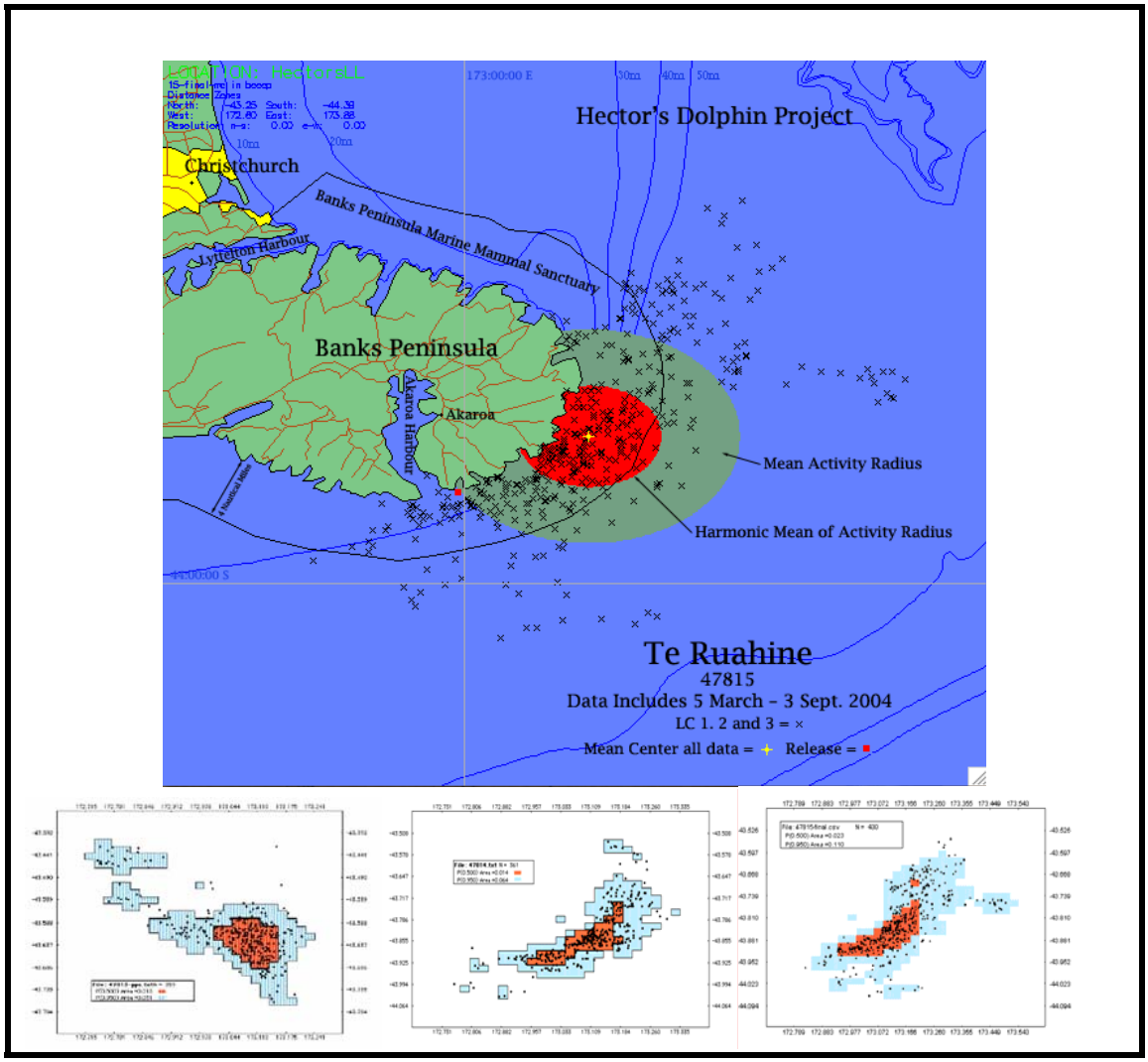
Hector's Dolphin (*Cephalorhynchus hectori hectori*) Satellite Tagging, Health and Genetic Assessment Project



1 June 2005

Final Report

Hector's Dolphin (*Cephalorhynchus hectori hectori*) Satellite Tagging, Health and Genetic Assessment

Final Report

By

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This research would not have been possible without the co-operation and support of the **Horomaka Runanga O Ngai Tahu**.

Submitted to the Department of Conservation (DOC), Auckland Conservancy, in fulfillment of a contract to the New England Aquarium, Central Wharf, Boston, MA, 02110, USA.

1 June 2005

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ACKNOWLEDGEMENTS

This work was conducted under a contract and a Marine Mammal Protection Act research permit issued to the New England Aquarium by the New Zealand Department of Conservation. However, this project would not have been possible without the extraordinary generosity and cooperation of all the project members and their home institutions. A project of this complexity, which provides a multidisciplinary view of Hector's dolphin biology, requires the broad-spectrum collaboration of a wide group of specialists. Such a group has never before been assembled for the study of this species, and the results of this trial have provided an unprecedented perspective on the biology of Hector's dolphin. Every project member contributed his or her time, either as a volunteer or with the support of their home institutions, or other sponsors; some researchers traveled from the other side of the world to help with this work. The Pew Fellows Program in Marine Conservation and the New England Aquarium Marine Conservation Action Fund provided additional support for this project and for the research and development leading to this work. Serology for *Brucella, Leptospira*, canine distemper, and influenza A, was conducted as an Exotic Disease Investigation, Project Number 1114, by the National Centre for Disease Investigation, Ministry of Agriculture and Food, Upper Hutt. Serology for calicivirus was carried out by Dr. Tao Zheng, AgResearch. We thank the Marsden Fund for the Major Histocompatibility Complex (MHC) analysis. This project would not have been possible without the cooperation and unfailing support of the Horomaka Runanga O Ngai Tahu. We thank Jo (nee Perano) and Rollie MacDonald of Picton for the loan of key field equipment. We thank New England Aquarium Director of Conservation, Heather Tausig, for help and support of this project. We also thank Cynthia Nichols, Catherine McGuirk, Al Barker, Carmine Tocci and Sandy Lane for their important help.

ABSTRACT

Three Hector's dolphins (*Cephalorhynchus hectori hectori*) were caught in the waters surrounding Banks Peninsula, New Zealand, in March 2004, and were released following attachment of lightweight satellite transmitters. The trial was intended to evaluate the efficacy and safety of satellite tagging for potential application to the critically endangered Maui's dolphin (*Cephalorhynchus hectori maui*). A complete health assessment was conducted on each captured dolphin prior to tagging and release, providing the first, extremely valuable baseline health data for this species. Blood samples collected from the dolphins during the brief live capture allowed extraction of high quality RNA to characterize functional genetic diversity involved in the immune system defense. All three satellite tags transmitted for more than three months, providing detailed information on the seasonal home range of each dolphin. There was no evidence that the dolphins experienced deleterious health impacts from the tagging, nor did they exhibit disruption to normal behaviours. The results exceeded contract expectations, and provided unprecedented insights into the movements, health and genetic diversity of the Hector's dolphin.

