Stress physiology and foraging of Diving petrels (*Pelecanoides urinatrix*) within the Hauraki Gulf.

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Diving petrels (*Pelecanoides urinatrix*) are an important local seabird species that was once found throughout New Zealand. Colonies within the Hauraki Gulf breed through the winter months and forage on zooplankton e.g. krill and copepods. However, higher densities of krill are found in the outer Gulf areas (i.e. near the Mokohinau Islands), with low densities occurring in the inner Gulf areas (i.e. near Tiritiri Matangi Island). Whether this results in inner Gulf colonies having greater levels of stress (and lower breeding success) as they work harder to obtain sufficient food for themselves and their chicks is unknown. Accordingly, we wish to test whether inner Gulf colonies are more stressed compared to outer Gulf diving petrel colonies.

To do this we wish to attach GPS devices to adult birds from Tiritiri Matangi and Mokohinau Islands to track foraging effort (trip length, duration and flight speed) during the breeding season. To measure stress, we wish to take blood and feather samples to measure stress hormones and stable isotopes (a measure of foraging quality) within both tissues. Measurements of chick weight and fledging success will also be determined in order to compare breeding success among colonies. Given that future conservation plans involve translocating diving petrels to islands deeper within the Gulf i.e. Rotoroa Island, understanding where diving petrels prefer to forage; and whether distance from prime feeding grounds results in increased stress and reduced breeding success is vital. By undertaking this study, our results will help optimise the location of these future colonies by allowing us to identify poor quality foraging areas where translocated colonies may fail to flourish.

Our hypothesis is: that "*Compared to the Mokohinau Islands, diving petrel colonies on Tiritiri Matangi Island are more stressed and not as successful in rearing chicks as they are further from the prime feeding grounds found at the outer Gulf areas."*

Therefore the aims of our experiments are to compare:

1) Foraging behaviour between adult diving petrels breeding on Mokohinau and Tiritiri Matangi Islands
2) levels of stress hormones in adult diving petrels breeding on Mokohinau and Tiritiri Matangi Islands
3) level of chick rearing success in terms of chick weight and fledging rates among the two colonies
4) Identify any inter-annual differences in foraging behaviour, stress physiology and chick rearing at these two sites

Diving petrels are a unique Procellariiform (petrels and shearwaters) seabird in that they are specialist predators of euphasid mesoplanktonic prey i.e. copepods and krill, that are accessed during day long foraging trips from their breeding colony (Prince and Morgan 1987). Diving petrels are noted for their poor flying ability as they have small wings adapted for reducing drag when diving underwater foraging for prey (Warham 1977). This poor flying ability is suspected to limit them to foraging near their breeding colonies. Indeed, preliminary GPD data collected by us for adult diving petrels breeding on Mokohinau Island (Figure 1) reveals that adults are restricted to foraging not more than 20 km from their colony. Thus if the foraging opportunities near their colony are poor then they may experience significant stress, particularly during breeding when adults have to feed a chick that weighs up to 20% more than the adult itself (akin to a 60 kg female human feeding a 72 kg baby!) Thus if diving petrels inhabit a colony where the nearshore waters offer a poor foraging environment (low krill and copepod numbers) then it may be that the colony also suffers, with adults having to

invest more energy into foraging and exhibiting higher levels of physiological stress (increased stress hormones) due to the effort involved.

