

Investigating the diet of Hector's dolphins from the top of the South Island with stable isotope analysis

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Introduction

Stable isotope analysis has emerged in the last two decades as a powerful tool for studying the diet of animals. Stable isotope analyses are widely used to investigate foraging ecology in cetaceans and other marine mammals (Bowen & Iverson, 2013; Codron et al., 2007; Hopkins & Kurle, 2016; Newsome et al., 2010; Nielsen et al., 2018; Whiteman et al., 2019). This method is beneficial for assessing the diet of rare, mobile, and otherwise difficult-to-observe species. It is a valuable approach for species where more traditional methods such as stomach content analysis may be unsuitable. Stomach content analysis is reliant on the examination of dead animals, only provides a snapshot of recently ingested prey, and may give biased results due to differential prey residency times in the stomach (Bowen & Iverson, 2013; Nielsen et al., 2018). Stable isotope analyses are used for assessing trophic relationships within a community of predators, and they can also provide insight into ontogenetic, geographic, and sex variations in diet and habitat use (Carlisle et al., 2015; Kiszka et al., 2014; Lesage et al., 2001; Phillips et al., 2011).

Stable isotope analysis operates on the assumption that a consumer's tissues will reflect the isotopic composition of their prey (DeNiro & Epstein, 1978; 1981), enabling us to make deductions about the trophic level of the consumer and prey, and origin of prey. Nitrogen stable isotopes can indicate trophic position whereas carbon stable isotopes can be used to determine the productivity of marine ecosystems. More positive carbon isotope values indicate productive, nearshore regions and more negative values indicate offshore, regions (Bowen & Iverson, 2013; Rounick & Winterbourn, 1986). Additionally they can be used to identify differences between pelagic and benthic foraging locations (Cherel & Hobson, 2007). For dolphins, stable isotopes reflect the prey consumed two to four months before sample collection (Browning et al., 2014).

Many traditional methods for estimating diet in marine mammals are unsuitable for the Hector's dolphin (*Cephalorhynchus hectori hectori*). Indirect visual techniques such as stomach content analysis are challenging and seldomly possible because these rely entirely

on the availability of dead animals, which are often opportunistic. One study assessing the diet of Hector's dolphins around the Te Waipounamu/ South Island using stomach content analysis showed that the most commonly consumed prey were red cod (*Pseudophycis bachus*), ahuru (*Auchenoceros punctatus*), arrow squid (*Nototodarus* sp.), sprat (*Sprattus* sp.), sole (*Peltorhamphus* sp.) and stargazer (*Crapatalus* sp.) (Miller et al., 2013). Hector's dolphins feed throughout the water column and typically target small or juvenile prey <10 cm in length. Significant differences were found between the diets of dolphins from the east and west coasts, reflecting the differing prey distribution between the two regions (Miller et al., 2013). This highlights how different populations of Hector's dolphins may alter their diet based on available prey.

Bulk stable isotope analysis is a suitable method for overcoming many of the limitations imposed by direct observation of feeding and stomach content analyses and is a practical method for beginning to understand the diet and foraging ecology of Hector's dolphins. Here we use stable isotope analysis to address knowledge gaps in the foraging ecology of Hector's dolphins from the top of the South Island (TOTS – Figure 1).

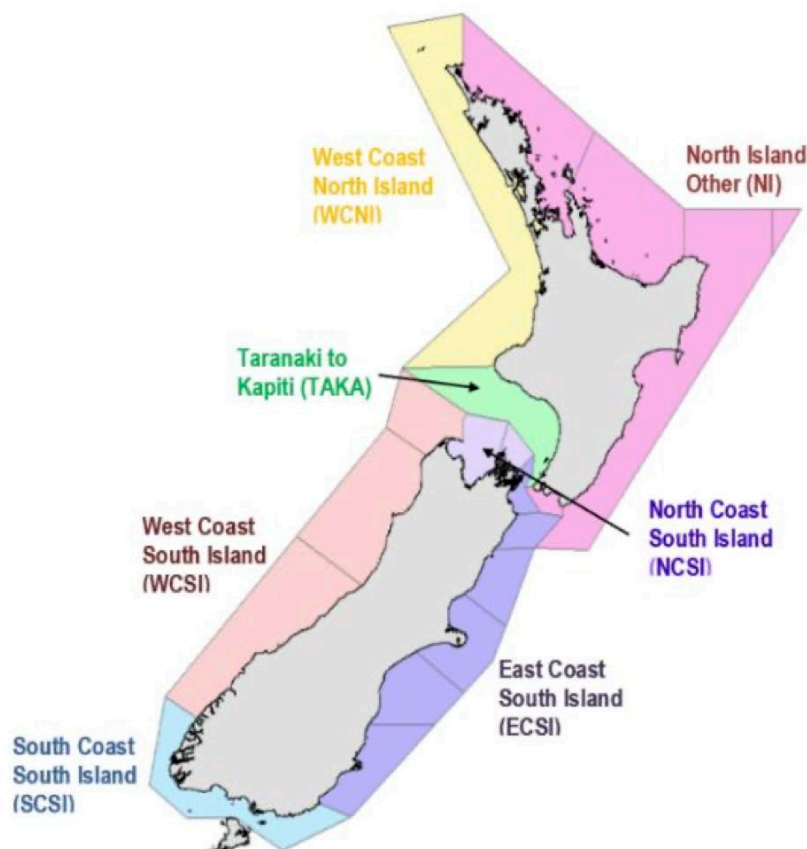


Figure 1: Spatial extent of Hector's and Māui dolphin sub-population areas (discrete colours) identified in the spatial risk assessment of threats to Hector's and Māui dolphins (Roberts et al., 2019). TOTS is referred to as North Coast South Island.

This is a complex region, including the east and west coast open waters (Te Koko-o-Kupe / Cloudy Bay), open shallow embayments (Mohua / Golden Bay and Te Tai-o-Aorere / Tasman Bay), and deep convoluted waters in the Marlborough Sounds - in particular Tōtaranui / Queen Charlotte Sound where Hector's are often sighted (Cross, 2019). The study by Miller et al. (2013) highlighted how diet differed between populations of Hector's dolphins, and little is known about diet and prey distribution for the sub-populations at the TOTS. Here we aimed to:

- Determine Hector's dolphin diet using stable isotope analysis and investigate any differences between dolphins from the west and east regions of the TOTS.
- Analyse the isotopic signatures of potential prey and estimate proportional contributions of prey to Hector's dolphin diet
- Discuss our findings in the context of aerial survey data from MacKenzie & Clement (2014) and with the assigned regions in the Roberts et al. (2019) review (Figure 1).

Methods

Sample Collection

Samples used in stable isotope analysis are a mixture of skin biopsy samples from live dolphins and samples from dead stranded dolphins. Skin biopsy samples were collected using a small, lightweight biopsy dart (PaxArms NZ Ltd) fired from a modified veterinary capture rifle (Krützen et al., 2002) during boat-based surveys conducted by the Department of Conservation – Te Papa Atawhai (DOC), Oregon State University and the University of Auckland – Waipapa Taumata Rau. All samples (excluding those collected in 2022) were genetically sexed and individuals were identified by genotype using standard PCR protocols, mitochondrial DNA and nuclear markers. Calves which were assumed to be less than one-year old based on size (< half the length of an adult and in close association with their mother (Webster et al., 2010)) were excluded from biopsy sampling. Skin biopsy samples were collected between 2011 – 2022 during genetic monitoring of Hector's dolphin populations in Queen Charlotte Sound, Golden Bay and Cloudy Bay, led by DOC. Stranded dolphin samples were collected opportunistically by DOC rangers in consultation with mana whenua and sent to the New Zealand Cetacean Tissue Archive (NZCeTA) for curation. All samples were stored in 70-90% ethanol until required for analysis. A summary of samples used in the analysis is provided in the Appendix (Table A1).

To determine the suitability of Hector's dolphin samples for inclusion in stable isotope analysis, we assessed the amount of sample available and its physical condition. To satisfy quality control criteria, there had to be at least 30mg of skin sample available to sub-sample.

Sample Preparation & Analysis

To mitigate the effect of ^{13}C depletion in lipid-rich tissues, all Hector's dolphin skin samples were lipid extracted with a 2:1 solution of chloroform and methanol, following the procedure in Newsome et al. (2018). Lipid extraction was deemed successful if the C:N ratio was between 3.0 and 4.0 (Post et al., 2007; Sweeting et al., 2006; Wilson et al., 2014; Yurkowski et al., 2015). Following lipid extraction, samples were sent to Isotrace NZ Ltd (University of Otago) for bulk stable isotope analysis of ^{13}C and ^{15}N .

Stable isotopes are reported in δ notation:

$$\delta^X = [(R_{\text{Sample}} / R_{\text{Standard}}) - 1] \times 1000$$

Where X is the isotope of interest (^{13}C or ^{15}N), and R is the ratio of heavy to light isotope (e.g. $^{13}\text{C}/^{12}\text{C}$). The internationally accepted standards for $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ are PeeDee Belemnite and atmospheric N_2 , respectively (Peterson et al., 1987). A correction for the Suess effect (0.011‰ yr^{-1}) (Eide et al., 2017) was applied to the $\delta^{13}\text{C}$ stable isotope values of the Hector's dolphin samples to allow the comparison of the $\delta^{13}\text{C}$ values from specimens from different time periods.

Statistical Analysis

The $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values for Hector's dolphin samples were plotted in R (v4.1.2) to visually inspect the data for any trends. Statistical analyses were carried out in R (v4.1.2). The Shapiro-Wilk test of normality was used to assess the distribution of $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values. The Kruskal-Wallis, one-way ANOVA and Post-hoc Dunn's multiple comparison tests were used to evaluate differences in $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values of Hector's dolphin skin samples according to location, year of sampling, and sex.

We assessed differences in the isotopic niche space of Hector's dolphins between sampling locations using the Stable Isotope Bayesian Ellipses (SIBER, version 2.1.6) package in R (Jackson et al., 2011). Bivariate ellipses of $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values with 95% confidence intervals were used to estimate isotopic niche space for Hector's dolphins from Cloudy Bay, Golden Bay, and Queen Charlotte Sound. Bayesian standard ellipse areas (SEA_B) were plotted using SIBER to show niche overlap and changes in isotopic niche space between time periods.

Results

A total of 116 Hector's dolphin skin samples were included in stable isotope analysis. This data set contained 111 biopsy samples and five samples from stranded animals (Tables 1 & 2). For analyses comparing isotope values between the three primary locations (Cloudy Bay,

Golden Bay and Queen Charlotte Sound), samples from the stranded animals were excluded as they were not collected from these locations. Metadata for each sample is provided in the Appendix (Table A1).

Table 1: Overview of Hector's dolphin biopsy samples included in stable isotope analysis. Median $\delta^{13}\text{C}$, $\delta^{15}\text{N}$ and standard deviations are provided for each sampling location. Genetically determined sex is provided where available.

Location	Median $\delta^{13}\text{C} \pm$ s.d. (range)	Median $\delta^{15}\text{N}$ \pm s.d. (range)	Year	Males	Females	Undeter- mined sex	Total (n)
Cloudy Bay	-16.7 ± 0.7 (-18.0 to -14.1)	15.2 ± 0.7 (14.3 to 17.7)	2011	15	14	0	29
			2012	12	15	0	27
Golden Bay	-17.8 ± 0.9 (-18.9 to -15.4)	15.8 ± 0.7 (14.7 to 17.4)	2014	2	2	1	5
			2015	2	1	0	3
			2021	2	0	0	2
			2022	-	-	4	4
Queen Charlotte Sound	-17.2 ± 0.4 (-17.8 to -16.2)	14.8 ± 0.4 (14.3 to 15.8)	2016	5	6	0	11
			2021	-	-	1	1
			2022	-	-	29	29
Overall	-17.1 ± 0.7 (-18.9 to -14.1)	15.1 ± 0.7 (14.3 to 17.7)	Total	38	38	35	111

Table 2: Overview of stranded Hector's dolphin samples included in stable isotope analysis. M = male, F = female and U = unknown sex.