Hector's dolphin is a suitable candidate for satellite telemetry studies. The risk to this species from capture, handling and tagging seems to be low, and useful new information for management has been provided relatively quickly. Confirming functional diversity in the relatively abundant Banks Peninsula population is critical to assessing the potential impact of inbreeding depression in the critically endangered Maui's dolphin.

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INTRODUCTION

The small coastal dolphin *Cephalorhynchus hectori* (Van Beneden 1881) is found only in New Zealand waters. There are two subspecies (Baker *et al.* 2003): the South Island subspecies, Hector's dolphin (*Cephalorhynchus hectori hectori*), is "endangered" as determined by the World Conservation Union; and the North Island subspecies, Maui's dolphin (*Cephalorhynchus hectori maui*), is "critically endangered" (Hilton-Taylor 2000; Reeves *et al*. 2003). Due to the proximity of their coastal habitats to human activity, both subspecies face numerous threats to survival, including entanglement in fishing gear, ship/boat strikes, and the unknown impact of unprecedented increases in tourist operations targeted at swimming with and watching the dolphins (Stone and Yoshinaga 1999, 2000; Dawson *et al.* 2001; Pichler *et al.* 2003). It is critical that information on dolphin movement and behaviour is obtained in order to implement appropriate conservation measures in an increasingly complex and dangerous marine environment.

The study of all cetaceans is restricted by the difficulties of working on the ocean and by human sensory limitations (Stone and Kraus 1998; Stone *et al*. 1999). Information gained from traditional observation platforms, such as boats and aircraft, provides only a glimpse of animal behaviour during the day, in calm weather, and at the ocean surface. Electronic telemetry tags attached to the animals are an excellent method to answer ecological questions about the species and to subsequently develop management strategies based on the detailed data provided from such telemetry studies. Hector's dolphins have been studied using suction cup VHF tags in the Banks Peninsula region, but this method can only provide information for a few days and within line of sight of land-based radio receivers (Stone *et al*. 1994, 1998).

The purpose of this project was to conduct a satellite tagging technique trial on three individuals within the Hector's dolphin population off Banks Peninsula on the east coast of the South Island. This project was a vital step in determining whether this telemetry technique can be successfully used as a tool to gain information for the management and research of the critically endangered Maui's dolphin on the west coast of the North Island. Satellite tagging has been identified as the only current methodology available to provide continuous dolphin movement and habitat information in the shortest possible time frame.

The first health assessments ever performed on Hector's dolphins were conducted on the three study animals, thereby providing crucial baseline data on the health and functional genetic diversity of this species. Such baseline studies are a key element in long-term recovery and management plans. This information will be of particular value should there ever be an unusual mortality event among Hector's or Maui's dolphins such as occurred among New Zealand sea lions (*Phocarctos hookeri)* in 1998, 2002 and 2003 (Baker 1999; Duignan *et al*. 2004).

METHODS

SATELLITE TAGGING

Dolphin Capture

The study dolphins were captured from a 5.8m rigid-hulled inflatable vessel using a tail grab device. This was the same device used by Dr. Alan Baker who caught 27 Hector's dolphins in the 1970s. The padded tail grab was fitted to an aluminum pole; as a dolphin rode the bow wave of the research vessel, the tail grab was placed over the dorsum of the caudal peduncle. Once placed over the peduncle, the grab device closed on the peduncle and the mechanism locked. The aluminum pole then automatically detached, leaving the captured dolphin connected to the researcher's vessel by a stretchable cord attached to the tail grab jaws. The length of the line was measured so that when the boat was powered in a forward direction the dolphin would come alongside the vessel, facing in tail-forward position three metres from the bow, where researchers stood ready with a customized sling hanging over the side. When the dolphin came alongside the boat, it was centered in the sling, raised horizontally from the water, and the tail grab removed. The sling was transferred to the second, larger boat where the health assessment and tag attachment procedures were conducted on a padded, custom-made foam examination platform.

Tag Design and Attachment

Satellite transmitters selected for this study were the Wildlife Computers (Seattle, Washington, USA) SPOT-3, cricket transmitter, with one flat M1 3V lithium battery. That configuration delivers approximately 25,000-30,000 transmissions, depending on temperature and tag attachment duration. The tag was designed to transmit at 0.5W; the battery voltage was sent with every transmission, allowing analysis of tag life.

The Wildlife Computers company worked closely with the research team to design a new tag mold specifically for a Hector's dolphin dorsal fin and to minimize drag. The tag was designed using a model of a Hector's dolphin dorsal fin, created from a plastic resin perfusion technique applied to a fin from a dead stranded animal, to show the pattern of vasculature. A saltwater switch (SWS) ensured that transmissions would occur only when the animal was at the surface. The tag electronics were encased in a contoured epoxy mold (mold #191-00) with dimensions of 10.5 x 3.5 x 1.7cm. The antenna projected up from the tag approximately 18cm. The tag weighed 55 grams in air, 10 grams in seawater, and had four 5mm tapped holes; however, only two holes were used for the attachment of the tags during this study. A specially developed 2mm-thick soft skin of hypoallergenic neoprene (TrackPack, Florida, USA) was glued to the back of the transmitters with ordinary aquarium silicone. For the backplate on the opposite side of the dorsal fin, a piece of 2mm rubber material, also lined with hypoallergenic neoprene, was used. The ID number (0,1, or 2) was printed with large numbers on the back plate for field identification.

Only animals considered to be in good health, as determined by the supervising veterinarian, and without calf, were selected for tagging. Local anaesthesia (xylocaine 5% ointment) was applied on both sides of the dorsal fin 10 minutes before the attachment holes were made. Systemic analgesia was administered intravenously using flunixin meglumine (0.25mg/kg) approximately 5 minutes prior to making the attachment holes. For each animal, the best placement position for the transmitter was determined so that the curve in the tag fitted the shape of the fin, and at the same time minimized the transmission shadow from the dorsal fin on the antenna. A rubber model of the tag with two holes for the attachment pins served as a template when the holes were made in the dorsal fin using a sterile 7mm surgical trocar fitted to a cordless drill running in low speed. Threaded POM pins, inserted into bioneutral silicone tubes to protect the tissue (total 7mm diameter), were glued (Locktite 414) into the threaded holes in the tag. The holes were slightly larger than the pins to avoid pressure necrosis and promote healing. Both the surgical trocar and the pins were disinfected in Betadine surgical scrub. The transmitter and its backplate were mounted onto the fin with the pins. The silicone tubes were cut to allow attachment of 2mm zinc eloxated nuts at the ends of the pins. The nuts were tightened and glued (Locktite 414) to the pins, while allowing for water to run between the tag and the skin. Reducing the rear pin's thickness where it emerged from the tag weakened it, allowing it to break easily when the front nut corroded and the pin released, thereby causing the tag to detach immediately.

