is not strictly true. However, any responsive movement must occur after detection by the observers, and such movement must be slow relative to the speed of the survey platform (13). In our case, the use of a fast-moving aircraft as the survey platform minimizes the chances of this being a significant issue. There is much more concern with vessel surveys, and this is generally not considered to be a problem for aerial surveys.

Assumption 3: No Distance Errors: Distances must obviously be measured accurately to avoid inaccuracies in the resulting estimates (13). However, in practice, distances are difficult to measure at sea, and it is likely that every marine mammal line-transect survey has suffered from some inaccuracy in distance measurement. However, small and random errors generally do not cause significant problems. It is large and/or directional errors that that cause large biases and are thus of more serious concern. We have strived to measure angles and distances as accurately as possible during this study. At this point, we have no indications that large or directional errors in distance measurement were an issue in this study, and we are conducting studies to further examine this potential bias.

Placing the Estimates into Context: Historically, patterns of cetacean relative abundance and presence in SOCAL waters are, in many cases, very different from what are currently observed (41). This is likely related to previous exploitation and depletion of these species, long-term changes in oceanographic conditions, and/or concomitant changes in prey distributions and densities. Peterson *et al.* summarized the anomalous conditions (including several El Niño and La Niña events) that have characterized the California Current System in the last several years (37). Henderson *et al.* have examined how these factors may affect small cetacean distribution and abundance in the SOCAL area (28). Below, we place the information obtained during the current study into the context of our historical knowledge.

Recent ship-based surveys of the SOCAL area using data collected from CalCOFI cruises have provided abundance estimates for cetaceans in an area overlapping ours. However, as these surveys used very different methods and did not produce estimates for the same strata and seasonal partitions as ours, the results are not directly comparable (14, 22). Carretta *et al.* conducted extensive year-round aerial surveys of an overlapping (although not completely so) area in 1998/1999, totaling 7,732 km of systematic line-transect effort (19). We flew 18,831 km of systematic line-transect effort. We followed very similar methods and used similar equipment to the surveys of Carretta *et al.*, including even using some of the same aircraft and pilots (19). Although, we cannot compare abundance estimates directly, since our study area boundaries differ somewhat (Figure 1), estimates of density from our study area can be reasonably compared with those of Carretta *et al.* (19). Comparisons to those estimates, in particular, can provide some useful information on potential changes in distribution and abundance of marine mammal species over the last 15 years. These data are discussed by species below.

Fin whale: The fin whale is one of the most common large whales off SOCAL and is seen in all seasons (16, 22, 25, 27). Fin whales were heavily hunted in the 20th century, but have been protected by the International Whaling Commission (IWC) since 1976. The species is listed as Endangered under the U.S. Endangered Species Act (ESA). Thus, the population would be predicted to have recovered somewhat since then (41). The fin whale was not mentioned in reports of cetacean surveys conducted in SOCAL waters in the 1950s (11, 36). Although there was no evidence of a population increase in the California/Oregon/Washington stock from traditional analysis of SWFSC line-transect surveys, a Bayesian analysis of the same dataset showed a significant increase in this species from 1991 to 2008 (18, 35). The past effects of illegal whaling, as well as ship strikes and gillnet entanglement, may have slowed recovery of the species. However, the current best estimate of stock size is 3,044 whales (CV = 0.18) (18). Carretta *et al.* sighted fin whales 21 times (6 in the cold- and 15 in the warm-water season), which for large whales was second only to the gray whale (sighted only in the cold-water season) (19). Densities of 0.27 animals/100 km^2 (CV = 0.34, cold) and 0.89 (CV = 0.33, warm) were calculated from the Carretta *et al.* surveys (19). Overall, our estimates (0.91 animals/100 km^2, warm; 0.93 animals/100 km^2, cold) are well above theirs, based on our 61 sightings. This is consistent with the documented increase in fin whale abundance along the U.S. west coast (35).

Gray whale: Gray whales migrate along the coast of California twice per year: once during their fall southward migration and again during their spring northward migration. They are commonly seen off the SOCAL coast during these times. The species was heavily exploited in the 19th and early 20th centuries and was subsequently protected from commercial whaling by the IWC in the mid-20th century. The ensuing recovery of the eastern North Pacific stock has been so successful that it has since been removed from the U.S. Endangered Species List. The current best estimate of the eastern North Pacific stock size is 19,126 (CV = 0.07), up from a low estimate of just a few thousand individuals (18, 38). Despite this overall increase, there have been several population ‘dips’ in recent years, thought to be mostly related to harsh environmental conditions on the northern feeding grounds and resulting detrimental effects on calf survival (1). Gray whales were observed 31 times by Carretta *et al.* all during the cold-water season (19). They calculated an overall density estimate of 5.1 animals/100 km^2 (CV = 0.29) for this species. We observed gray whales 39 times during the cold-water season, with a corresponding density of 1.16 animals/100 km^2 which is quite a bit lower than that of Carretta *et al.* (19).

Risso’s dolphin: Risso’s dolphins are currently one of the most common species of delphinids off the California coast, apparently due to significant changes in numbers and/or distribution over the last several decades (27, 41). Older reports from the mid-20th century did not identify these animals as common in SOCAL. In fact, they were not even mentioned by Brown and Norris or Norris and Prescott, who conducted extensive cruises in the SCB in the 1950s (11). Similarly, Risso’s were not discussed by Walker, who conducted many searches in the SCB in 1966-1972 to live-capture small cetaceans (36, 45). Leatherwood *et al.* stated that Risso’s were most abundant in SOCAL during periods of protracted warm water, and were considered to be primarily a tropical species (34). However, our current understanding of this species does not support this view. In contrast, greatest abundance generally appears to occur in areas with colder waters, such as central California (33). The California/Oregon/Washington stock of Risso’s dolphin is currently estimated at 6,272 individuals (CV = 0.30), which appears to be an underestimate (18). There is no empirical evidence of an overall trend in abundance from recent line-transect surveys conducted off the

U.S. west coast (18). However, we believe that this species has increased off SOCAL in recent years. In general, we found much greater densities in our study than were found by Douglas *et al.* for 2004-2008, though their study covered a much larger area (22).

