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ECOLOGY AND BIOLOGY OF THE ROUGH-TOOTHED DOLPHIN
(*STENO BREDANENSIS*)

**A DISSERTATION SUBMITTED TO THE GRADUATE DIVISION OF THE
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ECOLOGIE ET BIOLOGIE DU DAUPHIN A BEC ETROIT

STENO BREDANENSIS

**DISSERTATION CONJOINTEMENT PRESENTEE A L'UNIVERSITE DE HAWAII
ET L'UNIVERSITE DE LA POLYNESIE FRANCAISE EN VUE DE L'OBTENTION
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DUAL PH.D. PROGRAM BETWEEN THE UNIVERSITY OF HAWAI'I AND
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This dissertation is part of a dual Ph.D. program under a joint agreement between the University of Hawaii at Manoa and L'Université de la Polynésie Française. The dissertation committee is composed of an equal number of members to represent both Universities. Although the format of the dissertation document is not traditional, each of the three chapters follows similar formatting for journal submission. An overall abstract, *introduction and discussion* are also included in the dissertation document that follows more traditional formatting.

ABSTRACT

Greater knowledge of the rough-toothed dolphin, *Steno bredanensis*, is needed to effectively contribute to conservation and management efforts for this species. The primary purpose of this research was to describe ecological and biological parameters for *S. bredanensis* that will be useful in future assessments of population stress. Several approaches were used to study *S. bredanensis*, including investigations of free-ranging populations, dead specimens, and captive individuals. Free-ranging rough-toothed dolphins distributed near small oceanic island environments were found to be more commonly sighted in-shore than off-shore. In the Windward islands of French Polynesia, this species preferred water depths of 1000 to 2000 m and a distance of 1.8 to 5.5 km from the barrier reef. Group sizes of rough-toothed dolphins sighted in French Polynesia range between 1 and 35 individuals with a mean size of 12.1. Endocrinology data for *S. bredanensis* was established in captive healthy and stranded individuals. Ranges and means were provided for progesterone, testosterone, cortisol and thyroid hormones. Changes in thyroid hormone concentrations were reflective of health status and testosterone appeared to be suppressed in ill individuals. Reproduction in *S. bredanensis* was investigated by determining the size and age range that this species attains sexual and physical maturity. Female rough-toothed dolphins attain sexual maturity by 9 to 10 years of age and males between 5 and 10 years at a similar length of approximately 216 cm. Physical maturity is generally reached at an older age and larger size for both males and females. Ecologically healthy and unhealthy populations of *S. bredanensis* were described in this investigation and these findings will be useful in assessing future threats to this species.

RESUME

Une meilleure connaissance du dauphin à bec étroit, *Steno bredanensis*, semble cruciale pour améliorer l'impact des efforts de conservation et de protection de cette espèce. Le but principal de cette étude est de décrire les paramètres écologiques et biologiques du dauphin *Steno bredanensis*, afin d'avoir dans le futur une meilleure évaluation du niveau de stress de cette population. Plusieurs approches ont été utilisées dans cette étude du dauphin, *S. bredanensis*, incluant des observations en mer, des échantillons prélevés sur animaux décédés et des animaux vivant en captivité. Les populations sauvages étudiées dans un environnement insulaire ont été plus fréquemment observées près des côtes qu'au large. Dans les Iles du Vent en Polynésie française, cette espèce semble se situer préférentiellement dans des eaux présentant une profondeur de 1000 à 2000 mètres et dans une zone distante de 1,8 à 5,5 kilomètres de la barrière de corail. La taille des groupes de dauphins à bec étroit observés en Polynésie française va de 1 à 35 individus avec une moyenne de 12,1 par groupe. Des mesures de taux d'hormones pour *Steno bredanensis* ont été établies à partir de prélèvements faits sur dauphins en bonne santé en captivité et sur dauphins échoués. Les valeurs et leurs moyennes sont données pour la progestérone, la testostérone, le cortisol et les hormones thyroïdiennes. Les variations des taux d'hormones thyroïdiennes sont corrélées avec l'état de santé de l'animal ; la testostérone apparaît généralement basse chez les animaux malades. La physiologie reproductrice des *Steno bredanensis* a été étudiée en déterminant la taille et l'âge moyen à laquelle cette espèce atteint sa maturité sexuelle et morphologique. Les femelles atteignent la maturité sexuelle entre 9 et 10 ans, les mâles entre 5 et 10 ans, leur longueur corporelle ayant alors été évaluée à approximativement 216 cm. La maturité physique est logiquement atteinte plus tardivement, la taille des animaux étant alors supérieure, aussi bien chez les mâles que chez les femelles. La comparaison de

l'écologie des populations de *Steno bredanensis* en bonne ou mauvaise santé, décrite dans cette étude, sera très utile pour évaluer les menaces futures qui pèsent sur cette espèce.

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INTRODUCTION

Background

Marine ecosystems are being impacted by natural and anthropogenic sources on a world-wide scale. These changes are likely to cause stresses to cetacean populations and have the potential to harm individuals and lead to reductions in population sizes. The definition of stress developed in the early 1900's describes the biological response in three stages: 1) alarm and adaptation, 2) hormonal events, and 3) resistance, exhaustion and death, which are independent of the nature of the damaging agent (Neyan, 1998 in St. Aubin and Dierauf, 2001). The adaptation response apparent during this first stage is likely to reflect population level changes. For example, shifts in habitat preference may signify a first sign of impact as the source of known potential stressors all involve the immediate surroundings of these animals. As the biological stress response proceeds to the second and third stage, detectable physiological changes are likely to become evident on an individual level.

In a few of the relatively well-known species of cetacean, such as the bottlenose dolphin, beluga whale, and spinner dolphin, immunological and molecular biology marking techniques are currently being developed to study stress due to anthropogenic factors (Aldridge et al., 2001; De Guise et al., 1998; Romano et al., 1992; Romano et al., 1994; Southern, 2000; St. Aubin and Dierauf, 2001). In these species research is now focusing on defining precise biological perturbations on an individual level. Cetacean work in this area has included the development of specific monoclonal antibodies for a few species (Aldridge et al., 2001; De Guise et al., 1998) and molecular studies initiated

with the bottlenose dolphin are now being extended to beluga whales (Romano et al., 1992; 1999). Recent efforts to identify stress activated proteins by molecular analyses of tissues suggest promise for studying the effects of coastal pollution on individual beluga whales and tuna fishery stresses on individual spinner dolphins (Southern, 2000).

The effects of environmental impacts on wild populations of the cetacean species *Steno bredanensis* are unknown. It is undoubtable that changing marine ecosystems have some impact on dolphin species such as *S. bredanensis*. However, basic information and quantitative data for assessing impacts on either a population or individual level for this species is lacking. Defined distributional patterns for *S. bredanensis* would provide a starting point for investigating population level changes. Means to sense the adaptation of populations and eventually physiological responses to stress on an individual level would be a significant contribution to the future conservation of this dolphin species.

Impacts Affecting Some Small Dolphin Species

Very little is known about the rough-toothed dolphin when compared with other tropical and sub-tropical small dolphins with similar world-wide distributions. In the tropical Pacific ocean, species of the genus *Stenella* have been relatively well documented, and decades of research focusing on this genus provides a very comprehensive overview compared to the *Steno* genus. Most likely the only other species of dolphin where more information is available is the bottlenose dolphin, *T. truncatus*, which is the best known of all cetaceans (Reynolds et al., 2000; Wells and Scott, 1999).

Direct catch of small dolphins is one of several threats to populations throughout the world, and high numbers of take for human food consumption or other products can result in reduced population sizes. Direct catch of rough-toothed dolphins have been reported, but far more information on the extent of this impact is available for other small dolphin species. The tropical and subtropical spinner dolphin, *Stenella longirostris*, appears to inhabit similar waters to *S. bredanensis*. The distribution of *S. longirostris* is primarily pelagic but this species also occurs near islands throughout its range (Klinowska, 1991). Although the primary threat to this species is incidental by-catch in the tuna fishery, some direct catch of spinner dolphins does occur, and reported areas include the Solomon Islands, Japan and the Lesser Antilles (Klinowska, 1991). The pantropical spotted dolphin, *Stenella attenuata* is another small dolphin species found in the same water temperature range as *S. bredanensis*, and in both coastal and off-shore habitats (Klinowska, 1991). Similar to spinner dolphins, pantropical spotted dolphins are considered to be the most threatened by incidental catch but this dolphin is taken in direct fisheries in Japan, the Caribbean, Sri Lanka and the Solomon Islands (Klinowska, 1991). The striped dolphin, *Stenella coeruleoalba* is an example of another species found in a similar distributional range to *S. bredanensis*, but striped dolphins are also found in temperate waters and are more common in the Mediterranean Sea (Leatherwood and Reeves, 1983). A direct Japanese fishery for this species dates back centuries, and in the 1960's annual catches were estimated to be as high as 20,000 animals in this area. Direct fisheries are also present in Papua New Guinea and the Solomon Islands, and striped dolphins were harpooned in the Eastern Atlantic from fishing boats to provide crew with meat (Leatherwood and Reeves, 1983). *T. truncatus*, have a world-wide distribution and

are found in all tropical and temperate environments, absent only from cold waters (Leatherwood and Reeves 1983; Klinowska, 1991; Reynolds et al., 2000; Wells and Scott, 1999). The range of *S. bredanensis* is covered within that of *T. truncatus*, and there is concern regarding the effects of environmental impacts on inshore populations of *T. truncatus* as the nearshore habitat is especially susceptible to disturbances (Klinowska, 1991). Direct catch of bottlenose dolphins also poses a significant threat to this species and has resulted in population declines severe enough to close a Turkish fishery in 1983 (Klinowska, 1991). In the past, direct fisheries for bottlenose dolphin were common in small localized areas and in several countries including Russia, Bulgaria, the USSR, and Japan. Today, Japan and European countries remain the primary areas where bottlenose dolphins are taken to provide meat for human consumption (Klinowska, 1991). A relatively small number of *T. truncatus* are also taken for public display purposes in some parts of the world as this species tends to do well under captive conditions (Klinowska, 1991). However, no significant number of wild caught individuals have been brought into captivity since 1989 in the United States, and animals are not taken for public display from all regions (Andrews, 1999). There are a few selected areas where significant numbers of bottlenose dolphins are still taken for public display, for example hundreds have been removed from Cuban and Mexican waters (Krames, personal comm.).

Direct fisheries for the species *S. bredanensis* have been identified from the West Indies and West Africa, and specimens have been investigated from drive fisheries in Japan and in the Solomon Islands (Klinowska, 1991; Miyazaki, 1980). Numbers taken to supply food for human consumption are small from all areas, but the limited distributional information available for this species indicates that populations to draw

from are not especially large in any region identified to date (Klinowska, 1991). It is unknown what affect these direct fisheries have on the current population status of rough-toothed dolphins and in order to assess these potential effects, increased species knowledge is required (Young et al., 1993). Baseline distributional data for *S. bredanensis* would provide a means to determine aberrant distribution and eventual population decline resulting from potential threats to this species such as excessive direct catch.

Incidental death through fishery interactions poses a significant threat to the future of many dolphin populations. Perhaps the most well known example of this problem comes from large numbers of delphinid by-catch in the Eastern Tropical Pacific (ETP) tuna fishery. Rough-toothed dolphins are sometimes associated with tuna in the ETP and fishermen accounts indicate that this association occurs elsewhere as well (Carwardine et al., 1998; Leatherwood and Reeves, 1983). However, the total number of *S. bredanensis* individuals documented as incidental deaths in the ETP is not nearly as extensive as for species in the *Stenella* genus (Klinowska, 1991; Leatherwood and Reeves, 1983; Wade and Gerrodette, 1993). Tuna fisheries in the ETP annually reported hundreds of thousands of spinner and spotted dolphins as by-catch during the 1980's (Klinowska, 1991). As a result of intense exploitation pressure on spinner dolphins, populations in this area are well studied and information on distributional patterns over a large open ocean region is available. In the deep ETP waters, *S. longirostris* is often sighted in pods numbering in the hundreds or thousands, much greater group sizes than for *S. bredanensis* when found in this region (Perrin et al., 1987; Wade and Gerrodette, 1993). The exploitation of *S. attenuata* in the tuna purse seine fishery has led to detailed study of

this species in the ETP. Pod sizes of the pantropical spotted dolphin range from only a few individuals to thousands, and the smaller pod sizes are similar to that of *S. bredanensis* found in the ETP (Wade and Gerodette, 1993). *S. attenuata* is also incidentally lost in the hook-like fishery off of Japan where this species is believed to be in competition with fishermen and is blamed for interference (Perrin and Hohn, 1994). The striped dolphin experiences some incidental mortality in the ETP tuna fishery as well (Leatherwood and Reeves, 1983). *T. truncatus* is lost incidentally in fishery operations which include gill nets, purse seines, and beach netting for sharks in some regions (Leatherwood and Reeves, 1983; Klinowska, 1991). The bottlenose dolphin also has a reputation of being damaging to fishery profits in some European areas and this species has been known to be killed off of Japan due to apparent competition with fishermen (Leatherwood and Reeves, 1983; Klinowska, 1991).

In the 1970's by-catch information from the ETP indicated that although *S. bredanensis* was taken, numbers were not high when compared to the *Stenella* genus (Perrin and Henderson, 1984). At least some rough-toothed dolphins were killed in the purse-seine industry (Miyazaki and Perrin, 1994), and assessment of the dead specimen collection at the Smithsonian Institution in 2000 indicates a significant contribution of specimens from incidental catch in the Pacific during the 1980's and 1990's (personal observation). Tuna fishery interactions likely affect rough-toothed dolphin populations in other regions in addition to the ETP. In the early 1990's, although the total incidental take of *S. bredanensis* for the Indo-Pacific was not reported, the effect on the Indo-Pacific population of rough-toothed dolphins is believed to be insignificant (Young et al., 1993). No estimate of incidental catch is available for the Atlantic, and the potential effect on the

S. bredanensis population in the Western North Atlantic and Gulf of Mexico is considered unknown (Young et al., 1993). In the Indian ocean, a small number of rough-toothed dolphins are also lost in gillnets in Sri Lanka (Klinowska, 1991). On a world-wide scale, loss of this species in pelagic driftnets is unknown (Klinowska, 1991).

Environmental change can result in distributional shifts of small cetacean populations. For the species *S. bredanensis*, very limited distributional information is available, and not enough is currently known to assess potential changes in distribution due to impact effects. Oceanographic conditions during El Nino years indicate changing distributional patterns of spotted dolphins as well as other species of small cetaceans (Fielder and Reilly, 1994; Reilly and Fielder, 1994). Since the 1982/83 El Nino event, a northward range extension of *T. truncatus* has also been apparent off of the coast of California (Klinowska, 1991; Wells, 1990). The effect of either natural or unnatural environmental variations on *S. bredanensis* populations have not been previously investigated.

Habitat degradation is recognized as yet another example of a potential threat to small cetacean populations. The near-shore habitat is especially vulnerable to anthropogenic effects such as human encroachment, pollution and other disturbances. For spinner dolphin populations using near-shore waters as a resting area, human disturbances within the preferred habitat are believed to have resulted in distributional pattern shifts as the population adapts to the disturbance (Klinowska, 1991; Perrin, 1989). Changes in the distribution of bottlenose dolphin populations off of both the East and West coasts of the USA have been attributed to pollution, pollution linked decline in food availability, and increased boat traffic as likely causes (Klinowska, 1991). For the

species *S. bredanensis* it would be useful to establish expected distributional patterns in near-shore environments for future assessment of these types of threats to populations which are related to quality of habitat.

Impact on Reproductive Parameters

Extreme exploitation of dolphins can result in severe population stress when numbers experience great declines. A density dependent response may occur on a population level, where reproductive parameters of individuals shift in an attempt at compensation. This phenomenon provides another example of the biological stress response, and in this case the reproductive physiology is altered on an individual basis as part of the population adaptational phase. Age of sexual maturity attainment is an especially important reproductive parameter as it is believed to vary in individuals according to the degree of population exploitation (Perrin and Reilly, 1984; Smith, 1983). Other reproductive measures may also vary accordingly. Pressures due to low population numbers would be expected to result in younger age and smaller size of sexual maturity attainment in individuals, a higher rate of pregnancy, and shorter lactations (Perrin and Reilly, 1984).

Exploitation effects on reproductive parameters in populations of small delphinids have been investigated in *Stenella* populations from the ETP and Western Tropical Pacific (WTP) where heavy exploitation has occurred. Tuna fisheries in the eastern tropical pacific (ETP) annually reported hundreds of thousands of spinner and spotted dolphins as by-catch during the 1980's (Klinowska, 1991). Over a twenty year period, the spinner dolphin population in the ETP region was estimated to have been reduced to

only 20% of pre-exploitation abundances (Klinowska, 1991). This extreme exploitation is evident in the reproductive parameters of ETP spinner dolphins, believed to be responsive to the loss of huge numbers from this population (Perrin and Henderson, 1984). Attainment of sexual maturity in the ETP spinner dolphins were compared with an unexploited population in the Gulf of Mexico where both males and females reach sexual maturity at a longer and heavier size (Perrin and Henderson, 1984). Female spinner dolphins in the eastern tropical pacific reach sexual maturity at an average of 5.5 growth layer groups (GLG's) and at an average of 7-10 GLG's in the Gulf of Mexico (Klinowska, 1991; Perrin and Henderson, 1984). Striped dolphins off of the coast of Japan have been taken in high numbers as direct catch for centuries, and tens of thousands have been estimated as kill in this region on an annual basis (Leatherwood and Reeves, 1983). A shorter calving interval, a decrease in both the length of lactation and in the resting period were apparent in the exploited Japanese striped dolphins (Kasuya, 1984). Over 3000 individual striped dolphins were investigated in one of these studies, and over 4000 spinner dolphins in another (Miyazaki, 1984; Perrin and Henderson, 1984).

When thousands of individuals are available for reproductive studies and the level of exploitation is known, it is possible to compare reproductive parameters among populations of the same dolphin species. For the species *S. bredanensis*, reproductive information is lacking and it is not even known for any region at what age and size range these animals attain sexual maturity. A baseline to assess reproductive parameter shifts resulting from exploitation pressures into the future is needed for the species *S. bredanensis*.

Impact Effects on Hormonal Events

Adversely impacted cetacean populations are likely to reflect biological and physiological changes at the individual level. Hormonal events represent stage two of the biological stress response, directly following the alarm and adaptation response of populations. Hormonal change in relation to stressful events can be investigated by obtaining serum samples from individual dolphins to determine the circulating hormone concentrations (Hadley, 1994; St. Aubin, 2001). In order to be able to detect hormonal changes signifying that a species is experiencing significant stress, baseline endocrinology data from healthy animals is first required for comparative analyses.

Cortisol is a primary glucocorticoid secreted by the adrenal gland, and is often considered as a stress hormone. Glucocorticoids affect the metabolism of glucose, protein, and fat and the speed of these processes increase when the body is under stress. In the bottlenose dolphin, cortisol concentrations have been found to rise to more than three times baseline when the animal undergoes capture and handling stress (Thompson and Geraci, 1986). Thyroid hormone concentrations are likely to be affected by various factors in cetaceans, and the stress of capture and release in bottlenose dolphins and beluga whales has resulted in decreased thyroid hormonal concentrations (St. Aubin and Geraci, 1988; St. Aubin and Geraci, 1992; Schroeder and Keller, 1989). In beluga whales, studies of the stress response to capture and handling identified an interplay between changes in both cortisol and thyroid hormones and this has been suggested to define part of the biological mechanisms associated with stress for this species (St. Aubin and Geraci, 1992).

Hormonal concentrations are also effected by environmental surroundings and the topic of endocrine disruption has recently received a great deal of attention. Pollution levels are believed to be reflected in thyroid hormone values in marine mammals (St. Aubin, 2001). In the gray seal thyroid hormones act as biomarkers and in the common seal a thyroid deficiency has been found to be associated with environmental contamination, specifically polychlorinated biphenyls (PCB's) (Brouwer et al., 1989; Hall et al., 1998). Evaluation of thyroid glands from dead harbor seals in the North Sea are thought to be morphologically defective due to pollution in this area (Schumacher et al., 1993). Abnormal histological findings from beluga whale thyroid glands also correlate with high organochlorine contamination in the St. Lawrence estuary (De Guise et al., 1994). To be able to accurately assess the effects of environmental stressors such as contaminants on hormonal events, baseline concentrations from healthy populations on a species specific basis are first needed. No published values for any hormone are available for the rough-toothed dolphin, *S. bredanensis*.

Limited information for Steno bredanensis

Although it becomes clear that unprecedented change on marine ecosystems will ultimately affect free-ranging cetacean populations, information is lacking to assess the effects of environmental impacts on some species. In the relatively unknown rough-toothed dolphin, no immunological or molecular research has been undertaken, and there remains a need to obtain baseline information to aid in determining the potential impact of environmental changes on wild populations of this species. There is so little known about *S. bredanensis* that it is indeterminate whether this species is even endangered or

threatened or experiencing significant population stresses (Klinowska, 1991). For *S. bredanensis* there are no distributional or preferred habitat usage studies conducted to date in near-shore waters. There is almost no knowledge on reproductive parameters in this species, nor are any hormonal values available for *S. bredanensis*. This information is necessary to provide a base to aid in assessing large-scale population changes and individual perturbations that may be caused by environmental impacts. The rough-toothed dolphin is a difficult to access species, and opportunistic studies of both controlled confined populations and natural wild populations are needed to obtain this type of information.

History of Steno bredanensis

The species *S. bredanensis* was first identified from skulls in the Paris museum and originally named *Le Dauphin a bec mince* which translates to the dolphin with a thin beak. In the early 1800's this species was classified as *Delphinus frontatus* along with several other types of small dolphins (Cuvier, 1817). In 1823, an important addition was published indicating that this previously identified animal was in fact a new species (Cuvier, 1823). Lesson (1828) lists this species with a new name, *Delphinus bredanensis* and this is the first indication of a name change from *frontatus* in historical literature. This species was included in the broader *Delphinus* genus until Gray (1846) divided *Delphinus* and established the *Steno* genus. According to Gray (1846) the *Steno* genus included: The Steno, *Steno rostratus*, The Malay Dolphin, *Steno malayanus*, the Fronted Dolphin, *Steno frontatus*, the Compressed-Beaked Dolphin, *Steno compressus*, the Attenuated-Beaked Dolphin, *Steno attenuatus*, and the Cuban Steno, *Steno fuscus*. This

genus was originally named after a Danish naturalist and anatomist, Dr. Nikolas Steno (Gotch, 1995). In 1934 *Steno rostratus* was being used for this species, incorporating both the original name and the new *Steno* genus (Richards, 1934). In 1955 Muller and Kellog introduced the scientific name *Steno bredanensis* and Fraser and Purves (1960) classified the family Stenidae which included the *Steno*, *Sousa* and *Sotalia* genera. Today the rough-toothed dolphin, *S. bredanensis*, is classified in the Subfamily Stenidae of the Family Delphinidae. *S. bredanensis* is the only species in the *Steno* genus. The common name for *S. bredanensis*, the rough-toothed dolphin, is associated with wrinkled enamel found on the teeth of this species (Harrison and Bryden, 1988). *S. bredanensis* skulls are part of museum collections in many locations including Marseille, Florence, London, Washington D.C. and Honolulu.

Status of Wild Populations

The status of the species *S. bredanensis* is described as insufficiently known, and there is no estimate of worldwide population numbers (Klinowska, 1991; Leatherwood and Reeves, 1983). Only one true abundance estimate is available from extensive cetacean surveys in the eastern tropical Pacific. In this region, approximately 150,000 rough-toothed dolphins are present, with confidence limits ranging between 100,000 and 250,000 individuals (Wade and Gerrodette, 1993). A summary of the population status of *S. bredanensis* in other areas, including the North Pacific, Western North Atlantic, and Gulf of Mexico states that populations are unknown (Young et al., 1993).

S. bredanensis is described as a widely distributed species throughout warm waters of the world's oceans. Although isolated sightings of rough-toothed dolphins have

been reported from several regions, distribution of this species is centered in tropical and sub-tropical areas. Live, free-ranging animals are believed to generally inhabit seas with a water temperature greater than 25°C (Klinowska, 1991; Leatherwood et al., 1982; Leatherwood and Reeves, 1983; Miyazaki and Perrin, 1994). However, in the Canary Islands rough-toothed dolphins are quite commonly sighted where summer temperatures only range between 22 and 24°C and reach as low as 17-19°C in the winter (Ritter, 2002). Other than the presence of this species around the Canary Islands, a range extension has typically been described when sighted in cooler waters (Miyazaki and Perrin, 1994). For example, a few stranded rough-toothed dolphins have been reported off the Washington and Oregon coasts in the North Pacific, and a range extension documented in the South Atlantic off Brazil (Ferrero et al., 1994; Ott and Danielwitz, 1996). The rough-toothed dolphin is found throughout the eastern tropical Pacific, although at low densities, as indicated from cetacean survey work in this area (Au and Perryman, 1985; Hewitt, 1985; Wade and Gerrodette, 1993). *S. bredanensis* is also present around at least some island groups in the Pacific, such as Hawaii (Mazucca et al., 1999; Miyazaki and Perrin, 1994; Mobley et al., 2000). Leatherwood et al. (1982) may have been the first authors to recognize and draw attention to the presence of *S. bredanensis* specifically in Polynesia. Study by Gannier and Gannier (1998) has further indicated that French Polynesia represents an area where *S. bredanensis* is found in relatively high concentration compared to other cetacean species. Surveys of cetaceans in this region have ranked *S. bredanensis* as the second most common species to be sighted in these waters (Gannier, 2000).

Strandings of Steno bredanensis

There are certainly records of both mass and individual rough-toothed dolphin strandings from several areas (Chantrapornsyl et al., 1996; Ferrero et al., 1994; Gaspar et al., 2000; Mazucca et al., 1999; Miyazaki, 1989; Parsons, 1998; Smith and Whitehead, 1999; Stolen et al., 1999). However, this cetacean species is usually described as one that only occasionally strands, or where there are relatively few records of *S. bredanensis* strandings compared to that of other cetacean species (Ellis, 1982; Leatherwood and Reeves, 1983). It has been suggested that this may be due to the pelagic nature of rough-toothed dolphins, as off-shore species are less likely to be found washed ashore (Baker, 1983; Bruyus, 1971). Mass strandings of *S. bredanensis* have occurred, primarily in tropical or sub-tropical regions which is in agreement with the world-wide distributional information that is available for this species. Several events have been documented from the Florida area, dating back to 1965, and specimens investigated from three separate events during the 1990's (Stolen et al., 1999). In 1976, two mass strandings of rough-toothed dolphins occurred, one off of Hawaii and the other in Virginia. Rough-toothed dolphin skulls also indicate a smaller-scale event that either occurred in the Indonesian archipelago or from Senegal at some point in the past (Rudolph et al., 1997). From more temperate regions, generally only a single individual or very few animals have been documented to strand. For example, from the Mediterranean Sea, skulls are included in museum collections in France, England, and Italy (note by Schevill cited in Watkins et al., 1987). Along the Washington and Oregon coast, Balcomb (1980) reported a single individual found in Washington, and Ferrero et al. (1994) described three separate animals found in this area. Other regions where individual strandings have been known

to occur include Brazil, the Galapagos, Hong Kong, and Thailand (Chantrapornsyl et al., 1996; Hetzel and Lodi, 1998; Palacios, 1995; Parsons, 1998; Smith and Whitehead, 1999). From French Polynesia, there are accounts of rough-toothed dolphin strandings from the Windward Islands (Gaspar et al., 2000).

Biology of Steno bredanensis

The physical appearance of *S. bredanensis* is distinct from all other cetacean species. The rough-toothed dolphin has a long and slender beak, a unique head shape where the beak blends with the forehead and often the white coloration is apparent on the tip of the beak (Ellis, 1982; Leatherwood et al., 1976). This species tends to have large eyes, a dark gray coloration, and a high number of scars, marks and lighter colored splotches. External markings appear to increase with age (Leatherwood et al., 1982). Adult *S. bredanensis* range from approximately 210 to 240 cm in length, and weigh around 100 kg (Leatherwood et al., 1976). The *Steno* has a lean body shape compared with other cetaceans of a similar size. Previous studies focusing on the physiology of *S. bredanensis* include documentation of internal body temperature, where the core temperature of the rough-toothed dolphin was found to be lower than that of the spinner dolphin (Whittow et al., 1978).