Programming

Prior to deployment, the satellite tags were programmed to transmit on predetermined schedules. After deployment it was not possible to change the programming. Ideally, the tags would transmit 24 hours a day every day, but due to the limited battery life, a reduced programming schedule was designed to maximize initial information from the study dolphins while also allowing for longer-term information.

All three tags were programmed identically. The transmitters were programmed to give a maximum of 700 transmissions per day from 1 am to 11 pm or until the daily transmission allowance was reached. The tags were programmed to transmit every day for the first 5 days; thereafter every other day for 10 days; every third day for 15 days; every fourth day for 20 days; every fifth day for 25 days; every sixth day for 30 days and every seventh day for the rest of the battery life. Expected battery life was about 180 days. All tags had a transmission repetition rate of 45 seconds.

This schedule was designed to give maximum information in the early stages of deployment for several reasons: to determine the dolphins' initial reactions to the tag attachment, and to facilitate real-time locating and monitoring by the research team. The programming of the transmissions then methodically decreased as described, to one transmission per week, continuing until the tags detached or the battery power drained, whichever occurred first. The transmission days are listed on the tag program sheets for each dolphin (Appendix). Locations were collected via the ARGOS Location Service Plus system (Toulouse, France) and received online over the Internet and on CD-ROMs. The software program Satpak 3.0 (Wildlife Computers) was used for validating data received from Argos and transforming data into an ASCII format.

Figure 1. SPOT-3 tags with attachment pins and backplates.

Tag Accuracy and Land Tests

All three tags were programmed and tested onshore prior to deployment on dolphins (Table 1). Two tags provided location class 3 fixes while the third tag (47813), which was intentionally placed in an obstructed position, provided class 1 locations and therefore a higher variance in position agreement with GPS. This analysis does not account for any variance in GPS, which can vary due to a number of reasons.

Table 1. Land trials for position accuracy of satellite tags

Tag ID number	Argos position	GPS position	Average Difference between Argos and GPS
47813 Argos accuracy 1	43.820 S 172.935 E	43.818 S 172.943 E	680m
47814 Argos accuracy 3	43.805 S 172.973 E	43.804 S 172.973 E	80m
47815 Argos accuracy 3	43.756 S 172.972 E	43. 756 S 172.971 E	110m

Regarding tag accuracy in general, the more messages the satellite receives during a single pass over the tag, the more accurately a location is calculated. As with all location systems, there is some error in the determination of the location and the variables are numerous, including animal behaviour, geography, latitude and wave height, just to name a few. Using the doppler shift of the uplink frequency and the time of signal arrival, ARGOS calculates the tag location along with an error estimate. The error index is called Location Class and is a coarse estimate of accuracy. In order from most accurate to least accurate are Location Classes 3, 2, 1, 0, A, B, and Z. The majority of Class 3 locations are within $\pm$150 meters in latitude and $\pm$150 meters in longitude. Class 2, 1 and 0 locations show that at least four messages were received and that the accuracy of the majority of locations given are within $\pm$150 to 350 meters latitude and longitude (Class 2), within $\pm$350 to 1000 meters latitude and longitude (Class 1), and $\pm$1000 meters or greater in latitude and longitude (Class 0), respectively of the true position. Class A and B locations indicate that three or two messages respectively were received from the overpass. These locations have been validated by the location software, but are not assigned accuracy. Class Z locations are "rejected" locations and are thus "invalid." For this study, only the best quality Location Classes 3, 2 and 1 were used in the analyses; our land tests indicated that we were getting high quality fixes for these classes.

Monitoring and Data Analysis

In order to evaluate post-tagging effects on the dolphins, efforts were undertaken to visually locate and monitor the animals by either traveling to areas where the dolphins last transmitted or traveling to areas where other observers reported seeing the animals. Several times each day, researchers in Akaroa downloaded positions of tagged dolphins from the ARGOS website and manually plotted their locations on a chart. These positions were often less than two hours old and were used to identify potential survey areas for visual monitoring from boats. Once in an area where a dolphin was thought or reported to be, researchers used an ICOM R10 portable communications receiver and directional aerial, which could receive the tags' transmissions within a line of sight range.

Time series maps of the dolphin movements were generated at the New England Aquarium in Boston, Massachusetts, using mapping software GRASS 5.0.2 (open source) on a Powerbook G4 running OSX, and by the Danish National Environmental Research Institute in cooperation with CubiTech A/S (Denmark) using an interactive mapping program that automatically connected to the ARGOS data center every hour. The user-selected animal ID, period and location classes were mapped online over the Internet. Some maps were also edited/labeled with Adobe Photoshop version 7.0. Edits on text files for GRASS were completed with EMACS, and general data manipulations used MS Excel. Due to the expected ARGOS position errors and the scale of the map used, approximately 2-3 positions per dolphin over the entire study plotted on land, along the coastline. Since it is not possible for a dolphin to survive on land, those positions are not represented on these maps.

Statistical Values and Graphic Analysis

The **mean center** for each dolphin's distribution was calculated from all locations that were Argos LC 1, 2, and 3 using this equation:

Mean Center:

$$\overline{X} = \sum_{i=1}^{N} \frac{Xi}{N}$$

$$\overline{Y} = \sum_{i=1}^{N} \frac{Yi}{N}$$

$$S_x = \sqrt{\sum_{i=1}^{n} \frac{\left(X_i - \overline{X}\right)^2}{N-1}}$$

-for standard deviation of x (longitude)

$$S_y = \sqrt{\sum_{i=1}^{n} \frac{\left(Y_i - \overline{Y}\right)^2}{N-1}}$$

-for the standard deviation of y (latitude)

$$S_{xy} = \sum_{i=1}^{n} \frac{\left(d_{iMC}\right)^2}{N-2}$$

-standard distance deviation: where d_{iMC} is the distance between i and the mean center.

In addition to the mean center, a **mean activity radius** for each dolphin's distribution was calculated from all locations that were Argos LC 1, 2 and 3 using this equation:

Activity Radius:

$$r_i = \sqrt{(X_i - \overline{X})^2 + (Y_i - \overline{Y})^2}$$

Mean of Activity Radius:

$$\overline{r} = \frac{\sum ri}{N}$$

This is the mean of the radial distances from mean center of activity, a measure of area utilization around the estimated center of activity for each animal (Arvanitis *et al*. 1997).

The **harmonic mean of the activity radius** was also calculated to reduce the effect of outliers using:

$$\frac{1}{H_y} = \frac{1}{N}\sum\frac{1}{Y_j}$$

The **Minimum Convex Polygon** is a numerical method that draws a border around the most distant points enclosing the area within a polygon. This does not take into account utilization of different areas within the range and often intrudes into areas inaccessible to the animal.