Risso's dolphins were common in the late 1990s when the Carretta *et al.* surveys were conducted, and those authors observed 23 groups (16 of them during the cold-water season) (19). They calculated densities of 6.1 (CV = 56, warm) and 18.0 individuals/100 km^2 (CV = 40%, cold). Based on a total of 142 sightings, our calculated warm-water density (11.46 animals/100 km^2) is much higher; however, our cold-water density (7.85 animals/100 km^2) is quite a bit lower than that of Carretta *et al.* (19). These densities indicate that a substantial number of Risso's dolphins used the area during our study period (up to about 1,500 individuals). They were thus the third-most abundant dolphin species we saw, after the two common dolphin species. This may be generally indicative of increased use of the SCI area during the warmer season and decreased use during the colder season, although this remains to be determined.

Bottlenose dolphin: In the 1950s, bottlenose dolphins were considered uncommon north of Orange County, although they were often still seen inside SD Bay at that time (36). The NMFS currently recognizes two stocks of bottlenose dolphins in SOCAL. The coastal stock remains within 1 km from the mainland shore. Thus, animals observed in the present study around SCI would presumably belong mostly to the California/Oregon/Washington stock. So-called offshore bottlenose dolphins in California may actually comprise more than one stock, and there is some evidence of separate island-associated populations; however, this remains unconfirmed. Nevertheless, the currently recognized offshore (California/Oregon/Washington) stock is estimated to number 1,006 individuals (CV = 0.48), and there is no information on trends for this stock (18).

Older records of bottlenose dolphins in more offshore waters of SOCAL usually stated that they were almost always in the company of short-finned pilot whales (36, 45). Pilot whales were previously considered to be "quite common" in SOCAL waters considered to be "quite common" in SOCAL waters (11). This association was not seen in the present study, as pilot whales were never observed. Bottlenose dolphins were seen by Carretta *et al*. in both warm- and cold-water seasons (19). They estimated densities of 1.5 (CV = 0.67, warm) and 3.4 animals/100 km^2 (CV = 0.66, cold) from their late 1990s surveys. Their estimates were based on a total of 14 sightings, while we included 34 for this species. Our warm-water estimate of 2.58 animals/100 km^2 is higher. Our cold-water estimate of 1.51 animals/100 km^2 is lower than that of Carretta *et al.*, which may be expected as our surveys did not cover coastal waters extensively (19).

Short-beaked common dolphin: Until 1994, only a single species of common dolphin was considered to occur off the California coast, *D. delphis* (29). We now know that there are actually two species, *D. delphis* and *D. capensis*. Before 1994, the two species were erroneously lumped as *D. delphis*. Work conducted before the mid-1990s generally did not distinguish the two species. However, conclusions from these studies are probably mainly attributable to the more abundant short-beaked species. This species has long been known as one of the most abundant and widespread in the SCB (2, 11, 21, 22, 27, 36, 45). Although older records are sometimes contradictory, extensive aerial surveys for common dolphins in the 1980s showed them to be much more widespread and have much higher densities (0.8-2.4 individuals/km^2) in summer/autumn than during winter/spring (0.2-1.2 individuals/km^2) (11, 21, 36). The latter authors identified an influx of animals from the south into the SCB during the warm-water season.

Short-beaked common dolphins are extremely common and abundant in SOCAL waters. The current population estimate is 411,211 individuals (CV = 0.21), making it the most abundant cetacean in the SCB (18). There is some evidence of an increasing trend in SOCAL waters. This may be correlated with a decline in numbers of 'northern common dolphins' (which includes both species) in Mexican waters and the eastern tropical Pacific (18). Overall, the species' abundance off California is highly variable (2, 21, 25).

The short-beaked common dolphin was the

most frequently observed cetacean species during the Carretta *et al*. study (61 sightings) (19). They observed them in both seasons, with estimated densities of 465.0 (CV = 0.39, warm) and 178.0 animals/100 km^2 (CV = 0.37, cold). We observed both common dolphin species in our surveys (total 191 useable sightings). However, *D. delphis* was much more common: 17% of all common dolphin sightings were *D. delphis* vs. 6% *D. capensis*. The remaining 77% could not be reliably identified to species and were classified as *Delphinus* sp.

Warm-water densities of short-beaked common dolphins in our study (67.34 animals/100 km^2) were much lower than for Carretta *et al.*'s warm-water season (465 animals/100 km^2) (19). This may be at least partly related to colder water temperatures in recent years (for instance 2010 was a La Niña year, with unseasonably cold water temperatures). Our cold water estimate (126.10 animals/100 km^2) is more similar to that of Carretta *et al.* (178 animals/100 km^2) (19). Clearly, short-beaked common dolphins were very abundant in our study area (the most abundant species, by far) with an estimate of about 16,000 individuals present at the peak.

<u>Long-beaked common dolphin:</u> The long-beaked species of common dolphin is frequently observed in nearshore waters of SOCAL within 90 km of the mainland coastline (18, 27). Highest densities are found near the mainland coast and Channel Islands (22). There is little information on the historical status of the species, as it was not recognized as a separate species until 1994 (29). The California long-beaked common dolphin stock is currently estimated at 107,016 individuals (CV = 0.42) (18). This is much higher than the previous estimate of 27,046 (15). While no formal population trends analysis has been done for this species, their numbers do appear to be increasing off SOCAL (15). Oceanographic conditions (especially warming of local waters during El Niño conditions) cause density fluctuations among these dolphins in the SCB (15, 18, 29). Our abundance estimates suggest a ratio of about 2.5:1 (*delphis:capensis*), which includes a much higher proportion of *D. capensis* than reported by Douglas *et al.* (22). This is expected, as their study area was more offshore and extended further north, where *D. capensis* density is lower (29).

During the late 1990s, Carretta *et al*. did not report any sightings of this species, and all their identified common dolphins were considered to be *D. delphis* (19). We did identify 37 groups of long-beaked common dolphins to species (16 of which were were "useable" for density estimates). However, they were less frequent and in smaller groups than short-beaked common dolphins. We estimated densities of 26.19 animals/100 km^2 (warm), and 50.90 animals/100 km^2 (cold) for this species. This is consistent with the idea that long-beaked common dolphins are becoming much more abundant in SOCAL, as recently suggested by Carretta *et al*. (15). It should be noted that we observed a much higher proportion of *D. delphis* in our study (2.5:1) than Carretta *et al*. who encountered the two *Delphinus* species in nearly equal proportions during 2009 ship surveys conducted throughout the reported range for *D. capensis* (15). It is likely that if our study effort had focused more in coastal waters, we would have obtained a higher ratio of *D. capensis,* as this species' highest reported densities occur within several kilometers of the coast. Many of the local *D. capensis* schools in the San Diego area appear to be inshore of the eastern boundaries of our study area.