Year	Individual ID	Stranding Location	Sex	$\delta^{13}\text{C}$	$\delta^{15}\text{N}$
2014	Che14TM01	Golden Bay	M	-17.3	15.5
2015	Che15TM01	Nelson area	F	-16.7	15.4
2015	Che15TM02	Nelson area	M	-17.3	17.1
2017	Che17WC03	Westport	U	-17.7	14.3
2018	Che18MB01	Nelson area	F	-17.9	14.8
Mean (\pm s.d)				-17.3 \pm 0.5	15.5 \pm 1.0

The 116 Hector's dolphin skin samples, collected between 2011 and 2022, had a median $\delta^{13}\text{C}$ value of $-17.1 \pm 0.7\text{‰}$ (range -18.9 to -14.1 ‰) and a median $\delta^{15}\text{N}$ value of $15.1 \pm 0.7\text{‰}$ (range 14.3 to 17.7 ‰; Figure 2). The $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ distributions of the combined dataset were not normally distributed (Shapiro-Wilk W-test: $\delta^{13}\text{C}$: $n=116$, $p < 0.05$; $\delta^{15}\text{N}$: $n=116$, $p < 0.0005$).

In the Cloudy Bay data set from 2011-2012, there were 39 genetically identified individuals (Hamner et al., 2017). Of these, 21 were sampled in both 2011 and 2012. In the Golden Bay data set ($n=14$), two genetically identified individuals (Baker et al., 2017) were sampled in more than one year. Genotyping work to determine the sex and identity of samples from 2021 and 2022 is ongoing.

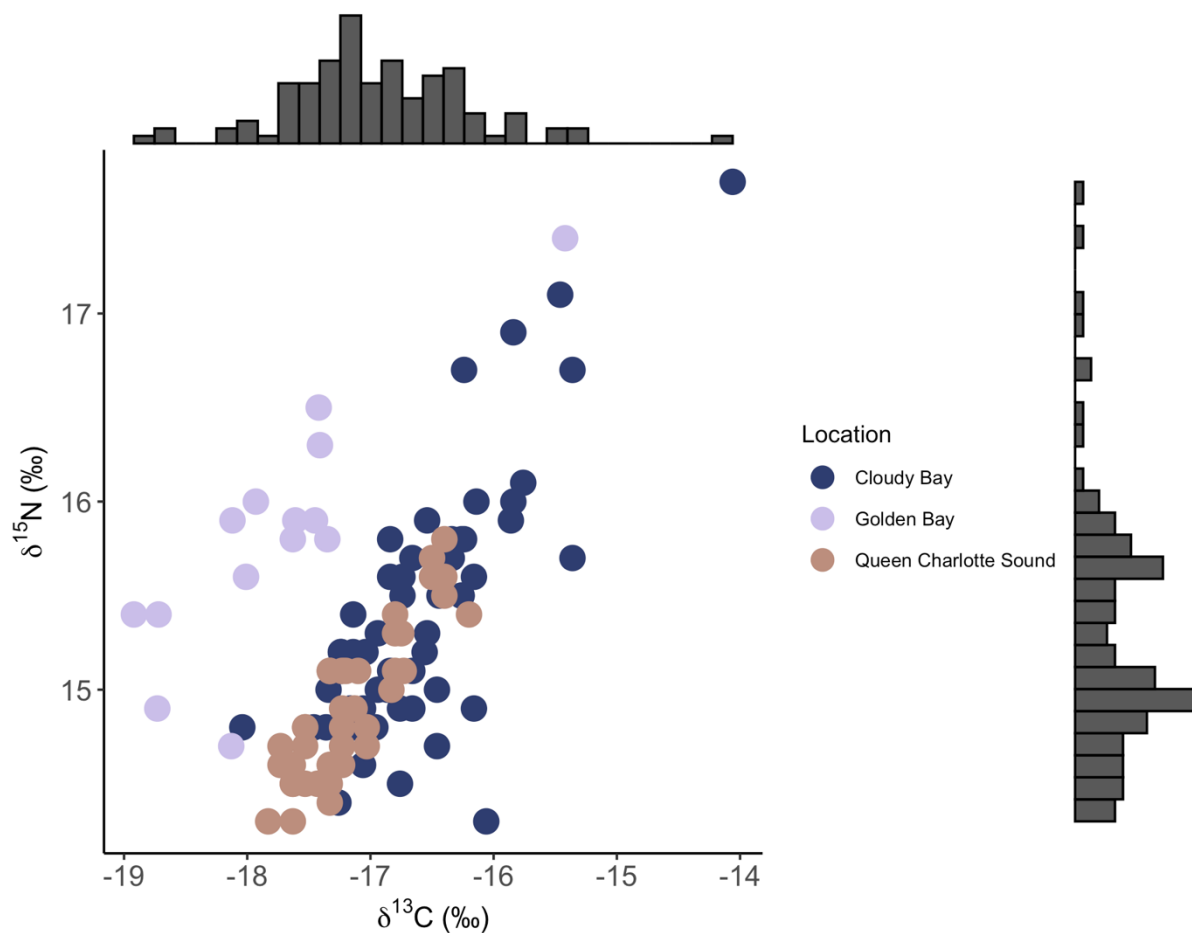


Figure 2: $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values of 111 Hector's dolphin skin biopsy samples. Isotope ratios are coloured according to sample location. Frequency distributions of $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ are shown as marginal histograms. Note that this figure does not include stranded samples (see Appendix Figure A1).

Differences between locations

Stranded samples were excluded from the analyses to test for differences between locations, as they could not be classified into the three primary locations of Cloudy Bay, Golden Bay and Queen Charlotte Sound. Future genetic work will aim to resolve this ambiguity. The distribution of isotope values in stranded samples compared with biopsy samples can be seen in Appendix Figure A1. Therefore, only biopsy samples ($n=111$) were included to test for differences between the three sampling locations. The data set was not normally distributed (Shapiro-Wilk W-test: $\delta^{13}\text{C}$: $p < 0.05$; $\delta^{15}\text{N}$: $p < 0.0005$). There were statistically significant differences in $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ between the three sampling locations ($\delta^{13}\text{C}$: Kruskal-Wallis (K-W) $\chi^2 = 38.4$, $p < 0.0005$; $\delta^{15}\text{N}$: K-W $\chi^2 = 27.1$, $p < 0.0001$). Post-hoc Dunn's multiple comparisons indicated that every location was statistically significantly different from the other (Appendix Tables A2 and A3). The $\delta^{13}\text{C}$ values in Cloudy Bay were statistically significantly higher than in Golden Bay and Queen Charlotte Sound (Figure 3A).

In Golden Bay, $\delta^{15}\text{N}$ values were statistically significantly higher than in Cloudy Bay and Queen Charlotte Sound (Figure 3B).

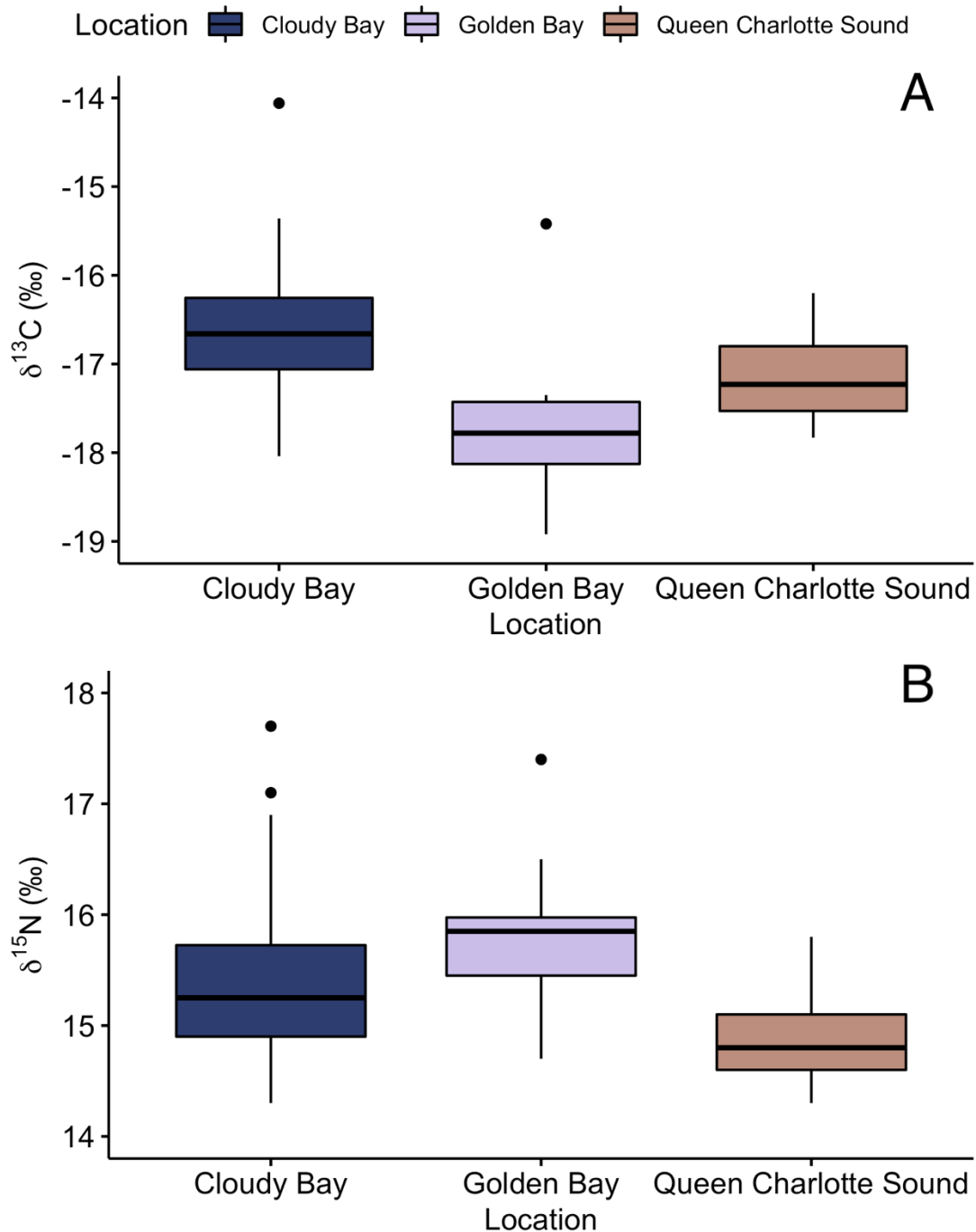
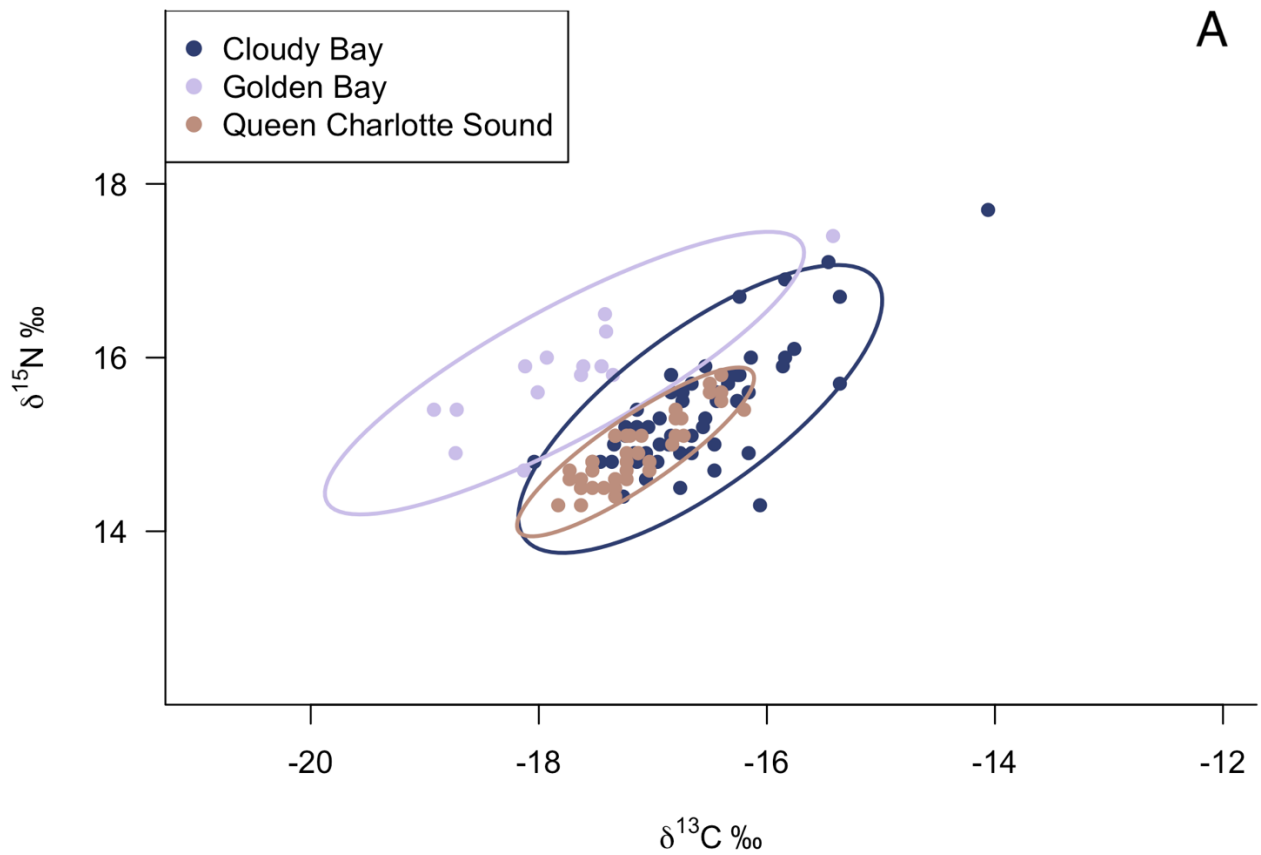