The **Anderson Fourier Home Range** is a non-parametric home range estimation technique taken from Anderson (1982). A non-parametric method was used as the data were positively auto-correlated by Moran's I and d^2/s^2 statistic (Turchin 1998). This method shows zones where there are 50% and 95% chance of dolphin occurrence. We used this spatial information to describe likely home ranges of the dolphins.

A preliminary analysis of **day and night mean** centers was conducted. Location classes 1,2 and 3 were divided between night and day and mean centers were calculated for each. The criteria used for night data were any position recorded after the local time of sunset and before the local time of sunrise. Day data were collected after the local time of sunrise and before the local time of sunset.

Genetic Analysis

Whole blood and tissue (dorsal fin biopsy plug) were collected for genetic analysis during the Hector's dolphin satellite tagging capture and release program. Dorsal plugs were collected from the three tagged dolphins and blood samples (for genetic analysis) were obtained from two (dolphins 47814 and 47815). A slough skin sample was obtained from a fourth dolphin that was caught and released without tagging.

Total RNA has been extracted from the blood samples with the "PAXgene Blood RNA extraction kit" and transcribed to cDNA by using oligo d(T) primers and the reverse transcriptase enzyme Superscript III. A reverse transcriptase Polymerase Chain Reaction (rtPCR) was used to generate cDNA and amplify class I and two class II genes of the Major Histocompatibility Complex (MHC). Total DNA was extracted from a subsample of the three dorsal plugs and one slough skin sample using a standard phenol-chloroform extraction method (Davis *et al*. 1987) and as modified by Baker *et al*. (1994). Field identification of the sex of each dolphin (see below) was confirmed molecularly by the paired amplification of X- and Y-chromosome markers (Gilson *et al*. 1998). The mitochondrial (mt) DNA control region of each individual was amplified and sequenced following methods described by Pichler and Baker (2000). Nucleotide substitutions at previously characterized positions of the control region were used to assign the individual dolphin to one of the 17 maternal lineages (*i.e*., mt haplotype) found across the entire species range of Hector's dolphins (Pichler 2002).

Veterinary and Health Assessment

After capture, the dolphins were lifted in a special sling and placed on a custom-designed plastic-covered foam mattress. The sex was determined by examination of the genital regions and the abdomens of females were palpated for signs of pregnancy. A Polar S810i heart rate monitor was placed around the thorax, caudal to the pectoral flippers, and the heart rate recorded for the remaining period of the procedure. Simultaneous monitoring of the respiratory rate was carried out by continuous videography of the blowhole. The mucous membranes of the mouth and eyes were examined and all orifices examined for discharges or unusual secretions. The skin was examined for pathology such as abrasions, wounds, parasites, and pox viral dermatitis. Following this examination, a series of standard measurements were made including snout to fluke length, snout to origin of dorsal fin, snout to blowhole, pectoral girth, umbilical girth, genital girth, and the horizontal length, height, and maximum length of the dorsal fin. In order to anaesthetize the integument of the dorsal fin, the entire surface was smeared with 5% Xylocaine gel. The body condition was assessed by ultrasonic measurement of the blubber depth at six locations: pectoral dorsal and lateral, umbilical dorsal and lateral, and genital dorsal and lateral. Within 10 minutes of capture, phlebotomy was carried out using a butterfly canula (22G, 1" 'Butterfly-19') placed into the dorsal fluke vein pre-swabbed using 70% ethanol swabs. Blood was withdrawn into 10ml syringes and decanted into two 5ml untreated serum vacutainers, one or two 5ml EDTA tubes, and one or two mRNA tubes. After the last blood was withdrawn, an analgesic, flunixin meglumine (0.25mg/kg), was administered intravenously through the canula and flushed using sterile saline (Walsh and Gearhart 2001). The analgesic was administered to suppress pain associated with tag attachment and to reduce potential tissue swelling due to oedema. During blood sampling the dolphin was covered, apart from the fluke and the head, using wet towels kept moist with seawater. The head was continually wet down using a bath sponge and seawater. On completion of blood sampling, the flukes were also wrapped in wet toweling. Following blood sampling, the expired air from the dolphin was sampled for pathogens by holding agar plates 20–30mm from the blowhole for three consecutive expirations. The agar plates used included Sabarauds agar for fungi such as *Aspergillus*, blood agar for aerobic pathogens, chocolate agar for anaerobes and Enterobacteriaceae, and anaerobic blood agar for organisms such as *Brucella*.

The position of the perforations on the dorsal fin was determined using a template. A sterile surgical trocar (7mm diameter) was used to biopsy the fin. The trocar was driven using a hand-held drill, which reduced the time taken to make the incision; a further benefit was that the velocity of the trocar assisted in cautery of the blood vessels. Ferric subsulfate granulate ("Sure Clot") and silver nitrate sticks were available for cautery but were not needed. During and following attachment of the tag, the respiration and heart rate were monitored closely.

Following tag attachment the dolphins were gently rotated to one side and genital and rectal swabs collected for bacteriology. After weighing, the dolphins were carefully lowered into the water and held in the sling for several full respiratory cycles. When the dolphins attempted to swim out of the sling, the outer side of the sling was lowered and the animal was allowed to swim free. All three dolphins were calm during the tagging procedure and swam off normally after release. During all three procedures, the other dolphins from the pod maintained normal activity, swimming near the boat. The study

dolphin movements were then observed for as long as possible after release, but the boat, to avoid further stress on the animals, did not follow dolphins.

Within two hours of returning to shore, the bacteriology plates and blood samples were delivered to Gribbles Veterinary Pathology Laboratories, Lincoln. Tests requested included culture and identification of all bacteria and fungi from the agar plates exposed to expired breath and from genital and rectal swabs. Total blood cell counts, differential white cell counts, erythrocyte parameters, packed cell volume, and serum chemistry were analyzed. Plasma was frozen (-20^{o}C) following completion of haematology tests and later submitted to the wildlife endocrinology laboratory (Dr. John Cockrem, IVABS, Massey University) for assay of stress (cortisol) and reproductive hormones (progesterone, oestrogen). The cortisol and progesterone assays have been validated for the measurement of these steroids in dolphin plasma. Serial dilutions of dolphin plasma were parallel to the standard curves for each steroid, and steroid added to dolphin plasma was quantitatively recovered.