<u>California sea lion:</u> California sea lions are very common in SOCAL waters and are the most abundant pinniped species along the California coast. The current best estimate of this single U.S. recognized stock is 296,750 individuals (18). The population has generally been increasing for many decades, although there have been several recently reported dips in abundance (18). The stock is considered to have reached carrying capacity, though this is currently unconfirmed (18).

Density in the water has not traditionally been estimated for pinnipeds in SOCAL. However, Carretta *et al.* provided the first such estimates based on several hundred sightings (19). Their California sea lion estimates ranged from 19.4 to 119.0 animals/100 km^2 during the cold-water season, and from 5.6 to 75.0 animals/100 km^2 during the warm-water season based on 371 total sightings. Our warm-water estimate of 5.83 individuals/100 km^2 and our cold-water estimate of 10.35 individuals/100 km^2

(based on 132 sightings) are generally much lower than those of Carretta *et al*. (19). The lower densities recorded in our study, vs. Carretta *et al.*, may be expected, as our surveys did not have extensive coverage in the nearshore shallow waters where California sea lions are most frequently observed (19). Carretta *et al.* focused their coverage in these waters specifically for pinniped surveys (19). California sea lion density at sea tends to be lower during summer months, when much of the population is ashore for the breeding season.

Conclusion

This report provides the most current (2008-2013), fine-scale estimates of density and abundance within portions of the offshore marine waters in SOCAL used by the USN. In particular, densities derived for the cold-water season represent information that has been largely absent from the region over the last 15 years. Abundance of marine mammals is known to fluctuate from year to year based on changing and dynamic oceanographic conditions in SOCAL (El Niño Southern Oscillation events, prey availability/distribution, etc.) (28). For instance, the NMFS in their spatial habitat models and density estimates generally prefers to pool multi-year survey data to reduce effects of inter-annual variation. Based on comparisons to historical data, such as Carretta *et al.*, we believe that our estimates reported herein are generally reflective of marine mammal numbers within the USN's SOCAL Range Complex during the 2008-2013 survey period (19). Although our study spans a nearly 6-year period, we did not attempt to evaluate trends in abundance, largely due to sample size limitations. We plan to further investigate this dataset through density modeling.

Overall, our results indicate that the study area continues to be used by a substantial number of marine mammal species during both the warm and cold water seasons. Although direct comparisons are problematic due to methodological, geographical, and temporal differences in the studies, the sometimes dramatic differences in the general patterns of seasonal density for some species suggest strong variability in occurrence and density patterns. These are most likely related to prey species shifts mediated by oceanographic events, and also anthropogenic impacts and recovery from such impacts (28, 38).

Our survey results, when compared to past studies, indicate that the relative density of some species has changed in the SCB since the 1950s and 1960s (41). Both increases and decreases have been indicated, depending on species (41). We hope that further survey work will facilitate continued estimation of abundance for all species occurring in the study area, allowing longitudinal refinement and updating of these estimates in the future. There are ongoing plans to synthesize data from this project with other data in an environmental modeling study to ultimately provide more accurate, fine-scale information and predictive capabilities for USN monitoring and assessment efforts relative to SOCAL marine mammals.

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Case Report

Behavioral patterns of a manatee in semi-captivity: implications for its adaptation to the wild

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Abstract

Rehabilitation of orphaned endangered Antillean manatees (*Trichechus manatus manatus*) enhances *in situ* conservation. We investigated the behavior of a five year-old male manatee rescued in Quintana Roo (Mexico) in relation to its failed rehabilitation. This is a unique case of a semi-captive manatee in the Caribbean, and the first endeavor to release a rehabilitated orphan in Mexico. Through 134 hours of direct and *ad libitum* observations, we described the manatee's behavior and assessed his behavioral time budget. The frequency of states was determined by instantaneous sampling, while the frequency of events was defined by the number of events per time unit. We designed an ethogram of 105 behaviors (56 states and 49 events), distributed in six behavioral categories. Compared with previous catalogs designed for manatees, the subject displayed 43 new behaviors (24 states and 19 events). The manatee showed indications of a daily rhythm; the animal consistently performed displacement behaviors in daytime hours, while engaged in comfort behaviors mainly at night. The use of space depended on the behavioral category and the time of day. The manatee showed dietary preference for the food provided by the caretakers, and virtually no consumption of native aquatic plants. This inadequate feeding behavior, along with a strong attachment with people, made the individual completely dependent on human care. Therefore, despite being free to explore natural areas, the animal remained close to the facilities after release. Future recommendations on the management of rescued manatees are discussed.
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Keywords: Trichechus manatus manatus, ethology, release, management, rehabilitation, diet

Introduction

The Antillean manatee (*Trichechus manatus manatus*), a subspecies of the West Indian manatee, is considered "Endangered" by the Red Data Book of IUCN (17). The main threats to manatees are related to anthropogenic activities such as hunting, habitat loss, environmental contamination, and collision with boats leading to injuries and fatalities (25). Another impact on the natural populations are orphaned calves that are left fending for themselves after injury or death of the mother (6). One of the best strategies for species conservation is through recovery and management plans, including the successful rehabilitation and release of orphans. Approximately 70 West Indian manatees are being rehabilitated in captivity, while another 100 remain permanently held (1). Evaluating the success of rehabilitation programs requires health assessments and behavioral documentation.

In 2003, Mexican authorities rescued an orphaned male Antillean manatee. After failed attempts to release the weaned manatee into the wild, it was kept in semi-captive conditions and used to promote public awareness and species education (1). This represents the first attempt of rehabilitation and release of a manatee in Mexico and a unique case of an Antillean manatee held in a semi-captive environment. The present work aims to describe the behavior of this individual in order to provide possible causes for his dependence on human care and unsuccessful release.