Figure 3: Box plots comparing (A) median $\delta^{13}\text{C}$ values and (B) median $\delta^{15}\text{N}$ values of Hector's dolphin biopsy samples from Cloudy Bay ($n=56$), Golden Bay ($n=14$) and Queen Charlotte Sound ($n=41$). The black line represents the median, shaded boxes show the interquartile range, and whiskers show the minimum and maximum isotope values. Black dots represent outliers.

Hector's dolphin isotopic niche space

The SIBER analysis of Hector's dolphin biopsy samples collected from Cloudy Bay, Golden Bay and Queen Charlotte Sound indicated distinct niche spaces between the east and west regions of the TOTS. Within the east region of the TOTS, the isotopic niche space of Queen Charlotte Sound was a subset of Cloudy Bay (Figure 4).



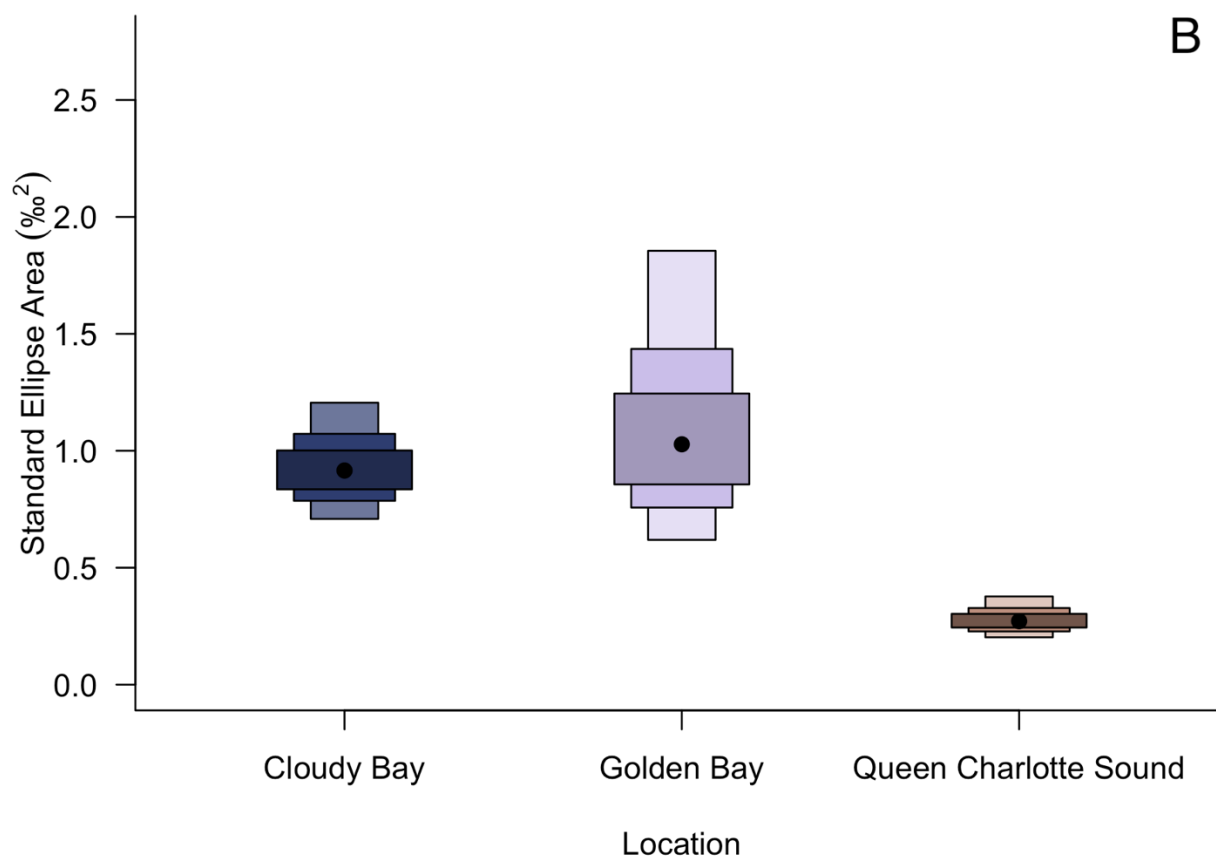


Figure 4: (A) Isotopic niche space indicated by 95% CI bivariate ellipses of Hector's dolphin biopsy skin samples collected from Cloudy Bay, Golden Bay and Queen Charlotte Sound. (B) Bayesian standard ellipse area (SEA_B) of isotopic signatures in Hector's dolphin biopsy skin samples collected from Cloudy Bay, Golden Bay and Queen Charlotte Sound. The black dot in the centre represents the mode, and shaded boxes represent the 50%, 75% and 95% credible intervals from dark to light.

Differences between sex

Sex has been genetically determined for 76 of the 111 Hector's dolphin biopsy samples, of which 38 were male and 38 female. The 35 remaining biopsy samples are from the 2022 Queen Charlotte Sound survey and are yet to be sexed genetically. This subset comprised 38 males and 38 females from the three sampling locations.

The subset of data where sex was known ($n = 76$) was not normally distributed with respect to $\delta^{13}\text{C}$ (Shapiro-Wilks test: $W = 0.96$, $p < 0.05$) or $\delta^{15}\text{N}$ (Shapiro-Wilks test: $W = 0.92$, $p < 0.005$). In the overall population ($n = 76$), there were no statistically significant differences in $\delta^{15}\text{N}$ between sexes (K-W: $df = 2$, $\chi^2 = 2.0$, $p > 0.05$), but $\delta^{13}\text{C}$ values were statistically significantly different (K-W: $df = 2$, $\chi^2 = 4.7$, $p < 0.05$).

To investigate this difference further, we tested for statistically significant differences between sexes in $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values for each location (Cloudy Bay, Golden Bay and Queen Charlotte Sound). In Cloudy Bay ($n = 56$), there were no statistically significant differences in

$\delta^{13}\text{C}$ (Shapiro-Wilks test: $W = 0.93$, $p < 0.005$; K-W: $df=1$, $\chi^2 = 2.1$, $p > 0.05$) and $\delta^{15}\text{N}$ (Shapiro-Wilks test: $W = 0.92$, $p < 0.005$; K-W: $df=1$, $\chi^2 = 3.5$, $p > 0.05$) values between sexes.

The Golden Bay subset ($n=9$) was normally distributed ($\delta^{13}\text{C}$: Shapiro-Wilks test: $W = 0.86$, $p > 0.05$; $\delta^{15}\text{N}$: $W = 0.87$, $p > 0.05$) and variance of the two groups was not statistically significantly different (F-test: $\delta^{13}\text{C}$: $p > 0.05$; $\delta^{15}\text{N}$: $p > 0.05$). There were no statistically significant differences in $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values between sexes (Student's two-sample t -test: $\delta^{13}\text{C}$: $t = 0.62$, $p > 0.5$; $\delta^{15}\text{N}$: $t = 0.86$, $p > 0.05$).

The Queen Charlotte Sound subset ($n=11$) was normally distributed ($\delta^{13}\text{C}$: Shapiro-Wilks test: $W = 0.92$, $p > 0.05$; $\delta^{15}\text{N}$: $W = 0.87$, $p > 0.05$) and variance of the two groups was not statistically significantly different (F-test: $\delta^{13}\text{C}$: $p > 0.05$; $\delta^{15}\text{N}$: $p > 0.05$). There were no statistically significant differences in $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values between sexes (Student's two-sample t -test: $\delta^{13}\text{C}$: $t = 0.30$, $p > 0.5$; $\delta^{15}\text{N}$: $t = 1.6$, $p > 0.05$).

Differences within locations: Cloudy Bay

There were 56 biopsy samples collected in Cloudy Bay in 2011 ($n=29$) and 2012 ($n=27$; Table 1). The subset of Cloudy Bay data ($n=56$) was not normally distributed (Shapiro-Wilks test: $\delta^{13}\text{C}$: $W = 0.93$, $p < 0.005$; $\delta^{15}\text{N}$: $W = 0.92$, $p < 0.005$). The Kruskal-Wallis test revealed no significant differences between years with respect to $\delta^{13}\text{C}$ ($\chi^2 = 2.0$, $p > 0.05$) but did show that years were significantly different with respect to $\delta^{15}\text{N}$ ($\chi^2 = 4.5$, $p < 0.05$; Figure 5). Of the 56 samples from Cloudy Bay, 18 genetically identified individuals (Hamner et al., 2017) were sampled once in 2011 and then again in 2012 (Appendix Figure A2) Please note the samples used here are a subset of the samples used in a genotype-based abundance estimate (Hamner et al., 2017). In our analysis we preferentially selected samples from this data set which were from individuals who had been resampled across 2011 and 2012, to assess individual variation in isotope values over time. In Hamner et al. (2017) there were 147 individuals identified across 2011 and 2012. Of these, 28 were sampled in both years. We have selected 18 of the 28 resampled individuals for stable isotope analysis.