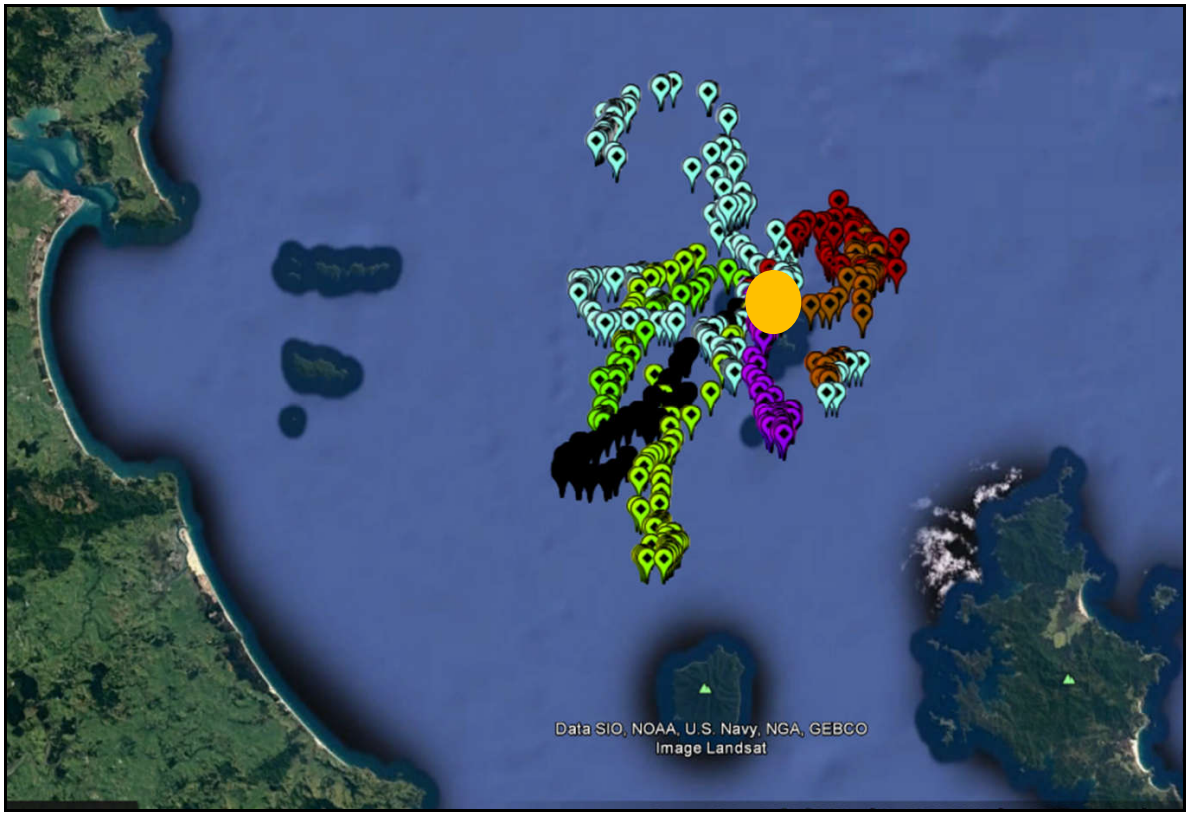

Figure 1: Preliminary GPD tracks of adult Diving petrels (*Pelecanoides urinatrix*) tracked from Mokohinau Islands during Oct 2016. Orange circle represents location of colony and n = 7 birds.

To achieve these aims we seek to combine data from miniaturised GPS, stable isotope analyses, ecophysiology and chick provisioning in two Hauraki Gulf populations of diving petrel (*Pelecanoides urinatrix*) with Figure 2 proving a schematic outlay of how our sampling procedures will occur.