Methods

History of the observed manatee:

The subject of this study was a male orphaned manatee rescued in 2003, at Guerrero Lagoon (18° 41' 25" N, 88° 15' 31" W), Quintana Roo, Mexico. The area is a freshwater aquifer, with salinities ranging between 3 and 4 psu which discharges into Chetumal Bay (7). This larger, interconnected system of coastal habitats, is considered to be a traditional use site for manatees (22). At the first stage of the recovery (September 2003 – May 2004), the calf remained in a plastic pool at El Colegio de la Frontera Sur (Ecosur). During the second phase (May 2004 – May 2005), the 1-2 year-old manatee was transferred to an enclosure in Guerrero Lagoon with the following objectives: (i) to familiarize the animal with his natural habitat, (ii) to

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facilitate the interaction of this individual with wild manatees, and (iii) to teach him to forage on natural aquatic vegetation (24). In July 2005, at approximately 2 years old, the manatee was tagged with a VHF radio and released near the enclosure. However, the animal followed people, including its caretakers, and preferred to stay near the pens. Despite the manatee's relocation by boat to the end of the lagoon (approx. 5 km), he returned to the release area within a few hours (24). After several days, the manatee was relocated to a pen in a closed channel designated for soft-release (a release strategy involving pre-release conditioning and training) with the intention of increasing its chances of survival and minimizing negative effects during readaptation to the wild. Soft released animals are kept within a shelter at the release location to allow acclimation to the new environment before they are completely independent and responsible for their own welfare. The individual was kept in the closed channel from July to September 2005, but was found stranded on several occasions, requiring caretakers to rescue and care for him. By mid-September, the animal was completely released. Five months later, it was seen in an emaciated state, displayed hyperkeratosis and a weight loss of 30 kg (33% of total weight) (24). In 2006, the 2.5 year old manatee was declared unfit for survival without human care and has since been maintained at the Center of Care and Rehabilitation of Aquatic Mammals (CARMA) (24). At the time of this study (August 2008 - December 2009), the five to six year old manatee required food provisioning from caretakers at the facility, but could move freely between captivity and the wild. Provisioned food consisted of fruits and vegetables such as apples (*Malus domestica*), pears (*Pyrus* sp.), squash (*Cucurbita maxima)*, carrots (*Daucuscarota* sp.), swiss chard (*Beta vulgaris L.)*, beetroot (*Beta vulgaris*), and lettuce (*Lactuca sativa*).

Behavior recording:

We conducted behavior observations from an observation platform, 2 x 5 m in surface area and 3 m in height. The selected observation method was 'instantaneous sampling', in which the observation time was divided in intervals (19). Each observation session lasted 30 minutes, with behavioral assessments occurring every minute. Observed behaviors were grouped into the following categories: breathing, displacement, feeding, elimination, comfort, and interaction with objects. The behaviors were organized, described, and codified in a catalog based on previous ethograms (8,15,28). Graphics of the cumulative number of behaviors were created in order to determine the sufficiency of observations. When the curve asymptote was reached, the possibility of observing a new behavior was considered low, and the catalog was considered complete.

The probability of finding a new behavior was also assessed by the formula:

$$\theta = 1 - \frac{N_1}{\ell}$$

Where N_1 is the number of behaviors observed once and ℓ is the total number of observed behaviors (19). To calculate the frequency of behaviors, 4 instantaneous samplings of 30 minutes each were carried out daily. Samples were equally distributed throughout the 24-hour period (day- and night- time) to detect temporal variation of the behavior. To facilitate detection during night sampling, a small plastic bottle equipped with two AA batteries and a small red LED light was attached to a belt on the manatee. To determine variations on space use within the enclosure, five imaginary quadrants were outlined inside the observation area (Figure 1a and 1b). The location of the manatee was identified concurrent with its associated behaviors. There were no differences in depth or any other condition between quadrants.

During each observation, details on the food offered and the animal's preference were annotated. Additionally, fecal samples were collected opportunistically and stored at room temperature in FAA (85% ethyl alcohol, 10% formalin, 5% glacial acetic acid). Fecal samples were observed under an optical microscope. Comparisons of plant epidermises with micro-histological descriptions and permanent collections of samples allowed for identification of consumed plants (5). Five glass slides per sample were examined under the microscope at 10X, 40X, and 100X magnifications. A total of 500 points was identified for each sample as recommended by Hurst and Beck (16). For each point, the plant fragment was identified using guides and a reference collection of plant tissues created from the provisioned food (16).