In terms of the reproductive biology of *S. bredanensis*, very few published reports are available. This presents a clear lack of necessary information for effective species conservation efforts, as definition of reproductive parameters is required for population modeling. The need for knowledge on reproductive biology has been emphasized as paramount for conservation measures aimed at the rough-toothed dolphin (Klinowska,

1991). Only one study has addressed reproduction in *S. bredanensis* to date. Miyazaki (1980) examined reproductive organs and aged adult specimens caught in a Japanese drive fishery. His findings indicate that at 14 GLG's and a length of 225 cm males are sexually mature, and females are mature at an estimated age of 17 years based on GLG's with a length of 225 cm. However, no immature specimens were aged, and even basic information such as what age or size range the rough-toothed dolphin attains sexual maturity is not yet known for this species. Miyazaki's work (1980) suggested that *S. bredanensis* may undergo several simultaneous ovulations by examination of corpora as described by Perrin and Donovan (1984) for cetaceans. However, no investigations have yet been made into reproductive seasonality, reproductive rate, or any other specific studies on reproduction in this species. Natural history information is also somewhat limited, as Miyazaki (1980) examined only adults, no growth curve from Japan is available. Stolen et al. (1999) aged rough-toothed dolphins from 3 stranding events in Florida, and these authors were able to generate preliminary growth curves for both males and females, indicating that males and females have attained maximum growth at 238 cm for males and 232 cm for females, both around 15 years of age. Unfortunately, no reproductive information is available to accompany these aged individuals.

Behavior of Captive Steno bredanensis

Most observations and study of rough-toothed dolphin behavior comes from animals held under captive conditions. During the 1960's and 1970's, 22 animals were captured in Hawaiian waters. These animals were on exhibit and trained at sea, some of which left during training sessions or were purposely released (Norris, 1965). In the

1970's and 1980's, Japan caught rough-toothed dolphins to be housed in oceanaria, and of these, 10 animals were measured for body length and weight during this time (Miyazaki, 1980). Rough-toothed dolphins have also been taken and maintained in captivity in Europe in the past, but not recently (Miyazaki and Perrin, 1994). In the 1990's, *S. bredanensis* was maintained in captive conditions in the lagoonal waters of French Polynesia where biological samples were collected from these individuals on a regular basis. In the late 1990's stranded animals were rehabilitated in the United States at Mote Marine Laboratory and many serum samples collected. Currently, only one rough-toothed dolphin is known to be held in captivity in the United States. Although the number of rough-toothed dolphins that have been held under captive conditions throughout history is very small compared to that of bottlenose dolphins, the unique behavior and advanced training skill of *S. bredanensis* has been well documented. The first training experiences with this species date back to the 1960's in Hawaii, and there are several accounts of surprising, spontaneous and creative behaviors, as well as contrasts provided between the rough-toothed dolphin and the more docile bottlenose dolphin (Norris, 1974; Pryor, 1975). The ability of this species to create its own novel behavior without traditional shaping methods led to its description as an intelligent, investigative animal that can become agitated at trainer mistakes and has the ability to manipulate its captive environment (Norris, 1974; Pryor, 1975). There have been a few captive situations where *S. bredanensis* has been housed with the bottlenose dolphin *Tursiops truncatus*. At Sea Life Park, the only known hybrid between *S. bredanensis* and *T. truncatus* was born healthy, and this animal lived until almost 5 years of age (Dohl et al., 1974; Pryor, 1975). Recently in French Polynesia, a stranded *S. bredanensis* calf was

documented to induce lactation in an adult female *T. Truncatus* that demonstrated adoptive behavior to this young calf (Gaspar et al., 2000). Notable behavioral characteristics in *S. bredanensis* do not appear to be limited to the captive environment. In the wild, care giving behavior among rough-toothed dolphins was observed in Brazil, where an adult continued to provide care for a young dead animal (Lodi, 1992). Intelligence and the ability to manipulate their environment also appears to be the case for free-ranging rough-toothed dolphins in Hawaii, where several attempts by National Marine Fisheries Service to solve the problem of bait stealing by this species have been unsuccessful (Schlais, 1984).

Statement of Purpose

Exploitation and environmental stressors pose potential threats to the future of *S. bredanensis* populations. This study on the rough-toothed dolphin was conducted to establish basic parameters associated with the biology and ecology of the species. The goal in accumulating this data was to provide species knowledge to aid in future population assessments and ultimately contribute to conservation of rough-toothed dolphins throughout the world. Several approaches are needed to define population and individual level characteristics which represent the current status of *S. bredanensis*. Once modalities are employed and baseline data becomes available on the ecology and biology of *S. bredanensis*, a starting point will be provided for comparing the population status of this species into the future. Regions of preferred habitat need to be identified for *S. bredanensis*, and surveys of free-ranging rough-toothed dolphin distributional patterns around oceanic islands were undertaken as part of this study. There is no evidence that rough-toothed dolphins in French Polynesia are currently under exploitation stress and this island archipelago was used to describe the distribution and group size of an ecologically healthy population of this species. Life-history parameters were investigated by examination of dead specimens to determine the age and size range that *S. bredanensis* attains sexual and physical maturity. *Steno* populations experiencing extreme exploitation stress have yet to be identified, and any measure of sexual maturity attainment is needed for determining impact effects. This information can then be utilized to assess population response to large scale pressures facing this species in times of rapid change to marine ecosystems. Opportunistic biological sampling was also conducted from seven individual rough-toothed dolphins in captive conditions to

investigate measures of stress and reproductive state in live animals. Some of these animals had stranded and were in ill health, while others served as controls for comparison of endocrinology data according to stress levels of individuals.

The overall goal of this study on the species *S. bredanensis* was to address the following questions: What are the biological and ecological characteristics of current free-ranging *Steno* populations believed to represent ecologically healthy animals ? How do physiological parameters compare between healthy rough-toothed dolphins and individuals experiencing stress? This research will provide basic tools to assess the health status of individuals and populations into the future. It will also provide scientific data to aid in determining if and when suspected environmental threats are harming wild *S. bredanensis* populations, allowing for proactive responses by wildlife managers to ameliorate the environmental stressors.

CHAPTER 1

Distribution of the rough-toothed dolphin (*Steno bredanensis*) around the Windward Islands (French Polynesia)

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ABSTRACT

The rough-toothed dolphin (*Steno bredanensis*) has been described as a primarily pelagic cetacean species, found in most oceans of the world. Little information is available on distribution patterns or habitat preference for this species from any region. This study reports on the distribution of *S. bredanensis* around the Windward Islands of French Polynesia. Data was obtained from vessel surveys between 1996 and 2000 where rough-toothed dolphins were sighted 38 times. Group sizes of rough-toothed dolphins ranged between 1 and 35 individuals with an average size of 12.1 individuals. When corrected for effort, results indicated that in French Polynesia, *S. bredanensis* is found over a wide area, but is more commonly distributed in-shore than offshore. Rough-toothed dolphins were most commonly sighted 1.8 to 5.5 km from the barrier reef, in water depths between 1000 and 2000 m. Our results also demonstrate the year-round presence of this species around Tahiti and Moorea. *S. bredanensis* has been reported in many oceanic archipelagos, and our findings may provide insight into preferred habitat and small scale oceanographic conditions associated with regions where this cetacean species is relatively abundant.

INTRODUCTION

Information on rough-toothed dolphin (*Steno bredanensis*) distributions are limited, and published reports which include this species are quite widespread. *S. bredanensis* is rarely the focus of field studies, but is often just mentioned from a few sightings. Present in almost all tropical and subtropical regions, sighting data suggests that *S. bredanensis* is not a particularly common species in any area (Au and Perryman, 1985; Hewitt, 1985; Mullin et al., 1994; Wade and Gerrodette, 1993;).

Previous reports on the distribution of rough-toothed dolphins are primarily from the eastern tropical Pacific, covering very large regions of open ocean waters. These studies have extended over 50 degrees of latitude, and in this open ocean environment, a large scale distribution map for *S. bredanensis* has been generated from approximately 40 sightings (Wade and Gerrodette, 1993). Other large scale surveys in the eastern tropical Pacific support the presence of *S. bredanensis* in relatively low abundances compared to other delphinid species in this area (Au and Perryman, 1985; Hewitt, 1985). Cetacean surveys covering large areas of the western tropical Indian Ocean have also mapped *S. bredanensis* sightings, where the percentage of cetacean abundance represented by this species appear similar to that of the eastern tropical Pacific and to the Gulf of Mexico where this species is also present (Ballance and Pitman, 1998). An aerial cetacean survey in the Gulf of Mexico documented only a single *S. bredanensis* sighting, but in a water depth very similar to the inshore areas of French Polynesian islands (~1000 m) (Gannier, 2000; Mullin et al., 1994).

S. bredanensis may also frequent coastal areas. Based on stranded animals and a few documented sightings, it appears probable that rough-toothed dolphins inhabit a

shallow, lagoon environment in Brazil (André et al., 1997; Ott and Danilewicz, 1996). Opportunistic sightings, as well as specimens from drive fisheries and strandings are available to indicate its presence, at least occasionally, in some areas such as the Mediterranean, off of the Oregon and Washington coasts (USA), and in Japan (Ferrero, 1994; Miyazaki, 1980; Watkins et al., 1987).

Cetacean surveys near island environments often indicate the presence of rough-toothed dolphins. On a similar scale to that of French Polynesia, *S. bredanensis* has been sighted during aerial surveys in Hawaii, but is one of the least common cetacean species found in this archipelago, and is rarely sighted within 5 miles of shore (Mobley et al., 2000). Dedicated or opportunistic surveys have indicated frequent sightings of *S. bredanensis* off the Canary Islands (Martin et al., 1998). Rough-toothed dolphins have also been sighted in the Indonesian Archipelago, although not frequently (Rudolph et al., 1997; Rudolph, 1998). From other archipelagos such as the Philippines, Galapagos, and the Solomon Islands, rough-toothed dolphins have not been sighted during cetacean surveys, although there is report of *S. bredanensis* as a species involved in Solomon Island drive fisheries and a stranded specimen has been recovered from the Galapagos (Dolar et al., 1997; Smith and Whitehead, 1999; Rudolph et al., 1997). Stranded specimens have also been recorded off of Hong Kong and in Thailand (Chantrapornsyl et al., 1996; Parsons, 1998). This suggests that rough-toothed dolphins are present around several sub-tropical/tropical archipelagos, but generally in low abundances.

Although *S. bredanensis* distributions have been assessed over the wide eastern tropical Pacific, which may allow for insight into preferred habitat associated with large scale oceanographic conditions, smaller scale studies on the distribution of this species

have yet to be undertaken. In French Polynesia, cetacean surveys have indicated that *S. bredanensis* is the second most commonly observed species after *Stenella longirostris* (Gannier, 2000). This provides an opportunity to determine the habitat preference of this species around small oceanic islands. Based on multiple sightings of rough-toothed dolphins, and on sampling effort spread over 4 years (of which 2 years the second author participated), this study reports on seasonal and temporal variation in distribution around the islands of Tahiti and Moorea.

METHODS

Area of study

The study area focused on Tahiti and Moorea, which are the main Windward Islands (Society Archipelago), located in the central tropical Pacific at a latitude of 17°S and a longitude of 150°W (Figure 1). Both inshore and off-shore areas were surveyed. Due to the 12°-18° slope of these volcanic islands, the water rapidly reaches depths in excess of 2000 m, within the inshore area. Although large scale primary production does not occur in the southern central tropical Pacific (Longhurst, 1999), passes may provide nutrients in the photic zone, due to the outflow of lagoon and river water. Edge effects may cause local eddy-induced vertical mixing, as shown around several tropical oceanic islands (Aristegui et al., 1997). The offshore area lies beyond 10 km of the barrier reef, with waters generally deeper than 3000 m. Its hydrobiology is dominated by oligotrophy and a very deep (200-300 m) and stable thermocline. However, the sea surface temperature features a significant seasonal change, with lower values of 25-26°C in August-September and higher temperatures of 29-30°C in February-April. These normal

seasonal temperatures may shift during an El Nino Southern Oscillation event, which refers to the warm phase of a naturally occurring sea surface temperature oscillation in the tropical Pacific.

The study area was sub-divided into 4 regions to investigate distribution of rough-toothed dolphins (Figure 2). The first region is the channel located between the islands of Tahiti and Moorea, the second covered the rest of Moorea up to 10 kilometers from shore which is essentially a leeward area. The third region comprised all areas off Tahiti, excluding the channel, mostly with a windward exposure, and extended to 10 km offshore. The last region included offshore waters, beyond 10 km of shore and extended to the small island of Maiao, west of Moorea (Figure 2).

A series of 11 surveys were conducted between March 1996 and May 2000 using a 12 m sailboat. Sampling took place when wind speed was less than or equal to Beaufort 4, primarily using diesel propulsion at an average speed of 9.5 km.h⁻¹. Both inshore and offshore strata were sampled, and zig-zag tracks were conducted around the islands whenever possible. A GPS system and an auto-steering device were used for positioning and navigation. During the surveys, two or three observers stood on the deck, about 2 meters above sea level and searched the side and frontal sectors. Position was recorded on average every 3 km, as well as wind, sea state and relative cloud cover. When cetaceans were sighted, a GPS position was immediately recorded and radial distance and bearing were estimated. Schools of rough-toothed dolphins were systematically approached to estimate school size and composition, and for recording of pod activity.

Data processing

Data obtained from sampling was entered into a database (*Dbase IV*), and made compatible with *Oedipe* ®Ifremer Geographic Information System (Massé and Cadiou, 1994). GIS was used for mapping, data post-stratification and effort calculations. Accurate bottom depth and distance to the barrier reef for each sighting were obtained from nautical charts issued by SHOM, the French Navy Hydrographic Service.

Numerous parameters may cause dolphin detectability to vary (Hiby and Hammond, 1989). However, for a given survey protocol (i.e. same platform and same number of observers) and a given species, the assumption of constant detectability holds if sighting conditions are constant. Sighting conditions may vary with the wind speed, the swell height and the luminosity. Because wind in excess of Beaufort 3 may have adverse effects on the detection of small cetaceans (Buckland et al., 1993; Hiby and Hammond, 1989), only survey effort in conditions between Beaufort 0 to 3 were used to estimate sighting rates.

As our survey effort was not homogeneous across the different strata (regions, periods, areas) distribution had to be effort-corrected. Hence a mean sighting rate (y_p) was computed, relating to the number of rough-toothed dolphins seen per kilometer. For estimating y_p , we retained the same sampling unit as that used for data recording and computed a sighting rate y_i for each sample,

$$y_i = n_i \cdot S_i / l_i$$

where n_i is the number of rough-toothed dolphin schools detected during the sample (in fact $n = 0$ or $n = 1$), S_i is the school size and l_i is the sample length.

Our data set included many 0 values due to the short sample length (2-5 km) used, and

the arithmetic mean was not adequate for estimating sighting rates. A Pennington estimator (Pennington and Berrien, 1984) was used instead to compute average sighting rates. This estimator is based on the log-normal distribution of the non-zero values of a serie of data, referred as delta distribution by Aitchison and Brown (1957). This estimator produces unbiased estimates of mean and variance when the data set includes many zero values, unlike the arithmetic estimator. For a distribution of n samples with m non-zero values ($m > 1$), with y_m the sample mean of the non-zero \log_e values and s^2 a sample variance of the \log_e values, Aitchison and Brown (1957) give the estimate of the mean, y_p :

$$y_p = m/n \cdot \exp(y_m) \cdot G_m(s^2/2)$$

Pennington (1983) gives the estimator of the variance, $\text{var}(y_p)$:

$$\text{var}(y_p) = m/n \cdot \exp(2 \cdot y_m) \cdot [m/n \cdot G_m(s^2/2) - (m-1/n-1) \cdot G_m((m-2/n-1) \cdot s^2)]$$

where $G_m(x)$ is defined by:

$$G_m(x) = 1 + (m-1/n) \cdot x + \sum_{j=2, \infty} [(m-1)^{2j-1} \cdot x^j] / [m^j (m+1)(m+3) \dots (m+2j-3) \cdot j]$$

These estimates were obtained by processing data with *Passtec* software (Ibanez and Etienne, 1994). T-tests were then used to compare the mean y_p estimates.

The seasonal variation of the rough-toothed dolphins sighting rate was investigated by processing the inshore data a second time: the data was split into three temporal strata, by considering a cold water season for data collected from September to November, an intermediate season from December to February and a warm water season for data obtained from March to May. Seasonal sighting rates were estimated with the same methodology as above. As our sampling effort was greater in Region 1 and 2, which represented the channel region and Moorea respectively, only these two areas were

considered for analyses of seasonal changes.

RESULTS

Effort

Our total survey effort over the entire study area was 6458 km (Table 1). An effective effort of 3441 km was conducted in the channel area, 1706 km around Moorea, 758 km around Tahiti, and 553 km in the off-shore region. For each region, effort was further expressed according to Beaufort conditions experienced during the survey (Table 1). In Region 1, 2 and 3 (the channel area, Moorea and Tahiti respectively), effort in excellent sighting conditions (Beaufort 0-1) represented 12.5-15% of the total effective effort, effort in very good sighting conditions (Beaufort 2) represented 32-40%, and effort in good sighting conditions (Beaufort 3) represented 45-53%. Hence, average sighting conditions were similar except for the offshore area, where Beaufort 3 conditions were more prominent, comprising 64.5% of the effective effort. Three different time periods during the year were investigated for seasonal effects in Regions 1 and 2: an effective effort of 2157 km of effort between September and November (Figure 3a), 559 km was obtained between December and February (Figure 3b), and 2475 km during March to May (Figure 3c).

Sightings and Group Size

The rough-toothed dolphin was observed 38 times over the study period. Smaller school sizes of *S.bredanensis* occur more commonly than larger group sizes (Figure 4), average school size was 12.1 (SE=10.2) individuals with a range between 1 and 35

individuals. Out of our 38 total sightings, 29% of rough-toothed dolphin groups had at least one calf present where a calf was defined as an animal that appears to be either newly born or still maternally dependent. Rough-toothed dolphin school size was found to be homogeneous across all four sub-regions. In the channel region (15 sightings) the average group size was 13.5 individuals (SE=12.2), off Moorea (11 sightings) the average group size was 12.8 (SE=10.9), off Tahiti, *S. bredanensis* was sighted on 9 occasions with an average group size of 8.3 individuals (SE=3.0) and in the off-shore region, the species was observed 3 times with an average group size of 14.0 (SE=12.5). In the Windward Islands, the rough-toothed dolphin was sometimes found in mixed schools, once associated with bottlenose dolphins, and twice with melon-headed and Fraser's dolphins. *S. bredanensis* was also observed interacting with humpback whales on two occasions. Although both the spinner dolphin and pilot whale are commonly found in French Polynesia (Gannier, 2000), rough-toothed dolphins were never seen associated with either of these species during our surveys.

Distribution

S. bredanensis was sometimes observed less than 100 meters from reef barrier, and occasionally more than 30 kilometers offshore, indicating a wide distribution range. When considering the entire study area, the total distributions corrected for effort indicate that *S. bredanensis* are found at a rate of 0.124 individuals/km. Inshore regions (sub-regions 1 to 3) indicated a higher number of *S. bredanensis* than the offshore area (sub-region 4), with 0.238 and 0.079 ind./km respectively (Table 2). However, sample size in the offshore region was small and this difference was not significant (T-test, $p>0.10$).

Effort-corrected distribution of rough-toothed dolphins was considered according to distance from the barrier reef, bottom depth, and different sub-regions sampled (Table 2). Overall, *S. bredanensis* was more frequently sighted 1.8-5.5 km off-shore, with 0.42 ind./km ($n=19$; SE = 0.17), than in the 0-1.8 km area, with 0.07 ind./km ($n=8$; SE = 0.03). This difference was statistically significant (T-test, $p<0.001$). Likewise, rough-toothed dolphins were more frequently sighted in depths between 1000 and 2000 m, with 0.19 ind./km ($n=13$; SE = 0.08), than in the 0-1000 m stratum, with 0.17 ind./km ($n=18$; SE = 0.06), although this difference was not significant (T-test, $p>0.10$).

Effort-corrected distributions were very similar off Tahiti and Moorea (sub-regions 3 and 2), with respective values of 0.33 ind./km (SE = 0.13), and 0.27 ind./km (SE = 0.13), and somewhat lower in the channel region (sub-region 1) with a figure of 0.19 ind./km (Table 2). Pairwise statistical comparison indicated that only Tahiti and the channel region were significantly different (T-test, $p<0.05$).

In summary, rough-toothed dolphins were found over an extensive range, but most commonly observed in inshore waters 1.8 to 5.5 km off the barrier reef, in a depth range of 1000 to 2000 m, and seemed to be evenly distributed around the Windward Islands.

Seasonal variation

Differences in distribution were apparent between the colder water season (September to November), medium water temperature range (December to February) and the warmer water season (March to May). During the cold water season (September to November), a sighting rate of only 0.10 ind./km ($n=8$; SE = 0.05) was obtained, much lower than for the two other periods. Between December to February the sighting rate

was 0.21 ind./km ($n=5$; $SE = 0.14$), close to the figure obtained for the warm season (March until May), with a sighting rate of 0.15 ($n=11$; $SE = 0.08$). However, the cold season estimate was probably influenced by an uneven effort distribution in that period (September-November), when sampling favored inshore waters as surveys were focused on humpback whales (Figure 3).

DISCUSSION

Our distribution results in the Windward Islands indicated that rough-toothed dolphins exhibit a specific habitat preference in French Polynesian waters. *S. bredanensis* is most frequently found in water depths between 1000 and 2000 m, approximately 1.8 to 5.5 km from shore. These results do not demonstrate a purely pelagic habitat usage in this region, in contrast to many general reports describing *S. bredanensis* as only a deep-water species (Leatherwood et al. 1982; Leatherwood and Reeves, 1983; Miyazaki and Perrin, 1994). In Hawaii *S. bredanensis* is apparently found farther offshore (Mobley, 2000) sometimes in water more than 20 miles offshore, but where depths are similar to those found closer inshore in French Polynesia (Schlais, 1984). However, water depth alone was not found to be a significant factor affecting sighting rates in our study (Table 2), despite a probable influence on prey availability. It is not known which specific species of cephalopods and fish *S. bredanensis* in French Polynesia feed upon, however during our study rough-toothed dolphins were observed feeding on epipelagic fishes (including flying fishes) during the day. In other regions of the world, the inferred diet of this species has been described from stomach contents, which have usually been classified as off-shore or pelagic species of fish, octopus, and squid (Leatherwood et al., 1982 ; Leatherwood and

Reeves, 1983). Inshore prey species have been suggested from stomach contents of stranded individuals in Hawaii, although an offshore distribution of *S. bredanensis* is apparent in this region (Shallenberger, 1991). By-catches reported by Monteiro-Neto et al. (2000) from coastal gillnet fisheries off northeastern Brazil also suggest that rough-toothed dolphins enter inshore waters at least periodically.

In the Windward Islands of French Polynesia, our overall average sighting rate of *S. bredanensis* was 0.124 individuals/km. Although comparisons are difficult as survey platforms and observation protocols are specific to each study, sighting rates in other oceans do indicate lower frequencies of *S. bredanensis* with estimates of 0.0047 individuals/km and 0.00258 individuals/km in the eastern tropical Pacific and western tropical Indian Ocean, respectively (Ballance and Pitman, 1998; Wade and Gerrodette, 1993). Rough-toothed dolphin importance in delphinid populations may better be compared in term of sighting frequency. *S. bredanensis* ranks second in the Society Islands with 34% of on-effort delphinid sightings (Gannier, 2000). In the Marquesas, the northernmost archipelago of French Polynesia, the species ranked sixth with a sighting frequency of 4.0% (Gannier, 1999). In the Solomons, rough-toothed dolphin ranked fifth among delphinids with a sighting frequency of 5.0% (Shimada and Pastene, 1995). In the Sulu Sea, in the Philippines, the species also ranked fifth, with a sighting frequency of 2.9% (Dolar et al., 1997). *S. bredanensis* was ranked as the ninth most common cetacean species to be sighted in both the eastern tropical Pacific and the western tropical Indian Ocean (Ballance and Pitman, 1998 ; Wade and Gerrodette, 1993). Hence from results available in the literature, French Polynesia, and in particular the Windward Islands, appear to be a favored area for *S. bredanensis*. Certainly, this species is present around

many small oceanic islands in warm temperate to tropical regions.

Our seasonal distribution results indicated surprising differences: the lowest seasonal sighting rate was obtained during the cold water season, and the highest sighting rate during medium water temperature values (December to February). This does not agree with relative abundance estimates of Gannier (2000) which indicated that delphinids are generally 3 times more likely to be sighted during cold water periods as opposed to the warm season in the Society Islands. However, the melon-headed whale and Fraser's dolphin account for two of these four delphinid species, and these species were sighted in very large groups during the cold water season, influencing the result presented for all delphinid species in Gannier (2000). It is also possible that our results on *S. bredanensis* seasonal distributions are at least partially effected by an unequal sampling effort regarding depth and distance from shore between the seasons. During the cold water period, our efforts were more concentrated on shallower areas within 1 mile of the barrier reef because surveys were sometimes focused on humpback whales during that portion of the year as compared to the other seasonal time periods (Figure 3 a, b, and c). This may influence the lower sighting rate of *S. bredanensis* during the cold season, as our results show that this species is more commonly found at a distance of 1.8 to 5.5 km from the barrier reef, and in deeper waters.