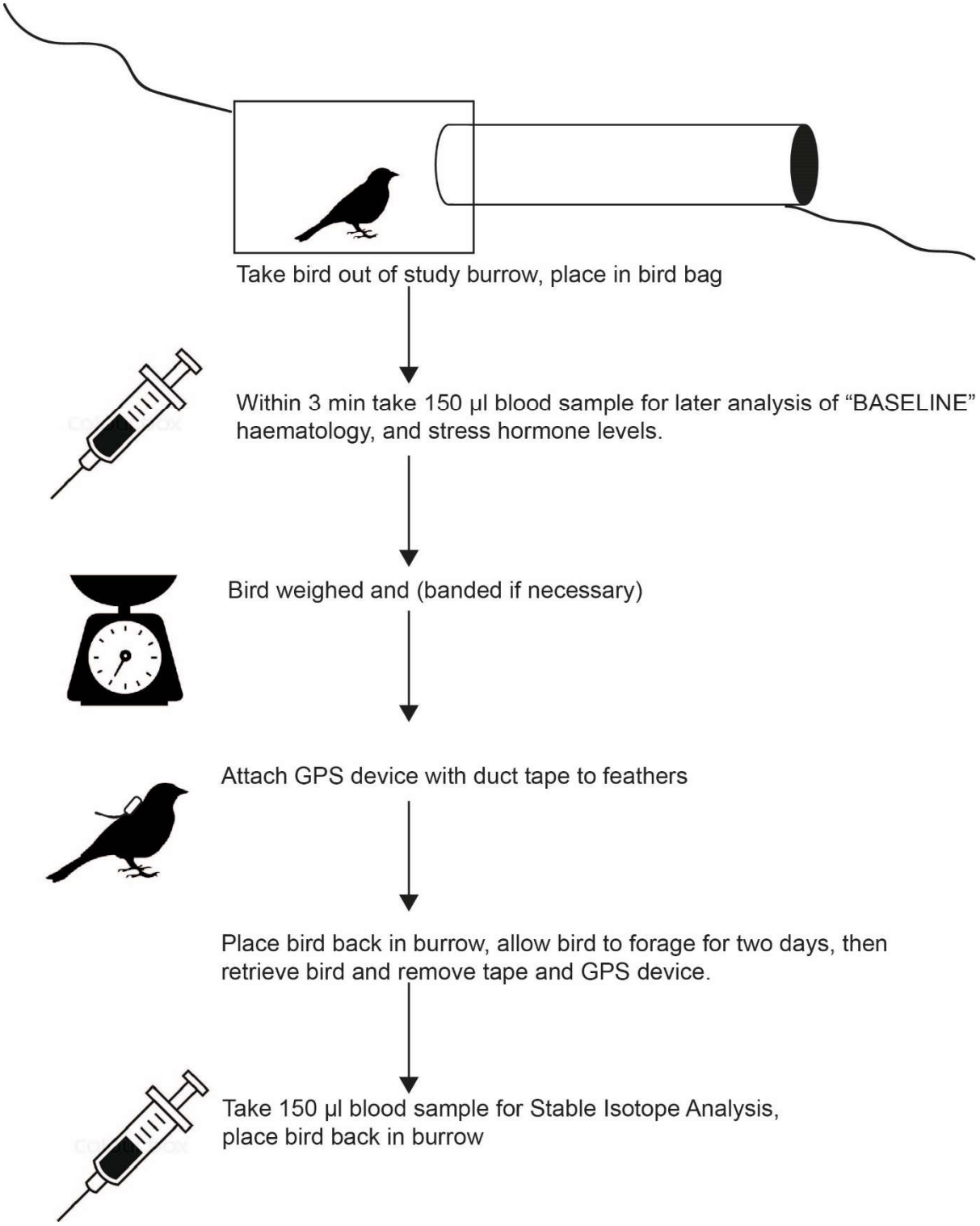

Figure 2: Schematic of sampling protocol to be used in attachment of GPS device and blood sampling of adult Diving petrels (*Pelecanoides urinatrix*).

Design of experiments

We will use adult birds already breeding in existing wooden study burrows on both Mokohinau and Tiritiri Matangi islands. These islands are ideal for our puroposes as suitable accommodation and field facilities are already existing to increase the likelihood of success. Moreover, established study burrows for this species are already in place on these islands.

During the 'incubating' and 'chick rearing' phases of the breeding cycle we will collect tracking data of foraging trips and blood samples for physiological parameters i.e. haematological variables (haemoglobin content, red blood cell count, haematocrit), stress hormones (CORT) and stable isotopes (see Figure 2).

Blood sampling protocol

To obtain blood samples, birds will be quickly and gently removed from the study burrow and placed within a dark bird bag. A blood sample will be obtained within 3 minutes of disturbing bird from the metatarsal vein by venipuncture. This will entail wiping with the leg with an ethanol swab and using a 29 gauge needle attached to a 1 mL syringe to remove 150 µl of blood, with the blood stored in a heparin lined tube for later analysis. Once sample has been taken a cotton swab will be held over the metatarsal vein to assist in stemming blood flow.

Bird weighing

Once blood sample has been obtained, the adult bird will be weighed within the bird bag using Pesola spring scales. At all times the bird will be supported, until the point where it is actually weighed whereupon it will be lifted gently off our hands by 2 mm so a weight can be obtained from the scale.

Also chicks will be weighed in the same manner (i.e. in a bird bag) when carefully removed from the study burrow.

GPS device attachment

Nanofix GPS devices by Path track (UK) will then be attached to the bird using 4 x 5 mm wide strips of duct tape. Devices will be attached to feathers on the shoulders of the bird i.e. between the wings and just behind the head. These devices weigh 2 g i.e. less than 3% of body weight. By using duct tape to attach the device we can then quickly peel off tape upon retrieval of bird and leave feathers intact. Once GPS device has been attached the bird will be put back into its burrow via the burrow entrance.

Total time take for above procedures

The above bleeding and attachment/removal of GPS devices will take no more than ten minutes. Should this time elapse then any remaining procedures will be abandoned for that bird, and another adult from a different burrow used.

Analysis of samples and collection of data

Once GPD devices have been collected, position fixes of the adult foraging tracks can be downloaded and foraging effort (trip duration, distance travelled, foraging events) between islands can be analysed by our colleague Jingjing Zhang at the School of Biological Sciences using *k*-means clustering models developed by her and colleagues (Zhang, O'Reilly et al. 2015).