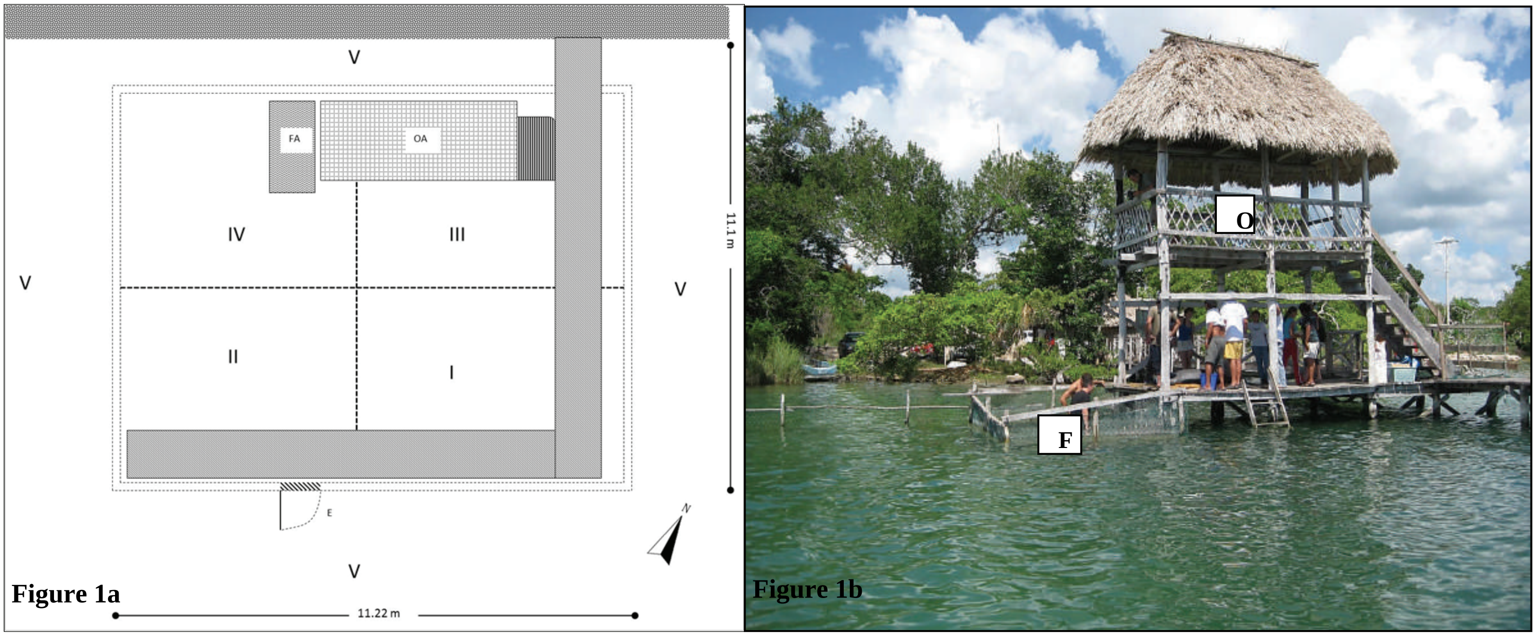


Figure 1: Facilities where the manatee was kept during the study. (a): Schematic view showing the location of the quadrants (I -V), limits of confinement (double dotted line), exit gate (E), feeding area (FA), and the observation area (OA). (b): Photo taken during a health assessment activity of the manatee, showing the feeding (F) and observation (O) areas. Reproduced with permission by Nataly Castelblanco.

Data Analysis:

Observed behaviors were constructed into a catalogue (19). Behaviors were classified as states or events: "events" were observations of relatively short duration, while "states", such as body postures, had relatively long durations (20). The frequency of occurrence, defined as the number of occurrences in time period, was calculated for behavioral events. The duration, in terms of proportion of time spent, was measured for behavioral states. The use of the space was explored as percentages of time in a determined quadrant. Differences in frequency of behavioral category and use of quadrants were tested with a Kruskal-Wallis one-way analysis of variance on ranks, followed by all pairwise multiple comparison procedure (Dunn's method). The Student's t-test was used to detect significant differences in the presence of the items found in fecal samples.

Results

A total of 151 observation sessions was completed, totaling 134 hours of effort distributed as follows: 36 hours in the morning (0500 - 1200); 49 hours in the afternoon (1300 - 1900) and 49 hours at night time (2000 - 0400). The calculated probability of finding a new behavior was low (θ= 0.91), and therefore, we considered the invested effort adequate to guarantee a satisfactory sample of the individual's behavioral repertoire (Figure 2). A total of 108 behaviors was registered, of which 43 (24 states and 19 events) were not recorded in previous catalogues; therefore, those were considered 'new behaviors' (Table 1). During the 14% of observations in which the individual was undetected, the behavior was considered 'absent'. The frequency of occurrence was statistically different between categories (Kruskal-Wallis: $H = 59.335$, df=3, $P<0.001$). The most frequent categories were comfort (44.60%) and displacement (39.50%), while feeding and interaction with objects were least frequent at 8.00 and 7.70% respectively (Figure 3).

The use of the space was significantly dependent on the quadrant (Kruskal-Wallis: $H = 174.126$, df=4, $P<0.001$) (Figure 4). The manatee used quadrants V (45.00%) and III (30.00%) a greater proportion of time. Quadrants I, II, and IV were used less often. Quadrant III was preferred for displacement behaviors, while quadrant V was used mainly for resting (comfort behaviors) (Figure 5). A preference for quadrant III was observed during daytime hours, when the interaction with people and feeding events occurred. The animal used quadrant V during nighttime hours and stayed

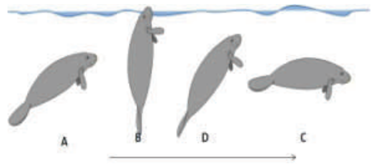
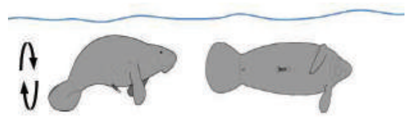
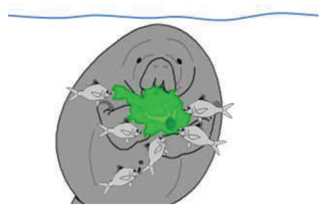
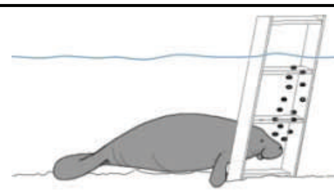
Category	Description	Example	Behaviors already reported*		New behaviors		Total of behaviors
			States	Events	States	Events	
Breathing	The manatee opens its nostrils for air at the moment of reaching the surface and closes them during submersion. Exhalation is followed by inhalation.	 "Vertical breathing" (198)	---	2	---	1	3
Displace-ment	Locomotive behaviors.	 "Spin 90°" (264)	14	15	4	5	38
Feeding	Includes foraging (searching for food) and grazing (consumption) behaviors.	 "Grazing + competition with fish" (380)	14	1	9	1	25
Elimination	Air expulsion.	 "Bubbles" (410)	---	6	---	1	7
Comfort	Relaxation periods.	 "Rubbing eye with fin" (571)	18	11	1	6	36
Interaction With objects	Contact with objects.	 "Playing with object" (674)	---	5	10	5	20
							Total=129

Table 1. Behavioral catalog for a West Indian manatee in semi-captivity. * Behaviors reported in the catalogs of Castelblanco-Martínez 2000, Charry 2002, and Mercadillo 2010. In parenthesis the code with name of the behavior in the example.