Acknowledgements

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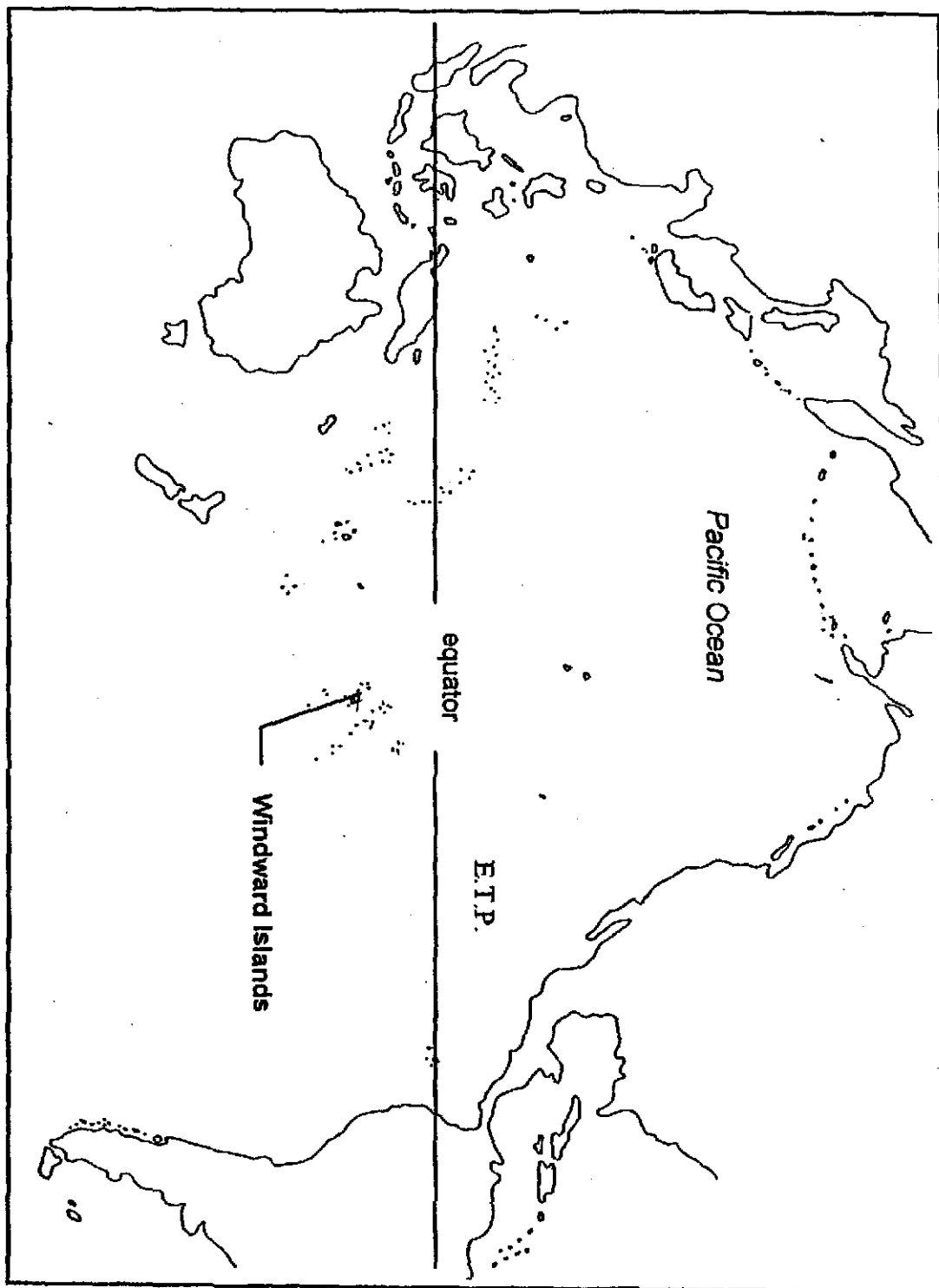
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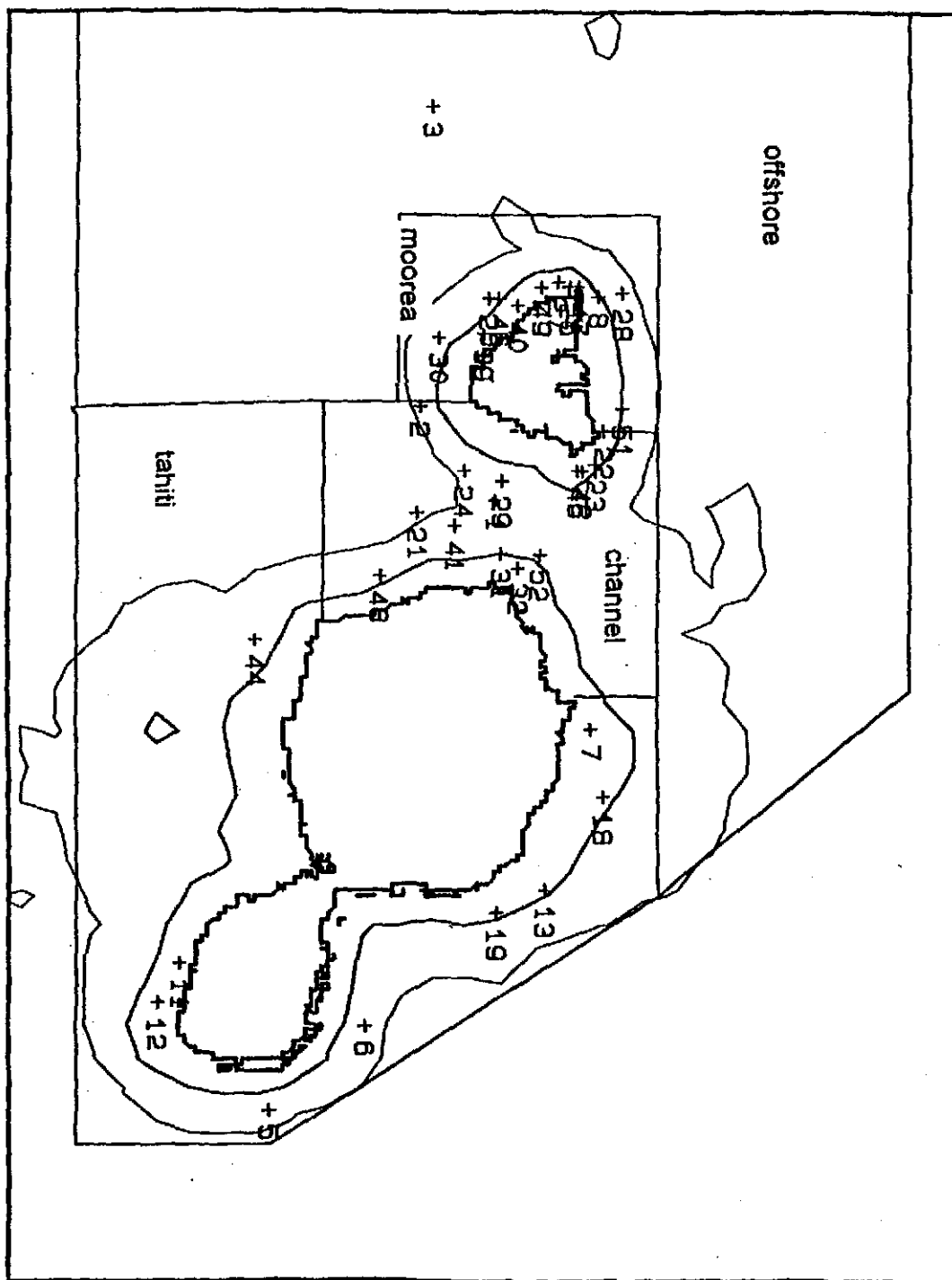
Table 1. Survey effort and sighting conditions where percentage of total in each column is given in brackets.

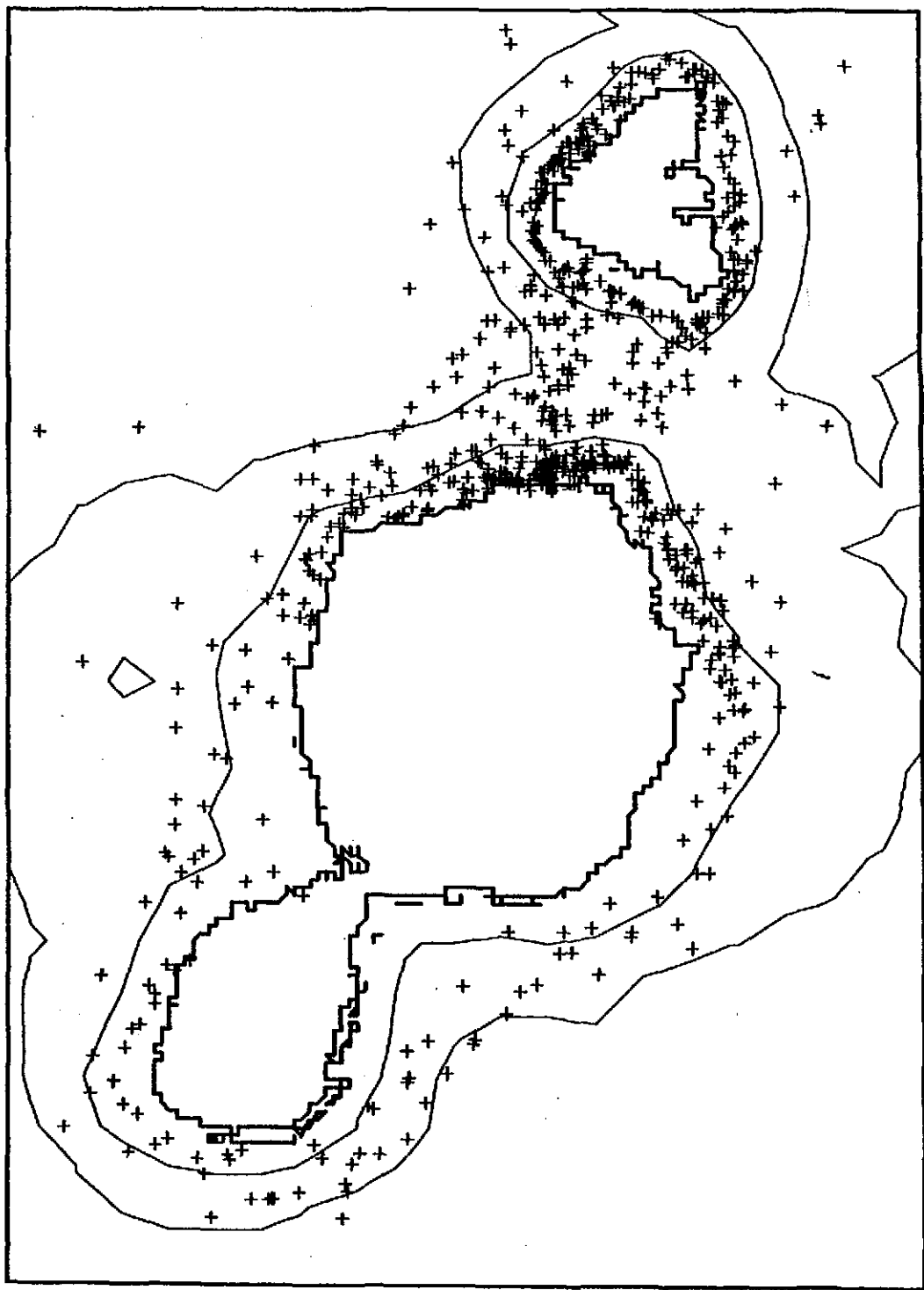
	Entire Study Area	Region 1 (channel)	Region 2 (Moorea)	Region 3 (Tahiti)	Region 4 (offshore)
Total Effective Effort (km)	6458	3441	1706	758	553
Effort in Beaufort 0-1 conditions (km)	864 (13.4%)	482 (14.1%)	213 (12.5%)	117 (15.0%)	52 (9.4%)
Effort in Beaufort 2 conditions (km)	2442 (37.8%)	1383 (40.2%)	678 (39.7%)	242 (31.9%)	139 (35.1%)
Effort in Beaufort 3 Conditions (km)	3152 (48.8%)	1576 (45.7%)	815 (45.7%)	399 (52.6%)	362 (65.4%)

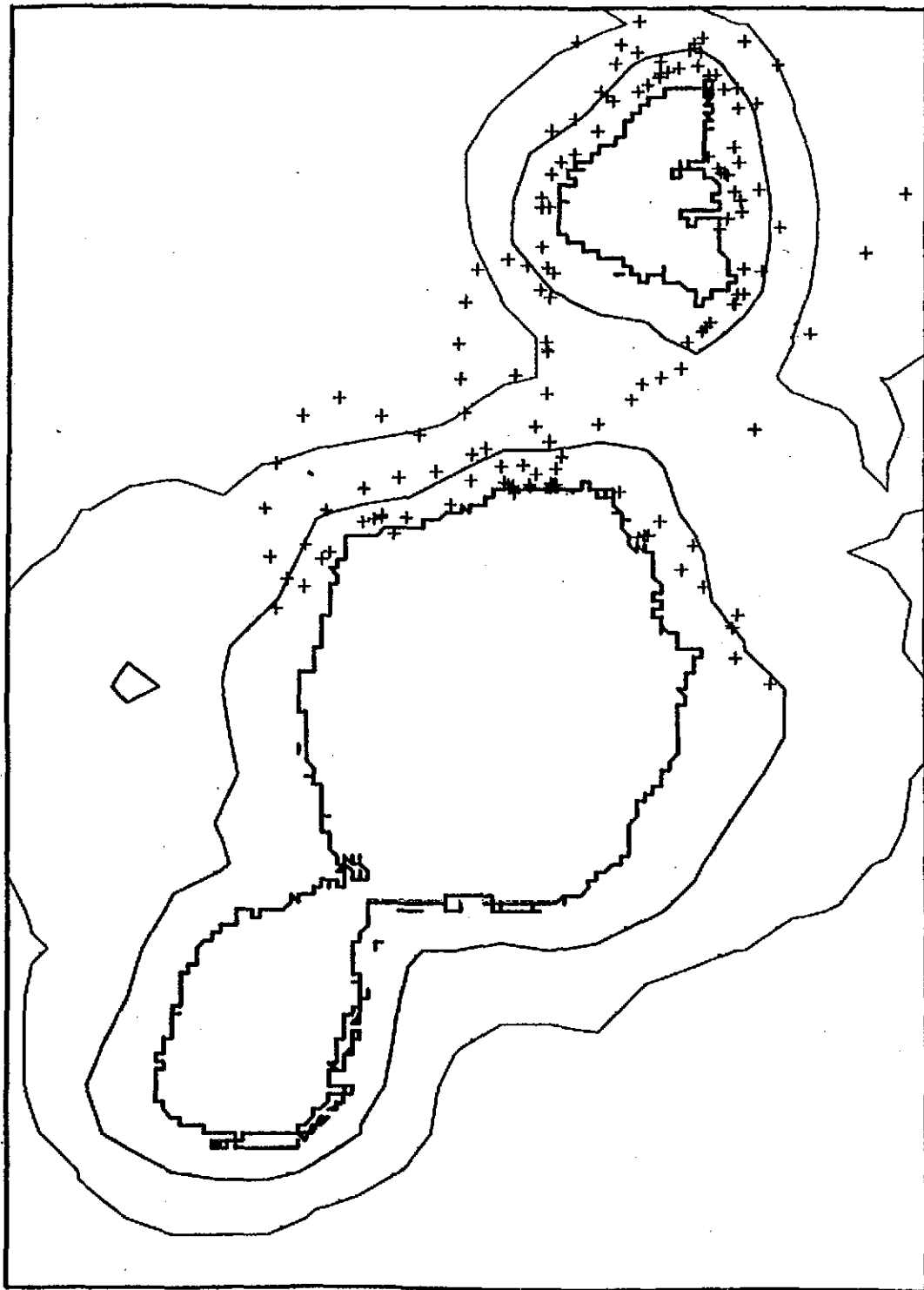
Table 2. Mean sighting rates for different strata (in dolphin/100km of effort).

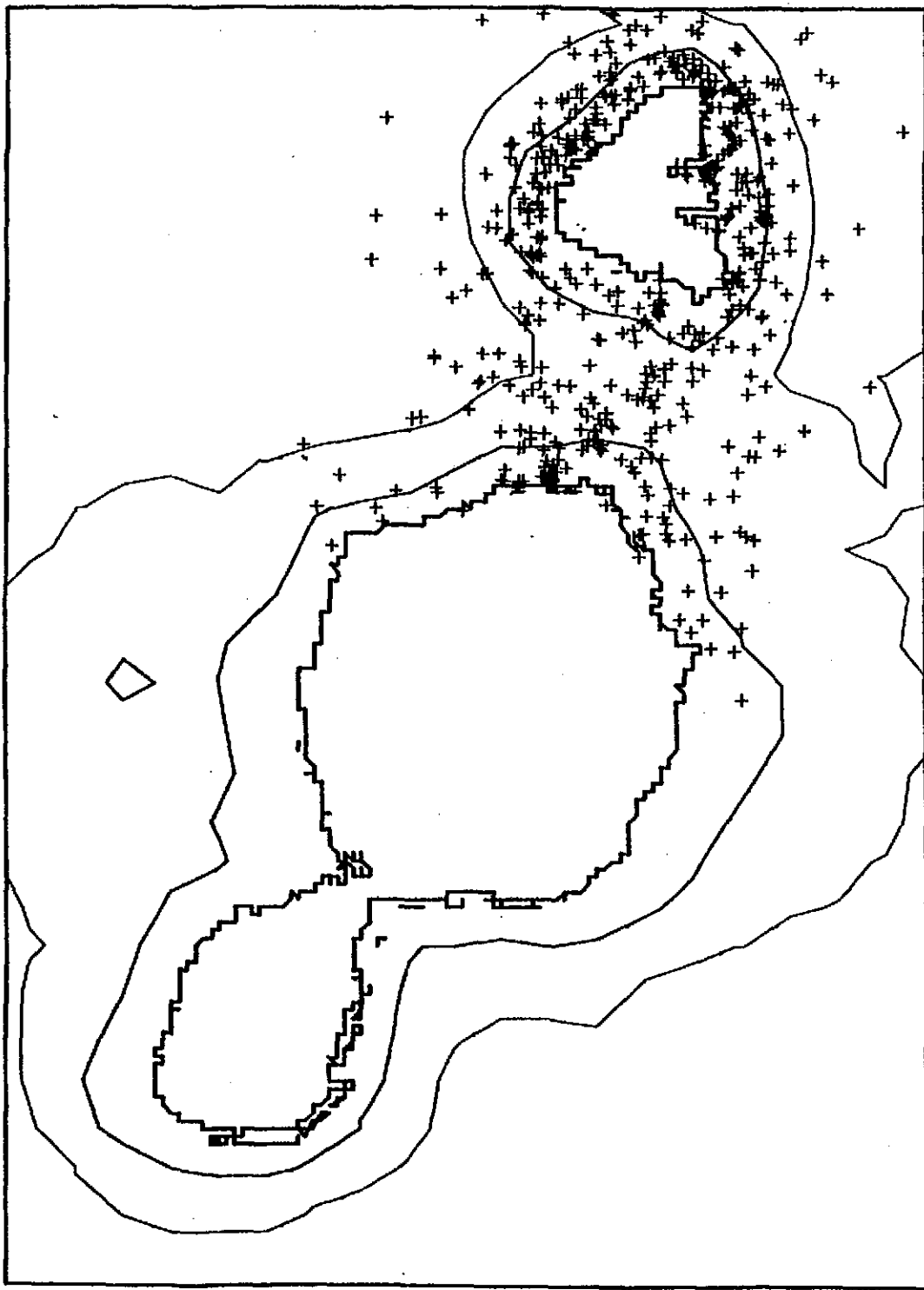
Mean sighting rates (n; SE)	Channel	Moorea	Tahiti	Inshore (both islands)
Whole region	18.62 (15 ; 8.51)	27.56 (11 ; 13.47)	32.59 (9 ; 13.16)	23.89 (35 ; 6.93)
Dist to shore 0 to 1.8km	-	12.49 (7 ; 6.73)	1.05 (1 ; 0.59)	6.29 (8 ; 3.12)
Dist to shore 1.8 to 5.5km	-	29.20 (8 ; 17.70)	47.80 (11 ; 20.17)	42.24 (19 ; 16.64)
Depth 0 to 1000m	-	19.87 (10 ; 10.07)	13.60 (8 ; 5.27)	17.34 (18 ; 6.12)
Depth 1000 to 2000m	-	23.95 (6 ; 13.37)	14.46 (7 ; 7.66)	18.98 (13 ; 7.90)











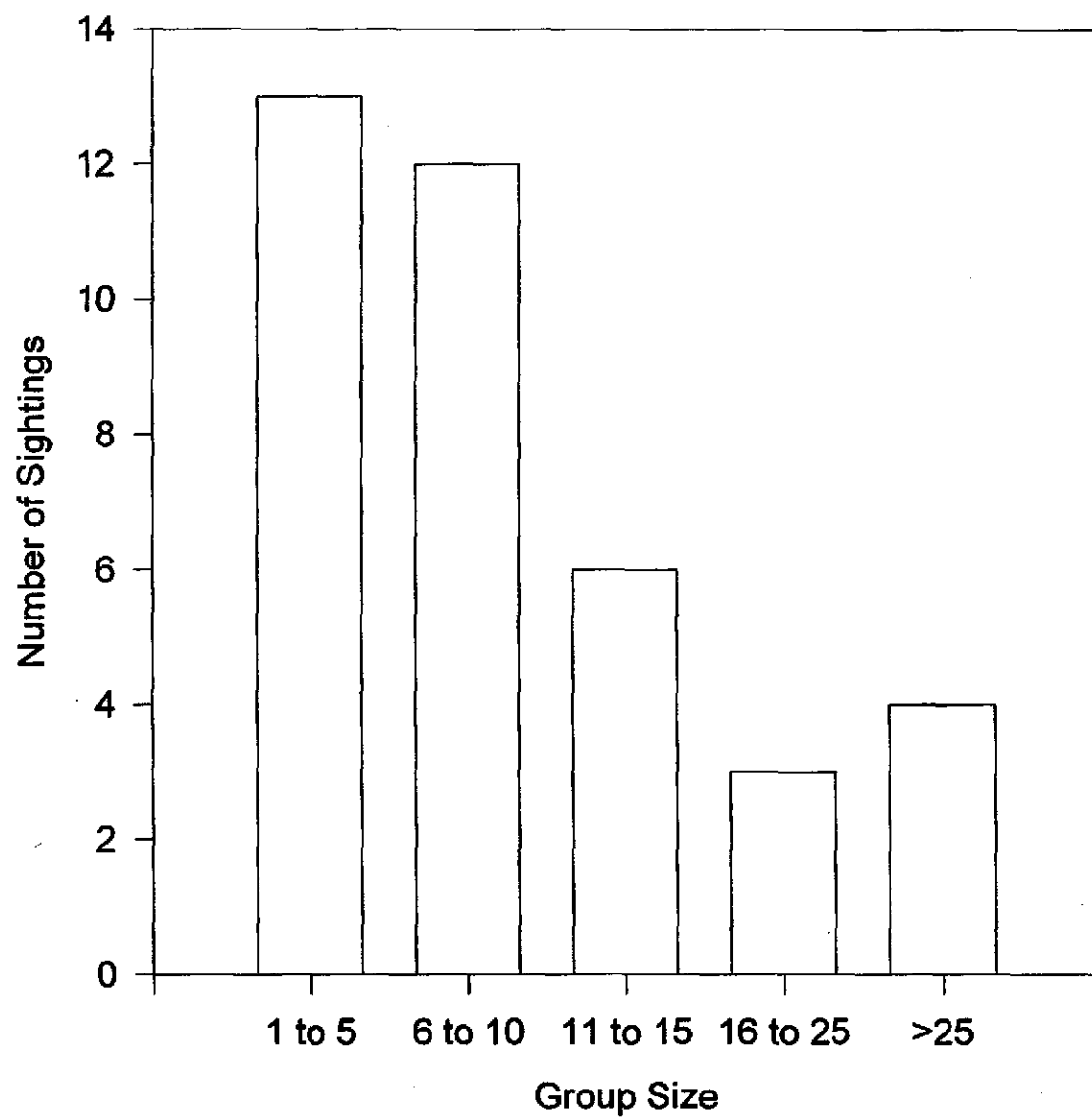


Figure 1. Study area located in the Windward Islands, French Polynesia.

Figure 2. Survey area was sub-divided into four regions for comparison of rough-toothed dolphin distribution. Regions include the channel, Moorea, Tahiti, and offshore areas.

Figure 3a, b, and c. Effective survey effort was corrected according to season. Effective effort is represented between September and November (a); December and February (b); and March to May (c).

Figure 4. Group size for each sighting of rough-toothed dolphins.

CHAPTER 3

Reproduction in the rough-toothed dolphin, *Steno bredanensis*:

Attainment of sexual and physical maturity

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ABSTRACT

The age and size range at sexual maturity is required for population modeling and effective management of cetaceans. In this study we report on the attainment of sexual maturity in the rough-toothed dolphin, *Steno bredanensis*. Twenty-seven females and 22 males collected from the Pacific and Atlantic oceans were examined at the Smithsonian Museum of Natural History. We estimated the age of these dolphins by counting growth layers in a prepared thin, longitudinal tooth section. We determined physical maturity according to fusion of vertebral epiphyses and reproductive status by histological and gross examination of reproductive organs. The histological examinations of uterine epithelial and mammary tissue indicate that female *S. bredanensis* begin the gradual process of sexual maturation between 3 and 6 years of age. Females reach sexual maturity by 9 to 10 years, at a length of 212 to 217 cm and a weight between 101 and 108 kg. Our youngest physically mature female specimen was estimated at 12 years of age, with a length of 210 cm and a weight of only 85 kg. Male rough-toothed dolphins attain sexual maturity between 5 and 10 years of age, at a length of 216 cm and a weight of 92 to 102 kg. Physical maturity in male *S. bredanensis* is attained between 5 and 14 years of age, at a length of 227 to 231 cm, and weight of 119 to 130 kg. Sexual dimorphism was evident in *S. bredanensis* and our findings provide the first report on sexual maturity attainment and characterization of different reproductive states for this species.

INTRODUCTION

Information pertaining to the life-history of *Steno bredanensis* is limited. Significant investigations into reproductive parameters in this species have only focused on populations found in Japanese waters (Miyazaki, 1980; unpublished data cited in Miyazaki and Perrin, 1994). Growth has been described as rapid during the first five years of life and the attainment of physical maturity in Japanese drive specimens estimated at 16 years of age and at a length of 231 cm (Miyazaki and Perrin, 1994). Miyazaki (1980) examined a small sample of individuals from two *S. bredanensis* schools where age and sexual maturity were assessed. A total of 39 specimens were aged, but testes were weighed for only nine males, and only two of these specimens examined histologically for sperm presence (Miyazaki, 1980). The youngest male examined was estimated at 14 years of age, and smallest testis weight recorded at 175 g, with no male specimens classified as immature. Miyazaki's (1980) examination of female specimens did not include the age or size range where sexual maturity is attained although females greater than 17 years of age were classified as mature. However, Miyazaki has estimated the attainment of sexual maturity for females found in Japanese waters to occur at about 10 years of age and between lengths of 210-220 cm (as cited as unpublished data in Miyazaki and Perrin, 1994). In addition to the work in Japan, a stranded male recovered in Oregon estimated at 7 years of age with a length of 209 cm was considered immature, based on a testis weight of 23.3g (Ferrero et al., 1994). In the Gulf of Mexico, Florida, 34 *S. bredanensis* specimens obtained since 1990 from strandings have also been aged using tooth growth layer groups (GLG's; Stolen et al., 1999), but no information on the reproductive status of these aged animals is yet

available. Asymptotic length based on a combination of both sexes was estimated at 239 cm for Florida populations which is slightly greater than the combined estimate of 231 cm for Japanese waters (Miyazaki and Perrin, 1994; Stolen et al., 1999).

It is not possible to obtain extensive life-history information for rough-toothed dolphins from the large sample sizes that have been available for other species such as the spinner, spotted and striped dolphin (Chivers and Myrick, 1993; Klinowska, 1991; Miyazaki, 1984; Myrick et al., 1986; Perrin and Henderson, 1984). *S. bredanensis* specimens collected since 1976 were examined at the Smithsonian Museum of Natural History. We examined teeth, reproductive tissue, and necropsy data from approximately 50 individuals. Primary objectives of the present study included aging of individuals and gross and histological examination of both male and female reproductive organs to determine the age and size range that *S. bredanensis* attains sexual and physical maturity. We also aimed to describe histological parameters in tissues representative of different reproductive states for this species.

METHODS

Collected Specimens

S. bredanensis collection specimens at the Smithsonian Museum of Natural History were studied, including 27 females and 22 males. Approximately two-thirds of these specimens were collected from one of two mass strandings events which occurred in 1976, one in Hawaii and the other in Virginia Beach. The remaining third of the total specimens were collected by the National Marine Fisheries Service as incidental by-catch in the Northwest Pacific. From all specimens, sex, total body length, a gross assessment

of sexual maturity, and information regarding weights and dimensions of reproductive organs were recorded at the time of necropsy. From most collected specimens, weight of animal, and measurements of mid-dorsal, mid-lateral, and mid-ventral blubber thickness were recorded. Vertebral epiphyses fusion was assessed in most individuals as either open, closed but visible, or fused and invisible. In most cases, reproductive organs were initially preserved in 10% formalin and later transferred to 70% ethanol for longer-term storage.

Estimated Ages

Ages of all *S. bredanensis* specimens were estimated by preparation of a thin, longitudinal tooth section for counting of growth-layer groups (GLG's; Hohn, 1985; Perrin and Merrick, 1980). Growth-layer groups have been previously validated in bottlenose dolphins by counting of layers upon the death of animals of known age (Hohn, personal communication). As deposition of growth layers may be seasonally dependent and not as pronounced in tropical regions, a captive rough-toothed dolphin from a tropical environment was used to validate the use of this method for the species *S. bredanensis*. In this known aged animal, yearly growth layers were clearly visible, and only mild seasonal change did not appear to affect clarity of deposited layers. In all samples, growth-layer groups in the dentine were counted, and each GLG was assumed to signify one year of growth. Counts were made independently by two readers to the nearest 0.5 of a year and then averaged. Key specimens were determined as those that had either recently attained sexual maturity or were thought to be near this stage. For these animals, GLG's were counted a second time by both readers to ensure the best

estimate possible. Photomicrographs of each tooth section were also taken to allow for further examination in cases where ages were questionable. In older individuals it was sometimes difficult to distinguish between GLG's and these animals were aged by the minimum countable layers and denoted with a + sign (i.e. 5++).

Gross Examination of Reproductive Organs

Sexual maturity in male specimens was initially assessed according to testicular size. Right and left testicular weights were taken and dimensions including length, width and depth measured for both testes. Testicular tissue was also prepared for further histological examination to confirm reproductive status of each individual.