Blood samples will be used to create blood smears for heterophil:lymphocyte ratios to check for compromised immunological parameters (Vleck, Vertalino et al. 2000). Also haematological parameters such as haematocrit, haemoglobin content and red blood cell counts will be used to check for changes in oxygen delivery (Dunphy, Taylor et al. 2015). Blood will also be spun to obtain plasma for measuring corticosterone (stress hormone) via commercially available ELISA kits (ENZO Biosciences) as laid out in Pitk, Tilgar et al. (2012). Finally a portion of plasma will be stored in ethanol and sent to NIWA laboratories for carbon and nitrogen stable isotope analysis of prey foraging quality. These will be quantified using an elemental analyser combustion furnace connected to a Delta^{Plus} continuous flow, IRMS (Thermo-Fischer Scientific, Bremen, Germany) with established operational protocols (Rayner, Hauber et al. 2008).

Chick weight and fledging rates will be compared by comparing weight of chicks and whether chicks in burrows fledged i.e. moulted down, grew flight feathers and left the burrow.

Replicates and statistical analysis

A schematic of our statistical analysis is given in Figure 3. To compare the foraging effort (mean trip length, duration and number of foraging events), haematology, stable isotope and stress hormone levels between islands a General Linear Mixed Model will be used to account for fixed (islands, breeding stage) and random (bird) statistical effects.

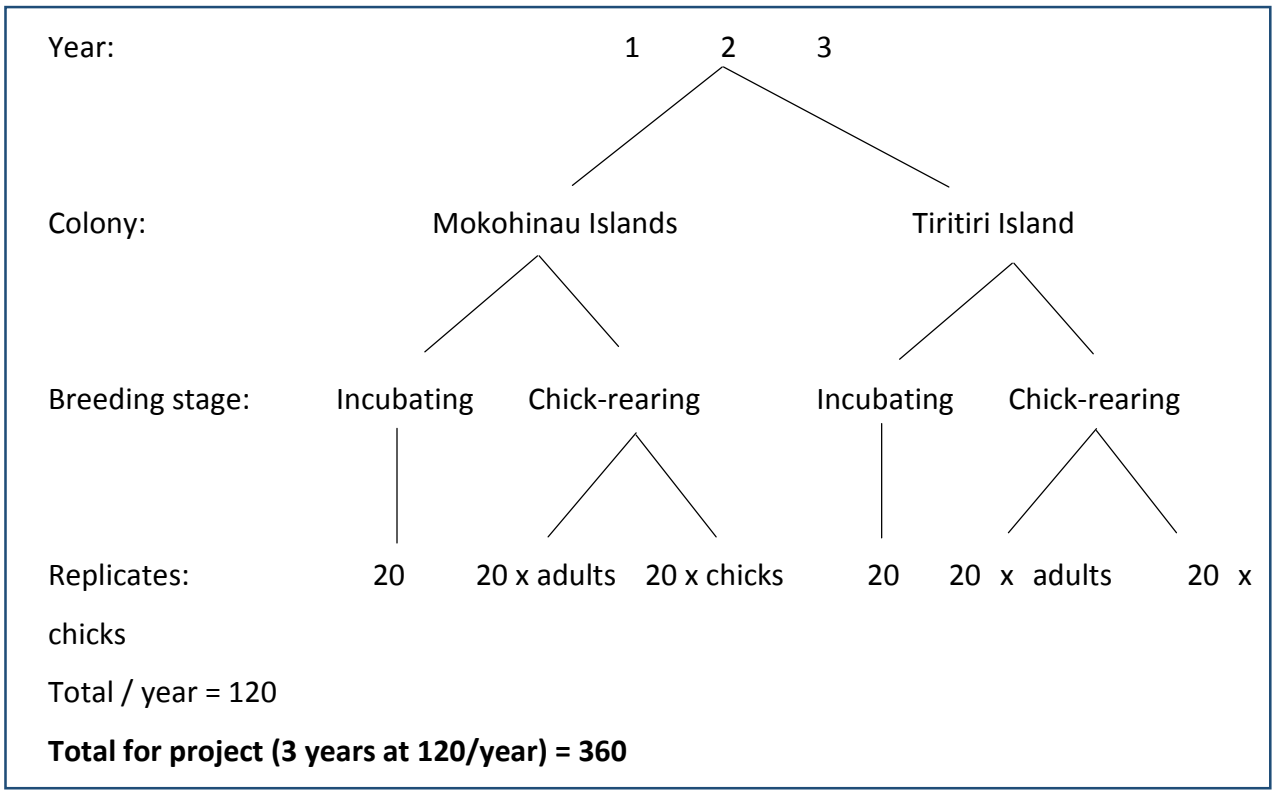

Figure 3: Schematic of statistical analysis and replicates used in this study.

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