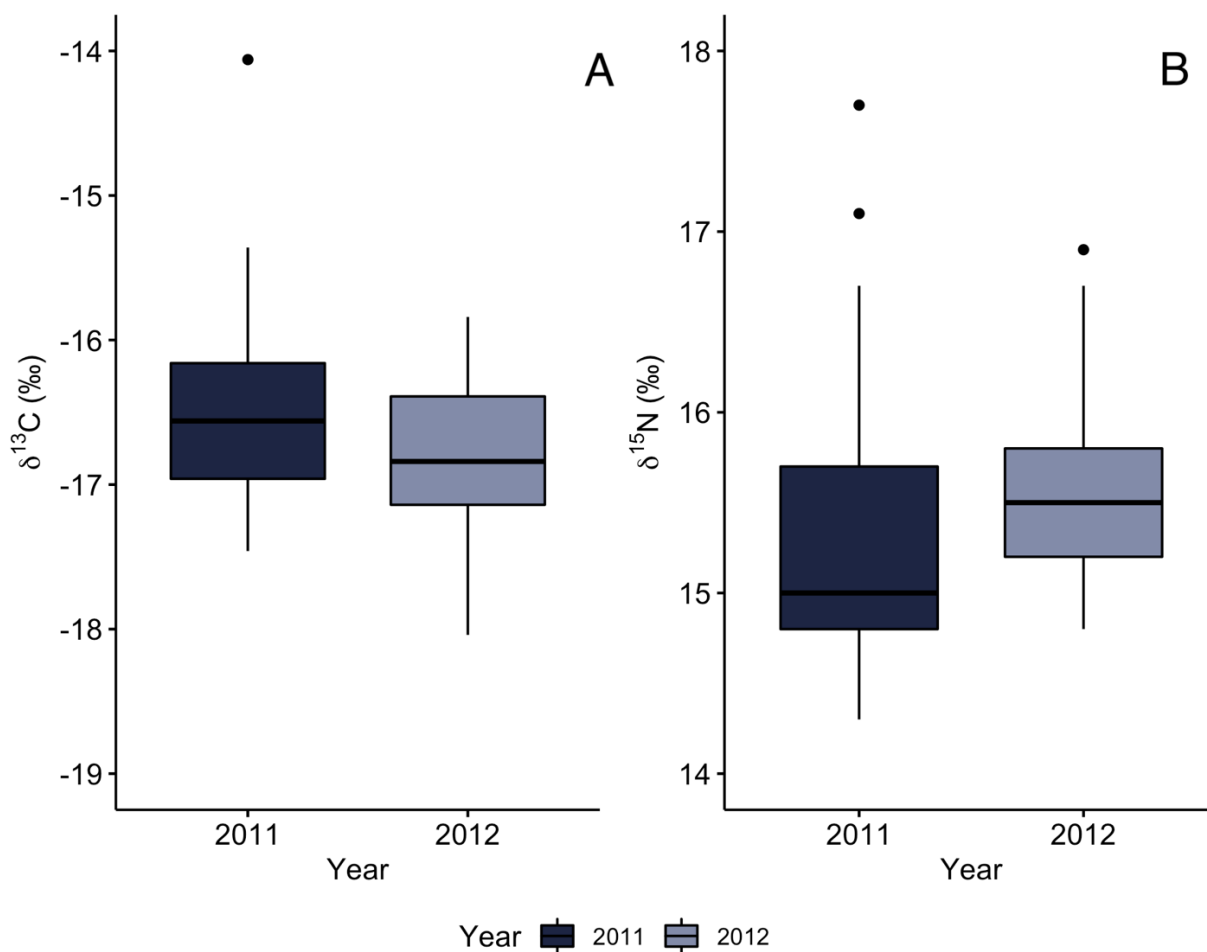


Figure 5: Combined box plots comparing (A) median $\delta^{13}\text{C}$ and (B) median $\delta^{15}\text{N}$ values between sampling years, in Cloudy Bay. The black line represents the median, shaded boxes show the interquartile range, and the whiskers show the minimum and maximum isotope values. Black dots represent outliers.

Differences within locations: Golden Bay

There were 15 samples from Golden Bay, including one sample from a stranded dolphin (Table 3). Of these, there were two individuals who were sampled in more than one year – Che14GB07 (2014 and 2015) and Che15GB03 (2015 and 2021) (Appendix Figure A3).

Table 3: Overview of Hector's dolphin skin samples from Golden Bay, including median $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values per year (s.d. = standard deviation).

Year	Stranded	Biopsy	Total (<i>n</i>)	Median $\delta^{13}\text{C} \pm \text{s.d.}$	Median $\delta^{15}\text{N} \pm \text{s.d.}$
2014	1	5	6	-17.8 ± 1.3	15.7 ± 0.8
2015	0	3	3	-17.6 ± 0.3	15.9 ± 0.4
2021	0	2	2	-17.4 ± 0.1	15.9 ± 0.1
2022	0	4	4	-18.0 ± 0.5	15.4 ± 0.6

The Golden Bay data are normally distributed with respect to $\delta^{15}\text{N}$ (Shapiro-Wilks test, $p > 0.05$) but are not normally distributed with respect to $\delta^{13}\text{C}$ (Shapiro-Wilks test, $p < 0.05$). To test for differences in $\delta^{13}\text{C}$ between years, a Kruskal-Wallis test was used and found there were no statistically significant differences ($\chi^2 = 2.9$, $df = 3$, $p > 0.05$). To test for differences in $\delta^{15}\text{N}$ between years, a one-way ANOVA was used and revealed no statistically significant differences between years ($df = 3$, $F = 0.9$, $p > 0.05$; Figure 8).

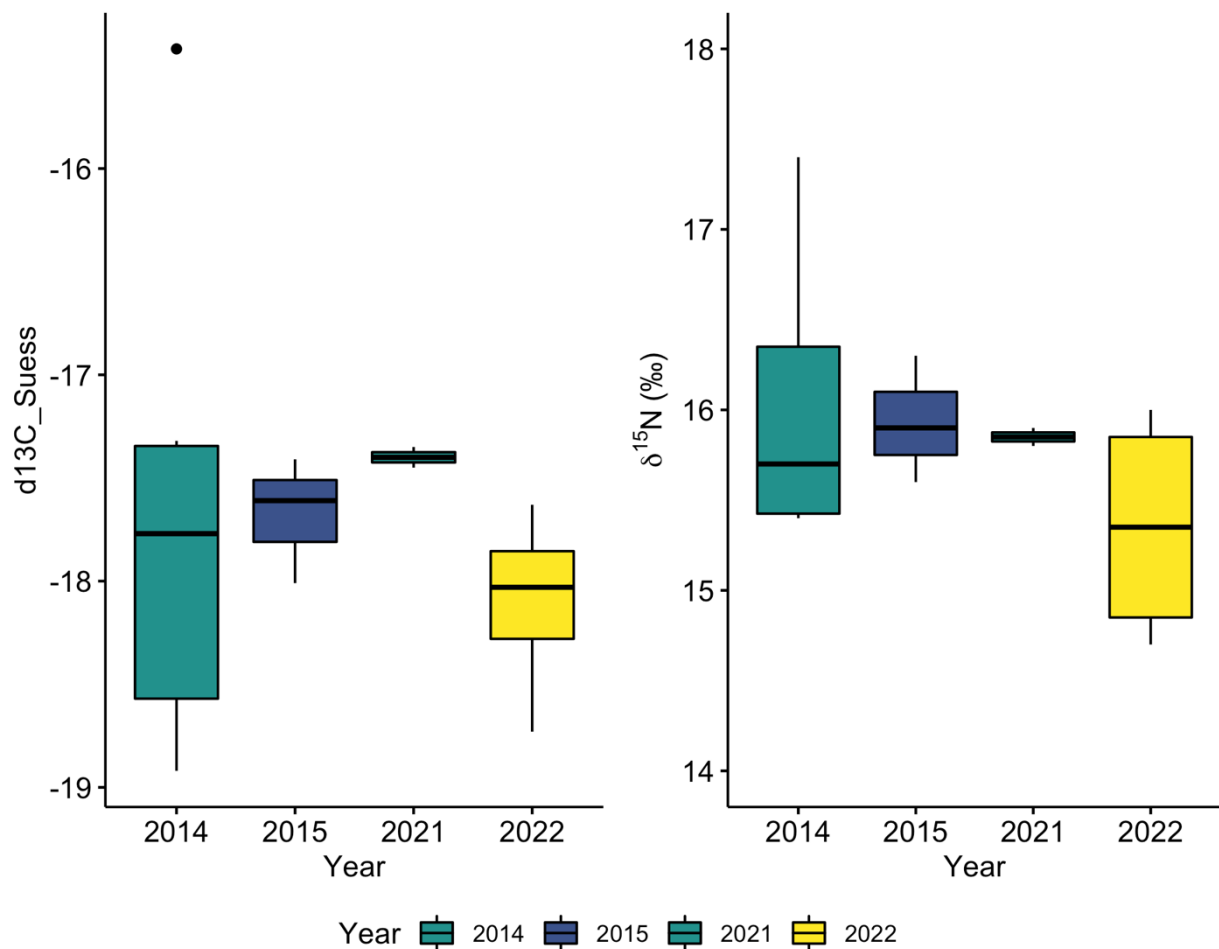


Figure 6: Combined box plots comparing (A) median $\delta^{13}\text{C}$ and (B) median $\delta^{15}\text{N}$ values between sampling years, in Golden Bay. The black line represents the median, the shaded boxes show the interquartile range, and the whiskers show the minimum and maximum isotope values. Black dots represent outliers.

Differences within locations: Queen Charlotte Sound

There were 41 biopsy samples from Queen Charlotte Sound, collected in 2016 ($n = 11$), 2021 ($n = 1$) and 2022 ($n = 29$). Samples from 2016 were collected in June and therefore reflect

the autumn diet of dolphins, whereas samples from 2022 were collected in April and reflect summer diet. One sample was collected in October 2021 and reflects the winter diet of Hector's dolphins. Work to genotype the samples collected in 2022 is ongoing.

Table 4: Overview of Hector's dolphin skin samples from Queen Charlotte Sound, including median $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values per year (s.d. = standard deviation).

Year	Total (<i>n</i>)	Median $\delta^{13}\text{C} \pm \text{s.d.}$	Median $\delta^{15}\text{N} \pm \text{s.d.}$
2016	11	-16.5 ± 0.3	15.4 ± 0.2
2021	1	-16.8	15.3
2022	29	-17.3 ± 0.3	14.7 ± 0.2

The Queen Charlotte Sound data are normally distributed with respect to $\delta^{15}\text{N}$ (Shapiro-Wilks test, $W = 0.9$, $p > 0.05$) but are not normally distributed with respect to $\delta^{13}\text{C}$ (Shapiro-Wilks test, $W = 0.9$, $p < 0.05$). There were statistically significant differences in $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ between sampling years (K-W test; $\chi^2 = 19.9$, $df = 1$, $p < 0.0005$, One-way ANOVA: $df = 1$, $F = 70.2$, $p < 0.0005$, respectively).

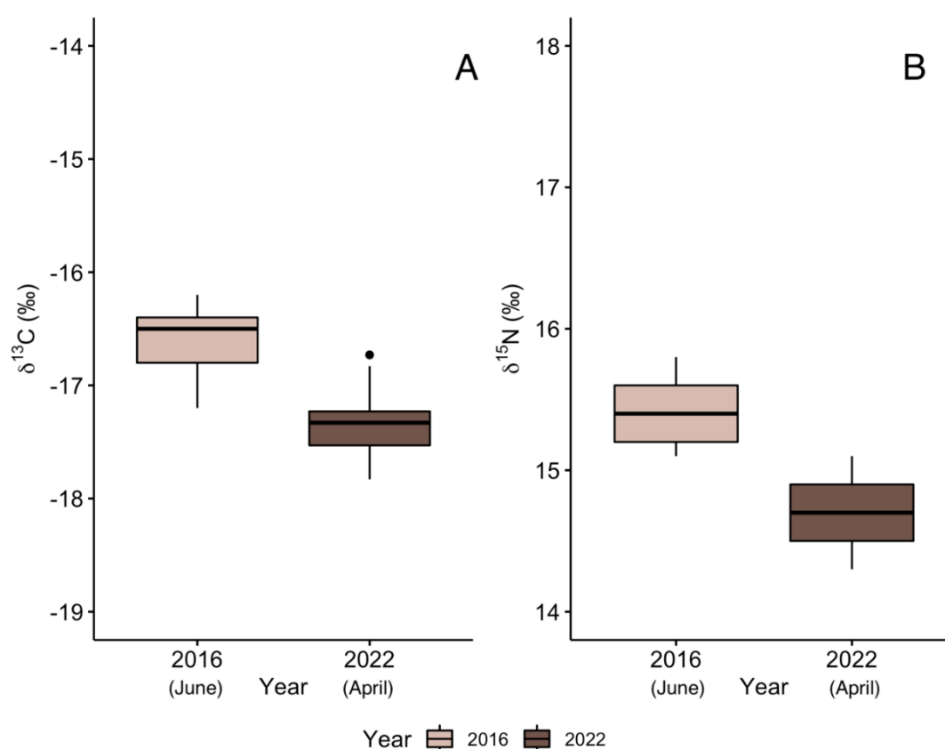


Figure 7: Combined box plots comparing (A) median $\delta^{13}\text{C}$ and (B) median $\delta^{15}\text{N}$ values between sampling years, in Queen Charlotte Sound. Month of sample collection is shown beneath the year. The median is represented by the black line, shaded boxes show the interquartile range, and the whiskers show the minimum and maximum isotope values. Outliers are represented by black dots.