Female specimens were first examined to determine if the animal was lactating and/or pregnant, and fetus size recorded when animals were found to be pregnant. Presence or absence of corpora were used to categorize females as sexually mature or immature, as it is assumed that if one or more corpora are present the animal has previously undergone at least one ovulation and is therefore considered mature (Perrin and Reilly, 1984). The number of corpora lutea and corpora albicantia were recorded and the diameter measured when possible. For each ovary, the weight and dimensions including length, width, and depth were also recorded. Ovaries were qualitatively described according to shape and degree of folding and wrinkling. Flat diameters of the left and right uterine horns were measured. Uterine condition was initially assessed on a gross basis as one of the following: immature, mature, mature and dilated, mature and inactive, pregnant, or vascular. Uterine epithelial tissue was prepared for further histological examination from *S. bredanensis* individuals of various reproductive states.

Mammary gland length, width and depth were measured and mammary tissue described according to shades of white, pink, tan, or brown coloration. Mammary tissue from individuals of various reproductive states was also prepared for histological examination.

Histological Examination of Reproductive Organs

Testicular, uterine and mammary tissues were prepared by embedding and staining in hematoxylin and eosin. A Modified Masson's Trichrome stain was also prepared for each of the tissues and an ocular micrometer used to take measurements from each of the slides. Male histological sections were tested for the presence or absence of spermatozoa using Berg's stain (Berg, 1963). Seminiferous tubule diameter was measured eight times to obtain an average for each specimen histologically examined. Uterine tissue was measured four times and averaged to obtain endometrium thickness and myometrium external muscle layer, myometrium vascular layer, and myometrium internal muscle layer thickness. Mammary tissue was measured four times to obtain an average lobule diameter, alveoli diameter, fat cell diameter and mammary duct length. Mammary slides were also quantitatively assessed for percent composition of mammary tissue, ducts, connective tissue, fat, veins, arterioles, blood vessels, and parasites.

Data Analyses

An initial analysis was performed to compare the morphological and physiological traits between individuals examined from the Pacific and Atlantic oceans. There were no significant differences in males from the Pacific and Atlantic ocean for all

11 traits examined: Seminiferous Tubule Diameter, Age, Body Length, Body Weight, Right Gonad Weight, Left Gonad Weight, Right Gonad Length, Right Gonad Width, Right Gonad Depth, Left Gonad Length, Left Gonad Width, Left Gonad Depth (all P's > 0.05). For females, there was no significant difference for 9 of 11 traits examined: Age, Right Ovary Weight, Left Ovary Weight, Right Ovary Length, Right Ovary Width, Right Ovary Depth, Left Ovary Length, Left Ovary Width, Left Ovary Depth, Flat Diameter of Right Uterine Horn, Flat Diameter of Left Uterine Horn, Mammary Gland Length, Mammary Gland Width, Mammary Gland Depth (all P's > 0.05). There were 2 significant differences among females from the Pacific and Atlantic Oceans: Body Weight ($t = 2.73$, $df = 14$, $P = 0.016$: Atlantic mean = 100.8 kg, Pacific mean = 68.2 kg) and Body Length ($t = 2.63$, $df = 22$, $P = 0.015$: Atlantic mean = 217.4 cm, Pacific 194.1 cm). However, female specimens were not equally represented between these two oceans, only 5 of the 27 specimens were from the Atlantic. The majority of the younger and smaller individuals sampled were from the Pacific which likely reflects these significant differences versus true population differences between the two oceans (Appendix 2). Overall, these results indicate that males and females from the Atlantic and Pacific Oceans are very similar for most characteristics measured in this study. Considering the relatively small sample sizes available for *S. bredanensis*, it was reasonable to assume that these two locations can be combined into one population for further analyses to obtain basic life-history information on this species.

Age-length curves were generated and the attainment of physical maturity in this species was characterized according to age, total body length and weight. Gompertz

growth curves were fitted to age-length data. The general Gompertz equation applied to *S. bredanensis* is:

$$S = A(\exp(-b\exp(-kt)))$$

The Gompertz equation applied follows that previously used for striped dolphins (Calzada et al., 1997) where S represents the size of the animal expressed as total body length, A is the asymptotic value, b is the integration constant, k is the growth rate constant, and t is the age. Second order regression analyses were performed for female length versus age, male length versus age, and a combination of sexes for length versus age. Weight-length curves were generated for both male and female *S. bredanensis* specimens and second order regression analyses performed for males, females and a combination of the sexes weight versus length. The age, total body length, and weight range that male and female *S. bredanensis* attain sexual maturity was determined. Gonad weights and dimensions were analyzed according to different reproductive states characterized in this study. Linear regression analyses were performed to examine the relationships among morphological traits and reproductive organ traits. Log transformations were performed on some morphological traits to obtain a linear relationship between some of the morphological and reproductive characteristics examined in both males and females. Means and ranges from histological measurements were used to describe characteristics of testicular, uterine, and mammary tissue for different reproductive states. All data obtained from individuals examined at the time of necropsy and gross assessment of reproductive condition is presented with histological findings (Appendices 1 and 2).

Sexual dimorphism of weight and length and gonadal traits for immature and mature individuals were analyzed using t-tests not assuming equal variances for the different traits in the age/sex classes. A t-test was also performed to determine if significant differences in seminiferous tubule diameters between immature and mature males was evident from the histology results. Regression and correlational analyses were performed to examine the relationships among age and morphological traits, including body size, weight, testis weight and length, and ovary weight and length. Log transformations were performed on some morphological traits to obtain a log-linear relationship for statistical analysis. Aged individuals denoted with a ++ sign were excluded from data analyses, but are listed in Appendices 1 and 2.

RESULTS

Males: Age-Length

Our male specimens were determined to be between 1 and 15 years of age but it is possible that these represent underestimates as some individuals could not be confidently aged after a certain number of GLG's (Appendices 1 and 2). Fitting of a Gompertz growth curve to the male length-age data yielded the following:

$$S = 231(\exp(-0.5671\exp(-0.3858t)))$$

The asymptotic value for this equation was established as the largest physically immature specimen total body length, this data point represents the upper limit of the range between smallest mature and largest immature. The development of this formula for male *S. bredanensis* required a body length value at time 0, or birth length. As this is not known for this species, an estimate of 100 cm was used. The largest recorded fetus of

this species is 93 cm, and the smallest animal aged at less than 1 year is 126 cm, and based on these available data, 100 cm was chosen as a reasonable value for equation development. The relationship between male age and male total body length was strongly positive and had high significance ($F = 10.7$, $df = 2,11$, $P < 0.003$, $R^2 = 66.0\%$). Male asymptotic length appears to be reached at approximately 230 cm and 13 GLG's (Figure 1).

Our data on vertebral epiphyses fusion in 15 individuals indicates attainment of physical maturity over a wide age range for males. Our smallest mature male was aged as 8+ years, and largest immature male aged at 14 (Table 1). However, attainment of physical maturity spanned a narrower range when assessed according to total body length and weight as opposed to age. Males reach physical maturity at a total body length of 227 to 231 cm and a total body weight between 130 and 137 kg (Table 1). This range in total body length at physical maturity attainment is consistent with the graphic illustration of asymptotic length (Figure 1).

Males: Weight-Length

The relationship between total body weight and total body length in *S. bredanensis* was examined from 14 males (Figure 2). In our male specimens, there is a lack of samples from very young animals, where no weight data was recorded at the time of necropsy. However, we still found a highly significant and positive relationship between male total body length and weight ($F = 58.7$, $df = 2,12$, $P < 0.001$, $R^2 = 90.7\%$).

Males: Sexual Maturity

Sexual maturity was assessed by gross examination of reproductive organs and status confirmed by histological sections of reproductive tissue whenever possible. Gross examination of testes was used to score male individuals as sexually mature or immature and our male sample set was equally representative of *immature and mature animals*. Male *S. bredanensis* attain sexual maturity at a total body length of 216 cm, and there was no overlap in our sample when comparing length to reproductive status (Table 2). Overlap was apparent when comparing estimated age and weight of males against reproductive status. Males of this species reach sexual maturity between approximately 5 and 10 years of age, our youngest mature specimen aged at 5 years (Table 2). Total body weight of our lightest mature specimen was 92 kg and 102 kg for our heaviest immature animal (Table 2).

Attainment of sexual maturity in males was investigated by comparing testis dimensions with total body length of individuals. From our 22 male specimens examined, right testis length and weight is provided for 20 and 18 specimens, respectively. Overall, there was a significantly positive relationship between the log of right testis weight and log of right testis length ($F = 526.4$, $df = 1, 17$, $P < 0.001$, $R^2 = 97.1\%$). There was also a significantly positive relationship between male total body length and both the log of male testis weight ($F = 66.9$, $df = 1, 17$, $P < 0.001$, $R^2 = 80.7\%$) and the log of male testis length ($F = 56.1$, $df = 1, 18$, $P < 0.001$, $R^2 = 75.7\%$). When comparing total body length with male gonad data, there is a distinct separation between sexually mature and immature animals (Figure 3). Male *S. bredanensis* reach sexual

maturity when testis length increases to approximately 20 to 25 cm at a weight of approximately 400 g (Figure 3). All of our individuals investigated that had smaller testis length and weight were classified as sexually immature and those with greater values mature. More detailed data on reproductive status and gonad parameters are provided in Appendix 1.

Histological examination of male specimens included Bergs stain of testicular material and measurements of seminiferous tubule diameter for 15 and 14 specimens, respectively. Males were scored as sexually mature or immature based on gross examination of reproductive organs, and histological results generally agreed with these findings. However, for two male specimens, Berg's stain was positive but these individuals were still classified as sexually immature. Measurements of seminiferous tubule diameters in immature *S. bredanensis* ranged between 35.4 and 167 μm , and all measured less than 72 μm except for one of the five specimens. The average seminiferous tubule diameter was 163.2 μm in mature males and 77.1 μm in immature males ($t = 3.22$, $df = 12$, $P = 0.018$). The range of tubule diameter in mature specimens varied between 82.9 and 209.4 μm , with no tubule diameter less than 82.9 μm for mature specimens. Comparison of Berg's stain and seminiferous tubule diameter results were consistent except in two of our examined specimens. One specimen scored as immature on gross examination (504491) had a negative Bergs stain but average tubule diameter of 167 μm . Another specimen (504495) classified as mature on gross examination stained positive for sperm presence but only had an average tubule diameter of 82.9 μm .

Females: Age-Length

Our 17 female specimens were aged between 1 and 18 years, and similar to male ages, this may underestimate age of some individuals (Appendix 2). Our male specimens were determined to be between 1 and 15 years of age but it is possible that these represent underestimates as some individuals could not be confidently aged after a certain number of GLG's (Appendices 1 and 2). Fitting of a Gompertz growth curve to the female length-age data yielded the following:

$$S = 221(\exp(-0.5475\exp(-0.31923t)))$$

As with the male data, the female asymptotic value for this equation was established as the total body length of the largest physically immature specimen. This data point represents the upper limit of the range between the smallest mature and largest immature animal. The development of this formula for female *S. bredanensis* also required a body length value at time 0, or birth length. As this is not known for this species, a reasonable estimate of 100 cm was used for female equation development as well. There was a highly significant and positive relationship between female body length and female age ($F = 18.9$, $df = 2, 12$, $P < 0.001$, $R^2 = 74.4\%$). Our sample suggests that female *S. bredanensis* attain asymptotic length at approximately 217 cm, and between 8 and 12 years of age.

Data on fusion of vertebral epiphyses was available from 13 female specimens. Like males, physical maturity attainment in females indicated a wide range when considered according to age, the largest immature female was estimated at 4 years of age, and the smallest mature aged at 13+ years of age. A narrower size range was apparent in

females as well, where physical maturity is reached between 217 and 221 cm and between 101 and 121 kg (Table 1).

Females: Weight-Length

For females, the weight-length curve indicates a statistically significant relationship in this species (body length and body weight regression: $F = 625.0$, $df = 2, 15$, $P < 0.001$, $R^2 = 98.8\%$). Females appear to reach a maximum weight of approximately 120 kg at their asymptotic length of 217 cm.

Females: Sexual maturity

Our female sample indicated that sexual maturity is attained between a total body length of 212 and 217 cm and at a body weight ranging between 101 and 108 kg based on our largest and longest immature specimen and smallest and shortest mature animal (Table 2). Unfortunately, our sample set lacked female individuals between the ages of 4 and 9, and accordingly our age results indicated the smallest mature specimen at 10 years of age and the oldest immature animal estimated at 9 years (Table 2).

In female *S. bredanensis* there was a significantly positive relationship between female body length and right ovary weight ($F = 30.1$, $df = 1, 15$, $P < 0.001$, $R^2 = 66.7\%$) but not right ovary length ($F = 2.2$, $df = 1, 13$, $P = 0.16$, $R^2 = 14.3\%$). Furthermore, there was no clear separation between immature and mature females for either ovary length or weight with total body length (Figure 4). However, our results are based on ovary lengths for only 12 specimens and ovary weights for 17 individuals. From our sample size, it does appear that ovary weight is a better indicator of sexual maturity status than

ovary length. All of our females with a right ovary weight greater than 4 g had already attained sexual maturity. Available weights and dimensions including length, width and depth of both right and left ovaries are provided in Appendix 2.

Uterine tissue slides were prepared for 6 female *S. bredanensis* specimens, representing several different reproductive states. Two immature females were investigated as well as two specimens clearly undergoing the process of sexual maturation. In the immature and maturing specimens, endometrium thickness was less than that of our mature and pregnant and mature and lactating individuals investigated (Table 3). A considerable difference in endometrium thickness was evident between the immature and maturing animals, with an average of 833 μm in the maturing animals, which was much closer to our mature and pregnant specimen at 853 μm , than the immature animals which only averaged 471 μm . Endometrium thickness was greatest in our mature and lactating animal, measuring 1128 μm . There was little difference in myometrium external, vascular and internal layer thickness between the immature and maturing individuals (Table 3). However, all myometrium measurements were higher in our mature and pregnant animal, and even greater in our mature and lactating individual (Table 3).

Examination of mammary tissue slides prepared for 8 individuals indicated clear distinctions among different reproductive states. Immature mammary tissue was obvious, as was a slide representative of a mature and lactating animal. However, there were no apparent differences in measured mammary tissue parameters for this species when attempting to distinguish between maturing and mature animals. Measurement results are therefore presented as a maturing and mature group when categorizing mammary

analyses (Table 4). In terms of overall mammary tissue composition in *S. bredanensis*, glandular mammary tissue was higher in our mature and lactating individual composing 77% of the tissue, 48% of the slide in mature/maturing animals, but only 16% in our immature individuals (Table 4). Connective tissue was the primary component of the mammary tissue in immature animals, comprising 73% versus 33% in mature/maturing animals and only 18% in the mature lactating specimen (Table 4). Fat percentage was highest in the mature/maturing animals at 14%, and the degree of ducts, arterioles, veins and parasites was not much different among the various reproductive states investigated. Measurements were also taken of lobules, alveoli, fat cells, and ducts in the mammary tissue. Lobule size was much greater in our mature and lactating individual, estimated at 1020 μm and less than 300 μm in both the mature/maturing individuals and the immature specimens (Table 4). Size of alveoli, fat cells, and duct sizes were not different among the reproductive states represented (Table 4).

Comparison between males and females

Sexual dimorphism is evident in *S. bredanensis* (Table 1; Figure 1), physically mature males were not significantly heavier ($t = 0.47$, $df = 5$, $P = 0.659$) but were significantly longer ($t = 5.86$, $df = 4$, $P = 0.004$) than mature females. There was no difference in the age of the physically mature males and females examined in this study ($t = 0.47$, $df = 5$, $P = 0.0659$). Physically immature males were significantly older ($t = 2.41$, $df = 12$, $P = 0.03$), heavier ($t = 2.45$, $df = 14$, $P = 0.03$) and longer ($t = 2.33$, $df = 10$, $P = 0.04$) than immature females.

Sexually mature males were heavier ($t = 2.91$, $df=19$, $P = 0.009$) and longer ($t = 3.00$, $df = 17$, $P = 0.009$) than sexually mature females. However, there was no difference in the age of the mature males and females ($t = 0.72$, $df = 6$, $P = 0.50$). Sexually immature males were significantly heavier ($t = 2.91$, $df = 20$, $P = 0.009$) than immature females. There was no significant differences in the length ($t = 1.81$, $df = 20$, $P = 0.085$) or age ($t=1.45$, $df = 17$, $P=0.164$) of sexually immature males and females.

DISCUSSION

The relationship between age and growth in this study of *S. bredanensis* supports that the most rapid growth in this species occurs during the first five years of life (Miyazaki and Perrin, 1994). This was evident for both males and females where some very young individuals of both sexes are represented (Figure 1). The relationship between length and weight was also investigated from a total of 34 stranded or live captured specimens in this study, which provides a more accurate estimate for a length-weight formula for this species than the previous data on 15 live caught animals (Miyazaki and Perrin, 1994). Our study presents the first available data for determination of the weight where physical maturity is attained in this species, which is considerably higher in males than females (Table 1).

Sexual dimorphism is apparent in *S. bredanensis* as males reach a larger size than females. Although a greater size in males was originally suggested by Miyazaki (1980), an asymptotic length of 231 cm was estimated as the same for both sexes in Japanese waters. This Japanese population consisted primarily of older animals between 14 and 32 years of age (Miyazaki, 1980). A study of 34 rough-toothed dolphins from Florida

stranding events represented a greater range of age classes, but again many of the animals were older, the oldest animal estimated at 48 years (Stolen et al. 1999). Our study population from both the Pacific and Atlantic oceans was comprised mainly of relatively young individuals, none of our animals were over 20 years of age. We did have some specimens noted with a + sign indicating that growth layer groups were not distinguishable after a certain age. In both of the previously investigated *Steno* populations from Japan and Florida, both males and females were combined to obtain an asymptotic length for the species. Our female asymptotic length was lower, ranging between 217 and 221 cm whereas our male estimate of 227 to 231 cm is very similar to the species estimate of 229 cm provided for Florida waters and 231 cm in Japan (Miyazaki, 1980; Stolen et al. 1999). However, we estimate an age range between 8 and 14 years where male *S. bredanensis* attain physical maturity, and between 4 and 13 years of age in females which is considerably lower than the overall 16 year old estimate previously provided for this species (Miyazaki and Perrin, 1994). Our 4 year old female specimen could be anomalous, but regardless, our study indicates that at least some individuals attain physical maturity at a younger age, and our varied results are most likely a function of the age classes which have been the focus of these different studies.

Sexual maturity in *S. bredanensis* has only been previously assessed in specimens from Japan and two stranded males, one off of Oregon and the other off of the coast of Washington (Ferrero et al., 1994; Miyazaki, 1980; Miyazaki and Perrin, 1994). From the Japanese sample set, a 14 year old male with a total body length of 225 cm and testis weight of 175 g was classified as sexually mature based on sperm presence (Miyazaki, 1980). However, reproductive organs were not examined in immature males (Miyazaki

1980). Our results provide the first report on the age and total size range where males attain sexual maturity as well as provide accompanying gonad weight and length data (Table 2; Figure 3). The male recovered from Washington was presumed to be immature based on a total body length of 197 cm and testis weight of 23.3 g, which agrees with our findings where males under 216 cm are sexually immature. The male specimen from Oregon is questionable however, as this animal was aged at 14 years which according to our results would suggest maturity, but had a total body length of 209 cm (Ferrero et al., 1994) which is less than our longest immature specimen.

Criteria employed for determination of sexual maturity in male cetaceans is variable among workers, and we considered both the gross testis size as well as presence of sperm using Berg's stain. Two of our animals were classified as sexually immature based on gross examination of reproductive organs although Berg's stain results were positive. As the required density of sperm necessary to successfully inseminate female dolphins is unknown, the immature classification of these two specimens was left unchanged by the histological results. In our study, males with a testis weight greater than 400 g were presumed to be sexually mature, and differences in seminiferous tubule diameter between immature and mature males obtained from histology can be used to accompany gross maturity assessments. Values for various parameters where sexual maturity is attained in males of this species will be a useful reference for cases where limited information is available to assess maturity in male *S. bredanensis* (Appendix 1).

Female *S. bredanensis* are believed to reach sexual maturity in Japanese waters around 10 years of age and at a total body length of 210 to 220 cm (unpublished data cited in Miyazaki and Perrin, 1994). Females greater than 225 cm and 17 years of age

have been assessed as mature based on presence of corpora (Miyazaki, 1980). Our results indicate that females reach sexual maturity between 212 and 217 cm, and our largest immature individual was aged at 9 years and smallest mature at 10 years of age (Table 2). Our study included histological examination of uterine and mammary tissue from several individuals as well as accompanying descriptive data on ovary size and shape. It appears clear that *S. bredanensis* undergo a process of sexual maturation which is evidenced both in the size and shape changes that occur in the ovaries as well as in changes in the uterine and mammary tissue. Many of our young individuals sampled were near the age of 3 or 4 years, and had already begun to exhibit some of these changes where ovaries were no longer flat, but folded, wrinkled, and larger in size. Additionally, when classifying results from our histological parameters, it became necessary to establish a "maturing" category for both uterine and mammary tissue. In fact, histological examination of mammary tissue showed no differences between parameters in a "maturing" female and a "mature" female that was not lactating (Table 4). These maturing animals did not show evidence of corpora, and accordingly were classified as sexually immature, but this stage was found to be significant in *S. bredanensis*. Our maturing animals aged at 3 to 4 years and the youngest mature animal aged at 9 years would suggest a sexual maturation process lasting for up to a six year period. This is doubtful, and it is probable that these findings instead demonstrate the dearth of samples from 4 to 9 year old females in our study. This stresses the value of young females of this species that may become available for future studies to determine how long females undergo the process of sexual maturation.

A primary goal of our study was to provide a working reference for scientists and managers interested in assessing sexual and physical maturity in the species *S. bredanensis*. There is very little information on reproduction in rough-toothed dolphins, and our determination of age, length and weight ranges of maturity attainment accompanied by gonad measurements, descriptions, and histology provide a significant contribution to the available knowledge for this species.

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Table 1. The Age, Body Length and Body Weight of <i>S. bredanensis</i> for physically mature and immature males and females.	Male (n=15)						Female (n=13)					
	Immature			Mature			Immature			Mature		
	X	SD	Range	X	SD	Range	X	SD	Range	X	SD	Range
Age (GLG's)	7.6	3.6	3-14	11.1	3.5	5-15	4.0	2.5	1-9	10.0	4.1	4-13
Body Length (cm)	214.1	7.6	206-231	230.1	3.1	227-235	197.9	19.4	157-217	215.3	4.5	210-221
Body Weight (kg)	95.1	16.4	79-130	136.0	10.4	119-149	73.2	20.5	34-101	105.3	18.4	85-121

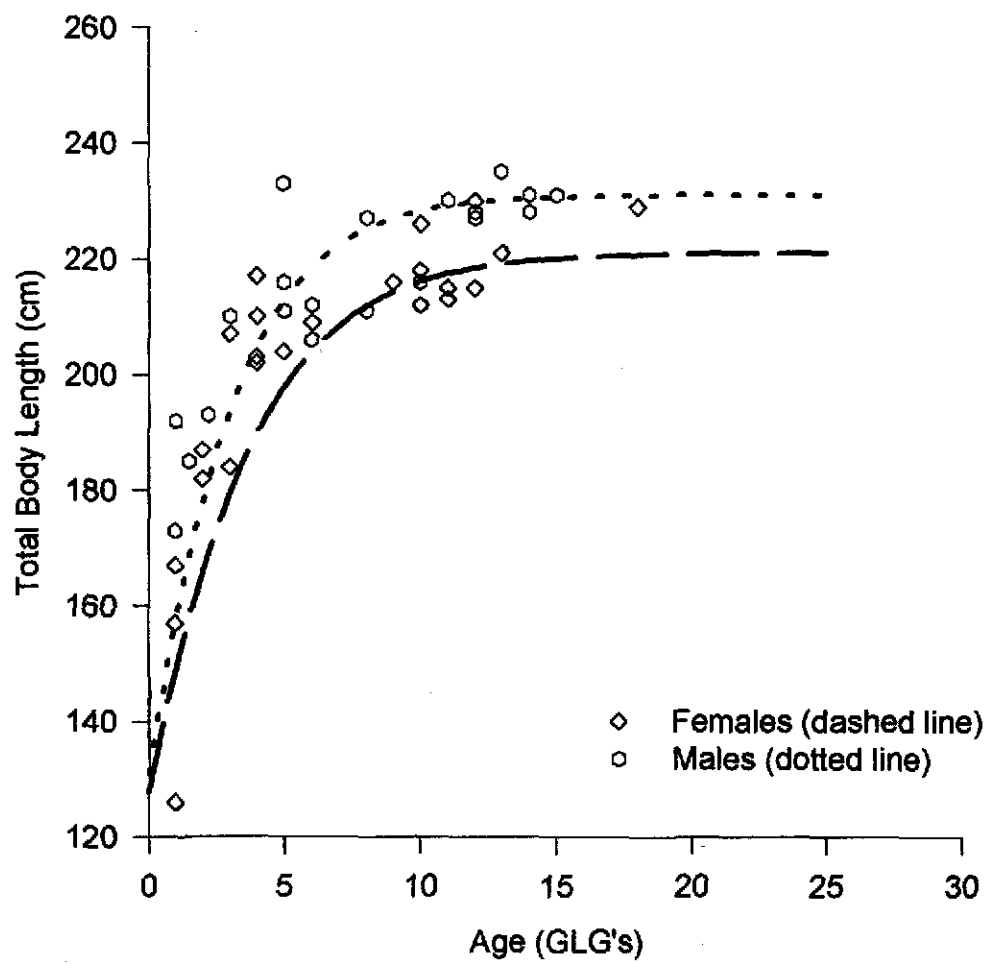
Table 2. The Age, Body Length and Body Weight of <i>S. bredanensis</i> for sexually mature and immature males and females.	Male (n=16)						Female (n=19)					
	Immature			Mature			Immature			Mature		
	X	SD	Range	X	SD	Range	X	SD	Range	X	SD	Range
Age (GLG's)	5.3	3.6	1-10	10.9	3.7	5-15	3.6	2.2	1-9	12.5	3.8	10-18
Body Length (cm)	203.9	13.9	173-216	228.61	5.2	216-235	185.7	34.8	93-217	220.5	6.8	212-230
Body Weight (kg)	87.9	14.0	62-102	128.3	17.1	92-149	62	27.7	85-101	112.4	5.0	108-121

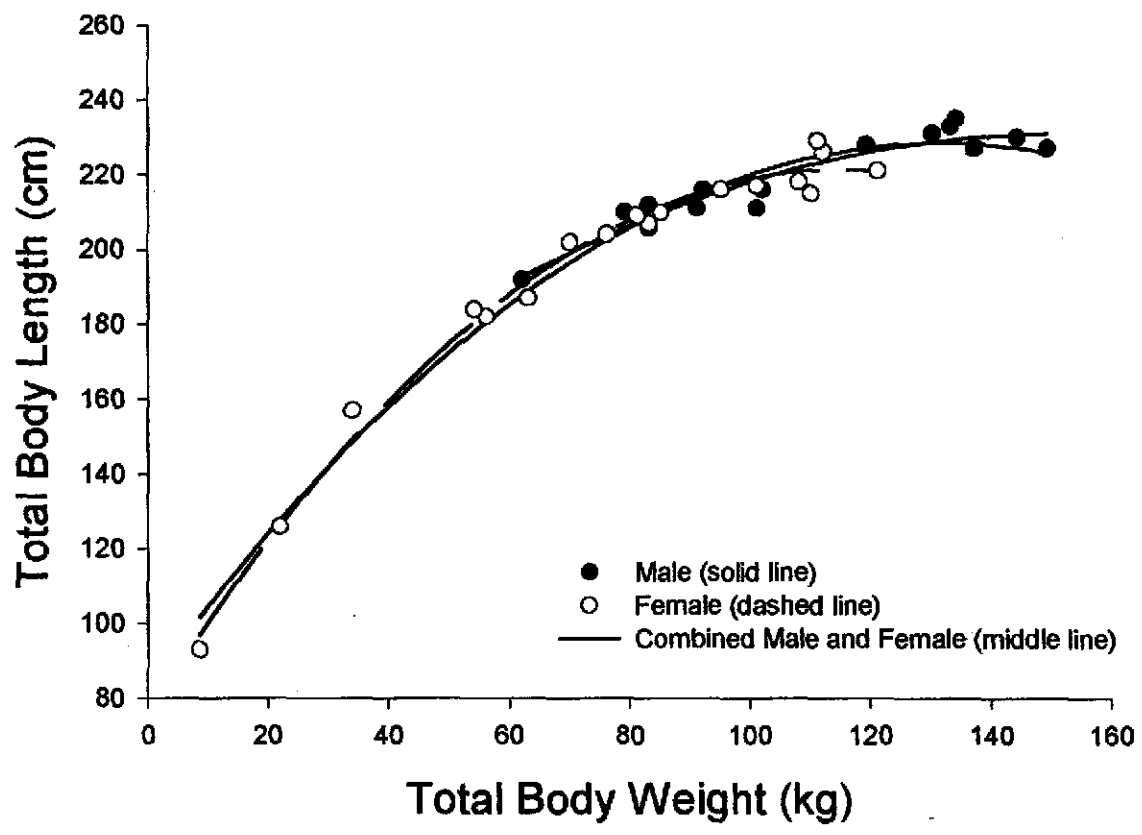
Table 3. Uterine tissue measurements among various reproductive states as measured from histological sections in *S. bredanensis*.