Frozen serum samples were submitted to the National Centre for Disease Investigation, Ministry of Agriculture and Food, Upper Hutt, for serology. Tests requested included:

Bacterial diseases:

- Brucellosis, *Brucella abortus.* Competitive ELISA (This cELISA works with all smooth *Brucella* species).
- Leptospirosis, *Leptospira interrogans*. MAT.
- Serotypes:
    - *L. interrogans* serovar Ballum
    - *L. interrogans* serovar Bratislava
    - *L. interrogans* serovar Canicola
    - *L. interrogans* serovar Copenhagenii
    - *L. interrogans* Grippotyphosa
    - *L. interrogans* Hardjo
    - *L. interrogans* Pomona

Viral Diseases:

- Canine Distemper Virus (Paramyxoviridae, genus *Morbillivirus*). VNT.
- Calicivirus. (Rabbit haemorrhagic disease). Competitive ELISA (test performed by Dr. Tao Zheng, AgResearch). The calicivirus serology test is a competitive ELISA (cELISA), based on rabbit haemorrhagic disease virus (RHDV) antigens. This assay was developed by RHDV OIE reference lab in Italy and has been used to detect RHDV cross-reactive antibodies in serum samples of many species (Zheng *et al*. 2003).
- Influenza A (Avian influenza, Orthomyxoviridae). Agar Gel Immunodiffusion Test performed by Dr. Wlodek Stanislawek, NCDI).

Figure 2. Supervising veterinarian Dr. Pádraig Duignan monitors heart rate of study dolphin during health assessment.

Assay of steroid hormones

Testosterone, estradiol, progesterone and cortisol concentrations in dolphin plasma were measured by direct radioimmunoassay using kits from MP Biomedicals, USA. A coated tube kit was used for cortisol and double antibody kits for the other steroids. Serial dilutions of plasma in assay buffer were parallel to the standard curves for each of the steroids. The quantitative recoveries of steroid were measured by adding different amounts of standard steroid (Sigma, USA) to plasma samples. The recoveries of added steroid were 100.4 $\pm$ 4.4%, 103.9$\pm$ 21.1%, 107.9 $\pm$ 8.3% and 98.7$\pm$2.2% for the four steroids. The sensitivity of each assay was the minimum hormone concentration that could be consistently distinguished from zero. It was determined as the hormone concentration at the mean - 2 standard deviations from the zero hormone point on the standard curves. The assay sensitivities were 0.013 ng/ml, 10.5 pg/ml, 0.55 ng/ml and 0.26 μg/dl for testosterone, estradiol, progesterone and cortisol respectively.

Samples were assayed in single assays for each steroid. Solutions of steroid in assay buffer at concentrations that gave approximately 80%, 50% and 20% binding on the standard curves were used as low, medium and high quality controls in every assay. The intra-assay coefficients of variation for these quality controls were less than 10% in all cases for the four steroids, except for the low quality control for progesterone for which the intra-assay coefficient of variation was 17.7%.

The cross-reactivities of the antisera with other steroids were tested by MP Biomedicals. Cross-reactions for testosterone were 5α-dihydrotestosterone (3.4%), 5α-androstane-3β, 17β-diol (2.2%), 11-oxotestosterone (2.0%), 6β-hydroxytestosterone (0.9%), 5β-androstane-3β, 17β-diol (0.7%), 5β-dihydrotestosterone (0.6%), androstenedione (0.6%), epiandrosterone (0.2%) and 11β-hydroxyandrostenedione, 11β-hydroxytestosterone, androsterone, 5α-androstane-3, 17-dione, 5β-androstane-3, 17-dione, 5α-androstane-3α, 17β-diol, dehydroepiandrosterone, oestrone, oestradiol-17β, oestriol, progesterone, corticosterone and desoxycorticosterone (<0.01%). Cross-reactions for estradiol were oestrone (20.0%), oestriol (1.5%), oestradiol-17α (0.7%) and ethinyl oestradiol, androstenedione, dehydroepiandrosterone, 5α-dihydrotestosterone, 20α-

dihydroprogesterone, DOC, progesterone, testosterone, pregnenolone, 17-hydroxypregnenolone, DHEA-sulphate, aldosterone, cortisol, 11-desoxycortisol, 17α-hydroxyprogesterone and cholesterol (<0.01%). Cross-reactions for progesterone were 20α-dihydroprogesterone (5.4%), desoxycorticosterone (3.8%), corticosterone (0.7%), 17α-hydroxyprogesterone (0.7%), pregnenolone (0.4%), androstenedione (0.2%), testosterone (0.2%) and 11-desoxycortisol, pregnenolone sulphate, cholesterol, dehydroepiandrosterone, ethiocholanolone, oestradiol-17α, oestradiol-17β, oestrone, oestriol, andosterone, aldosterone, cortisol and DHEA-S (<0.1%). Cross-reactions for cortisol were prednisolone (45.6%), 11-desoxycortisol (12.3%), corticosterone (5.5%), prednisone (2.7%), cortisone (2.1%), 17α-hydroxyprogesterone (1.0%), progesterone (0.25%) and dexamethasone, dihydrotestosterone and testosterone (<0.10%).

RESULTS

The field research team included:

- Gregory Stone, Ph.D., New England Aquarium, Boston, Massachusetts, USA
- Alistair Hutt, Department of Conservation, Akaroa, NZ
- Pádraig Duignan, M.V.B., Ph.D., New Zealand Wildlife Health Centre, Institute of Veterinary, Animal and Biomedical Sciences (IVABS), Massey University, Palmerston North, NZ
- Katja Geschke, D.V.M, Ph.D., Wellington Zoo Trust, Wellington, NZ
- Jonas Teilmann, Ph.D., National Environmental Research Institute, Department for Arctic Environment, Rescaled, Denmark
- Alan N. Baker, Ph.D., Private consultant, NZ
- Kirsty Russell, University of Auckland, Auckland, NZ
- Rob Suisted, Department of Conservation, Wellington, NZ
- Marie Haley, Department of Conservation, Akaroa, NZ
- David Higgins, Department of Conservation, Christchurch, NZ
- Austen Yoshinaga, M.P.H., New England Aquarium, Boston, Massachusetts, USA

Due to poor weather conditions and delays in issuance of permit and contract for this research, actual tagging did not commence until 4 March 2004. On that day, one dolphin was captured and tagged on the north side of Banks Peninsula. On 5 March 2004, two dolphins were caught and tagged on the south side of Banks Peninsula.

Chronology Of Field Work

Key Dates:
20 February 2004: Final unsigned contract is sent to G. Stone from DOC
21 February 2004: Dr. Jonas Teilmann arrives from Denmark
24 February 2004: Contract signed by New England Aquarium is delivered to DOC
25 February 2004: Marine Mammal Permit is signed for project
26 February 2004: Contract signed by DOC is returned to G. Stone
27 February 2004: First day of fieldwork
28-29 February and 1-3 March 2004: Bad weather kept team onshore
4 March 2004: First dolphin tagged
5 March 2004:Second and third dolphins tagged

27 February 2004
The vessels left the Duvauchelle wharf at 7:15 am and headed out of Akaroa Harbour to the east, finding the first pod of dolphins off Pompeys Pillar. At first the tail grab device was used from the DOC vessel Ranger II, but the dolphins were not approaching closely enough to attempt capture. Consequently, the tail grab and the dolphin catcher were transferred to the 5.8m vessel SeaFox. Dolphins approached this vessel more closely and attempts were made to secure the tail grab onto a dolphin. However, the tail grab device's thick padding prevented the jaws from closing on the dolphin's peduncle.