Discussion

In this preliminary investigation we focused on documenting the $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values in the skin of Hector's dolphins from the TOTS, collected from Queen Charlotte Sound, Golden Bay and Cloudy Bay between 2011 and 2022. We reveal that there are statistically significant differences in isotope values between the Hector's dolphins from Golden Bay, Cloudy Bay, and Queen Charlotte Sound. Isotopic niche space was distinct and had minimal overlap between the western Tasman Bay and eastern Marlborough regions. Within the eastern region of the TOTS, isotopic niche space of Queen Charlotte Sound was a subset of Cloudy Bay.

Spatial differences in isotope values

We observed statistically significant differences in $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values in Hector's dolphins from the three sampling locations. In particular, there were differences between the eastern (Cloudy Bay, Queen Charlotte Sound) and western (Golden Bay) regions (Figure 2). Within the eastern region (Marlborough), there were statistically significant differences in isotope values of dolphins from Queen Charlotte Sound and Cloudy Bay.

The differences observed could be driven by a combination of prey preferences and variation in the isotopic baseline across the TOTS, which has complex topography and biogeochemistry (McMullin et al., 2021; Nicol, 2011; Trewick & Bland, 2012; Ulrich & Handley, 2020). In general, the productivity of marine ecosystems can be inferred by $\delta^{13}\text{C}$ values; more positive values indicate nearshore, productive regions, whereas more negative values are indicative of less productive, offshore regions (Newsome et al., 2010). Here the most negative $\delta^{13}\text{C}$ values were observed in the Tasman region (Golden Bay, Figure 3), suggesting that Hector's dolphins in this region may consume more pelagic or offshore sources of prey which are typically depleted in ^{13}C (Cherel & Hobson, 2007; Gaden & Stern, 2010), compared to Hector's dolphins from the Queen Charlotte Sound, which had more positive $\delta^{13}\text{C}$ values.

Nitrogen isotopes ($\delta^{15}\text{N}$) serve as indicators of trophic position, where $\delta^{15}\text{N}$ values increase as you move up the trophic web (Newsome et al., 2010). Here we observed Golden Bay had statistically significantly higher $\delta^{15}\text{N}$ values than the Marlborough region (Figure 3). This suggests that prey consumed by Hector's dolphins in Golden Bay are of a higher trophic level than prey in the Marlborough region, but this can also indicate an elevated $\delta^{15}\text{N}$ baseline in Golden Bay. To better understand the drivers of higher $\delta^{15}\text{N}$ values in Golden Bay, we would need to compare the isotopic baselines of both regions, ideally for the period when the Hector's dolphin samples were collected. This requires samples representative of the baseline from each region (e.g., green-lipped mussels, *Perna canaliculus*) which were unavailable for this study.

Isotopic niche space can be thought of as a proxy for ecological niche. Isotopic niche space is defined by the consumer's isotopic distribution in two dimensions, where $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values are presented on the x and y axes, respectively (Jackson et al., 2011; Newsome et al., 2007; Newsome et al., 2012). There was a complete overlap of isotopic niche space between Queen Charlotte Sound and Cloudy Bay, with the niche space of the former was essentially a subset of the latter. The overlap of niche space in Queen Charlotte Sound and Cloudy Bay suggests common prey sources for Hector's dolphins from these areas. The niche space of Golden Bay did not overlap at all with Queen Charlotte Sound and had minimal overlap with Cloudy Bay, suggesting differing prey sources between the eastern and western regions of the TOTS. Cloudy Bay had the most positive $\delta^{13}\text{C}$ values, which suggests diet consists of a high proportion of inshore prey items compared to Golden Bay and Queen Charlotte Sound. Golden Bay has the most negative $\delta^{13}\text{C}$ value which reflects a higher proportion of offshore prey items compared to Cloudy Bay and Queen Charlotte Sound. However, the absolute difference in $\delta^{13}\text{C}$ values between each location is $\sim 1\text{‰}$, and if there was an extensive difference between offshore and inshore feeding, it is likely the absolute difference would be substantially greater than observed here.

The overlap of niche space between Hector's dolphins from Queen Charlotte Sound and Cloudy Bay is of particular interest. We hypothesise that this may reflect a seasonal distribution pattern, where individual dolphins in Queen Charlotte Sound and Cloudy Bay may move between these two areas. Research on Hector's dolphins in Queen Charlotte Sound from 2011-2014 found shifts in seasonal distribution and density patterns, where dolphin distribution was more widespread and occurred in greater densities nearshore during summer and autumn, the latter of which the isotope data reflects. During winter, nearshore distribution was restricted to more central regions of Queen Charlotte Sound and occurred in lower densities. The reduced density of dolphins in winter suggested the possibility that the dolphins were moving further offshore and along the east coast of the South Island towards Cloudy/Clifford Bays during winter (Cross, 2019). The samples from Queen Charlotte Sound were collected in June 2016 and April 2022 (Figure 7). The isotopic turnover of dolphins is 2 – 4 months, so the isotope values presented here reflect diet over summer/early autumn for both sampling years.

Seasonal density patterns of Hector's dolphins have also been identified in Cloudy/Clifford Bay during aerial surveys carried out from 2006 -2009 (DuFresne & Mattlin, 2009). Abundance estimates were highest in summer and autumn, and a clear preference for offshore waters during winter was identified. This is not unusual for Hector's dolphins from the ECSI (Brough et al., 2019; Rayment et al., 2010; Slooten et al., 2006). Aerial surveys of Hector's dolphins along the whole east coast South Island (Mackenzie & Clement, 2014) revealed similar seasonal distribution patterns to DuFresne and Mattlin (2009). A higher density of dolphins was found offshore during winter in Cloudy Bay and Clifford Bay, and

there were strong indications of regional shifts in Hector's dolphin distribution between summer and winter, with fewer dolphins found in Cloudy/Clifford Bay over winter.

All Cloudy Bay samples in this study were collected in February, so reflect the diet of dolphins during late spring/early summer. Here we observe the most positive $\delta^{13}\text{C}$ values of the three sample locations in Cloudy Bay; suggesting these dolphins were feeding comparatively inshore. As the samples reflect summer diet of these dolphins, this is in agreement with the aerial surveys of 2006 – 2009, where dolphins were sighted in nearshore areas during summer (DuFresne & Mattlin, 2009).

In addition to the seasonal distribution patterns reported by Cross (2019), DuFresne & Mattlin (2009) and MacKenzie & Clement (2014), there is also preliminary genetic evidence to support the notion of dolphin movement between Queen Charlotte Sound and Cloudy Bay. A genetic analysis of 11 individuals from Queen Charlotte Sound in 2016 revealed genetic similarity with Hector's dolphins from Cloudy Bay (Baker et al., 2017). All individuals from Queen Charlotte Sound shared a single mtDNA haplotype 'Ca' which is also characteristic of dolphins from the east coast of the South Island. In contrast, individuals from Golden Bay shared haplotypes in common with dolphins from the west coast of the South Island (Baker et al., 2017). Furthermore, in 2011 and 2012 abundance estimates for Cloudy Bay were carried out using genotype and photo-identification methods. The values obtained from both methods were different; it was suggested this difference may be due to movement of Hector's dolphins in and out of Cloudy Bay, either alongshore or offshore (Hamner et al., 2017). Genetic connectivity has also been identified in Hector's dolphins from north of Kaikōura and Cloudy Bay, suggesting that some movement along the east coast of the South Island (north of the Kaikōura canyon) has occurred (Hamner et al., 2016). This work is currently being expanded upon in an update of the genetic analysis of all Hector's dolphin samples around the South Island to elucidate genetic connectivity between regions, including the TOTS.

The comparison of Hector's dolphin isotope values between regions from the TOTS does not account for temporal variation. Due to difficulty in obtaining tissue samples, the data set presented here spans 11 years and samples from each region were collected at different times. However, the statistically significant differences between regions presented in this preliminary analysis are still likely to be evident even if the effect of temporal variation could be accounted for. Hector's dolphin diet composition, assessed in different populations of the east and west coasts of the South Island, has also shown substantial differences in composition of prey items which likely reflect differing prey distributions between the two coasts (Miller et al., 2013). This supports the theory that Hector's dolphin diet is influenced by local prey distributions and differs between regions, as we observe in this study.

*Temporal differences in foraging ecology within locations**Queen Charlotte Sound*

There were statistically significant differences in $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ between sampling years within Queen Charlotte Sound. This is unsurprising given that samples were collected six years apart (2016 and 2022) and temporal variability in isotope values of marine mammals is common (Beltran et al., 2015; Borrell et al., 2018; Marcoux et al., 2012; Ogilvy et al., 2022; van den Berg et al., 2020; Watt & Ferguson, 2015) and has been recorded in Māui dolphins (Ogilvy et al., 2022). The $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values of Hector's dolphins from Queen Charlotte Sound decreased over time (Figure 7). This could be due to a shift in prey distribution or a shift in the isotopic baseline of the region. Decreasing $\delta^{15}\text{N}$ values have been observed in other species (Hempson et al., 2017; Inger et al., 2006; Wise et al., 2006) linked to a new prey source that has relatively lower $\delta^{15}\text{N}$ values than earlier prey. Alternatively, decreasing $\delta^{15}\text{N}$ values over time can reflect changes in the ecosystem that have resulted in a shift of the isotopic baseline of primary production. It is possible a combination of these factors can result in the change in $\delta^{15}\text{N}$ values observed here. To determine if the isotopic baseline has shifted in Queen Charlotte Sound, we would require samples representative of the base of the trophic web collected throughout the study period which were unavailable for this study. Collection of these samples (e.g., annual and seasonal collection of mussels) should be considered for future investigations of diet in marine species, including dolphins, from the TOTS.

We also observed statistically significantly lower $\delta^{13}\text{C}$ values in 2022 compared to 2016 (Figure 7). In other species (Fleming et al., 2016; Hilton et al., 2006; Johnson et al., 2019; Marcoux et al., 2012), decline in $\delta^{13}\text{C}$ values over time can be attributed to a reduction in the net primary productivity of an ecosystem, or a shift in foraging activity to more pelagic prey which are typically depleted in $\delta^{13}\text{C}$ (Cherel & Hobson, 2007; Gaden & Stern, 2010), or a combination of both. There is limited supply of macroalgae-derived organic matter to temperate reef fish biomass in the Marlborough Sounds (Udy et al., 2019a; Udy et al., 2019b). Marine heatwaves, increased sediment loading (e.g. from the conversion of natural forests to agriculture) and loss of sea urchin predators have been linked to a loss of kelp bed habitats in the Marlborough Sounds (Salinger et al., 2019). This may explain the decrease in $\delta^{13}\text{C}$ values observed in Queen Charlotte Sound from 2016 to 2022, especially as the Hector's dolphin samples from 2022 were collected during a marine heatwave (de Burgh-Day et al., 2022). Decreasing $\delta^{13}\text{C}$ values can also be attributed to the oceanic Suess effect, a phenomenon where $\delta^{13}\text{C}$ values in the biosphere have decreased exponentially since the beginning of the industrial revolution due to the burning of fossil fuels (the carbon dioxide (CO_2) introduced into the biosphere from fossil fuel burning has a lower $\delta^{13}\text{C}$ value than background atmospheric CO_2). Due to the increased concentration of aqueous CO_2 in the ocean since the beginning of the industrial revolution, the Suess effect also influences the

$\delta^{13}\text{C}$ values of the world's oceans (Gruber et al., 1999; Hilton et al., 2006). However, we have corrected our data to account for the Suess effect so it is unlikely that the observed decreasing $\delta^{13}\text{C}$ values can be attributable to this.