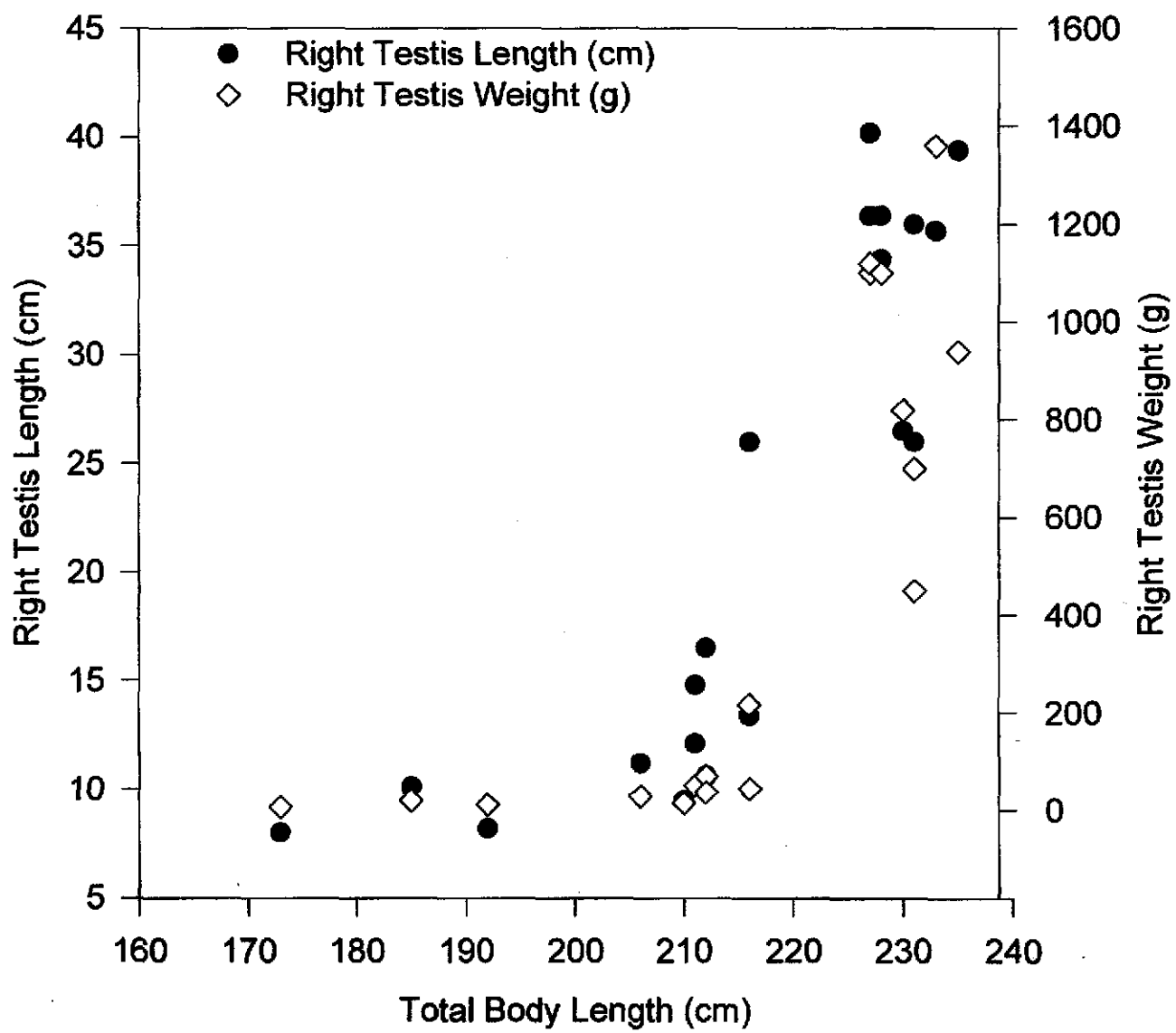
	Uterine Tissue Measurements (microns)			
	Myometrium: External Layer	Myometrium: Vascular Layer	Myometrium Internal Layer:	Endometrium: Thickness
IMMATURE (n=2) Mean: Range:	130 116-144	136 72-199	271 230-312	471 423-519
MATURING (n=2) Mean: Range:	109.5 62-157	81.5 57-106	332 265-399	833 763-903
MATURE and PREGNANT (n=1) Mean: Range:	194 -	339 -	495 -	853 -
MATURE and LACTATING (n=1) Mean: Range:	583 -	465 -	887 -	1128 -

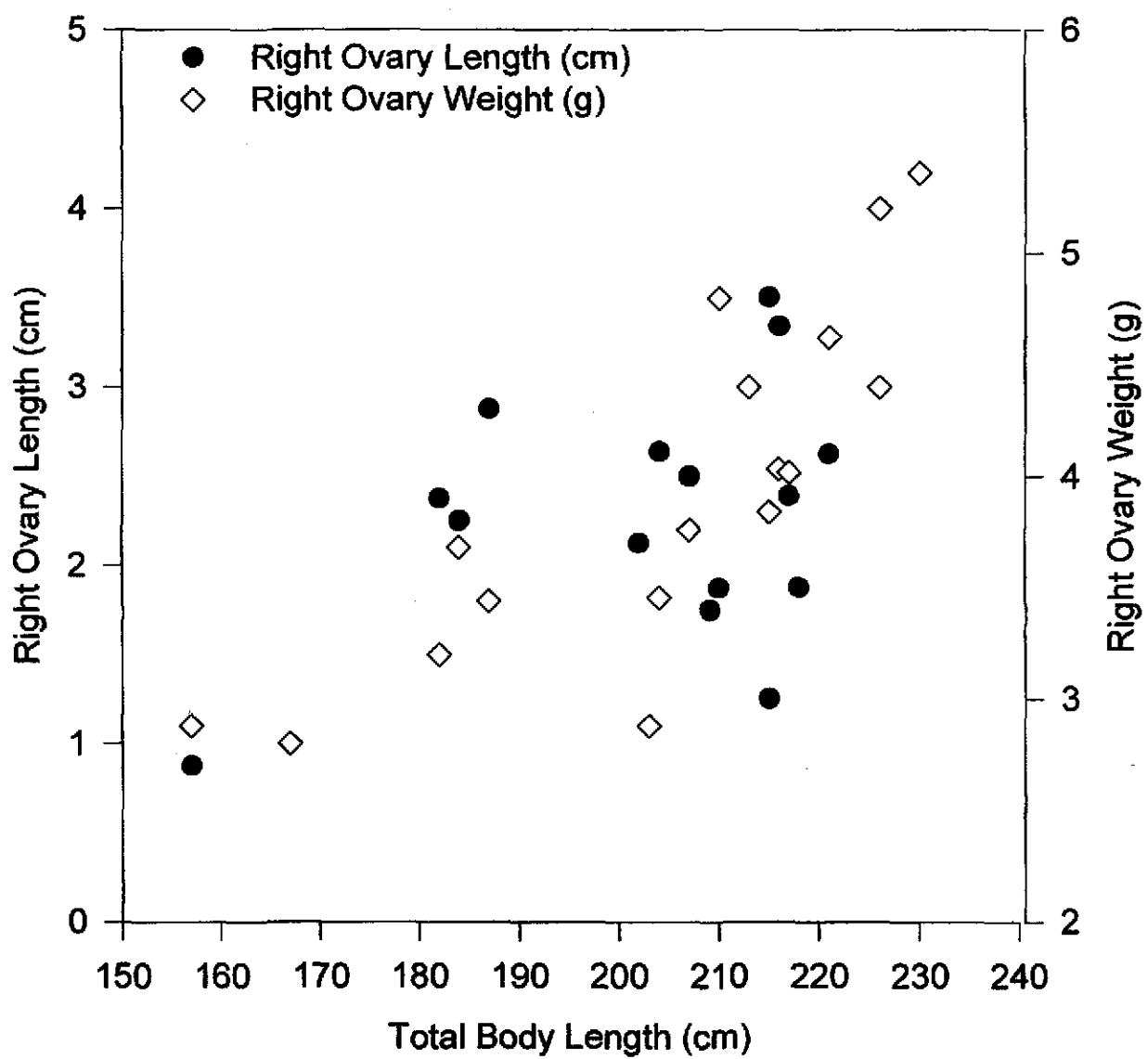
Table 4. Mammary tissue composition and structure measurements among various reproductive states in *S. bredanensis*.

	Composition of mammary tissue (%)							Measurements of mammary tissue components (microns)			
	Glandular	Fat	Ducts	CT	Arterioles	Viens	Parasites	Lobule	Alveoli	Fat Cell	Duct
IMMATURE (n=3) Mean: Range:	16 10-20	4 0-7	5 2-7	73 62-80	0.3 0-1	0 0	1.3 0-4	208 74-325	26 23-29	23 17-29	478 56-1658
MATURING or MATURE (n=4) Mean: Range:	48 43-53	14 2-35	3.5 1-8	33.3 10-45	0.5 0-1	0.3 0-1	0.5 0-2	268 202-383	50 39-60	37 29-52	428 278-617
MATURE and LACTATING (n=1) Mean: Range:	77 -	0 -	3 -	18 -	0 -	2 -	0 -	1020 -	61 -	- -	310 108-1113









									Gonad Weight		Gonad Dimensions						Vertebral Epiphysis
Specimen ID	North Ocean: Pacific or Atlantic (NP/NA)	Bergs Stain (Y/N)	Seminiferous Tubule Diameter (μ)	Age (GLG's)	Body Length (cm)	Body Weight (kg)	Maturity Status (gross)	Sperm In Epid. (Y/N)	R. (g)	L. (g)	R.L (cm)	R.W (cm)	R.D (cm)	L.L (cm)	L.W (cm)	L.D (cm)	Fused (Y/N)
550918	NP	-	-	1	173	-	IM	-	6.5	-	8	-	-	-	-	-	-
PMT001	NP	-	-	1.5	185	-	IM	-	19.5	-	10.1	-	-	-	-	-	-
RCR153	NP	-	-	1	192	62	IM	-	11.2	12.3	8.2	-	-	-	-	-	-
550369	NP	-	-	2.2	193	-	IM	NE	19	NE	-	-	-	-	7.8	-	NE
504486	NA	N	52.1	6	206	83	IM	NE	29.3	NE	11.2	2	1.8	11.3	1.9	1.6	N
504488	NA	-	59.1	3	210	79	IM	N	14.7	12.9	9.5	1.6	1.3	9.4	1.6	1.1	N
504464	NP	Y	35.4	5	211	91	IM	N	NE	NE	12.1	2	1.4	11.7	2.1	1.2	N
504491	NA	N	167.1	8	211	101	IM	NE	50.1	54.3	14.8	2.6	1.9	13.9	3	1.8	N
550182	NP	Y	-	10	212	83	IM	N	70	66	16.5	3.4	1.8	16	3	1.8	N
BAH0008	NP	-	-	6++	212	-	IM	-	36	-	10.6	-	-	-	-	-	-
550217	NP	Y	127.9	5	216	92	M	Y	217	218	26	5	3	24	5.5	3.1	N
504495	NA	N	72.1	10	216	102	IM	NE	44.5	47.1	13.4	2.6	2	13.4	2.5	2	N
504468	NP	Y	82.9	8+	227	137	M	Y	1100	1080	40.2	10	3.4	38.8	10.4	3.4	Y
504492	NA	Y	209.4	12+	227	149	M	Y	1120	1170	36.4	8.9	6.7	34.4	9.4	7	Y
504465	NP	Y	169.7	14+	228	119	M	Y	1100	280	36.4	8.8	6.5	27.3	5.9	4.3	Y
550837	NA	Y	165.7	12+	228	-	M	N	NE	NE	34.4	7.6	6.1	32.5	7.2	4.6	NE
504489	NA	Y	191.7	11+	230	144	M	Y	820	1000	26.5	8.8	6.3	30.1	9	6.3	Y
504490	NA	Y	145.2	14	231	130	M	Y	450	420	26	6	4.5	26.5	6	5	N
504499	NA	-	-	15	231	-	M	Y	700	670	36	8	5.5	34	8	5.5	Y
504494	NA	Y	196.3	5	233	133	M	Y	1360	1300	35.7	8	6	34	8.5	6.3	Y
504467	NP	Y	180.2	13+	235	134	M	Y	940	1060	39.4	8.3	3.2	39.7	9.4	3.4	Y

[illegible]

Specimen ID	Uterine Horn Flat Diameter		Mammary Glands				Vertebral Epiphysis
	R (cm)	L (cm)	Color	L (cm)	W (cm)	D (cm)	Fused (Y/N)
504463	-	-	-	-	-	-	-
550219	-	-	-	-	-	-	-
550181	1.0	1.0	Pink	19	2.5	0.3	N
550183	-	-	-	-	-	-	-
550220	1.2	1.2	-	11.5	-	0.2	N
550179	1.6	1.4	Pink	24	40	0.5	-
550218	1.8	-	-	15	-	0.3	N
504461	1.3	1.3	Pink	26	5.5	0.5	N
PEL0010	-	-	-	-	-	-	-
504497**	1.0	1.0	Pink	22	4	0.7	N
550343	-	-	-	-	-	-	-
550180**	1.8	1.7	Pink/Brown	24	4	0.6	N
550368	2.1	1.9	Pink/Brown	31	5.5	0.9	Y
504469	1.0	1.2	Pink	30	2	0.5	N
550370	-	-	NE	-	-	-	-
BAH007	-	-	-	-	-	-	-
550221	9.0	24	Pink/Brown	36	6	1.5	Y
504462	-	-	Dark red/Brown	31	8	1.5	Y
504496**	1.6	1.6	Pink/Tan	26	4.5	1.4	N
504493**	1.3	1.3	White/Pink	24	3.8	0.9	N
504466	2.5	4.7	Brown	28	7.5	2.5	-
504487	2.3	2.6	Brown	35	7.4	2.6	Y
BAH009	-	-	-	-	-	-	-
RCR151	-	-	-	-	-	-	-
504498	33	10	Tan/Cream	-	-	-	-
550371	-	-	-	-	-	-	-
Pea	-	-	-	-	-	-	-

Figure 1. Total body length of male and female *S. bredanensis* plotted against age (GLG's). The regression equation for male body length = $181 + 6.27 \text{ male age} - 0.247 (\text{male age})^2$; female body length = $168 + 8.44 \text{ female age} - 0.302 (\text{female age})^2$. The regression equation for combined males and females body length = $174 + 7.60 \text{ age} - 0.274 (\text{age})^2$.

Figure 2. Total body weight of male and female *S. bredanensis* plotted against total body length. The regression equation for female body length = $76.1 + 2.53 \text{ female body weight} - 0.0110 (\text{female weight})^2$; male body length = $121 + 1.44 \text{ male body weight} - 0.00475 (\text{male weight})^2$. The regression equation for combined males and females body length = $83.4 + 2.20 \text{ weight} - 0.00836 (\text{weight squared})^2$.

Figure 3. Right testis length (cm) and weight (g) plotted against total body length in male *S. bredanensis*. The regression equation for male right testis length = $-105.316 + 0.592889 \text{ male total length}$; log male right testis weight = $-1.59273 + 0.0133394 \text{ male total length}$.

Figure 4. Right ovary length (cm) and weight (g) plotted against total body length in female *S. bredanensis*. The regression equation for female right ovary length = $1.40717 + 0.0119194 \text{ female body length}$; female right ovary weight = $-529757 + 0.0378120$.

Appendix 1. Reproductive parameters for all male *S. bredanensis* individuals investigated. STD refers to average seminiferous tubule diameters.

Appendix 2. Reproductive parameters for all female *S. bredanensis* individuals investigated.

CHAPTER 3

Hormone concentrations during rehabilitation and in healthy rough-toothed dolphins, *Steno bredanensis*

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ABSTRACT

Endocrinology parameters were investigated in the relatively unknown rough-toothed dolphin, *Steno bredanensis*. This study reports on cortisol, thyroid, progesterone and testosterone hormone concentrations. One hundred samples from seven different individuals housed at Mote Marine Laboratory and Aquarium and Dolphin Quest French Polynesia were analyzed for concentrations of cortisol and thyroid hormones, including Total and Free thyroxine, and Total and Free tri-iodothyronine. Testosterone concentrations were also determined in the four male rough-toothed dolphins and progesterone concentrations in the three females. Hormone concentrations were profiled from stranded animals during rehabilitation and over time in healthy captive individuals. Of five rehabilitated dolphins that were serially sampled for 62-100 days, two were released back to the wild, one continued to thrive in captivity, and two died. All of these individuals initially had high cortisol concentrations that declined during treatment regardless of outcome. Generally, thyroid hormone concentrations reflected the outcome of treatment for each stranded individual, increasing in the successfully rehabilitated animals, and declining in those that died. Thyroid hormone concentrations profiled over several years in the 3 captive individuals generally declined with time. Testosterone concentrations appeared to increase during treatment in the 2 successfully rehabilitated males as compared to the male that died. Testosterone concentrations increased over a 6 year time period in the captive male. Progesterone concentrations were low in a juvenile and adult female. Progesterone concentrations reached a maximum of 5.25 ng/ml in the other female which may reflect pubertal status in this individual.

INTRODUCTION

Steno bredanensis is wide-spread throughout most tropical and sub-tropical waters of the world, but few studies have focused on this species of cetacean, especially its biology (Leatherwood and Reeves, 1983; Miyazaki and Perrin, 1994). The number of rough-toothed dolphins held in captive conditions throughout history is very small compared to bottlenose dolphins, which may be partially responsible for its poorly known status.

Cetaceans have been known to strand throughout history, and relatively recent efforts to establish stranding networks involving professionals and volunteers have concentrated on increasing the survival of stranded dolphins and whales. Hematology findings indicate that most of these animals are in ill health at the time of the event (Walsh et al., 1990). Although *S. bredanensis* only occasionally strands (Ellis, 1982), there are records of both mass and individual rough-toothed dolphin strandings from several areas. Mass strandings of *S. bredanensis* have been reported from Florida (Stolen et al., 1999; Wells et al., 1999), Hawaii (Mazzuca et al., 1999), and Virginia (West et al., in prep). At least a few individuals have stranded in Washington and Oregon (Balcomb, 1980; Ferrero et al., 1994), Japan (Miyazaki, 1989), Brazil (Hetzl and Lodi, 1998), the Galapagos (Palacios, 1995; Smith and Whitehead, 1999), Hong Kong (Parsons, 1998), Thailand (Chantrapornsyl et al., 1996), French Polynesia (Gaspar et al., 2000) and the Indonesian archipelago or from Senegal (Cadenat, 1959; Duguy, 1975). However, no accompanying hematology or information pertaining to the endocrinology of this species has been previously investigated in association with these events.

Hormonal monitoring of captive animals provides a means to enhance our understanding of biological and reproductive parameters in marine mammals. Serum analysis is a reliable method that is widely utilized for determining circulating hormone concentrations (Hadley, 1994; St. Aubin, 2001). Cortisol, a primary glucocorticoid secreted by the adrenal gland is often considered as a stress hormone. Cortisol has been studied in the bottlenose dolphin (St. Aubin et al., 1996; St. Aubin, 2001), and several other odontocete species (Suzuki et al., 1998), but levels have not been reported in *S. bredanensis*. Glucocorticoids have many functions, including involvement in immune response, reproduction, growth and development (Fox, 1999). However, investigations into the relationship between cortisol concentrations, health and comparison with other hormones in cetaceans is limited, especially on a species specific basis (St. Aubin, 2001).

Thyroid hormones are secreted by the thyroid gland and are involved in many physiological functions (Ganong, 1999). The principal thyroid hormones are thyroxine (T_4) and tri-iodothyronine (T_3), with the free forms being active at the cellular level. Circulating concentrations of T_4 are higher than T_3 , but T_3 is believed to be the active form (Ganong, 1999). Total T_3 decreases with acute illness or starvation in humans (Larsen and Ingbar, 1992), and both T_3 and T_4 values have been found to be higher in neonates of several pinniped species (Engelhardt and Ferguson, 1980; Haulena et al., 1998; Stokkan et al., 1995; Woldstad and Jenssen, 1999). Consistently higher thyroid hormone concentrations in juvenile bottlenose dolphins versus adults is apparent (West et al., in prep.). A wide range of Total T_4 concentrations have been reported for the bottlenose dolphin, pacific white-sided dolphin, river dolphin, pilot whale, killer whale, harbor porpoise and in beluga whales (St. Aubin, 2001). Thyroid hormone

concentrations (Total T₄, Free T₄, Total T₃ and Free T₃) all decline as pregnancy progresses (West et al., 2001). However, thyroid hormone trends sometimes differ according to the hormone of interest. St. Aubin et al. (1996) determined that wild bottlenose dolphins had higher Total T₄, Free T₄, and Free T₃ than captive animals, but this trend was opposite for Total T₃. Thyroid mechanisms are complex, involving several hormones, and a great deal is yet to be learned about thyroid function in cetaceans.

Testosterone is a primary reproductive hormone secreted by cells in the testes following puberty in males. In cetaceans, baseline testosterone concentrations are only available for a few species, the bottlenose dolphin, spinner dolphin, and beluga whale (Calle et al., 2000; Kirby, 1990). Baseline testosterone concentrations in cetaceans appear to be very low in immature animals, less than 0.1 ng/ml, and range between approximately 1 ng/ml and 70 ng/ml in mature adult bottlenose dolphins (Kirby, 1990). Testosterone concentrations vary according to season in the bottlenose dolphin, as well as in spinner dolphins and beluga whales (Calle et al., 2000; Kirby, 1990; Wells, 1984). For example, in beluga whales mean testosterone concentrations obtained from greater than 20 samples were highest in March at 4.95 ng/ml and only 0.9 ng/ml in September (Calle et al., 2000). Spinner dolphin testosterone values range between 0.1 ng/ml and 65 ng/ml. Testosterone concentrations in bottlenose dolphins are also known to decrease with illness, and appear to be affected by the health of individuals (Kirby, 1990). No testosterone concentrations are available for *S. bredanensis*.

Female reproductive hormones secreted by the ovaries include progesterone and estrogens. Monitoring progesterone concentrations in serial plasma or milk samples have been used to assess ovarian activity in captive bottlenose, spinner, striped and common

dolphins, pilot whales and false killer whales (Atkinson et al., 1999; Kirby and Ridgway, 1984; Wells, 1984; West et al., 2000; Yoshioka et al., 1989). Although baseline progesterone concentrations have been established for six species of odontocetes, the rough-toothed dolphin is not among this group (Kirby, 1990). In *Tursiops truncatus*, baseline progesterone concentrations are considered as anything below 1 ng/ml, ovulatory as fluctuations greater than 1 ng/ml, and pregnancy characterized by progesterone concentrations sustained over 3 ng/ml for an extended period (Kirby, 1990). However, existing data does appear to indicate variations in progesterone concentrations according to different cetacean species. For example, progesterone values related to reproductive status in striped dolphins and short-finned pilot whales caught in Japanese cetacean fisheries indicated a range of 30 to 40 ng/ml during pregnancy for the striped dolphin, but only 3 to 9 ng/ml in the pilot whale (Yoshioka et al., 1989).

Total estrogens have also been measured as an indicator of reproductive condition in bottlenose dolphins. Total estrogen baseline values are estimated at 20 pg/ml, ranging between 23 and 90 ng/ml in pregnant dolphins (Kirby, 1990). Estradiol widely fluctuates in dolphins, and a greater serum sample is required to detect estradiol concentrations than progesterone values in bottlenose dolphins (Yoshioka et al., 1986; West, unpublished data).

METHODS

Study Animals

Serum samples were collected from 7 individual rough-toothed dolphins to determine baseline hormonal values. From each of these individuals, a minimum of 6

samples were included to establish means and ranges for this species. Four of the individual rough-toothed dolphins (Dolphins F-1, F-2, F-3, and F-4) were part of mass stranding events occurring in Florida during late 1997 and 1998. Rehabilitation was attempted for these stranded animals at the Dolphin and Whale Hospital at Mote Marine Laboratory and Aquarium (MOTE) in Sarasota, Florida. During medical treatment and observation blood samples were collected from each of these four individuals on approximately a weekly basis for a 3 to 4 month period. Dolphins F-1, F-2, and F-3 were adult males and F-4 was an adult female. Dolphins F-1 and F-2 were released back to the wild and Dolphins F-3 and F-4 died. While under care, testes for Dolphins F-1 and F-2 were described as sexually mature during ultrasound examination. Upon release, Dolphins F-1 had a body weight of 152.5 kg and body length of 236 cm and Dolphin F-2 weighed 160.4 kg with a length of 247 cm. Upon necropsy, Dolphins F-3 weighed 122.5 kg with a length of 236 cm and Dolphin F-4 weighed 107.2 kg with a length of 237.5 cm. Examination of tooth sections indicated 21+ growth layer groups (glg's) for F-3 and 18-19 glg's for F-4. All length, weight, and glg measurements indicate that Dolphins F-1, F-2, F-3 and F-4 were sexually mature individuals (West et al., in prep).

Three of the sampled animals, Dolphins P, M, and T, were housed at Dolphin Quest French Polynesia (DQFP) which is a natural oceanic environment located within the barrier reef on the island of Moorea, French Polynesia. Dolphin P was initially a young stranded female estimated at less than a year of age that fully recovered and thrived in captivity for three years. Dolphin M was a male that likely reached sexual maturity during the study period based on the length and weight range of sexual maturity attainment for *S. bredanensis* (West et al., in prep.). Dolphin T was an older juvenile

female where sexual maturity had not been attained at the time of sample collection based on absence of corpora upon necropsy. Serum samples were collected on an opportunistic basis from these three animals between the years 1994 and 2000.

Dolphin P was estimated at less than a year of age at the beginning of the sampling period (1997) and weighed approximately 40 kg with a length of 178 cm. This juvenile female grew steadily throughout the study period and by November of 2000 Dolphin P weighed 82 kg and was 208 cm in length. Dolphin M weighed approximately 81 kg with a length of 200 cm at the start of sampling (1994). Dolphin M increased in weight and length to 103.5 kg and 232 cm by the year 2000. Dolphin T was estimated at 91 kg with a length of 211 cm at the start of sampling (1994) and weighed approximately 94 kg with a length of 222 cm at the end of sampling in 1996.

Serum Samples and Analyses

Serum samples were centrifuged to separate constituents and the serum was analyzed for a panel of hormonal values for each of the dolphins. Direct radioimmunoassays (Diagnostic Products Corp., Los Angeles, CA) were performed on each sample to obtain concentrations of cortisol, Total T₄, Free T₄, Total T₃, and Free T₃, progesterone, estradiol and testosterone. All samples were analyzed in duplicate. Aliquots of 50 ul of serum were analyzed to obtain cortisol concentrations, between 25 and 100 ul of serum were used for the thyroid hormones, 100 ul for progesterone, 200 ul for estradiol, and 50 ul for testosterone. Serum samples were not extracted and mean nonspecific binding was <1% for all assays. Inter and intrassay coefficients of variation were <15 and <5%, respectively. Assay sensitivity for the cortisol, Total T₄, Free T₄,

Total T₃ and Free T₃ assays was 0.02 ng/ml, 0.025 ng/ml, 0.1 pg/ml, 7.0 pg/ml, and 0.2 pg/ml, respectively. Sensitivity of each reproductive hormone assay was 0.02 ng/ml for progesterone, 1.4 pg/ml for estradiol, and 0.04 ng/ml for testosterone. Concentrations of all hormones were read from a log-logit transformed standard curve (Rodbard, 1974).