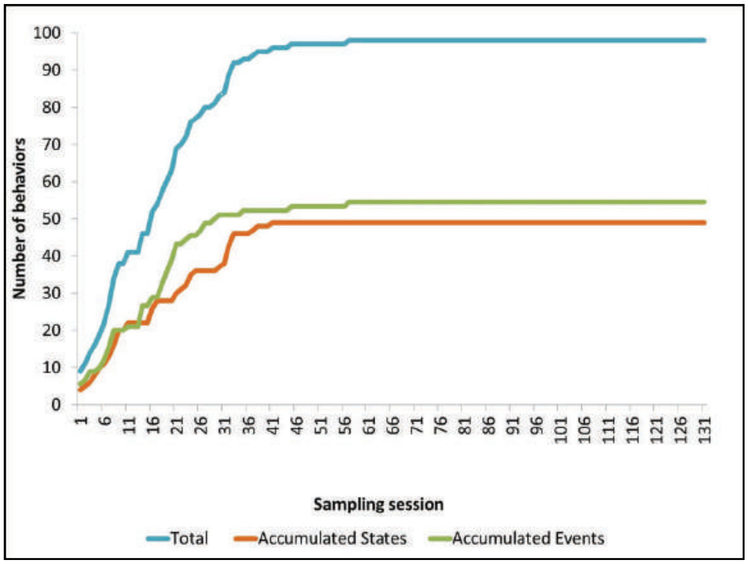


Figure 2: Cumulative curve of behaviors displayed by a semi-captive Antillean manatee.

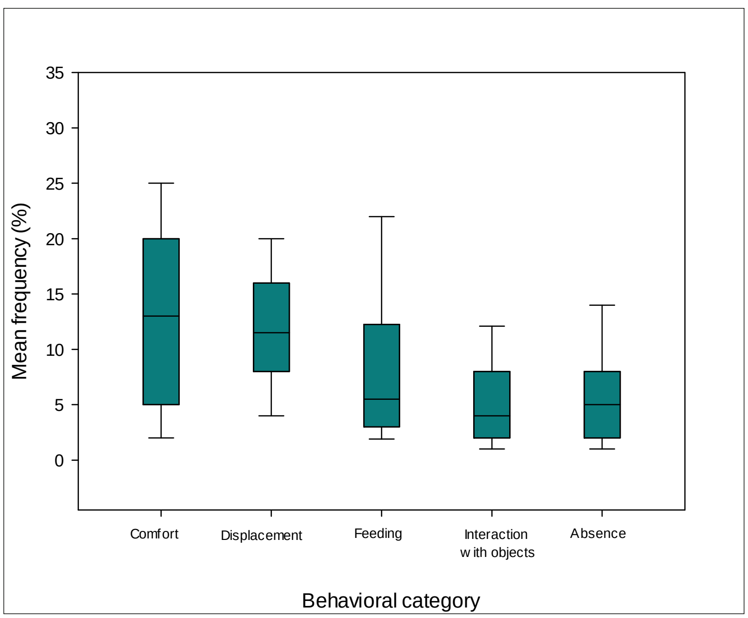


Figure 3: Frequency of behavioral categories displayed by a semi-captive Antillean manatee.

submerged at the bottom the majority of the time. The manatee showed a greater breathing and displacement frequency during daytime hours, while comfort behaviors were most often seen during the night (Figure 6).

Feeding behaviors occurred mainly during daytime observations (60.17%) and were recorded in areas where keepers and visitors were constantly present. The manatee showed preference for tubercles

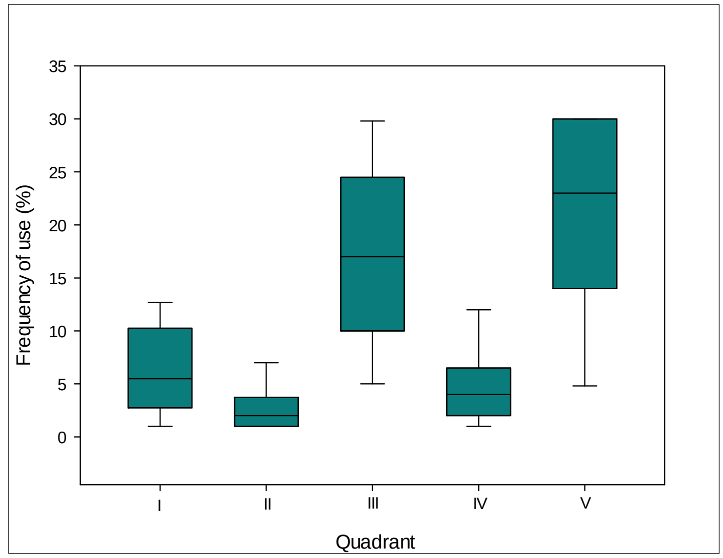


Figure 4: Occupancy of quadrants by a manatee in semi-captivity.

such as beetroot (30.19%), carrot (14.03%), and jicama (6.23%). Direct observations did not reveal consumption of any vegetation naturally occurring in the region. Fecal sample collection was difficult due to the presence of fish that opportunistically and efficiently consumed the excrement. However, we were able to collect and analyze three samples, which contained seven items offered by caregivers (Table 2). Lettuce was the most frequently consumed plant (31.44%), followed by beetroot and broccoli. The turtle grass (*Thalassia testudinum*) was the only species native found in the feces, representing 11.79% of the detected items. There were no significant differences between the frequency of items within fecal samples ($Tc = 1.14$, $P<0.05$).

Discussion

We consider our sampling effort sufficient at identifying new behavior since the probability of finding a new behavior is low ($\theta=0.91$); similar to what was found in previous studies: 0.94 and 0.96 (9). The manatee's behavior was somewhat predictable, showing higher breathing rates during daylight hours (between 0600 and 1800). Breathing behavior is considered a response to the level of metabolic activity; behaviors requiring high energy investment (displacement, interaction with an object, and feeding) showed the same increased rhythm pattern, while low-energy activities, such as resting, were