A decision was made to return to shore and modify the tail grab. The boat returned to the Duvauchelle wharf at 11:00am. The tail grab's padding was decreased in order to allow the jaws of the device to close around a dolphin's peduncle.

Both vessels left the same wharf again and headed out of Akaroa Harbour to recommence capture operations. At 2:25 pm a group of dolphins was located west of the entrance to Akaroa Harbour; a suitable dolphin was selected from the group and the tail grab was placed over the peduncle at 2:40 pm. While calm at first, the dolphin then began resisting capture and rotated its peduncle in the tail grab. The dolphin struggled and was brought onboard the vessel by two researchers who lowered their arms around the dolphin's body, at which point the animal became calm and breathed normally. At that time it was noted that the dolphin had a skin laceration on the peduncle with minor bleeding caused by contact with the capture device where rubber padding had come off during the capture. The dolphin was examined and released. After the animal was lowered into the water, it swam away to rejoin the pod from which it had been caught at 2:42 pm. The released dolphin was observed for approximately 15 minutes, swimming normally, until the pod dispersed heading in the direction of a commercial dolphin swim boat that had entered the area. Further captures this day were abandoned in order to reexamine the capture method; it was decided to reapply additional padding to the tail grab to avoid possible injury to an animal.

It was also discovered that tags 0 and 1 had turned themselves off during the boat operations. The seas had become very rough and possibly the tags were jostled, thereby triggering the on/off mechanism. We decided to contact Wildlife Computers before any tags were attached in order to explore this unexpected event.

Upon returning to shore, Alistair Hutt and Greg Stone telephoned Jan Coates (DOC) and reported the capture event. Besides reassessing the tail grab design, it was decided to bring Dr. Alan Baker to Akaroa to consult on further capture techniques. Wildlife Computers was contacted by email, alerting them of the tags turning off and seeking their advice. Once onshore, the tags were reprogrammed and restarted, and thereafter worked normally.

28-29 February and 1-3 March 2004
Bad weather kept the team onshore. During these days the tail grab technique was reviewed in detail with Dr. Alan Baker. Wildlife Computers could not explain the

problem with the tags; however, other studies have experienced similar problems when tags underwent strong impacts. The tags were thereafter kept in a more protected place until tagging.

4-5 March 2004

On 4 March, the weather finally improved and vessel operations were conducted on the north side of Banks Peninsula, launching from Pigeon Bay. Ranger II and Seafox surveyed south and east and found a group of 10 dolphins off Okains Bay. After working with this group for 20 minutes, it was decided to attempt a capture. Within five minutes a dolphin was captured and subsequently tagged with ARGOS tag #47813. Throughout the procedure, between five to nine other dolphins were milling around the vessels and exhibited particular boat-positive behaviour; the animals swam within one meter of the stern of the vessel during the tagging and health assessment procedures.

After tagging, the dolphin appeared healthy and was released, whereupon it swam away energetically. The vessels circled the area several times, but could not visually relocate the animal.

After this tagging procedure, the vessels returned to shore to refuel and headed out again, this time departing from Duvauchelle wharf at the upper end of Akaroa Harbour, on the south side of Banks Peninsula. The second effort was on the southeastern side of the peninsula, to the east outside of Akaroa Head, but conditions were too windy and the research vessels returned to shore with no dolphins captured or tagged.

On 5 March, the research team again launched from the Duvauchelle wharf at the upper end of Akaroa Harbour at 7:00 am and headed out to Akaroa Heads, where we found the wind too high for safe boat operations; we headed back inside Akaroa Harbour and waited onboard for the winds to diminish. At 9:00 am the winds abated; we surveyed to the east outside of the entrance to Akaroa Harbour, where we found a pod of eight dolphins off Damon's Bay. The dolphins swam in the bow wave of the vessels, and within five minutes a dolphin with no obvious marks on the dorsal fin was selected from the pod and was captured. During the health assessment and tagging procedures, the other seven dolphins swam closely around the boat, similar to the dolphins from the previous day's tagging. Upon release, the tagged dolphin dived and swam off in the direction of the pod. No further sightings were made of the study animal that day.

The next and last study dolphin, also without visible marks on its dorsal fin, was captured from another group. The other dolphins from this pod also remained in the area during the health assessment and tagging procedures. Upon release, the dolphin dived and swam off in the same manner as the two previous dolphins. No further sightings were made of the study animal that day.

Study Dolphin Details

ARGOS #: 47813
Field ID #: 0 (on backplate)

Captured animal details:

PUARI, a mature female who had likely given birth in the past based on the size and development of her genital slit and mammary glands. Permit did not allow tooth extraction for exact aging.

Body Weight:	45kg

Measurements

Snout-fluke:	142 cm
Snout-dorsal fin (posterior edge):	67 cm
Snout-blowhole:	20 cm
Girth pectoral:	90 cm
Girth umbilicus:	85 cm
Girth genital:	51 cm
Horizontal length dorsal fin:	22 cm
Height dorsal fin:	11 cm
Maximum length dorsal fin:	23 cm

Blubber thickness

Dorsal pectoral:	23 mm
Dorsal umbilical:	23 mm
Dorsal genital:	21 mm
Lateral pectoral:	23 mm
Lateral umbilical:	23 mm
Lateral genital:	21 mm

Capture time:	10:05.52
On-bed-time:	10:06.31
Heart rate monitor on-time:	10:09.32
Release time:	10:35.29
Time out of water:	28.58 min

4 March 2004 capture date
Position of capture and release: 43^{o} 39.906 S., 173^{o} 05.507 E.

ARGOS #: 47814
Field ID # 1 (on backplate)

Captured animal details:

TIMU TIMU, a male appearing to be a young adult. Permit did not allow tooth extraction for exact aging.

Body Weight:	32 kg
Measurements	
Snout-fluke:	123 cm
Snout-dorsal fin (posterior edge):	54 cm
Snout-blowhole:	18 cm
Girth pectoral:	79 cm
Girth umbilicus:	80 cm
Girth genital:	43 cm
Horizontal length dorsal fin:	21 cm
Height dorsal fin:	10 cm
Maximum length dorsal fin:	22 cm
Blubber thickness	
Dorsal pectoral:	19 mm
Dorsal umbilical:	18 mm
Dorsal genital:	16 mm
Lateral pectoral:	17 mm
Lateral umbilical:	18 mm
Lateral genital:	18 mm
Capture time:	09:31.13
On-bed-time:	09:33.33
Heart rate monitor on-time:	09:37.00
Release time:	09:58.26
Time out of water:	24.53 min
5 March 2004 capture date	
Position of capture and release:	43^{o} 53.67’ S., 172^{o} 59.39 E.