Analyses included establishing overall means and ranges for each of the separate hormone concentrations assayed. Hormonal profiles were also separated according to individual, and each hormone except estradiol plotted during rehabilitation of the stranded animals. Female samples analyzed for estradiol concentrations were too low to be detectable and were therefore not profiled over time. From the three individuals housed at DQFP, reproductive hormone concentrations were profiled over a two to five year interval, but frequency of serum sampling during this much longer period was substantially less than the dolphins at MOTE. Thyroid hormones, progesterone and testosterone concentrations were profiled over time for Dolphins P, M and T. For Dolphin P, cortisol concentrations were profiled during the first 100 days of rehabilitation for comparison with the individuals at MOTE. However, thyroid hormones and progesterone concentrations in Dolphin P were profiled over a longer time interval, between 1997 and 2000.

Statistical Analyses

Using MINITAB, a scientific statistical software package, regression analyses were performed on the concentration of thyroid and cortisol hormones in stranded individuals from MOTE to examine the influence of the length of rehabilitation on hormonal changes. For the 3 DQFP individuals sampled over a longer time period,

regression analyses were performed on thyroid hormone concentrations. MINITAB was used for ANOVA analysis of thyroid hormone concentrations between individuals and the Tukey's multiple comparisons test used to determine if thyroid hormone concentrations in the juvenile *S. bredanensis*, Dolphin P, were significantly different than the adult individuals and two animals that likely were pubertal during part of the sampling period. Regression analyses were also performed on testosterone and progesterone concentrations to examine rehabilitation efforts at MOTE and time at DQFP on reproductive hormonal changes.

RESULTS

Hormone Analyses:

Hormone concentrations measured in *S. bredanensis* are summarized in Table 1. Cortisol concentrations averaged 18.6 ng/ml and ranged between 0 and 130.4 ng/ml when including both stranded and healthy individuals (Table 1). Thyroid hormone concentrations also showed a large range of values, averaging 84.1 ng/ml for Total T₄, 10.2 pg/ml for Free T₄, 1071.7 pg/ml for Total T₃, and 1.06 pg/ml for Free T₄ (Table 1).

Five of the 7 rough-toothed dolphins were initially stranded individuals. These 5 animals were in ill health at the start of the sampling period and rehabilitation efforts attempted. In all of the stranded animals, cortisol concentrations were higher at the beginning of the sampling period and dropped as rehabilitation continued (Figure 1a and b). For Dolphins F-1, F-2, F-3, F-4 and P there was a general decline in cortisol concentrations over time. This trend was statistically significant for Dolphin F-4 ($F = 16.6$, $df = 1,16$, $P < 0.001$) and Dolphin P ($F = 8.0$, $df = 1,20$, $P = 0.01$) and nearly

significant for Dolphin F-1 ($F = 4.2$, $df = 1,6$, $P = 0.087$), Dolphin F-2 ($F = 4.9$, $df = 1,8$, $P = 0.057$) and Dolphin F-3 ($F = 4.8$, $df = 1,8$, $P = 0.059$). Peak cortisol concentrations reached a high of 130.4 ng/ml in one individual soon after arrival at the Dolphin and Whale Hospital, but this value declined during treatment and this animal ultimately survived and was released. In all 5 animals, whether surviving or not, cortisol concentrations had dropped to below 50 ng/ml by day 50 of rehabilitation. Cortisol concentrations on a longer time scale (years) from the DQFP animals were low for Dolphin P, M, and T, the highest value was less than 6 ng/ml.

The different thyroid hormone concentrations, measured as Total T_4 , Free T_4 , Total T_3 or Free T_3 , tended to follow the same general pattern in each of the individuals profiled during rehabilitation from MOTE (Figure 2a and b, 3a and b, 4a and b, and 5a and b). The overall health of each rehabilitated animal was closely reflected in thyroid hormone concentration trends. Dolphins F-1 and F-2 showed increasing thyroid hormone concentrations through the study period. This trend was statistically significant for F-1 with Total T_4 ($F = 13.0$, $df = 1,5$, $P = 0.015$) and nearly significant for Total T_3 ($F = 5.2$, $df = 1,5$, $P = 0.072$); the trends for Free T_4 and Free T_3 were not statistically significant (P 's > 0.05). For F-2 the trend was statistically significant for Free T_4 ($F = 10.6$, $df = 1,8$, $P = 0.012$) and Total T_3 ($F = 8.6$, $df = 1,8$, $P = 0.019$); the trends for Total T_4 and Free T_3 were not statistically significant (P 's > 0.05). Both of these individuals were successfully treated and released back to the wild (Figure 2a and b, and 3a and b). Dolphins F-3 and F-4 were also treated throughout the study period, but both of these animals died, and accordingly a declining trend in thyroid hormone concentrations over the duration of sampling was apparent (Figure 4a and b, Figure 5a and b). There was a

significantly negative trend throughout treatment for F-3 with Free T₄ ($F = 10.6$, $df = 1,8$, $P = 0.012$) but the trends for Total T₄, Total T₃ and Free T₃ were not statistically significant (P 's > 0.05). For F-4 there was a statistically significant negative trend for Total T₄ ($F = 13.8$, $df = 1,16$, $P = 0.002$) and Free T₄ ($F = 4.6$, $df = 1,16$, $P = 0.048$); there was no statistically significant trend for Total T₃ or Free T₃ (P 's > 0.05).

Thyroid hormone concentrations indicated a general dropping tendency with time in Dolphins P, M, and T (Figures 6a and b, 7a and b, and 8a and b). This trend was statistically significant for Dolphin P for Free T₄ ($F = 8.1$, $df = 1,16$, $P = 0.012$). For Dolphin M the negative trend was statistically significant for Total T₄ ($F = 7.7$, $df = 1,13$, $P < 0.016$) and Free T₃ ($F = 5.70$, $df = 1,14$, $P = 0.032$). For Dolphin T the negative trend was statistically significant for Free T₃ ($F = 8.64$, $df = 1,4$, $P = 0.042$). The other trends were not statistically significant for the number of samples-days measured (Figures 6, 7 and 8).

There were highly significant differences in thyroid hormones concentrations among the 7 individuals (Total T₄: $F = 16.5$, $df = 6,80$, $P < 0.001$; Free T₄: $F = 10.6$, $df = 6,75$, $P < 0.001$; Total T₃: $F = 35.1$, $df = 6,83$, $P < 0.001$; Free T₃: $F = 17.6$, $df = 6,80$, $P < 0.001$). In all cases, Dolphin P had higher hormone values that were statistically significant when compared to Dolphins F-1, F-2, F-3, and F-4 using the Tukey's multiple comparison test following the ANOVA analysis. Dolphin P also had significantly higher hormone values than Dolphins M and T for Total T₄, Total T₃, Free T₃ but not Free T₄ using the Tukey's multiple comparisons test.

Testosterone values for male *S. bredanensis* averaged 1.93 ng/ml and ranged between 0.04 and 6.33 ng/ml (Table 1). For Dolphins F-1 and F-2 testosterone

concentrations appeared to increase over rehabilitation and decrease for Dolphin F-3 (Figure 9a). This trend was only statistically significant for Dolphin F-1 ($F = 7.1$, $df = 1,5$, $P = 0.045$). In the rehabilitated males, testosterone concentrations only reached a value greater than 5 ng/ml for Dolphins F-1 and F-2. Testosterone concentrations were profiled over a 6 year period for Dolphin M and increased significantly ($F = 10.2$, $df = 1,31$, $P = 0.003$). The maximum recorded testosterone concentration for Dolphin M was 5.79 ng/ml (Figure 10).

Recorded progesterone concentrations in *S. bredanensis* ranged between 0 and 5.25 ng/ml with an average value of 0.38 ng/ml (Table 1). Progesterone concentrations profiled during rehabilitation for Dolphin F-4 and over time for Dolphins P and T did not exhibit a clear trend (Figure 9b and Figure 11a and b). Values did not rise above baseline in either Dolphin F-1 or Dolphin P but fluctuated for Dolphin T, reaching a recorded maximum of near 5 ng/ml on two occasions (Figure 9b and Figure 11a and b).

DISCUSSION

Cortisol concentrations in rough-toothed dolphins showed a large range of values, with concentrations as high as 130 ng/ml which is higher than bottlenose dolphin ranges (St. Aubin et al. 1996), and much higher than averages reported for several odontocete species studied from Japanese fisheries (Suzuki et al., 1998). In the bottlenose dolphin cortisol concentrations have been found to rise to more than three times baseline when the animal undergoes capture and handling stress (Thompson and Geraci, 1986). Our average value of 18.6 ng/ml for the 89 samples analyzed for cortisol concentrations in *S. bredanensis* does not differentiate between animals in good versus poor health nor

according to time spent in captivity (Table 1). A more useful baseline value for cortisol concentrations in this species is obtained from values profiled over long time periods in the 3 healthy animals at DQFP. During sampling of the healthy animals, cortisol concentrations were generally $< 1\text{ ng/ml}$ which agrees with the lower values reached in rehabilitated dolphins as treatment progressed and the initially high cortisol values fell (Figure 1a and b). General health problems in the stranded *S. bredanensis* individuals and profiled cortisol concentrations during rehabilitation are consistent with the relationship between high cortisol and leukocytosis in most mammals (St. Aubin, 2001). Veterinary evaluation of the rehabilitated dolphins at the Dolphin and Whale Hospital indicated neutrophilia and elevated white blood cell counts in these animals upon arrival. The stranded rough-toothed dolphin housed at Dolphin Quest French Polynesia was also in ill health at the time of arrival and clearly appeared to have undergone starvation. Higher cortisol concentrations found in all of these individuals at the start of rehabilitation is not surprising, and it is possible that cortisol does reflect a stress response in *S. bredanensis*, at least in relation to either physiological demands associated with ill health or acclimation to new environmental surroundings. Cortisol concentrations at the start of rehabilitation may provide expected values for this species when experiencing acute medical stresses or starvation. However, although Dolphins F-3 and F-4 eventually died, cortisol concentrations had stabilized about halfway through treatment and did not rise near death (Figure 1b). It is unclear if the decline in cortisol concentrations as treatment progressed in our study animals was related to health status, captivity acclimation or a combination of both factors. Further study to determine

cortisol concentrations in healthy cetaceans immediately after captive exposure is needed for clarification.

Thyroid hormone concentrations obtained for *S. bredanensis* were compared with published values for other odontocete species as summarized by St. Aubin (2001). Total T₄ averages are available for 7 other species; T₃ values have been previously reported for only two species. Total T₄ values among the different species widely range between 15 and 192 ng/ml and Free T₄ between 13.6 and 39.9 pg/ml (St. Aubin, 2001). Our findings which include both healthy and unhealthy *S. bredanensis* individuals are within the range of all the other species, between 26.2 and 197.7 ng/ml for Total T₄, but are in the lower than the end of the range available for Free T₄ in other species (Table 1). Total T₃ and Free T₃ values in *S. bredanensis* also exceeded the higher and lower end of the range available for cetaceans, but this is in comparison to only 2 other species (St. Aubin, 2001). In order for cetacean values to have optimum usefulness from a diagnostic perspective, the wide ranges available for all thyroid hormones need to be investigated according to factors likely to affect thyroid function.

Many factors may affect baseline values as the thyroid gland is involved in a large number of physiological functions such as metabolism and growth. Even for the bottlenose dolphin, *T. truncatus*, which is often considered the model cetacean species, values have only been presented as overall averages and ranges except in one study by St. Aubin et al. (1996). The difficulties involved in obtaining large numbers of serum samples from cetaceans makes distinctions between factors challenging, but averages from at least 50 samples from each age/sex class in bottlenose dolphins have shown considerable differences (West et al., in prep.). In *T. truncatus*, male and female

concentrations are very similar for Total T₄, Free T₄, and Total T₃, but concentrations are significantly higher in both male and female juveniles compared with adults (West et al., in prep.). In our study of *S. bredanensis* we had between 81 and 89 serum samples, depending on the thyroid hormone, which provides a good starting point for establishing baseline values in this species. Although we only had one young juvenile rough-toothed dolphin for comparison with adults and animals likely of pubertal status, higher juvenile values were found to be statistically significant for *S. bredanensis* which is similar to the bottlenose dolphin trends (West et al., in prep.). Overall average Total T₄ concentrations in the juvenile female *S. bredanensis* was 120 ng/ml compared with an average of 77.2 ng/ml in the adults and pubertal animals. Findings were similar for Free T₄ and Total T₃, 14.2 pg/ml vs. 9.2 pg/ml, and 1694 pg/ml vs. 916 pg/ml, respectively, in the juvenile versus the adults and pubertal animals. Our findings in healthy *S. bredanensis* individuals sampled over several years also indicated a slight decline in thyroid hormone values as each animal aged which was statistically significant for Free T₄ in Dolphin P, Total T₄ in Dolphin M, and Free T₃ in Dolphin T (Figures 6, 7 and 8). None of these 3 individuals were considered adults at the start of sampling. Further studies to establish values according to age and sex classes in healthy individuals of various species will aid in providing narrower thyroid hormone baselines.

Thyroid hormones in cetaceans are likely to reflect clinical conditions, but there is only one report of this in a bottlenose dolphin where a T₄ drop was believed to be associated with candidiasis (Greenwood and Barlow, 1979). Determination of cortisol concentrations and thyroid hormones in the harbor seal have suggested studies to compare clinical states with hormonal changes which would be useful in monitoring the

health of captive seals (Riviere et al., 1977). Stress certainly suppresses thyroid hormone concentrations in the beluga whale, believed to be due to capture and handling (St. Aubin and Geraci, 1988). Low thyroid values were maintained over time in the beluga, and reduced caloric intake may also be a possible explanation as to why thyroid feedback mechanisms did not re-elevate concentrations in this study of thyroid function (St. Aubin and Geraci, 1988). Physiological stresses are difficult to distinguish from each other, and altered nutritional state and specific clinical conditions have similar effects on thyroid hormone values in humans (Larsen and Ingbar, 1992). Both chronic or acute illnesses and starvation change human thyroid hormone economy as indicated by declines in T_3 concentrations. In humans, the response of various thyroid hormones is sometimes different according to the organ system affected, but T_3 concentrations consistently drop with illness (Larsen and Ingbar, 1992).

Our findings for rehabilitated *S. bredanensis* individuals demonstrate a general relationship between health status and thyroid hormone concentrations. The only individual where no significant relationship was apparent is within the first 100 days of sampling of Dolphin P, while this stranded dolphin was rehabilitated. This is likely due to less samples and greater intervals between days sampled in this specific animal. In the other 4 rehabilitated dolphins, thyroid hormone values did fluctuate, but increasingly rose in those individuals that were successfully treated and deemed releasable back to the wild (Figure 2a and b, Figure 3a and b), and decreased in those that died (Figure 4a and b, Figure 5a and b). Free T_3 values followed the same overall pattern as the other thyroid hormones investigated during rehabilitation (Figure 2a and b, Figure 3a and b, Figure 4a and b, Figure 5a and b), but was the only thyroid hormone of the four investigated that

did not show a statistically significant incline or decline in at least one of the animals. Total T₄ and Total T₃ trends agreed with patterns in Free T₄, which was statistically significant in most cases. Out of all thyroid hormone measurements, perhaps Free T₄ is one of the better indicators of general health status for *S. bredanensis*. Certainly, Free T₄ is the most commonly used indicator of the thyroid hormones in human medicine for diagnosing various forms of thyroid disease (Fitzgerald, 2000; Ganong, 1999).

Testosterone concentrations for *S. bredanensis* exhibited a similar range to that available for other species such as the beluga whale and bottlenose dolphin, often measuring between 0 and 5 ng/ml (Calle et al., 2000; Kirby, 1990). In *T. truncatus* testosterone levels in mature males tend to fluctuate between 2 and 5 ng/ml, and rise to greater than 10 ng/ml during the breeding season (Robeck et al., 2001). For *S. bredanensis* all 4 males had values within this range but breeding seasonality was not investigated. The 3 rehabilitated dolphins were only under care between the months of December and March, and testosterone levels reached maximum values in March for the animals that were released but a low in the dolphin that died (Figure 9a). In the individual sampled opportunistically over several years, concentrations greater than 5 ng/ml were reached in October and November in two out of three years which may suggest seasonality trends. Regular sampling throughout the year for several years is needed to clarify potential seasonal patterns in this species. Dolphins F-1, F-2, and F-3 were classified as mature adults according to both testicular size and length and weight measurements consistent with sexual maturity in this species (West et al., in prep). Dolphin M likely attained sexual maturity during the study period, puberty in *T. truncatus* is described by an increase in testosterone levels from less than 1 ng/ml to 10

ng/ml and the first rise to 2.79 ng/ml for Dolphin M occurred in 1994. Dolphin M testosterone levels were consistently recorded above 1 ng/ml towards the end of the study period (Figure 10).

Although our sample size was small, findings from the 3 rehabilitated *S. bredanensis* males suggest that testosterone concentrations are suppressed with illness in *S. bredanensis* as has been found for *T. truncatus*. Dolphins F-1 and F-2 were initially in ill health, Dolphin F-1 presented with neutrophilia, elevated liver values, tachycardia and bilateral congestion of both lungs. Testosterone values were below 2 ng/ml during the initial treatment of Dolphin F-1 but had increased to 5 ng/ml immediately prior to release. Necropsy findings from Dolphin F-3 indicated cardiac and intestinal disease, and the maximum testosterone concentration of 3.4 ng/ml was reached relatively early in the rehabilitation process, prior to serious deterioration.

In *S. bredanensis* progesterone concentrations ranged between 0 ng/ml and 5.25 ng/ml and this suggests follicular secretion of small amounts of progesterone in Dolphin T (Figure 11b). It is difficult to detect ovarian cycling using only progesterone concentrations unless samples are collected on a frequent basis. Serum samples were collected regularly during rehabilitation efforts for Dolphin F-4. Frequency ranged between 3 days and one week for almost four months in this adult female but concentrations did not rise above baseline. It is unknown what effect the ill health of Dolphin F-4 may have on reproductive activity, this individual was euthanized due to extreme pain from a non-responsive intestinal infection. In the juvenile female, Dolphin P, only baseline progesterone concentrations were apparent which is in agreement with an immature animal. Progesterone concentrations fluctuated above baseline for Dolphin

T, spiking to 4.67 ng/ml in January which dropped and rose again in June of the same year to 5.25 ng/ml before dropping to baseline again. Unfortunately, samples were only opportunistically collected for this individual on an infrequent basis. At the time of necropsy, no corpora were obvious, but a follicle was observed which may be responsible for small amounts of progesterone secretion. It is possible that the progesterone concentration spikes above 1 ng/ml are associated with the gradual attainment of sexual maturity in *S. bredanensis*. The body length at the time of death in Dolphin T was within the length range of 212-217 cm where sexual maturity is reached in this species (West et al., in prep.).

Hormonal values presented for healthy and ill individuals of *S. bredanensis* will provide a useful reference for clinicians and scientists. Additionally, baseline values determined in our study contribute towards a greater understanding of the endocrinology of this relatively unknown species of cetacean.

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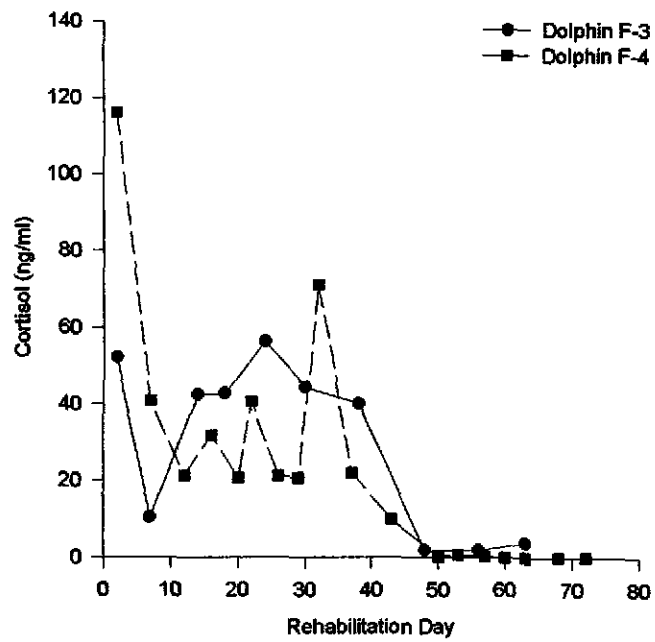
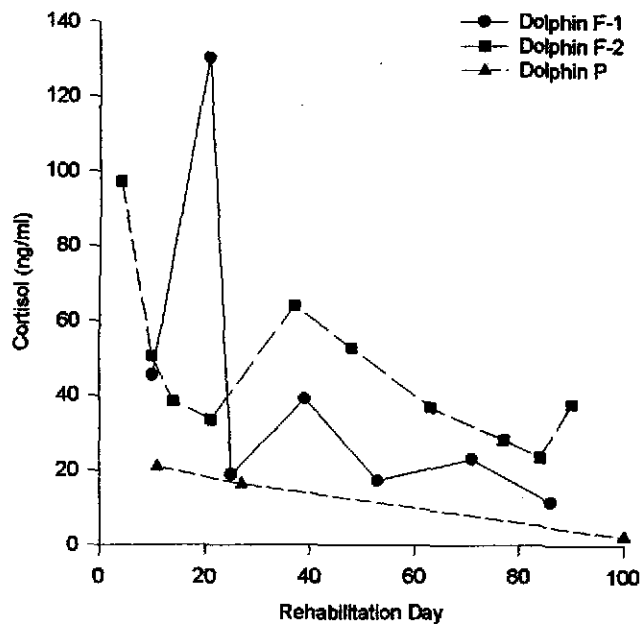
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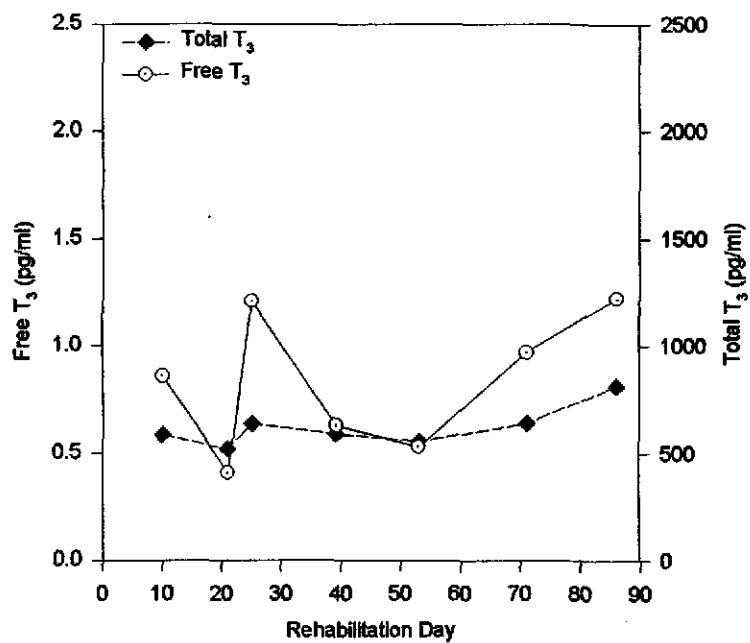
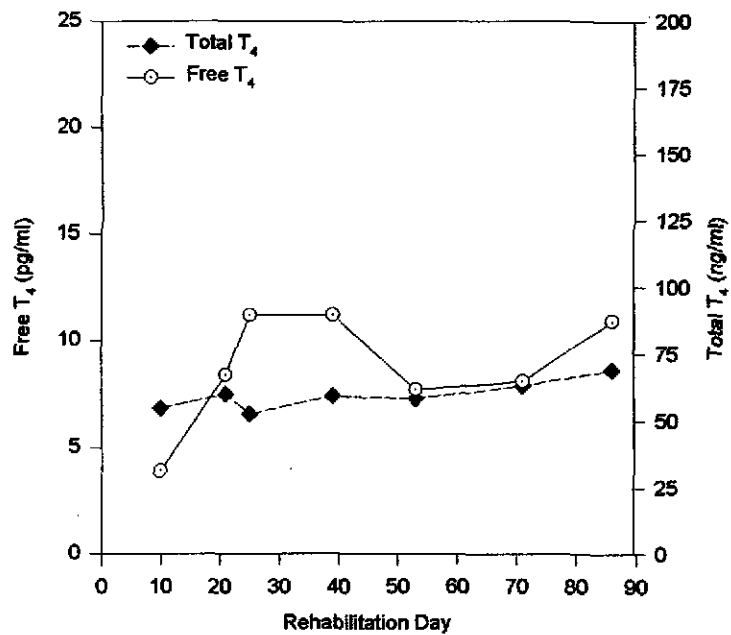
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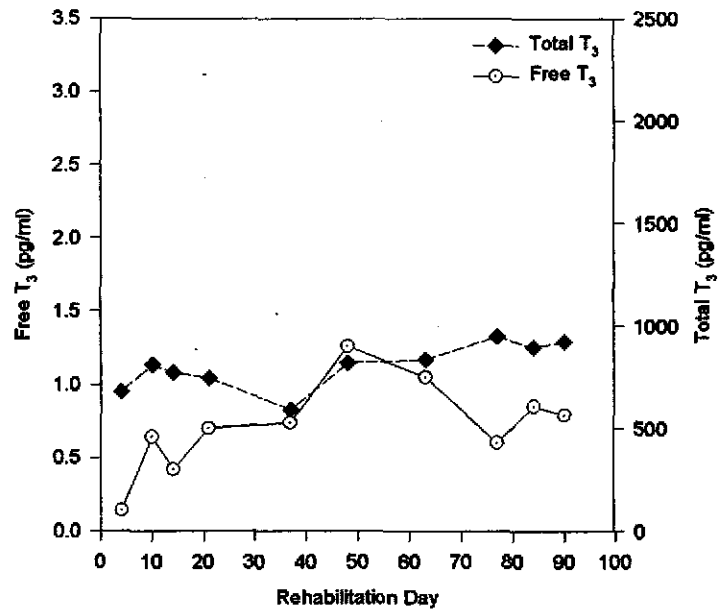
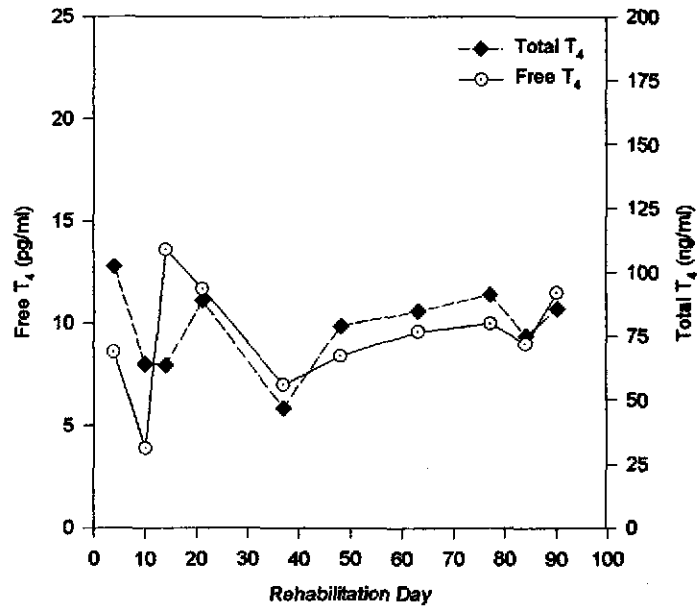
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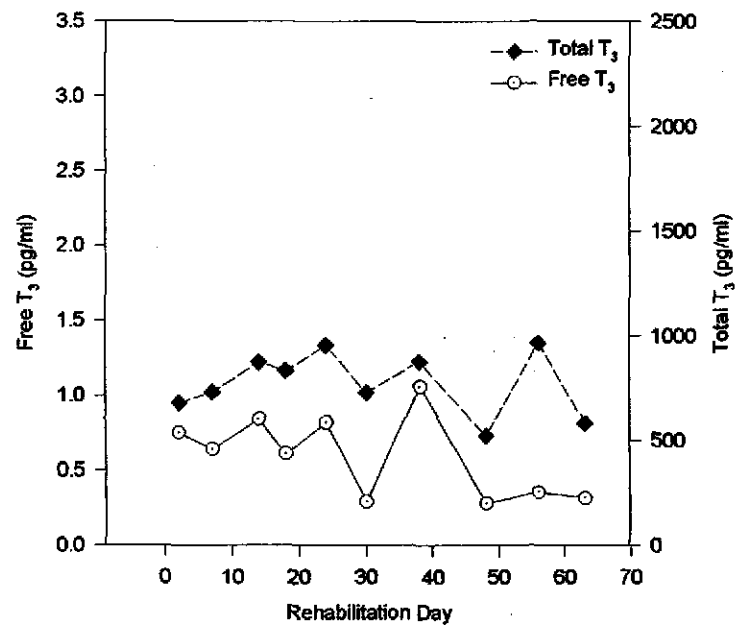
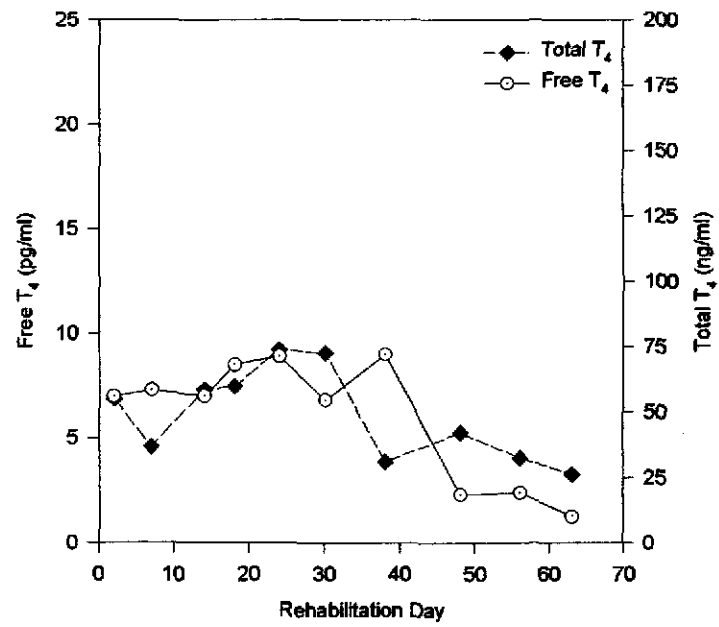
Table 1. Cortisol, Total T₄, Free T₄, Total T₃, and Free T₃, progesterone and testosterone in serum samples collected from seven *Steno bredanensis* individuals.