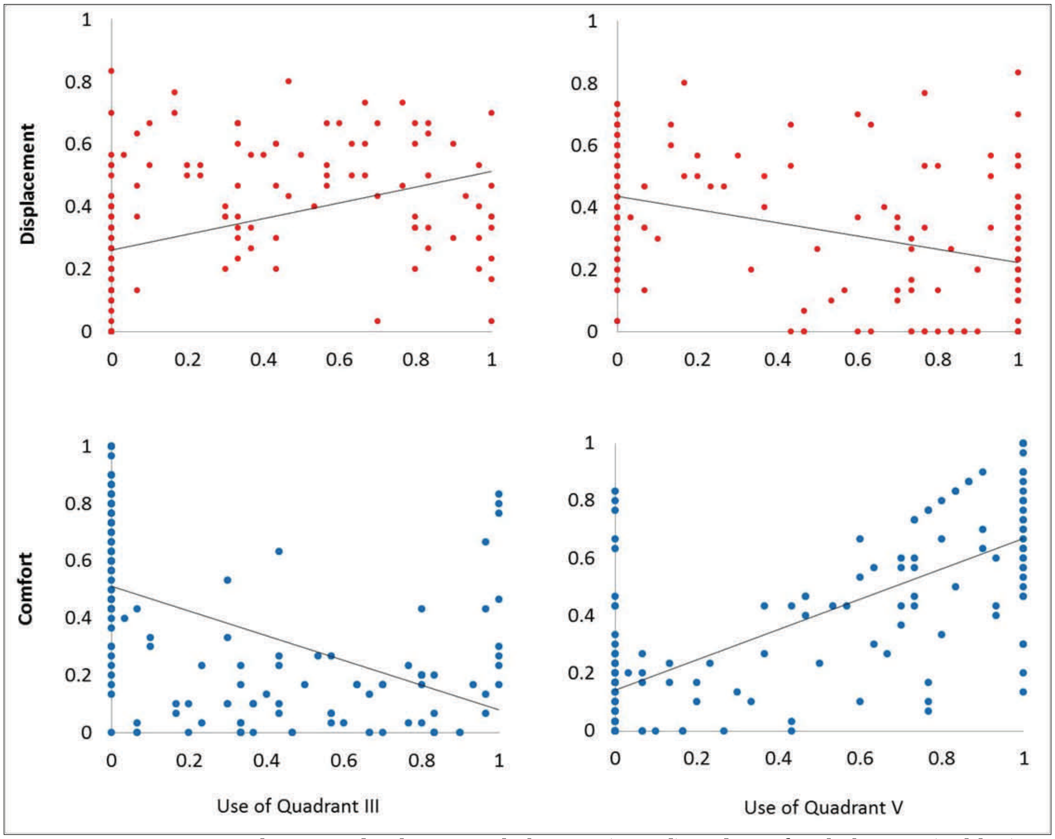


Figure 5: Comparison between displacement behavior (in red) and comfort behavior (in blue), and the relationship with the occupancy of the space.

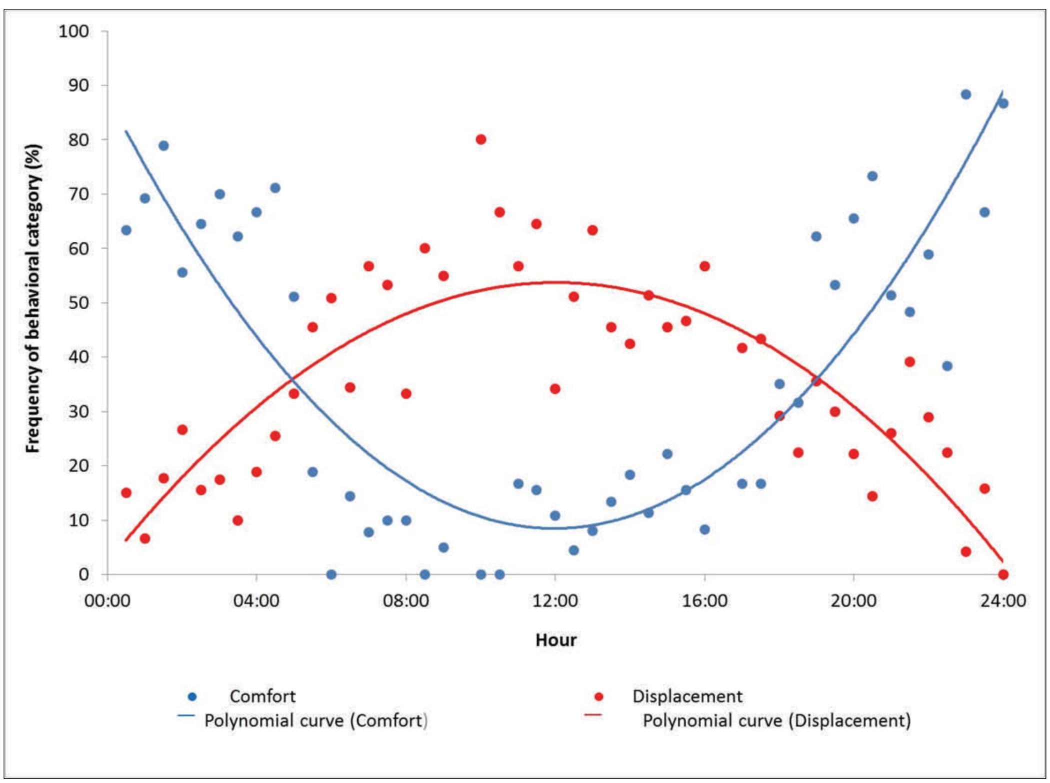


Figure 6: Daily variation of displacement and comfort activities displayed by a manatee in semi-captivity.

	Species	Consumption observed directly	Average consumption observed in feces
Artificial supply	Lettuce	Yes	31.44%
	Carrot	Yes	6.98%
	Apple	Yes	5.24%
	Pear	Yes	---
	Banana	Yes	---
	Beetroot	Yes	23.14%
	Broccoli	Yes	17.03%
	Cauliflower	Yes	---
	Chard	Yes	4.36%
Natural supply	*Thalassia testudinum*	No	11.79%
	Halodule sp.	No	---
	Batophora sp.	No	---
	Chara sp.	No	---
	Ruppia sp.	No	---

Table 2. Dietary preferences of a manatee in semi-captivity through direct observation and analysis of fecal samples.

were more frequent during the afternoon. In Florida and Puerto Rico, increased activity rate in the manatees' nocturnal behaviors seems to be quite common, however, it is unclear if wild manatees in the study area normally exhibit a daily rhythm of activities (10). Captive manatees have been observed to alter their activity rates from day to night, showing displacement and foraging behaviors during the day, and rest during the night (2,8,14,28). The authors speculate that the activity rhythm could be artificially influenced by their daytime feeding schedule. Conditioned responses to food provisioning are poorly understood for wild manatees outside of Florida, making it challenging to speculate on its effects on survival in other regions. Additionally, the occupancy of quadrants was also affected by the presence of visitors. The manatee actively sought human contact during the visits, which always took place during daylight hours.