In addition to the decrease in isotope values over time we also observe very small isotopic niche space in Queen Charlotte Sound. Reduction in niche space can occur for several reasons. It may reflect an increase in prey availability, although this is sometimes observed in regions where marine protected areas (MPAs) have been implemented and is likely due to a lower density of high-quality prey outside of the MPA, which can increase interspecific competition and force individuals to broaden their diet (Davis et al., 2019). As no MPA is present in Queen Charlotte Sound, this is not a plausible explanation. Anthropogenic disturbance can also reduce trophic diversity and decrease isotopic niche space. For example, an increase in sediment deposition has been associated with decreased niche space (Burdon et al., 2020). Narrow niche width is also observed in species which are dietary generalists at the population level, but are composed of a group of individuals which use a subset of available resources and exhibit high site-fidelity (e.g. Anderson et al., 2008; Ceia & Ramos, 2015; Woo et al., 2008). This individual specialization may have serious ecological consequences; these groups are more vulnerable to location-specific habitat degradation and this has been observed in populations of bottlenose dolphins (*Tursiops truncatus*) (Gonzalvo et al., 2014). However, we do not believe the narrow niche width in Queen Charlotte Sound reflects a subset of specialized individuals with high site-fidelity, as there is preliminary genetic evidence to suggest that movement of Hector's dolphins between Queen Charlotte Sound and Cloudy Bay may occur.

Environmental conditions such as the El Niño Southern Oscillation can also affect the relative size of niche space. This has been observed in Humboldt penguins (*Spheniscus humboldti*) where niche space varied significantly between years of differing El Niño intensities. The largest isotopic niche space reflected opportunistic feeding behaviour during unfavourable oceanographic conditions (i.e. strong El Niño intensity). Conversely, comparatively small isotopic niche space was observed in years with mild environmental conditions (i.e. moderate to neutral El Niño conditions) (Chiu-Werner et al., 2019). Queen Charlotte Sound samples from 2016 were collected in June, so reflect the autumn diet of dolphins, as the isotopes represent the dolphins' diet two to four months prior (Browning et al., 2014). Southern Oscillation Index data for this period fluctuates between neutral and El Niño conditions between February and May 2016 (Statistics New Zealand, 2020) so the diet of dolphin samples collected in 2016 is not a strict reflection of their diet under severe El Niño conditions. To better understand the drivers of niche space and temporal fluctuations of isotope values in Queen Charlotte Sound, long-term isotopic baseline monitoring of this region would need to be undertaken.

Cloudy Bay & Golden Bay

No statistically significant differences in $\delta^{13}\text{C}$ values between 2011 and 2012 were observed in Cloudy Bay. This was expected due to the shorter time in between sample collection. There was a statistically significant difference in $\delta^{15}\text{N}$ values, where $\delta^{15}\text{N}$ was significantly higher in 2012 (Figure 5). This could be a result of consuming higher trophic level prey in 2012, or an increase in the $\delta^{15}\text{N}$ baseline during the study period. There were 18 genetically identified individuals sampled in both 2011 and 2012, but only small differences in $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values per individual between the two time periods (Figure A2) where the average difference within individuals across years (0.3 ‰) was less than the overall difference between years (0.5 ‰).

No statistically significant differences $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values were observed between years (2014, 2015, 2021 and 2022) in Golden Bay. This is potentially due to the small sample sizes present in each year (Table 3), but there is no evidence here to suggest temporal changes in prey distribution or isotopic baseline have occurred in Golden Bay. Of the two individuals from Golden Bay sampled in more than one year (Figure A3); there was minimal difference observed in $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values from the individual where samples were collected six years apart. This is further evidence to suggest no change in prey distribution or isotopic baseline has occurred during this period.

Sex-related differences in foraging ecology

Males and females consume similar prey and no sex-dependent foraging strategies are evident in our results. This was not unexpected as we see the same in Māui dolphins (Ogilvy et al., 2022), and Commerson's dolphin (*Cephalorhynchus commersonii commersonii*) (Ricciardelli et al., 2013), although sex-segregation among social groups of the *Cephalorhynchus* genus does occur in some larger populations. For example, Hector's dolphins in Te Pātaka-o-Rākaihautū / Banks Peninsula, along the east coast of the South Island have a high degree of sex-segregation reflected in lower $\delta^{15}\text{N}$ values of males compared to females.

Spatial differences in prey

Due to a lack of available prey data for the Marlborough and Tasman regions, we were unable to carry out a mixing model analysis to determine the contribution of different prey types to TOTS Hector's dolphin diet as per the contract deliverable. As a result of COVID lockdowns and DOC staff availability to catch fish in key regions where there are no commercial fisheries, we did not receive fish samples to analyse. However, we have just recently obtained some prey data from the literature (Kolodzey, 2021; Udy et al., 2019b) which covers a range of fish species from the Marlborough region (Figures A4, A5). Given these samples were not lipid extracted, we are currently determining whether we can apply mathematical lipid correction models (Lesage et al., 2010; Logan et al., 2008; Sweeting et al.,

2006; Yurkowski et al., 2015) to compare the data with Hector's dolphin isotope values. If lipid correction can successfully be applied, it may be possible to carry out a mixing model analysis. However, the prey data obtained was collected only from the Marlborough region. Given the variation in diet between regions highlighted in this study (Figure 4) it would not be appropriate to use the prey data to infer the diet of Hector's dolphins from the Tasman region (e.g. Golden Bay). Additionally, the prey samples were collected in a different year (2017-2018) to the Hector's dolphin samples of Queen Charlotte Sound and Cloudy Bay, so caution should be applied when comparing these isotope values with Hector's dolphin isotope values collected during a different period, as there is no data available to assess how the isotopic baseline of the region may change over time.

Summary and future directions

This study aimed to investigate differences in Hector's dolphin diet between key regions of the TOTS. Here we have demonstrated that $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ isotope values in dolphins were statistically significantly different between east (Golden Bay) and west regions (Cloudy Bay, Queen Charlotte Sound) of the TOTS. Furthermore, isotopic niche space analysis revealed minimal overlap of niche space between east and west regions, which supports the hypotheses that the east and west regions of the TOTS have different prey sources. Within the eastern regions of the TOTS, the niche space of Queen Charlotte Sound overlapped entirely with Cloudy Bay. This overlap suggests dolphins from Queen Charlotte Sound and Cloudy Bay may share similar prey sources, and/or alongshore movement of dolphins between the two areas may be occurring. However, to fully determine the drivers influencing isotope values of Hector's dolphins in the TOTS region, isotopic baseline sampling of key areas (Golden Bay, Queen Charlotte Sound and Cloudy Bay) is required.

Ecosystems may have multiple food sources with distinct $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ isotope values, that occupy a single trophic level. To accurately compare isotope values of animals from different regions and/or periods, isotopic baseline (i.e. the isotopic composition of primary producers) must be accounted for. Important ecological questions such as dietary tracing cannot be satisfactorily answered without accounting for baseline changes. Baselines are temporally dynamic and can be influenced by many physical and chemical variables such as rainfall, sedimentation, depth and distance from shore (Casey & Post, 2011). To accurately monitor temporal changes in isotope values of key species, and compare diets of species between regions, a clear picture of how the baselines vary spatially and temporally is needed.

This preliminary analysis has revealed differences in isotope values between regions. To investigate this further, we are currently assessing the feasibility of comparing Hector's dolphin isotope values with published prey data from the Marlborough region (Kolodzey, 2021). With limited data from surveys on Hector's dolphin distribution in the northernmost waters (i.e., Golden Bay, Tasman and Marlborough Sounds, Baker et al., 2017; MacKenzie et

al., 2014; Roberts et al., 2019) except for Queen Charlotte Sound (Cross, 2019), our findings suggest that the TOTS region may require a more fine-scale approach to determine genetic connectivity and manage potential threats.

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Appendix

Table A1: Metadata for Hector's dolphin biopsy and stranding samples included in stable isotope analysis

Individual ID: the unique identifier for every Hector's dolphin as determined by genotyping;

Date: the date the sample was collected, in the format dd-mmm-yy;

Location: The area within the top of the South Island, where the sample was collected;

Sex: genetically determined sex (F = female, M = male, TBD = genetic analysis is ongoing, U = sex unable to be determined);

Latitude and Longitude: GPS-determined location of sample collection;

Type: The type of tissue sample, either biopsy or stranded;

$\delta^{13}\text{C}$: The stable isotope ratio of $^{13}\text{C}/^{12}\text{C}$ determined by Isotracer NZ Ltd;

$\delta^{15}\text{N}$: The stable isotope ratio of $^{15}\text{N}/^{14}\text{N}$ determined by Isotracer NZ Ltd.

Individual ID	Sex	Date	Location	Latitude	Longitude	Type	$\delta^{15}\text{N}$	$\delta^{13}\text{C}$
Che11CB006	M	22-Feb-12	Cloudy Bay	-41.58528	174.17375	Biopsy	15.3	-16.5
Che11CB007	F	01-Feb-11	Cloudy Bay	-41.53654	174.14415	Biopsy	15.5	-16.2
		20-Feb-12	Cloudy Bay	-41.48584	174.08492	Biopsy	15.6	-16.8
Che11CB009	F	03-Feb-11	Cloudy Bay	-41.53653	174.13292	Biopsy	17.1	-15.4
Che11CB012	F	03-Feb-11	Cloudy Bay	-41.54252	174.14508	Biopsy	14.9	-16.1
		20-Feb-12	Cloudy Bay	-41.48782	174.08965	Biopsy	15.6	-16.4
Che11CB015	F	03-Feb-11	Cloudy Bay	-41.54218	174.14613	Biopsy	16.1	-15.7
Che11CB017	F	20-Feb-12	Cloudy Bay	-41.4846	174.0835	Biopsy	15.6	-16.7
Che11CB022	F	04-Feb-11	Cloudy Bay	-41.40863	174.12783	Biopsy	14.5	-16.7
		20-Feb-12	Cloudy Bay	-41.49996	174.11969	Biopsy	15.1	-17.2
Che11CB028	F	04-Feb-11	Cloudy Bay	-41.41959	174.08593	Biopsy	15.0	-16.8
Che11CB031	F	05-Feb-11	Cloudy Bay	-41.4596	174.11871	Biopsy	14.9	-16.7
		20-Feb-12	Cloudy Bay	-41.5446	174.17753	Biopsy	14.8	-18.0

Che11CB034	M	05-Feb-11	Cloudy Bay	-41.45668	174.12009	Biopsy	14.4	-17.2
		21-Feb-12	Cloudy Bay	-41.49213	174.11369	Biopsy	14.8	-17.1
Che11CB035	M	05-Feb-11	Cloudy Bay	-41.45717	174.11813	Biopsy	14.6	-17.0
Che11CB040	F	08-Feb-11	Cloudy Bay	-41.41883	174.07259	Biopsy	14.8	-17.3
Che11CB045	F	08-Feb-11	Cloudy Bay	-41.43682	174.05852	Biopsy	14.9	-17.1
Che11CB052	F	10-Feb-11	Cloudy Bay	-41.50099	174.09397	Biopsy	15.7	-15.3
		21-Feb-12	Cloudy Bay	-41.49292	174.11488	Biopsy	16.0	-15.8
Che11CB057	M	10-Feb-11	Cloudy Bay	-41.51354	174.11415	Biopsy	15.1	-16.6
		21-Feb-12	Cloudy Bay	-41.54367	174.14142	Biopsy	15.4	-17.1
Che11CB059	M	10-Feb-11	Cloudy Bay	-41.51519	174.123	Biopsy	14.7	-16.4
		22-Feb-12	Cloudy Bay	-41.5848	174.17407	Biopsy	15.0	-16.9
Che11CB061	F	10-Feb-11	Cloudy Bay	-41.51545	174.12811	Biopsy	14.9	-17.0
Che11CB063	M	10-Feb-11	Cloudy Bay	-41.51549	174.13165	Biopsy	14.3	-16.0
Che11CB066	M	10-Feb-11	Cloudy Bay	-41.49486	174.08983	Biopsy	15.2	-16.5
Che11CB067	M	10-Feb-11	Cloudy Bay	-41.49526	174.08967	Biopsy	15.0	-16.4
		21-Feb-12	Cloudy Bay	-41.4938	174.1147	Biopsy	15.3	-16.9
Che11CB073	F	10-Feb-11	Cloudy Bay	-41.43143	174.04159	Biopsy	15.6	-16.1
		18-Feb-12	Cloudy Bay	-41.47064	174.05515	Biopsy	15.9	-16.5
Che11CB083	M	12-Feb-11	Cloudy Bay	-41.43351	174.04963	Biopsy	14.9	-16.6
		18-Feb-12	Cloudy Bay	-41.45803	174.06055	Biopsy	15.2	-17.0
Che11CB090	M	12-Feb-11	Cloudy Bay	-41.45436	174.05811	Biopsy	14.9	-17.1
		21-Feb-12	Cloudy Bay	-41.49176	174.10743	Biopsy	15.2	-17.2
Che11CB092	M	13-Feb-11	Cloudy Bay	-41.44808	174.04886	Biopsy	14.8	-16.9
		20-Feb-12	Cloudy Bay	-41.48454	174.08338	Biopsy	15.2	-17.1
Che11CB095	F	13-Feb-11	Cloudy Bay	-41.45571	174.04951	Biopsy	17.7	-14.0
		24-Feb-12	Cloudy Bay	-41.52482	174.11511	Biopsy	16.0	-16.1
Che11CB097	M	13-Feb-11	Cloudy Bay	-41.44418	174.04151	Biopsy	15.5	-16.2