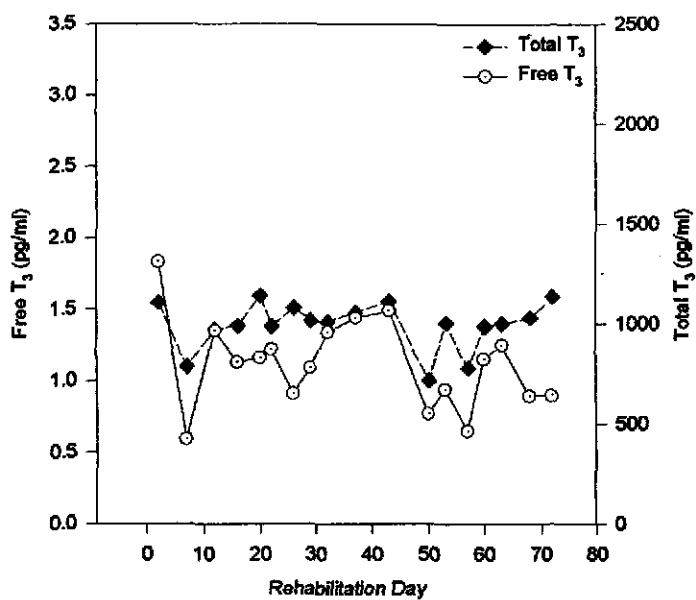
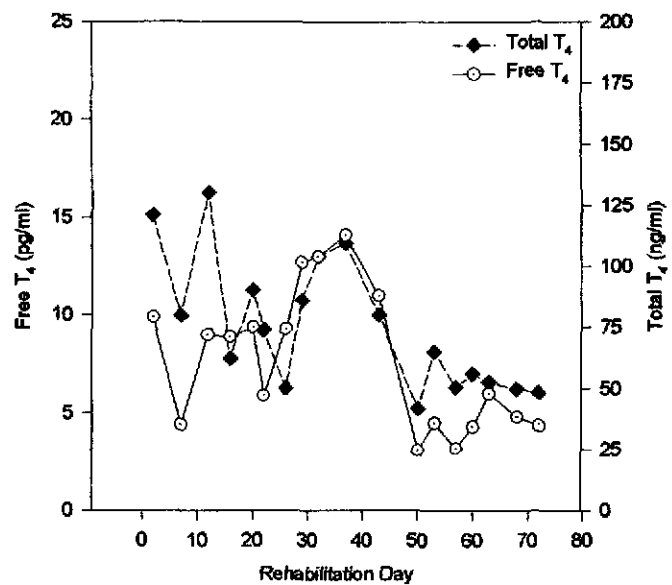
	Cortisol (ng/ml) n=89	Total T ₄ (ng/ml) n=86	Free T ₄ (pg/ml) n=81	Total T ₃ (pg/ml) n=89	Free T ₃ (pg/ml) n=86	Progesterone (ng/ml) n=35	Testosterone (ng/ml) n=59
Mean:	18.6	84.1	10.2	1071.7	1.06	0.38	1.93
Standard Deviation:	25.4	32.1	4.3	425.1	0.56	1.15	1.76
Range:	0 -130.4	26.2 - 197.7	1.3 - 22.4	521.0 - 2451.0	0.04 - 2.3	0-5.25	0.04-6.33

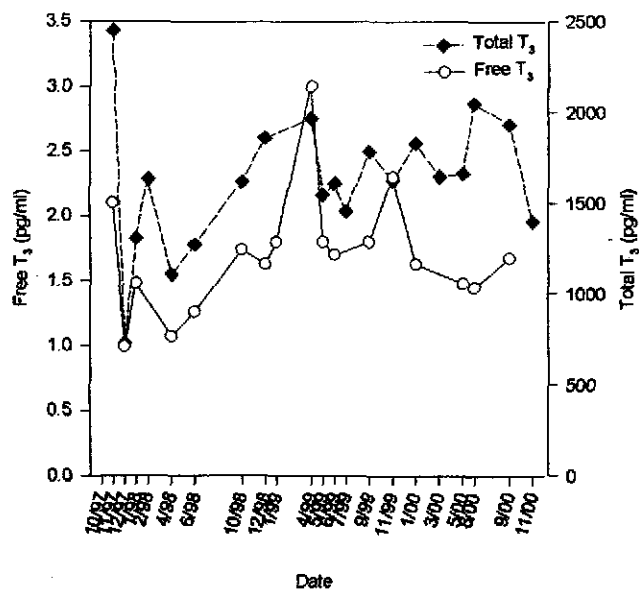
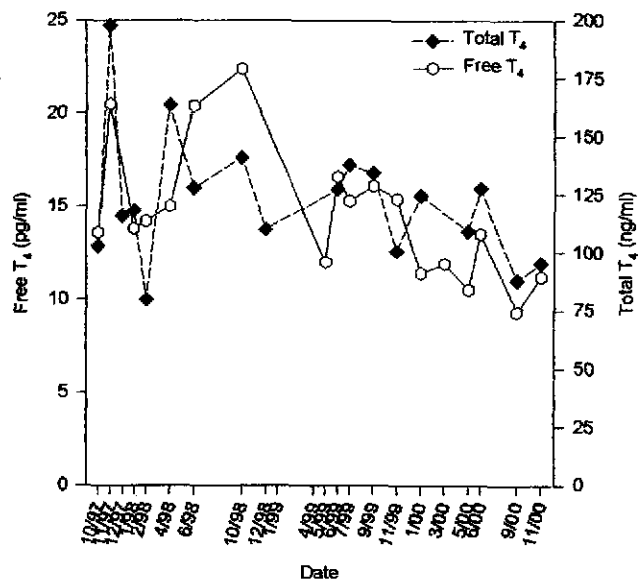


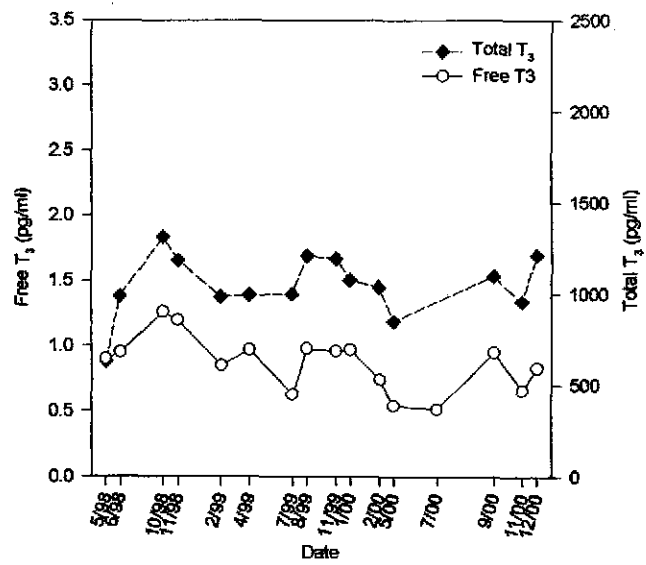
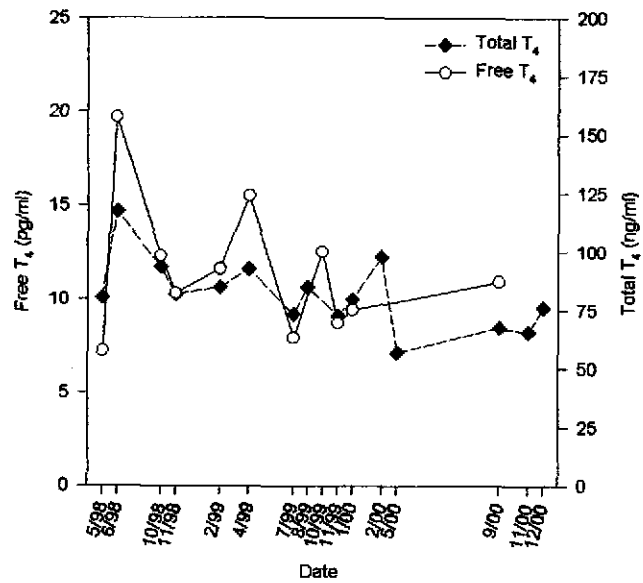


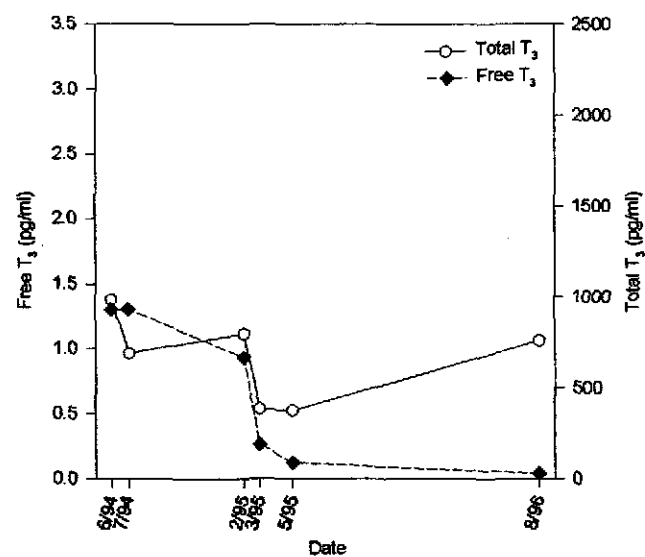
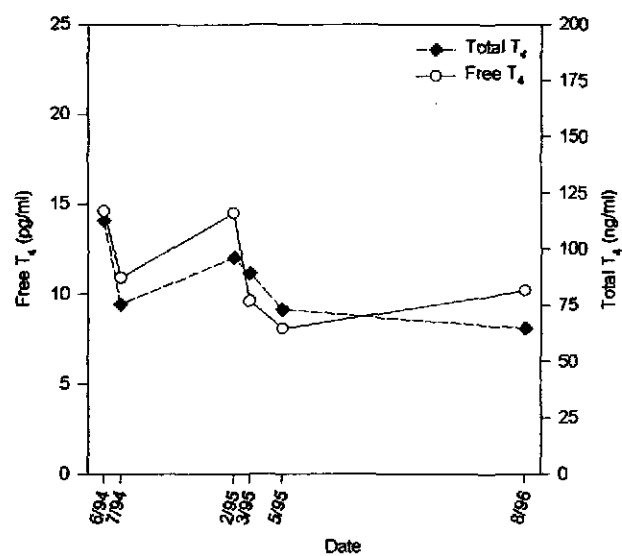


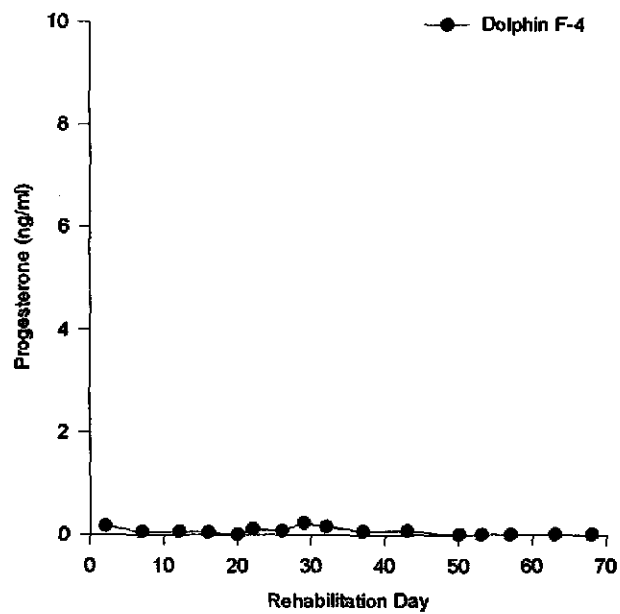
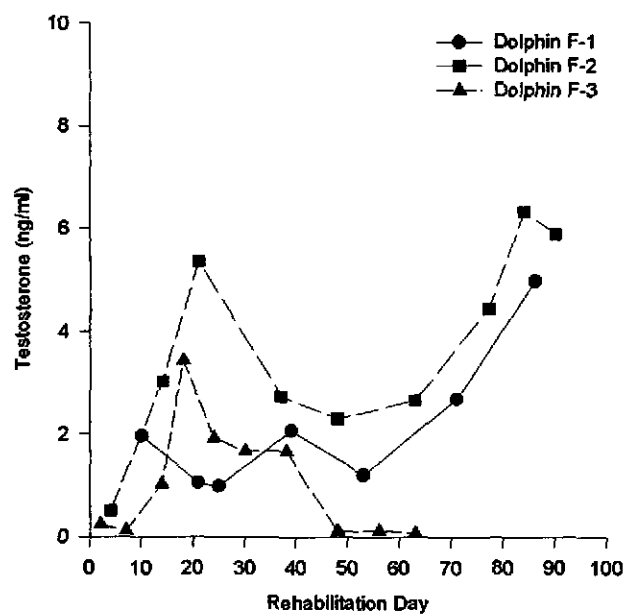


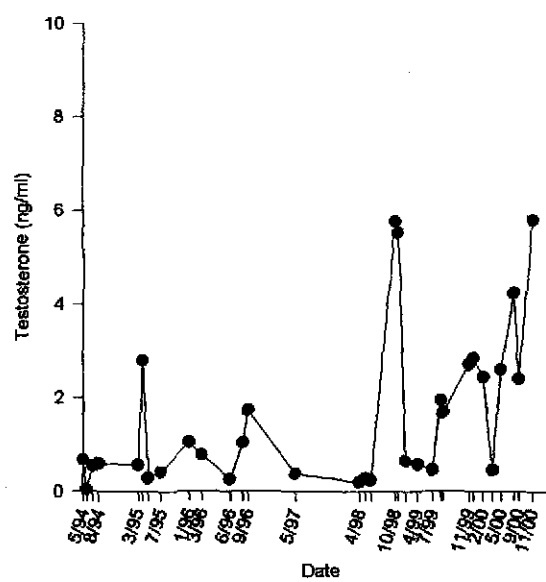












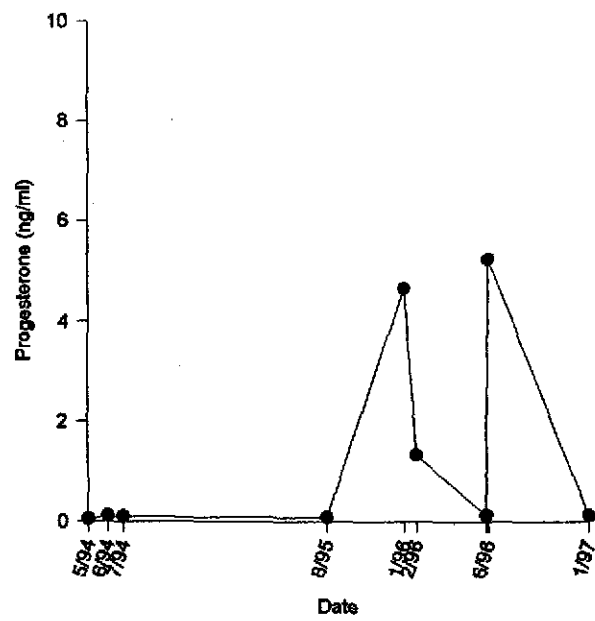
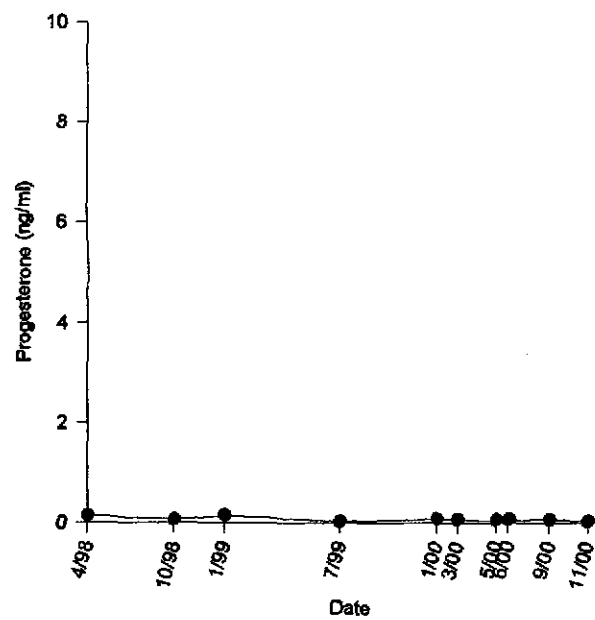


Figure 1: Cortisol concentrations profiled against time in rehabilitation for five stranded *S. bredanensis* individuals. Animals which survived (a) and those that died (b) following rehabilitation efforts. The regression equation for Dolphin F-1 cortisol = $33871.6 - 0.944358 \text{ date}$; Dolphin F-2 cortisol = $14595.6 - 0.406131 \text{ date}$; Dolphin P cortisol = $318.066 - 0.0086683 \text{ date}$; Dolphin F-3 cortisol = $22725.8 - 0.627146 \text{ date}$; Dolphin F-4 cortisol = $33739.3 - 0.931488 \text{ date}$.

Figure 2: Thyroid hormone concentrations profiled against time in rehabilitation in Dolphin F-1, released back to the wild from Mote Marine Laboratory and Aquarium. Thyroxine concentrations against time (a) and tri-iodothyronine concentrations against time (b). The regression equation for Dolphin F-1 Total $T_4 = 52.2365 + 0.167031 \text{ date}$; Free $T_4 = 7.10583 + 0.0382268 \text{ date}$; Total $T_3 = 514.319 + 2.44515 \text{ date}$; Free $T_3 = 0.646245 + 0.0042829 \text{ date}$.

Figure 3: Thyroid hormone concentrations profiled against time in rehabilitation in Dolphin F-2, released back to the wild from Mote Marine Laboratory and Aquarium. Thyroxine concentrations against time (a) and tri-iodothyronine concentrations against time (b). The regression equation for Dolphin F-2 Total $T_4 = 74.8109 + 0.0749809 \text{ date}$; Free $T_4 = 8.57937 + 0.0267552 \text{ date}$; Total $T_3 = 691.561 + 2.45624 \text{ date}$; Free $T_3 = 0.489810 + 0.0051158 \text{ date}$.

Figure 4: Thyroid hormone concentrations profiled against time in rehabilitation in Dolphin F-3. This animal later died at Mote Marine Laboratory and Aquarium. Thyroxine concentrations against time (a) and tri-iodothyronine concentrations against time (b). The regression equation for Dolphin F-3 Total $T_4 = 62.2815 - 0.451051 \text{ date}$; Free $T_4 = 9.22222 - 0.105741 \text{ date}$; Total $T_3 = 809.320 - 1.20067 \text{ date}$; Free $T_3 = 0.811967 - 0.0071656 \text{ date}$.

Figure 5: Thyroid hormone concentrations profiled against time in rehabilitation in Dolphin F-4. This animal later died at Mote Marine Laboratory and Aquarium. Thyroxine concentrations against time (a) and tri-iodothyronine concentrations against time (b). The regression equation for Dolphin F-4 Total $T_4 = 106.247 - 0.836680 \text{ date}$; Free $T_4 = 10.5310 - 0.0772159 \text{ date}$; Total $T_3 = 1001.09 - 1.89330 \text{ date}$; Free $T_3 = 1.30326 - 0.0050205 \text{ date}$.

Figure 6: Thyroid hormone concentrations profiled against time for Dolphin P. This dolphin thrived in captivity at Dolphin Quest French Polynesia beyond initial rehabilitation efforts. Thyroxine concentrations against time (a) and tri-iodothyronine concentrations against time (b). The regression equation for Dolphin P Total $T_4 = 135.1980 - 0.728710482 \text{ date}$; Free $T_4 = 17.8965 - 0.1681965959 \text{ date}$; Total $T_3 = 1446.8482 - 9.13187 \text{ date}$; Free $T_3 = 1.5360 - 0.009083 \text{ date}$.

Figure 7: Thyroid hormone concentrations profiled against time for Dolphin M. This captive dolphin was sampled for thyroid hormone concentrations for 2.5 years at Dolphin Quest French Polynesia. Thyroxine concentrations against time (a)

and tri-iodothyronine concentrations against time (b). The regression equation for Dolphin M Total $T_4 = 95.8264 - 0.9267 \text{ date}$; Free $T_4 = 12.8212 - 0.1183710534 \text{ date}$; Total $T_3 = 995.0345 + 3.4012401694 \text{ date}$; Free $T_3 = 1.05498 - 0.0118243728 \text{ date}$.

Figure 8: Thyroid hormone concentrations profiled against time for Dolphin T. This captive dolphin was sampled for thyroid hormone concentrations for 2 years at Dolphin Quest French Polynesia. Thyroxine concentrations against time (a) and tri-iodothyronine concentrations against time (b). The regression equation for Dolphin T Total $T_4 = 98.0426 - 1.2615 \text{ date}$; Free $T_4 = 12.5866 - 0.1249145461 \text{ date}$; Total $T_3 = 713.2837 - 4.8803646031 \text{ date}$; Free $T_3 = 1.1858 - 0.0518837828 \text{ date}$.

Figure 9: Testosterone and progesterone concentrations profiled against time in rehabilitation for 4 stranded dolphins at Mote Marine Laboratory and Aquarium. Dolphins F-1 and F-2 survived and were eventually released and F-3 and F-4 died. Testosterone concentrations against time (a) and progesterone concentrations against time (b). The regression equation for Dolphin F-1 Testosterone = $0.4505 + 0.0386774749 \text{ date}$; Dolphin F-2 Testosterone = $1.7246 - 0.0400074405 \text{ date}$; Dolphin F-3 Testosterone = $1.4806 - 0.0148206048 \text{ date}$; Dolphin F-4 Progesterone = $0.1203 - 0.0016844240483 \text{ date}$.

Figure 10: Testosterone concentrations profiled against time for Dolphin M. This captive dolphin was sampled for testosterone concentrations for 6 years at Dolphin Quest French Polynesia. The regression equation for Dolphin M Testosterone = $0.2708 + 0.0010516426106 \text{ date}$.

Figure 11: Progesterone concentrations profiled against time for Dolphins P and T. These captive dolphins were sampled for progesterone concentrations for 2 years for Dolphin P (a) and 2.5 years for Dolphin T (b). The regression equation for Dolphin P Progesterone = $0.12219 - 0.000079344973259 \text{ date}$; Dolphin T Progesterone = $0.1203 - 0.0016844240483 \text{ date}$.

SYNTHESIS

The overall goal of this study on *S. bredanensis* was to provide scientific data that will directly contribute to quantification of characteristics representative of ecologically healthy and unhealthy populations of this species. Establishing basic information on a relatively unknown dolphin and advancing the current state of knowledge for *S. bredanensis* will lead to assessment means and ultimately proactive measures for addressing environmental impacts to this cetacean. This study included research into live wild populations, dead specimens, and captive individuals. Current knowledge on distributional patterns and status of free-ranging populations of *S. bredanensis* is compared with information available for other relatively well known species of small dolphins. The adaptational response of delphinid populations to exploitation pressures and environmental stressors is illustrated with examples of distributional and reproductive parameter shifts that have been demonstrated in other species. A discussion of population level effects is followed by an analysis of endocrinology data from individuals of the species *S. bredanensis*. The individual rough-toothed dolphins investigated include both healthy animals and those experiencing physiological stress, providing an opportunity to obtain comparative data. Establishing baseline biological and ecological parameters for *S. bredanensis* on a population and individual scale contribute to needed species knowledge for this relatively unknown cetacean.

Adaptation of Wild Populations

The first response phase which includes alarm and adaptation is a stage where definition of species specific characteristics to stressors is extremely valuable. Stressors

do not cause the same degree of stress among cetacean species (St. Aubin, 2001). If it is possible to detect suspected stressors early in the overall biological response pattern of a species, the likelihood of implementing preventative measures to avoid the final stage of this destructive process increases. However, at the time of the actual effect of a given stressor on individuals, direct application of techniques for assessing the initial alarm response presents difficulties. Cardiac tissue contraction band necrosis and ischemic injury to organs in many species of cetacean suggests initial alarm but histological analyses only become possible once these animals have already died and carcasses are subsequently recovered (Cowan, 2000). Additionally, histological investigation into the alarm response has not yet been undertaken for the species *S. bredanensis*. The adaptation response which is also apparent during this first stage may provide an easier means to assess population responses in live, wild individuals. Once patterns and normal behaviors for the species *S. bredanensis* are determined, populations can be monitored and comparative research techniques used to detect changes in these established patterns.

It is probable that changes in the distributional pattern of cetacean populations in response to environmental stressors commonly occurs, but is not documented at the time of the event. It is rare to find available baseline data prior to impact effects. Additionally, it is difficult to prove cause and effect when environmental changes are suspected as the causative agent resulting in population pattern shifts. A combination of these factors likely manifests in undetected stress to cetacean populations during early stages of the biological stress response, in which case the problem does not become apparent until it has reached a more advanced and severe stage.

It is likely that the adaptation response of wild populations of *S. bredanensis* to environmental stressors would be reflected in changes of habitat use. As cetaceans spend their entire lives in the marine environment and the source of known potential stressors all involve the immediate surroundings of these animals, a shift in habitat preference should be detectable on a population level. Defined distributional patterns for *S. bredanensis* provide a starting point for initial assessments of the population adaptation response to environmental changes.

This research on *S. bredanensis* describes preferred habitat characteristics and distributional patterns for this type of dolphin in a small oceanic island environment. This provides a significant contribution to available species knowledge since establishing distributional patterns for *S. bredanensis* is challenging as live, wild populations of this dolphin tend to be somewhat inaccessible for study. The consistent presence of rough-toothed dolphins in Polynesian waters was first identified by Leatherwood et al. (1982). The relatively frequent sighting rate of *S. bredanensis* in French Polynesia provided a unique opportunity for wild population study as in these waters this species is ranked as the second most commonly sighted cetacean (Gannier, 2000). The ability to generate a distributional data set for *S. bredanensis* in French Polynesia is advantageous to wildlife managers in this area and will additionally provide quantitative baselines for other oceanic regions with similar oceanographic conditions.