According to previous studies, at least six species of plants typically found in the diet of manatees are naturally present in Guerrero Lagoon: *Rhizophora mangle*, *Thalassia testudinum*, *Halodule wrightii*, *Batophora* sp., *Chara* sp., and *Ruppia* sp. (4). However, the manatee was never observed consuming naturally occurring vegetation, and only one species (*Thalassia* sp.) was identified in his feces in low proportions. In the current investigation, we did not register any indication of coprophagy, however, previous studies have observed that some captive manatees eat their own feces (8,9,15). In this study, the manatee may not have had the opportunity to eat his own feces since fish rapidly consumed all that the animal passed. Previously studies were conducted in captivity, where the absence of fish allowed the animals access to consume their own feces. Therefore, it is possible that coprophagy in the wild occurs less frequently than in captivity where fishes are present.

Maintaining a captive manatees' natural behavior, including foraging, is key to the success of rehabilitation and release programs. A mortality analysis developed with released Antillean manatees in Brazil indicated that adaptation to a natural diet is important in the release process (23). Therefore, the ability to forage on local vegetation is vital for adult manatees and a dependency on human food provisioning compromises an individual's ability to survive on its own. Previous rehabilitation experience with other mammal species demonstrated the importance of providing naturally occurring vegetation during the pre-release stage to ensure the development of adequate foraging skills, as well as acclimating the animals to their future diet in the wild

(11,13,21,26). Furthermore, the need for a permanent food supply such as apples, pears, and lettuce, represents a significant cost for the managers and entities in charge.

Animals reared in captivity may form unnatural attachments to people because of the strong learning that sometimes occurs during sensitive, early periods in development. Our observations indicate that this individual was prone to search for human contact such as swimmers, canoes, visitors, and keepers, which led to dangerous situations for both the animal and the people involved. On one occasion, a fisherman wounded the manatee with his spear, causing a severe lesion on the animal's back. Although the cause of the attack is unknown, it is speculated that the fisherman was trying to avoid contact with the animal. While injuring a manatee is considered illegal, the affiliation of the manatee with people promotes human-related interactions that can harm both parties. Humans constitute a major threat to manatees in their natural environment through poaching, injuries from boat strikes, capture in fishing nets, and harassment. If manatees are habituated to humans and/or associate humans with rewards, they would most likely approach or at the very least not actively avoid them. The manatee in this study was most likely habituated to humans due to the regular presence of people during feeding episodes, health assessment procedures, and public visits. During the early stages of release, behavioral flexibility is critical to survival, as animals adapt their current behavioral strategies to meet the demands of the wild environment (27). Thus, dishabituation to people in the wild can be expected because of the substantial differences in context or following the simple passage of time (3). For example, post-release observations of tracked captive-reared manatees in Florida revealed an initial period of weeks or months in which the individuals did not travel far from the release site. However, over time they began to explore their surroundings and travel farther from the release site. If they do join local manatees, captive-reared animals seem to conform to their wild type behavior (12). In the case of the manatee in this study, attachments to humans in conjunction with a lack of normal learning experiences about the natural environment, particularly food resources, may adversely have affected the dishabituation process. Hand feeding represents a close temporal-spatial association and correlation between human presence and food consumption (3). Under such circumstances human presence is predictive of food, leading to a strong, excitatory association between humans and food rewards (3). For example, because food was not available at night, there was an extended period when a ¨no human, no food¨ association could be developed. In the long-term, these associations may also strongly modify a manatee's natural circadian rhythm.

There are several factors to consider within any rehabilitation or training plan. Animals must be able to successfully forage and process locally available food, locate freshwater, avoid predators, interact appropriately with conspecifics, find shelter, orient, and navigate in a complex environment (18). By the completion of this study, the manatee showed a satisfactory health status for release but displayed abnormal behaviors that could have jeopardized his successful release. The manatee demonstrated a total dependence for human care and strong habituation to humans, confirmed by: (i) the preference for food provided by humans over naturally occurring items, (ii) the presence of the manatee in the observation area despite the opportunity to leave and explore natural environments, and (iii) the high frequency of interaction behaviors with humans. Additionally, while anecdotally the manatee has been seen interacting with conspecifics, during this study there were no observed encounters between the individual and wild manatees.

The persistence of abnormal behaviors induced by captivity could reduce survivorship of released animals, as has been demonstrated for terrestrial mammals such as bears and orangutans (29). The possibility that management decisions can potentially impair the normal behavior of the manatees designated for release, suggesting that further protocols need to be applied in order to address the best ways to prevent such detrimental behavioral displays. The literature suggests that training can improve the behavioral skills of captive animals, and facilitate a potentially successful release (18). The preparation of animals prior to release should include training in behaviors that are likely to contribute to survival (e.g. feeding on natural vegetation) and extinguishment of undesirable behaviors (e.g. approaching or not avoiding humans). Manatees are completely herbivorous, semi-social, and apparently, do not have natural predators in this study area. Therefore, learning of hunting behaviors, highly complex social behaviors or predator avoidance behaviors might not be necessary for survival. During rehabilitation processes of manatees with intentions of release to the wild, it is highly recommended to feed them with the vegetation found within their natural habitat. Although this could imply some extra effort in the short/medium time, this investment will be compensated by the rapid and successful weaning of the animal towards natural vegetation. Also, special attention has to be given to the contact between a manatee in rehabilitation and humans. It is undesirable to create human-food associations that elicit a conditioning of manatee behavior to human presence.

Rehabilitation programs are established for several reasons, including conservation of the species, increasing public awareness, supporting local economies, and satisfying political concerns (1). The manatee in this study is a symbol for manatee conservation in the Laguna Guerrero community, and during the completion of this study, was considered a major attraction for visitors. However, rehabilitation has greater worth when it contributes to species conservation and population growth (1). Given the current degradation of manatee habitat, along with a sustained prevalence of various other threats to the species, most manatee populations seems destined to decline and the rehabilitation and release of rehabilitated orphans as a conservation strategy is becoming increasingly necessary (6). However, considerable infrastructure is needed for maintaining captive animals as technology for monitoring released individuals is essential to assess post-release success, as well as veterinary interventions that may be necessary during any phase of the release. In our case, the human and infrastructural resources were limited, making it difficult to take appropriate measures. Finally, it is important to recognize that rehabilitation efforts *per se* will not reverse the impact of human-related activities on manatee survival. Clear management plans, education programs, and basic research are also necessary to promote the species conservation.

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