ARGOS #: 47815
Field ID#: 2 (on backplate)

Captured animal details:

TE RUAHINE, a female appearing to be a young adult. Permit did not allow tooth extraction for exact aging.

Body Weight:	38kg

Measurements

Snout-fluke:	126 cm
Snout-dorsal fin (posterior edge):	64 cm
Snout-blowhole:	18 cm
Girth pectoral:	81 cm
Girth umbilicus:	81 cm
Girth genital:	47 cm
Horizontal length dorsal fin:	22 cm
Height dorsal fin:	9.5 cm
Maximum length dorsal fin:	22 cm

Blubber thickness

Dorsal pectoral:	19 mm
Dorsal umbilical:	20 mm
Dorsal genital:	19 mm
Lateral pectoral:	19 mm
Lateral umbilical:	22 mm
Lateral genital:	19 mm

Capture time:	11:03.25
On-bed-time:	11:05.55
Heart rate monitor on-time:	11:07.06
Release time:	11:26.21
Time out of water:	20.26 min

5 March 2004 capture date
Position of capture and release: 43^{o} 53.64' S., 172^{o} 59.67 E.

Genetics Results

The two blood samples collected during the tagging yielded high quality RNA, with clear evidence of full-length mRNA suitable for cDNA amplification of expressed genes. rtPCR reactions have confirmed the expression of a class I and two class II. Patterns of sequence variation in at least two of the expressed MHC genes showed evidence of over dominant selection typical of genes involved in disease resistance. Analyses are underway to compare the functional diversity of the expressed MHC genes from the Banks Peninsula population with that of the critically endangered Maui's dolphin.

Molecular sexing of the samples confirmed the field identification of the tagged dolphins, and identified the untagged dolphin as a female (Table 2). Sequences variation in the first 420 base pair of the mtDNA control region matched two of the 17 previously described haplotypes. Two of the tagged dolphins (Puari #47813 and Timu Timu #47814) and the untagged dolphin were identified as haplotype Che-C, the most common maternal lineage in the East Coast population (Pichler 2002). The third tagged dolphin (Te Ruahine) was identified as haplotype Che-I, a relatively rare haplotype (5.5%) in this population.

Table 2. Sex and mitochondrial haplotype of four Hector's dolphins captured and released during satellite tagging trials.

Haplotype codes follow Pichler (2002).

Name	Tag #	Sample code	Sex	mt haplotype
Puari	47813	CheBP04-02	Female	Che-C
Timu Timu	47814	CheBP04-03	Male	Che-C
Te Ruahine	47815	CheBP04-04	Female	Che-I
None	None	CheBP04-01	Female	Che-C

Health Assessment Results

***PUARI (*47813)**

This adult female was in excellent body condition with a deep blubber layer. There were no significant integumentary lesions apart from a large pox tattoo lesion (70mm diameter) on the right side of the head. The dolphin appeared to have a mature genital slit and well developed mammaries but she was not lactating or detectably pregnant. A plasma progesterone level of 26.9 ng/ml suggests that she may have been in early pregnancy by comparison with progesterone levels reported for captive bottlenose dolphins, *Tursiops truncatus* (Sawyersteffan *et al*. 1983). Estradiol and testosterone levels were low (Table 3). Cortisol level in this dolphin was 0.59 µg/dl or 5.9 ng/ml (Table 3). This level is similar to those reported for bottlenose dolphins and killer whales, *Orcinus orca*, maintained in a captive facility and accustomed to people, handling, and blood sampling (Suzuki *et al*. 2003). It is also lower than levels reported for free-living bottlenose dolphins captured, restrained for varying time periods, and blood sampled (Thompson and Geraci 1986; Ortiz and Worthy 2000) and

much lower than in free-living beluga whales, *Delphinapterus leucas*, captured for blood sampling in the Canadian Arctic (St. Aubin and Geraci 1989). This result suggests that the Hector's dolphin did not mount a stress response to capture and handling during the 10 minutes post-capture when the blood was sampled. However, based on experimental studies on bottlenose dolphins, cortisol levels would not be expected to rise within the first hour and should have returned to baseline within four to five hours after release (Thompson and Geraci 1986; St. Aubin and Geraci 1990). The mean heart rate was 145±19 beats per minute (bpm) with a brief (2 second) excursion to 238 bpm following biopsy of the dorsal fin (Table 4). The heart rate, respiratory rate and cortisol level were all higher in this dolphin than in the two younger animals (Table 4).

No significant bacterial or fungal growth was recovered from the agar plates exposed to expired breath. *Bacillus sp*. grew on the blood agar plate but most bacteria in this genus are environmental saprophytes so no significance was attributed to this finding.

There are no reference values for Hector's dolphin haematology or serum chemistry. Comparisons were therefore made with values from captive healthy Commerson's dolphins (*Cephalorhynchus commersoni*) (Bossart *et al*. 2001). Based on this, all of the parameters relating to red blood cells were within expected ranges. The white blood cell count (15.6 x 10^9/L) was markedly elevated. This was caused by a markedly elevated eosinophil count and a mildly elevated neutrophil count. The former is most likely caused by endoparasitism such as lung worm infection or gastric nematodes. Both forms of parasitism are common findings in beached or bycatch Hector's dolphins. Elevated neutrophils usually indicate active acute inflammation but the site of this was not determined. It may be related to tissue damage caused by parasites. The blood urea level (17.8mmol/L) was higher than in Commerson's dolphins (5.5 to 7.2 mmol/L) but because the creatinine and albumin levels were low, there is no suggestion of renal failure or dehydration in this dolphin. Urea levels are often high in marine mammals because of their protein- and fat-rich diet, and the levels in the other two Hector's dolphins were also high. Creatinine phosphokinase (CPK), a muscle specific enzyme, was slightly elevated as is consistent with capture and handling. Alanine aminotransferase (ALT) is used as a marker of hepatocellular injury and increased levels are associated with hepatic necrosis, parasitism, neoplasia, and also with hepatic or muscular trauma. The level in this animal was lower than in Commerson's dolphins indicating no hepatic or significant muscular injury or disease. Aspartate aminotransferase (AST) is also released into the blood from injured muscle or liver. The level in this dolphin was within the normal range reported for Commerson's dolphins. Alkaline phosphatase (ALP) when elevated in the blood can indicate skeletal, hepatic or renal injury. It may also be elevated physiologically in young growing marine mammals. The level in this animal was within the range reported for Commerson's dolphins.

This dolphin tested positive in the *Brucella abortus* competitive ELISA for serum antibodies with a value of 66% inhibition (cut-off is at 30% inhibition). This is consistent with exposure to, and infection by, an organism antigenically similar to *B. abortus* which is a pathogen of cattle and terrestrial mammals. In cattle, this bacterium causes abortion late in pregnancy with autolysis *in utero* of the infected foetus. Subsequent pregnancies may be carried to term but the female may remain

infected for life. *B. abortus* can also cause mastitis in infected cows and orchitis in bulls and thus have a negative effect on milk production and male fertility.