		20-Feb-12	Cloudy Bay	-41.47764	174.08329	Biopsy	15.8	-16.8
Che11CB101	M	13-Feb-11	Cloudy Bay	-41.44798	174.04234	Biopsy	15.7	-16.6
		20-Feb-12	Cloudy Bay	-41.47955	174.08368	Biopsy	16.9	-15.8
Che11CB105	F	13-Feb-11	Cloudy Bay	-41.46839	174.05048	Biopsy	16.7	-15.3
		18-Feb-12	Cloudy Bay	-41.46413	174.05927	Biopsy	15.7	-16.3
Che11CB111	M	13-Feb-11	Cloudy Bay	-41.43814	174.04163	Biopsy	14.8	-17.4
Che11CB113	M	14-Feb-11	Cloudy Bay	-41.47351	174.05269	Biopsy	15.8	-16.2
		21-Feb-12	Cloudy Bay	-41.50651	174.11804	Biopsy	16.7	-16.2
Che11CB115	M	14-Feb-11	Cloudy Bay	-41.475	174.05307	Biopsy	15.9	-15.8
Che12CB002	F	18-Feb-12	Cloudy Bay	-41.46463	174.05953	Biopsy	15.5	-16.4
Che12CB003	M	18-Feb-12	Cloudy Bay	-41.46883	174.05756	Biopsy	15.1	-16.8
Che12CB010	F	20-Feb-12	Cloudy Bay	-41.47663	174.08352	Biopsy	15.0	-17.3
Che12CB013	F	20-Feb-12	Cloudy Bay	-41.48397	174.08078	Biopsy	15.5	-16.7
Che12CB026	F	20-Feb-12	Cloudy Bay	-41.49655	174.1117	Biopsy	15.8	-16.2
Che12CB030	F	20-Feb-12	Cloudy Bay	-41.53374	174.16854	Biopsy	15.2	-17.2
Che12CB139	F	24-Feb-12	Cloudy Bay	-41.51019	174.10119	Biopsy	15.8	-16.3
Che14GB03	U	28-Mar-14	Golden Bay	-40.80949	172.86123	Biopsy	15.9	-18.1
Che14GB04	F	28-Mar-14	Golden Bay	-40.80949	172.86123	Biopsy	15.4	-18.7
Che14GB05	M	28-Mar-14	Golden Bay	-40.80949	172.86123	Biopsy	15.4	-18.9
Che14GB06	F	28-Mar-14	Golden Bay	-40.80949	172.86123	Biopsy	17.4	-15.4
Che14GB07	M	28-Mar-14	Golden Bay	-40.80949	172.86123	Biopsy	16.5	-17.4
	M	27-Mar-15	Golden Bay	-40.81392	172.83851	Biopsy	15.6	-18.0
Che14TM01	M	30-Oct-14	Pakawau Beach, Golden Bay	-43.53091	172.74162	Stranded	15.5	-17.3
Che15GB01	F	27-Mar-15	Golden Bay	-40.81709	172.84353	Biopsy	16.3	-17.4
Che15GB03	M	27-Mar-15	Golden Bay	-40.81958	172.8541	Biopsy	15.9	-17.6
	M	22-Jan-21	Golden Bay	-40.81966	172.87648	Biopsy	15.9	-17.5

Che15TM01	F	09-Jan-15	Rocks Road, Nelson	-42.63438	171.06659	Stranded	15.4	-16.7
Che15TM02	M	11-Jan-15	Waimea Inlet, Nelson	-43.8695	172.3044	Stranded	17.1	-17.3
Che16QCS03	M	13-Jun-16	Queen Charlotte Sound	-41.21303	174.08762	Biopsy	15.1	-16.8
Che16QCS04	F	13-Jun-16	Queen Charlotte Sound	-41.21303	174.08762	Biopsy	15.8	-16.4
Che16QCS05	F	13-Jun-16	Queen Charlotte Sound	-41.19862	174.25317	Biopsy	15.1	-17.1
Che16QCS06	F	13-Jun-16	Queen Charlotte Sound	-41.19862	174.25317	Biopsy	15.6	-16.5
Che16QCS07	M	13-Jun-16	Queen Charlotte Sound	-41.19862	174.25317	Biopsy	15.3	-16.8
Che16QCS09	M	13-Jun-16	Queen Charlotte Sound	-41.19862	174.25317	Biopsy	15.1	-17.2
		13-Jun-16	Queen Charlotte Sound	-41.19862	174.25317	Biopsy	15.4	-16.2
Che16QCS13	F	14-Jun-16	Queen Charlotte Sound	-41.21913	174.14741	Biopsy	15.7	-16.5
Che16QCS14	M	15-Jun-16	Queen Charlotte Sound	-41.25225	173.98033	Biopsy	15.6	-16.4
Che16QCS01	F	13-Jun-16	Queen Charlotte Sound	-41.21303	174.08762	Biopsy	15.5	-16.4
Che17WC03	M	13-Dec-17	Nine Mile Beach, Westport, Buller	-40.8163	172.8464	Stranded	14.3	-17.7
Che18MB01	F	09-Feb-18	Ure Stream mouth, South Marlborough	-41.904243	174.119386	Stranded	14.8	-17.9
Che21GB02	M	22-Jan-21	Golden Bay	-40.81966	172.87648	Biopsy	15.8	-17.4
Che22GB01	TBD	14-Apr-22	Golden Bay	-40.82117	172.88622	Biopsy	14.9	-18.8
Che22GB02	TBD	14-Apr-22	Golden Bay	-40.82236	172.88675	Biopsy	14.7	-18.2
Che22GB03	TBD	14-Apr-22	Golden Bay	-40.82236	172.88585	Biopsy	15.8	-17.7
Che22GB04	TBD	14-Apr-22	Golden Bay	-40.82227	172.86536	Biopsy	16.0	-18.0
Che22QCS01	M	05-Apr-22	Queen Charlotte Sound	-41.251	173.98106	Biopsy	14.5	-17.7
Che22QCS02	M	05-Apr-22	Queen Charlotte Sound	-41.25001	173.98035	Biopsy	15.1	-17.3
Che22QCS03	M	05-Apr-22	Queen Charlotte Sound	-41.24872	173.98037	Biopsy	14.6	-17.8
Che22QCS04	F	05-Apr-22	Queen Charlotte Sound	-41.24686	173.9806	Biopsy	14.7	-17.6
Che22QCS05	F	05-Apr-22	Queen Charlotte Sound	-41.24763	173.98031	Biopsy	14.8	-17.6
Che21QCS01	M	19-Oct-21	Queen Charlotte sound	-41.312598	172.08515	Biopsy	15.3	-16.8

Che22QCS06	TBD	05-Apr-22	Queen Charlotte Sound	-41.24645	174.00026	Biopsy	14.6	-17.7
Che22QCS07	TBD	05-Apr-22	Queen Charlotte Sound	-41.25221	174.00357	Biopsy	14.8	-17.6
Che22QCS08	TBD	05-Apr-22	Queen Charlotte Sound	-41.25348	174.00072	Biopsy	15.0	-16.9
Che22QCS09	TBD	05-Apr-22	Queen Charlotte Sound	-41.25862	173.99209	Biopsy	14.6	-17.4
Che22QCS10	TBD	05-Apr-22	Queen Charlotte Sound	-41.25192	173.98601	Biopsy	14.6	-17.4
Che22QCS11	TBD	05-Apr-22	Queen Charlotte Sound	-41.21891	174.03306	Biopsy	15.1	-17.4
Che22QCS12	TBD	05-Apr-22	Queen Charlotte Sound	-41.21945	174.03333	Biopsy	14.3	-17.9
Che22QCS13	TBD	05-Apr-22	Queen Charlotte Sound	-41.23054	174.03559	Biopsy	14.7	-17.8
Che22QCS15	TBD	05-Apr-22	Queen Charlotte Sound	-41.22727	174.03567	Biopsy	14.8	-17.1
Che22QCS16	TBD	05-Apr-22	Queen Charlotte Sound	-41.21348	174.06899	Biopsy	15.1	-17.4
Che22QCS17	TBD	06-Apr-22	Queen Charlotte Sound	-41.24346	173.97884	Biopsy	14.9	-17.2
Che22QCS18	TBD	07-Apr-22	Queen Charlotte Sound	-41.25708	173.99133	Biopsy	14.3	-17.7
Che22QCS19	TBD	08-Apr-22	Queen Charlotte Sound	-41.2573	173.99135	Biopsy	14.6	-17.3
Che22QCS20	TBD	08-Apr-22	Queen Charlotte Sound	-41.25563	173.99159	Biopsy	14.4	-17.4
Che22QCS21	TBD	08-Apr-22	Queen Charlotte Sound	-41.25232	173.98661	Biopsy	14.9	-17.3
Che22QCS22	TBD	08-Apr-22	Queen Charlotte Sound	-41.25454	173.97345	Biopsy	14.9	-17.2
Che22QCS23	TBD	08-Apr-22	Queen Charlotte Sound	-41.25999	173.95696	Biopsy	14.8	-17.3
Che22QCS24	TBD	08-Apr-22	Queen Charlotte Sound	-41.25956	173.95693	Biopsy	14.7	-17.1
Che22QCS25	TBD	08-Apr-22	Queen Charlotte Sound	-41.25949	173.957	Biopsy	14.5	-17.7
Che22QCS26	TBD	08-Apr-22	Queen Charlotte Sound	-41.25905	173.95805	Biopsy	14.5	-17.6
Che22QCS27	TBD	08-Apr-22	Queen Charlotte Sound	-41.25909	173.95839	Biopsy	14.5	-17.4
Che22QCS28	TBD	08-Apr-22	Queen Charlotte Sound	-41.25699	173.96824	Biopsy	14.5	-17.5
Che22QCS29	TBD	08-Apr-22	Queen Charlotte Sound	-41.25527	173.97455	Biopsy	14.7	-17.3
Che22QCS30	TBD	08-Apr-22	Queen Charlotte Sound	-41.23759	174.03549	Biopsy	15.1	-16.8