A description of preferred habitat characteristics is needed for any given cetacean species. This information can then be used in comparative analyses to assess changes in natural habitat use that may occur due to factors such as habitat degradation, increased human encroachment, or pollution. Certainly, cetaceans can be negatively impacted by

their surroundings and adapt to environmental disturbances. For example, cetaceans are likely to flee from areas when alarmed, noise pollution from oil rigs has been shown to have this effect on beluga whales, and tuna fishery operations alter movements of spinner dolphins (Norris and Dohl, 1980; Thomas et al., 1990).

A significant number of studies have focused on defining home ranges and normal activity patterns for other species of small dolphins that are relatively well known such as the bottlenose and spinner dolphin from many different regions (Ballance, 1990; Corkeron, 1990; Hansen, 1990; Kenney, 1990; Klinowska, 1991; Norris and Dohl, 1979; Pryor and Norris, 1991; Reynolds et al., 2000; Saayman and Tayler, 1973; Scott et al., 1990; Shane, 1980; Shane, 1990; van Waerebeek et al., 1990; Wells et al., 1990; Wells and Scott, 1999; Wursig and Harris, 1990; Wursig et al., 1994). Coastal habitats have been the focus of these investigations because of researcher accessibility and concern for habitat vulnerability as the inshore waters are especially susceptible to anthropogenic impacts. For spinner dolphin populations using near-shore waters as a resting area, human disturbances within the preferred habitat are believed to have resulted in distributional pattern shifts as the population adapts to the disturbance (Klinowska, 1991; Perrin, 1989). For bottlenose dolphins, a population in San Diego Bay illustrates dramatic distributional changes believed to be attributable to pollution effects. Until the 1960's, a bottlenose dolphin population was consistently present in San Diego Bay before disappearing from the area for almost a 10 year period. The return of these resident animals coincided with improved water quality of the Bay, and although other factors may have been responsible or contributed to these observed distributional changes, pollution is the likely culprit (Klinowska, 1991). Prior to the current study on *S.*

bredanensis, no distributional or preferred habitat usage findings have been available from near-shore waters for this dolphin species.

The only detailed distributional map for *S. bredanensis* is from the Eastern Tropical Pacific (ETP) region, where survey effort covering over 25° of latitude and longitude over several years in large and expensive NOAA ships were used to obtain this data (Au and Perryman, 1985; Hewitt, 1985; Wade and Gerrodette, 1993). *S. bredanensis* was sighted on a relatively infrequent basis in the ETP, ranked as the ninth most likely cetacean to be sighted at a density of only 0.0047 individuals/km. Similar large-scale survey efforts have included study of *S. bredanensis* distributional patterns for other wide regions of open ocean such as the Western Tropical Indian Ocean (Ballance and Pitman, 1998). *S. bredanensis* has been described as a primarily pelagic species found in off-shore waters throughout tropical and subtropical oceans of the world (Klinowska, 1991; Leatherwood et al., 1976; Leatherwood et al., 1982; Leatherwood and Reeves, 1983; Miyazaki and Perrin, 1994), and accordingly species distributional data in off-shore environments is useful. Although not to the extent of the spinner and bottlenose dolphin, the rough-toothed dolphin has been sighted in near-shore waters in addition to the described off-shore habitat (Klinowska, 1991; Leatherwood et al., 1976; Leatherwood et al., 1982; Leatherwood and Reeves, 1983; Miyazaki and Perrin, 1994). *S. bredanensis* is also present around oceanic islands throughout its range, for example the Canary Islands have been identified as a region where this species is frequently found (Ritter, 2002).

In French Polynesia, rough-toothed dolphins are most commonly found in water depths between 1000 and 2000 m at distance of 1.8 to 5.5 km from the reef barrier.

Results also indicate the frequency of sighting at various distances from shore and that the average group size of *S. bredanensis* is 12.1 individuals. This species was observed in French Polynesian waters throughout the year and no clear variations in seasonal distribution were apparent. These findings provide reference values and insight into the habitat preference of *S. bredanensis* in an oceanic island environment. However, many questions on rough-toothed dolphin ecology remain, and further investigations of live wild populations in this region could lead to a determination of site fidelity, home ranges, social structure fluidity and reproductive seasonality in this species.

Through continued population monitoring of French Polynesia's rough-toothed dolphins, comparisons can be made to these established distributional patterns in order to assess potential threats. Fishery interactions have been identified by fishery biologists in French Polynesia, for example video footage documents rough-toothed dolphins stealing bait at depth in excess of 200 m (Bach, P., pers. comm.). It is not known which specific species of cephalopods and fish that *S. bredanensis* feeds upon in French Polynesia but any marine ecosystem threats in this area are likely to affect all levels of the food chain. Direct impacts on rough-toothed dolphin populations may also result from human activities associated with the tourism industry in French Polynesia. Dolphin watches are a popular tourist attraction in many regions of the world. The focus of this activity in French Polynesia is currently on spinner dolphins, but the preferred habitat of rough-toothed dolphins overlaps with that of *S. longirostris*. *S. bredanensis* has been held under captive conditions and the removal of this species for public display purposes may present a future threat to populations. Defined distributional patterns and group characteristics of *S. bredanensis* in French Polynesia will aid wildlife managers in

determining if population adaptational responses are occurring as a result of these types of potential stressors into the future.

Adaptation of Reproductive Parameters

The adaptation of populations to stressors include biological and physiological changes on an individual scale. Severely depleted population numbers may result in a density dependent response, where individual reproductive parameters change in an attempt at compensation for greatly reduced population sizes. Evidence of the density dependent response is reflected in birth rate, age of first birth, and juvenile and adult survival. This response is based on changes in recruitment processes and the interplay between reproduction and survival, and has been documented to occur in 16 species of marine mammals (Fowler, 1984). For most cetaceans, the density dependent response usually manifests in a change of the age that females give birth to their first calf and in overall birth rates (Fowler, 1984). In this event, physiological mechanisms come into play to increase pregnancy rate by decreasing age of sexual maturity attainment, decreasing lactation length, and decreasing the resting period. The density dependent response of cetacean populations appears to depend not only on predation and available resources, but the social and behavioral characteristics of a species also appear to play an important role (Fowler, 1984).

A density dependent reproductive response to exploitation pressures has been documented in the killer whale and sperm whale which represent two species of cetacean with highly organized social structures (Fowler, 1984). The killer whale is considered to be a gregarious species where strong social bonds and the maintenance of complex relationships is paramount in group structuring (Leatherwood and Reeves, 1983).

Comparisons were made between killer whales found off of the West coast of Canada, and birth rate was found to be higher in pods where young animals had been taken for display as opposed to pods where no animals had been removed (Biggs, 1982; Fowler, 1984). The suggested explanation for this difference was an increase in the reproductive activity of older females as a direct result of social and behavioral changes which occurred in response to the removal of the young animals (Biggs, 1992; Fowler, 1984). Sperm whales have showed a similar reproductive response to exploitation pressures that has been demonstrated in killer whales (Best et al., 1984; Fowler et al., 1984). Sperm whales off of South Africa were compared during two different time periods where the later investigations followed exploitation of the population. Similarly for sperm whales, an increase in pregnancy rate followed exploitation effects and was most evident in the older female age classes (Best et al., 1984). This increase in pregnancy rate reflects in a decreased calving interval, and in this case the calculated decline was from an average of 6 to 5.2 years (Best et al., 1984). Sperm whales exhibit a highly organized social structure, and provide another example of the density dependent reproductive response for a species of cetacean with a high interdependence of school members (Best et al, 1984).

Not all exploited cetacean species that have been previously investigated have demonstrated the density dependent reproductive response to population declines. The fin whale is an example of a species where greatly reduced populations resulted from extensive commercial whaling. Reproductive parameters in fin whales were described in detail in the 1950's as this baleen whale became the focal whaling species, constituting a major portion of the world's catch after blue whale stocks were depleted (Macintosh and

Wheeler, 1952; Mizroch and York, 1984). Pregnancy rate in the fin whale has been analyzed, and a high degree of variability was detected among regions, time periods, and seasons. No increase in pregnancy rate for fin whales was apparent according to exploitation effects and if a trend is present, it is masked by high variability (Fowler, 1984; Mizroch and York, 1984). As fin whales are often sighted as solitary individuals or in relatively small group sizes rarely exceeding 7 animals, it is possible that the lack of evidence to support a density dependent reproductive response is related to social structures that differs from that found in odontocetes such as the killer whale and sperm whale. Regardless of the reason why heavily exploited fin whale populations did not demonstrate a density dependent reproductive response, clearly reproductive parameters need to be investigated on a species specific basis. The population response to exploitation is not consistent among all cetaceans but needs to be determined for effective species management efforts aimed at the species of interest.

The spinner dolphin provides an example of a small dolphin species that inhabits similar environments to the rough-toothed dolphin where heavy exploitation has been documented and responsive reproductive parameters investigated (Leatherwood and Reeves, 1983; Klinowska, 1991). Spinner dolphin pod sizes range a great deal, sometimes numbering into the thousands, and this species exhibits fluid social groupings (Klinowska, 1991; Leatherwood and Reeves, 1983). In the ETP, life-history parameters in *S. longirostris* have been documented to shift to the extent that the age of sexual maturity attainment in females is decreased by several years (Klinowska, 1991; Perrin and Henderson, 1984). Over a twenty year period, the spinner dolphin population in the ETP region was estimated to have been reduced to only 20% of pre-exploitation

abundances (Klinowska, 1991). This extreme exploitation is evident in the reproductive parameters of ETP spinner dolphins, believed to be responsive to the loss of huge numbers from this population (Perrin and Henderson, 1984). Attainment of sexual maturity in the ETP spinner dolphins were compared with an unexploited population in the Gulf of Mexico where both males and females reach sexual maturity at a longer and heavier size (Perrin and Henderson, 1984). Female spinner dolphins in the eastern tropical pacific reach sexual maturity at an average of 5.5 growth layer groups (GLG's) and at an average of 7-10 GLG's in the Gulf of Mexico (Klinowska, 1991; Perrin and Henderson, 1984). Potential problems with this data set need to be taken into account although a comparison of these spinner populations with varying degrees of exploitation do support a density dependent reproductive response to extreme population decline. Firstly, as opposed to a direct comparison between exploited and unexploited populations, in this specific study of spinner dolphins, both populations had experienced different levels of pressure. The degree of population exploitation was estimated to have resulted in a much lower fraction of the original size in one population (17-25%) versus the other (58-72%). Another consideration with this data set is that the two spinner dolphin populations investigated represent two different forms from different geographical locations. There are at least four different forms of spinner dolphin stocks in the eastern tropical pacific, with morphological and size variations between these stocks (Gray, 1846). However, this still provides a measure of comparison for a small species of dolphin and supports manifestation of the density dependent reproductive response evidenced by decreased age of sexual maturity attainment. In another small dolphin species, a shorter calving interval, an excessively short lactation period and short

resting period have been suggested as an exploitation result for striped dolphins exploited off of Japan (Kasuya, 1984). For the small species of cetacean where exploitation effects have been investigated, findings indicate that reproductive parameters are altered, which is also in agreement with the generally social nature of small dolphins.

When thousands of individuals are available for reproductive studies and the level of exploitation is known for a given dolphin species, it becomes possible to compare reproductive parameters among populations. For the species *S. bredanensis*, reproductive information is lacking and it has not previously been known for any region at what age and size range these animals attain sexual maturity. No estimates of calving interval, length of lactation, length of resting period or other important reproductive parameters are available from any region (Klinowska, 1991; Leatherwood and Reeves, 1983; Miyazaki, 1994). Reference baselines are needed to assess potential shifts in reproductive parameters that may result from future exploitation pressures. The difficulty in obtaining a sufficient sample size to investigate reproduction in *S. bredanensis* has precluded extensive study of this species.

The need for knowledge on reproductive biology has been emphasized as paramount for conservation measures aimed at the rough-toothed dolphin (Klinowska, 1991). Only one study has addressed reproduction in *S. bredanensis* to date. Miyazaki (1980) examined reproductive organs and aged adult specimens caught in a Japanese drive fishery. His findings indicate that at 14 years of age and a length of 225 cm males are sexually mature, and females at an age of 17 years of age and length of 225 cm. However, no immature specimens were aged, and even basic information such as what age or size range the rough-toothed dolphin attains sexual maturity is not yet known for

this species. Miyazaki's work (1980) suggested that *S. bredanensis* may undergo several simultaneous ovulations by examination of corpora, but there have been no investigations into reproductive seasonality, reproductive rate, or any other specific studies on reproduction.

Results from this study provide basic knowledge on reproductive parameters and characterize the age and size range where sexual maturity is attained in *S. bredanensis*. Sexual dimorphism is apparent as *S. bredanensis* males grow to a longer and heavier size, males attain sexual maturity between 5 and 10 years of age. Females reach sexual maturity by at least 9 years of age, and undergo the gradual process of sexual maturation which may begin as early as 3 or 4 years of age. Due to the difficulty in obtaining access to a large enough sampling of dead specimens to investigate reproduction in this species, specimens were combined from both the Atlantic and Pacific oceans to establish baseline values. Significant differences between the Pacific and Atlantic populations were not obvious, but the combination of sampling needs to be recognized. It is also possible that the age and size range of sexual maturity attainment characterized by this study may represent populations already experiencing some degree of environmental or exploitation stress. However, this is the first study to investigate sexual maturity attainment in this species, and these reference values serve as the only baseline value available. Monitoring of these established parameters will provide a means to aid in the detection of future changes to the current status of populations found in both oceans.

Hormonal Events

The second phase of the biological stress response in individuals following alarm and adaptation is hormonal events. In order to be able to detect hormonal changes which signify that a species is experiencing significant stress, baseline endocrinology data from healthy individual animals is first required. Originally from French Polynesian waters, 3 individual rough-toothed dolphins housed in a natural lagoon environment in this same region allowed opportunity to obtain serum samples over time. Two of these animals were healthy and the third was originally a stranded individual that was rehabilitated and thrived in captivity for three years. In addition to the ability to establish distributional information from live, wild rough-toothed dolphins in French Polynesia, captive animals from this same area were accessible for this study to determine biological parameters in healthy individuals. Although captive animals may not be entirely representative of live, free-ranging individuals, this still provides a starting point for establishing baselines in individuals from a species of cetacean where so little is known.

There is no indication that French Polynesia's rough-toothed dolphins currently represent an ecologically unhealthy population. This relatively isolated island environment in the South Pacific does not have continental scale impacts from land use change, nor does the human population on these small islands exceed a million people like some other oceanic islands of similar size (West and van Woesik, 2001). Human activities in this region very well may be affecting *S. bredanensis* populations, however, baseline distributional and hormonal data established from this area are assumed to represent a population that was not under significant stress at the time of data collection.

This assumption is based on the lack of evidence to support a high mortality rate for *S. bredanensis* in French Polynesia. If *S. bredanensis* populations in French Polynesia were under extreme stress, phase three of the biological stress response would be apparent and dead individuals would be much more prevalent. The rough-toothed dolphin has been identified as the second most abundant cetacean species to be sighted in French Polynesian waters, yet there have been only a few individual stranding reports for this area (Gaspar et al., 2000). This area has not been identified as one where rough-toothed dolphins are taken for human consumption like Japan, the West Indies, and the Solomon Islands (Klinowska, 1991). This supports the likelihood that *S. bredanensis* populations in French Polynesia are ecologically healthy, at least in comparison to other regions where live rough-toothed dolphins are either taken or rarely sighted and only stranded individuals are generally reported (Ferrero et al., 1994; Klinowska, 1991; Mazucca et al., 1999).

To define characteristics representative of an ecologically healthy population of this species, French Polynesia provided the ideal location. French Polynesia may be regarded as a pristine environment for many cetacean species. Both live, wild population distributional patterns could be determined and hormonal baselines established from healthy captive animals in these waters. Investigation of hormonal events in *S. bredanensis*, which signify the second stage of the biological stress response, requires comparative hormone concentrations. Serum samples are required for hormonal analyses, and collection of blood samples from free-ranging animals is not possible without implementation of capture procedures. It is necessary to take advantage of biological sampling opportunities that arise when cetaceans are held in a captive

environment. Very few individuals of the species *S. bredanensis* have been held under captive conditions throughout all of history, and currently there is only one individual housed within the United States. This necessitates opportunistic sampling, and this study included serum sampling of 4 individuals in Florida, housed at Mote Marine Laboratory and Aquarium (MOTE) over a 3-4 month period. At the time of early sampling, the *S. bredanensis* individuals at MOTÉ were representative of generally unhealthy animals that were part of mass stranding events where the majority of pod members died. This provided an opportunity to obtain endocrinology data from an unhealthy population under considerable stress. Hormonal data from animals in French Polynesia and Florida allowed for comparisons between individuals believed to be representative of both ecologically healthy and unhealthy populations of *S. bredanensis*.

Cetaceans are often in ill-health at the time of mass stranding events (Walsh et al., 2001). The Florida study animals that survived the initial mass stranding events and were transported to the Dolphin and Whale Hospital at MOTÉ for rehabilitation are no exception. From one of these stranding events in December of 1998 a total of 12 *S. bredanensis* individuals stranded, 2 died enroute to the Dolphin and Whale Hospital and only two which became study animals survived long enough to be medically treated. The two individuals were diagnosed with elevated white blood cell counts and elevated liver values upon arrival at the Dolphin and Whale Hospital. Although treated with antibiotics and antifungal medications, both animals eventually died and necropsy results revealed gastrointestinal disease, cardiac fibrosis, and bronchopneumonia in one animal and intestinal and cardiac disease in the other. The other two stranded study animals housed at MOTÉ initially exhibited neutrophilia, elevated liver values, bilateral congestion of

both lungs, and tachycardia. Both of these animals were treated throughout rehabilitation and eventually released. Early veterinary diagnoses certainly indicate compromised health in all MOTE animals at the beginning of rehabilitation efforts and that biological samples during this timeframe are reflective of dolphins in ill-health. Physiological stresses associated with clinical state and transport and acclimation to the treatment facility was evident. Frequent serum samples collected from unhealthy *S. bredanensis* individuals allowed for determination of hormonal values representative of this species while experiencing significant stress.

Endocrinology data from the captive rough-toothed dolphins housed in French Polynesia and at MOTE in Florida was separated according to health status for direct comparison of values. The initially stranded individual from French Polynesia was not included in these analyses. Cortisol, thyroid, progesterone and testosterone values for unhealthy individuals were defined as those concentrations obtained within one month following the Florida mass stranding events. All 4 individuals were in ill-health during this period of the rehabilitation process. Healthy values were categorized as concentrations obtained within the last month of rehabilitation for the 2 Florida individuals that were successfully treated and released. Healthy values also included all concentrations obtained from the 2 healthy captive rough-toothed dolphins in French Polynesia. T-tests assuming unequal variances were performed between these two groups, and cortisol and Free T₄ concentrations were found to be significantly different between healthy and unhealthy individuals. For cortisol, mean = 8.6 ng/ml in healthy individuals and mean = 46.2 ng/ml in unhealthy individuals ($t = 5.24$, $df = 24$, $P < 0.05$). For Free T₄, mean = 11.2 pg/ml in healthy individuals and mean = 8.7 pg/ml in unhealthy

individuals ($t = -2.89$, $df = 40$, $P < 0.05$). Total T_4 , Total T_3 , Free T_3 , progesterone and testosterone concentrations were not significantly different between the two groups (P 's > 0.05). These direct comparisons support results obtained by profiling cortisol and thyroid hormonal concentrations during rehabilitation in the stranded animals. Cortisol was initially high which likely indicates a stress response due to compromised health, acclimation to captivity or a combination of these two influences. Results from this study on *S. bredanensis* suggest that baseline cortisol concentrations are less than 10 ng/ml, and values over 25 ng/ml likely signify an individual under stress. Thyroid hormone results also demonstrated a relationship with health status of individuals, and acclimation to captivity did not appear to affect these hormones. From the four rehabilitated dolphins, thyroid hormones generally increased as medical condition improved in the two that were released. The other two rough-toothed dolphins that died demonstrated a declining trend in thyroid hormone concentrations as clinical state deteriorated. Free T_4 values immediately before death reached a low of only 1.3 pg/ml which is well outside of the range available for other species. Free T_4 values between 16.6 and 39.9 pg/ml have been summarized by St. Aubin (2001) as the overall range available for cetaceans which includes the pacific white-sided and bottlenose dolphin and beluga, pilot and killer whale. This study is the first to investigate differences in thyroid hormone concentrations according to health status. Only immediately prior to death were extremely low levels apparent for *S. bredanensis*, most samples were within the range given for other species of cetacean. Results from this investigation provide cortisol and thyroid reference values and will have diagnostic usefulness in assessing clinical state and relative stress level of *S. bredanensis*.

Hormonal concentrations are also effected by environmental surroundings and the topic of endocrine disruption has recently received a great deal of attention. Pollution levels are believed to be reflected in thyroid hormone values in marine mammals (St. Aubin, 2001). In the gray seal thyroid hormones act as biomarkers and in the common seal a thyroid deficiency has been found to be associated with environmental contamination, specifically polychlorinated biphenyls (PCB's) (Brouwer et al., 1989; Hall et al., 1998). Evaluation of thyroid glands from dead harbor seals in the North Sea are thought to be morphologically defective due to pollution in this area (Schumacher et al., 1993). Abnormal histological findings from beluga whale thyroid glands also correlate with high organochlorine contamination in the St. Lawrence estuary (De Guise et al., 1994). To be able to accurately assess the effects of environmental stressors such as contaminants on hormonal events, baseline concentrations from animals from an ecologically viable population are first needed. Concentrations from captive *S. bredanensis* individuals from French Polynesia provided the most feasible alternative to multiple captures for obtaining the necessary biological samples for establishing baselines relating to endocrinology. Although many factors influence hormonal concentrations, further research is underway to establish to what degree natural factors play a role in the thyroid hormone concentrations provided by this investigation (St. Aubin et al., 1996; West et al., 2001). Bottlenose dolphin adults tend to have lower thyroid hormone values than juveniles which is in agreement with findings for neonatal seals of several species (Engelhart and Ferguson, 1980; Haulena et al., 1998; Stokkan et al., 1995; West et al., 2002; Woldstad and Jenssen, 1999). The healthy individuals which served as the control samples for reporting thyroid hormones in *S. bredanensis* were

either all adults or appeared to have approached sexual maturity during the sampling period. Although the pubertal status of 2 individuals may have slightly affected the baseline means reported, differences among all individuals sampled was only apparent in the concentrations of the very young juvenile when compared to the other 6 study animals. Regardless, these results do provide a starting point for assessing the status of potential environmental stressors on hormonal change in *S. bredanensis*. This is the only cetacean species to date where direct comparisons in endocrinological parameters have been made according to physiological health.

Testosterone concentrations are negatively related to blubber PCB's and DDE, which is transformed from DDT concentrations in Dall's porpoise (Subramanian et al., 1987). Decreases in testosterone concentrations were found to be statistically significant with increasing levels of DDE in this species of small cetacean from the North Pacific (Subramanian et al., 1987). Implications of altered reproductive hormones in cetaceans is far reaching, any factor which threatens the ability of populations to reproduce could have devastating effects on maintenance of population numbers. Determination of baseline reproductive hormone concentrations according to various reproductive states are required to be able to accurately assess the estrogenic effect of environmental stressors such as contamination. For the species *S. bredanensis*, this study provides ranges for testosterone and progesterone concentrations, and will allow for further studies and future comparisons with these newly established baselines. Testosterone values in adult *S. bredanensis* indicated suppressed values in ill animals which was evidenced by low concentrations that increased with successful rehabilitation but remained low in adults that died. Suppression of testosterone concentrations with illness has been

previously documented in the bottlenose dolphin, as an individual close to death and another with Lobo's disease had significantly lower reproductive hormone levels (Kirby, 1990). The ability to impregnate females is a key component in reproductive success, and in bottlenose dolphins testosterone levels profiled throughout the year showed a similar pattern to sperm production although spermatogenesis lagged behind by several weeks (Schroeder and Keller, 1989). In the *T. truncatus* individual with suppressed testosterone during Lobo's disease, a female was impregnated while this male was ill (Kirby, 1990). Perhaps this was due to the lag in sperm production, further studies are needed to quantify concentrations of reproductive hormone concentrations needed in cetaceans for successful reproduction. This would be useful data in determining the ultimate effect of environmental stressors that alter reproductive hormonal events. For females, this study of *S. bredanensis* did not demonstrate any unusual findings for the reproductive hormone progesterone, where values were often at levels that indicate a lack of ovarian activity. It is unfortunate however, that estradiol concentrations were undetectable in this species, as female estrogens may serve as key hormones for studying pollution effects and endocrine disruption. Assay development in dolphins for other forms of estrogen would be useful in further study of reproductive hormones in cetaceans, as total estrogens and estradiol are the only forms previously reported, and only for the bottlenose dolphin (Kirby, 1990; Yoshioka et al., 1986).

It is likely that reproductive hormone concentrations obtained from live animals are reflective of population life-history parameters determined by the analyses of large sample sizes of dead specimens. Testosterone concentrations obtained from the 5 captive males provide ranges between 0 and 6 ng/ml for *S. bredanensis* adults. Adult status in

these individuals was established by comparison of individual length and weight measurements with dead specimen data determined in this study of *S. bredanensis*. Available gonad data from the captive males was also compared with dead specimen data from 22 males and the relationship to total body length, total body weight and sexual maturity in the species. Results agreed that by the end of the sampling period, all males had attained sexual maturity. Both gonad measurements and testosterone concentrations suggest that the captive male in French Polynesia reached sexual maturity during the study period. Testosterone concentrations have been demonstrated to increase at the time of sexual maturity attainment in many species of seals (Atkinson and Gilmartin, 1992; Noonan et al., 1991), similar to the pattern in this *S. bredanensis* individual. Over the 6 year study period, testosterone concentrations fluctuated, but increased to values consistently greater than 2 ng/ml near the end of sampling, demonstrating similar concentrations to those of mature adult bottlenose dolphins (Kirby, 1990). Dead specimen data from *S. bredanensis* males indicates that individuals with testes lengths greater than 25 cm are sexually mature. An increase in testes length from the captive male from less than 25 cm to 33 cm was observed on ultrasound near the end of the study period.

Female reproductive data obtained from 27 dead specimens suggests a gradual process of sexual maturation for *S. bredanensis*. This process is described by gross physical changes in the structure of the ovary as it becomes more folded and wrinkled and shape changes occur. Findings indicate that sexual maturation in females may take up to 5 years, although this is probably an overestimate due to a lack of available female dead specimens representing young adult age classes in this study. In the healthy captive

female *S. bredanensis*, progesterone values fluctuated above baseline on a few occasions. This individual died suddenly and no corpora were apparent when ovaries were examined at the time of necropsy, and accordingly this animal was classified as sexually immature (Perrin and Reilly, 1984). However, small amounts of progesterone are secreted by follicles, and a follicle was apparent upon examination of the ovary. It is possible that these reported progesterone fluctuations are due to follicular activity. The reproductive state of this individual may represent an animal undergoing the gradual process of sexual maturation. Total body length and weight ranges for this individual are also in close agreement examined female dead specimens that were categorized as maturing animals upon gross and histological examination of reproductive organs.

This investigation provides a significant contribution to characterizing baseline values and hormonal events associated with the biology and clinical state of *S. bredanensis*. This foundation will be useful for further studies of individual *S. bredanensis* and these reference values for various reproductive states and conditions will be valuable in assessing stressor effects to this species. Much remains to be learned about the species *S. bredanensis* and baseline findings resulting from this study emphasize areas where future research would be especially useful to aid in conservation. Wild population research focusing on the long-term study of photographically identified individuals should be possible in near-shore areas of relatively high species abundance such as French Polynesia. Photographic identification would allow for a greater understanding of behavioral characteristics, social fluidity and relationships within *S. bredanensis* pods. The application of technology advancements used for other species of cetaceans such as tagging and tracking techniques would lead to definition of movement

patterns, home range, and activities at depth. This information would contribute significantly to available knowledge on the ecology of this species. On a world-wide population level, biopsy sampling of wild individuals would provide the necessary tissue for genetic analyses. A great deal is yet to be learned for *S. bredanensis* and the baseline species data generated by this research should serve to stimulate further work in areas needed for more effective conservation efforts.

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