B. maris or *B. delphinii* are the tentative names for a new strain(s) of *Brucella* isolated from pinnipeds, cetaceans, and an otter. Under experimental conditions one marine mammal isolate was found to cause abortion in cattle. Marine mammal strains have also been isolated from humans who have come in contact with infected cetaceans.

Evidence for infection in marine mammals was first recorded in 1994. Reports from the United Kingdom, Norway, the United States, Canada, the eastern Pacific and Antarctica described either the isolation of a *Brucella* organism or the identification of antibodies against it in tissues or serum from a variety of free-living pinniped and cetacean species (Foster *et al*. 1996; Nielsen *et al*. 1996, 2001; Ross *et al*. 1996; Garner *et al*. 1997; Jepson *et al*. 1997; Clavereau *et al*. 1998; Forbes *et al*. 2000; Ohishi *et al*. 2003). Clinical illness with abortion was reported in captive bottlenose dolphins in the United States (Miller *et al*. 1999).

Diagnosis of *Brucella* infection in marine mammals is difficult. Culture of the bacterium from tissues requires special media and culture conditions and can be a very lengthy process (Miller *et al*. 1999). The organism was not cultured from expired air or from genital swabs of this Hector's dolphin. However, the samples may not have been sufficient for detection of the bacterium. Serological diagnosis based on the presence of circulating specific antibody in the animal's serum is also challenging and controversial in that there is not always agreement on the correlation between newer enzyme based tests (such as the test used on this dolphin) and the classical test methods. The latter may result in more false positives when used on marine mammal sera. Cross reactivity between *Brucella* antigens and those from some other bacteria may also cause false reactions in some older tests (Weynants *et al*. 1995). The cELISA test employed at NCDI is, however, the test of choice and the same as reported in other studies (Nielsen *et al*. 1996, 2001). The remaining serology tests for leptospirosis (7 serovars) , morbillivirus (CDV), calicivirus (RHD), and influenza A (avian influenza) were negative.

***TIMU TIMU* (47814)**

This animal was a juvenile or sub-adult male in good body condition based on blubber depth. Testosterone levels were low but slightly higher than in the two females (Table 3). Plasma progesterone levels were negligible (1.83 ng/ml) and consistent with the sex of this animal. Cortisol levels were very low (0.26 μg/dl or 2.6 ng/ml) and much lower than in tagged animal Puari (47813). These levels are also similar to the baseline levels reported for captive bottlenose dolphins and killer whales (Suzuki *et al*. 2003) and calmly captured bottlenose dolphins (Thompson and Geraci 1986). The mean heart rate was 123±30 bpm and there was no change in pattern in response to any procedures (Table 4). The respiratory rate and cortisol level were the lowest of the three dolphins captured (Table 4).

There were no significant lesions apart from occasional dolphin pox tattoos on the head and thorax. A single colony of *Klebsiella oxytoca* was cultured from a genital swab, but this is a non-pathogenic commensal organism. No pathogens were isolated on the agar plates exposed to expired air. The erythrocyte parameters for this dolphin were within the ranges reported for Commerson's dolphins apart from the mean corpuscular haemoglobin concentration (MCHC) which was slightly lower. As with the previous dolphin, the white blood cell count was elevated but only mildly to moderately so in this case. The elevation was again caused by elevated eosinophils and also by slightly elevated lymphocytes and basophils. This again suggests parasitism with some chronicity rather than the more acute inflammation seen in the previous dolphin. Reticulocytes (immature red blood cells) were elevated slightly and there were nucleated red blood cells on the blood smear. This along with the lowered MCHC may indicate either some low grade chronic blood loss with regeneration or may be a feature of immature dolphins. The serum chemistry profile was almost identical to the previous dolphin, with elevated urea but low albumin and creatinine. Enzyme levels were either within the range for Commerson's dolphins (ALP, AST) or lower as in the case of ALT indicating no significant hepatic, muscular, skeletal or renal injury. CPK was again slightly elevated as is consistent with capture and handling.

Serology tests for pathogenic bacteria (leptospirosis and brucellosis) and viruses (morbillivirus, calicivirus, influenza A) were negative. In the case of *Brucella abortus* competitive ELISA for serum antibodies, this dolphin had an inhibition of only 28% (the cut-off for positivity is 30% inhibition).

***TE RUAHINE* (47815)**

This dolphin was a female and probably not yet reproductive as the genital slit was small and the mammary slits not prominent. Plasma progesterone levels were negligible (0.55 ng/ml) further suggesting that this female was either immature or not cycling. Estradiol levels were slightly higher than in Puari, the older female (Table 3). Plasma cortisol levels were intermediate between those of Puari and Timu Timu (0.36 µg/dl or 3.6 ng/ml). The mean heart rate was 117$\pm$20 bpm with no appreciable change in pattern following any procedure (Table 4). The respiratory rate and cortisol level were intermediate between the other two dolphins (Table 4).

The dolphin was in good body condition based on blubber depth. There were no integumentary lesions apart from occasional pox-like tattoos on the thorax. Approximately 15 whale lice (Crustacea, Cyamidae) up to 3mm diameter were present on the skin of the trunk and head. Specimens were collected for identification. A faecal swab was cultured for *Salmonella* but no bacteria were isolated. *Klebsiella oxytoca* and a Vibrio, probably *V. parahaemolyticus*, were isolated from a genital swab. Both are common in the marine environment and are likely commensal in this case.

The red blood cell parameters were all within normal ranges as compared to Commerson's dolphins. The white blood cells were again elevated with most of the elevation due to increased eosinophils and mildly increased basophils. As with the previous animals, this is most consistent with endoparasitism. The serum chemistry

was consistent with the previous two dolphins and shows no indication of injury or disease in any of the internal organs.

Serology tests for pathogenic bacteria (leptospirosis and brucellosis) and viruses (morbillivirus, calicivirus, influenza A) were negative. In the case of *Brucella abortus* competitive ELISA for serum antibodies, this dolphin had an inhibition of only 12% (the cut-off for positivity is 30% inhibition).

Figure 3. SPOT-3 tag on Hector's dolphin dorsal fin using two-pin attachment.

Hormone analysis results for study dolphins.

Table 3. Hormone analysis results for study dolphins.

Dolphin	Sex	Testosterone ng/ml	Estradiol pg/ml	Progesterone ng/ml	Cortisol µg/dl	Cortisol ng/ml	Cortisol nmol/l
Puari (47813)	older female	0.013	16.8	26.9	0.59	5.90	16.3
Timu Timu (47814)	male	0.182	12.0	1.83	0.26	2.57	7.08
Te Ruahine (47815)	younger female	0.013	24.4	0.55	0.36	3.60	9.93

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