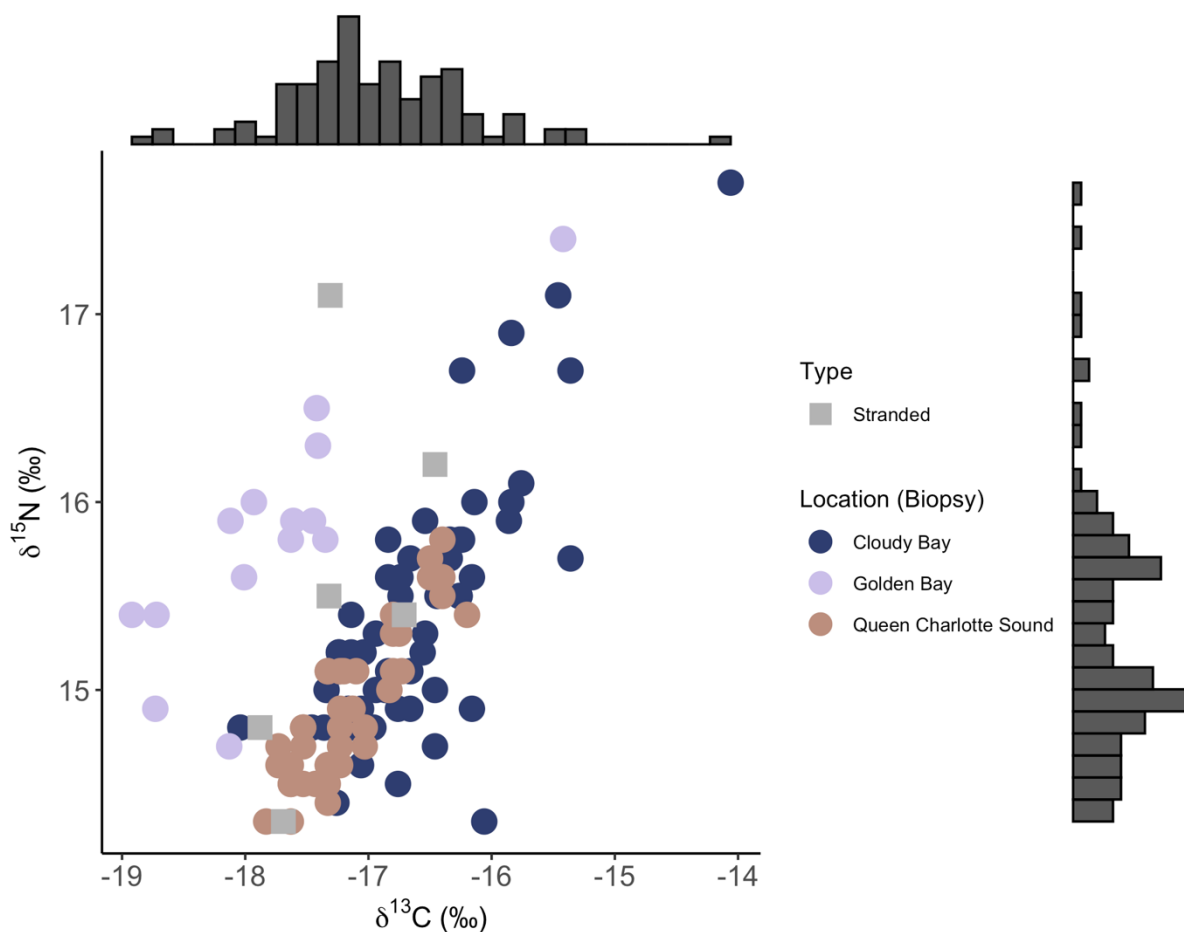


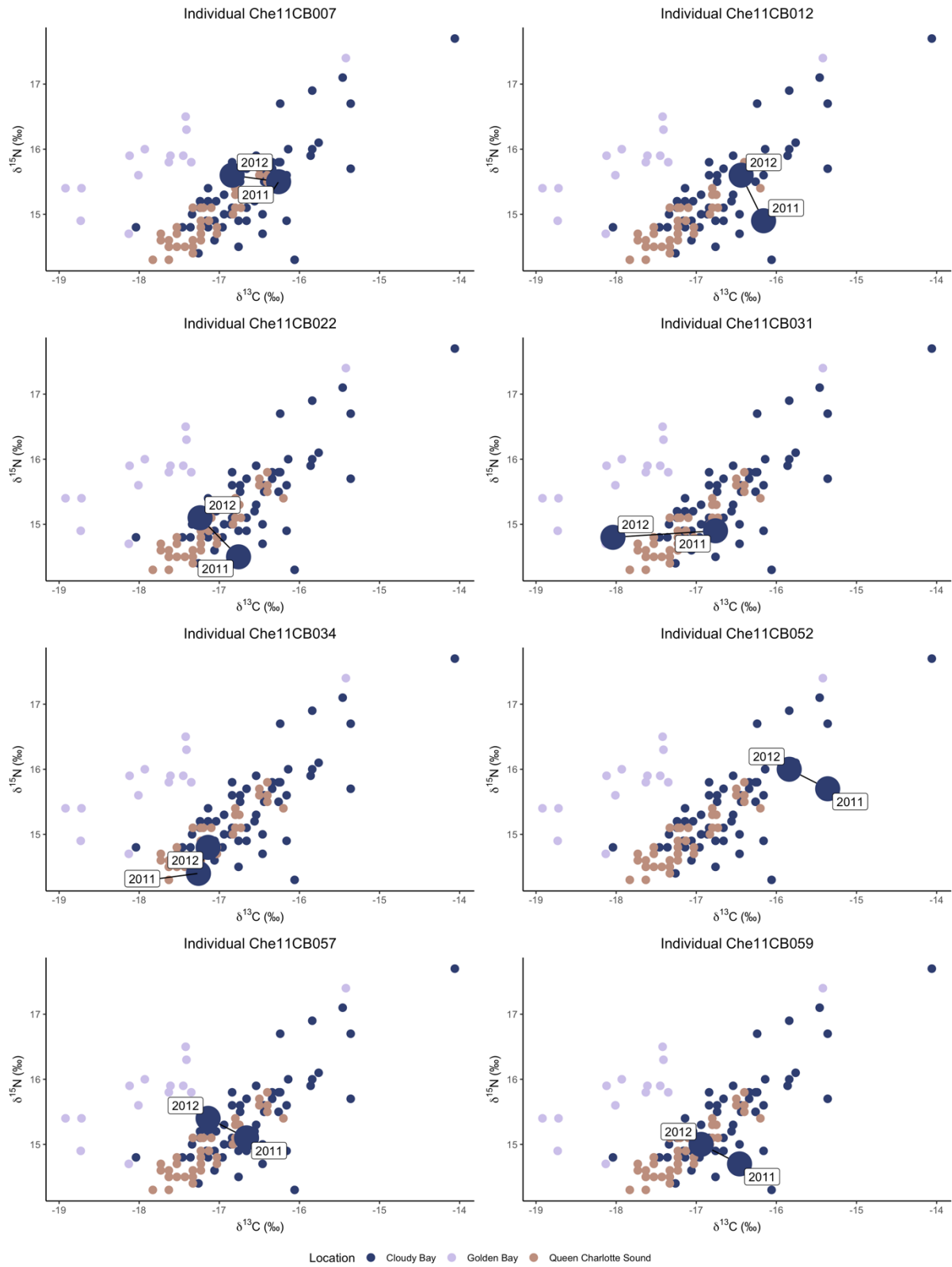
Figure A1: $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values of 116 Hector's dolphin stranded and biopsy skin samples. Isotope ratios of biopsy samples are coloured according to sample location. Stranded samples are not coloured according to location and are instead shown by grey squares, independent of location. Frequency distributions of $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ are shown as marginal histograms.

Table A2: Post Hoc Dunn's multiple pairwise comparisons test comparing $\delta^{13}\text{C}$ values of 111 Hector's dolphin skin samples by location. Within each cell is the Dunn's pairwise z-test statistic (above) and the associated p -value (below), with statistically significant differences indicated by asterisks.

$\delta^{13}\text{C}$	Cloudy Bay	Golden Bay
Golden Bay	5.580967 0.0000*	-
Queen Charlotte Sound	4.195659 0.0000*	-2.601389 0.0046*

Table A3: Post Hoc Dunn's multiple pairwise comparisons test comparing $\delta^{13}\text{C}$ values of 111 Hector's dolphin skin samples by location. Within each cell is the Dunn's pairwise z-test statistic (above) and the associated p -value (below), with statistically significant differences indicated by asterisks.

$\delta^{15}\text{N}$	Cloudy Bay	Golden Bay
Golden Bay	-2.137084 0.0163*	-
Queen Charlotte Sound	3.923115 0.0000*	4.667930 0.0000*



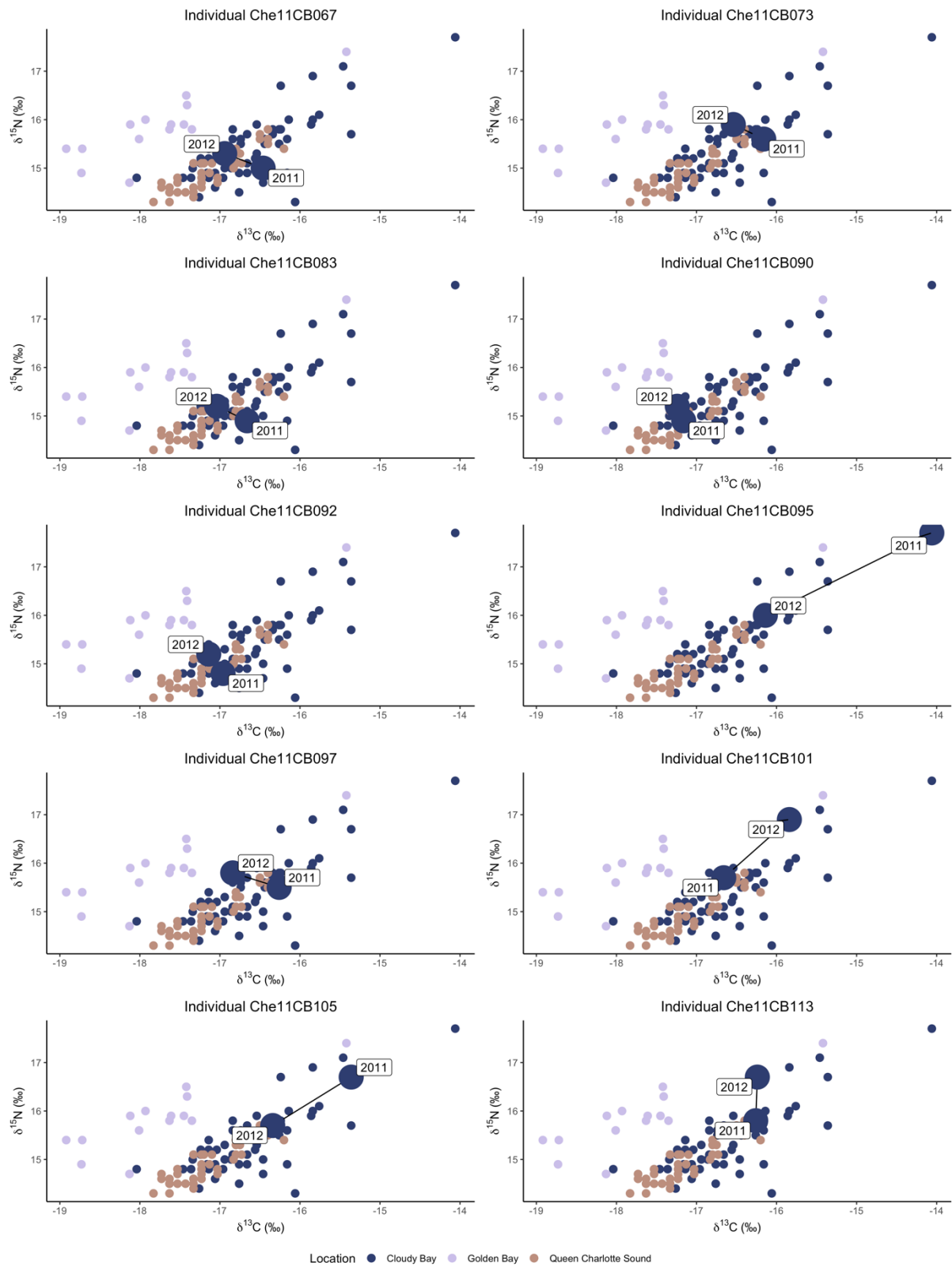


Figure A2: Isotope values ($\delta^{13}\text{C}$ and $\delta^{15}\text{N}$) of genetically identified individuals sampled in Cloudy Bay in 2011 and 2012. Samples from the same individual are connected by a black line. Isotope values of Hector's dolphins from Golden Bay (purple) and Queen Charlotte Sound (brown) are also shown for context.

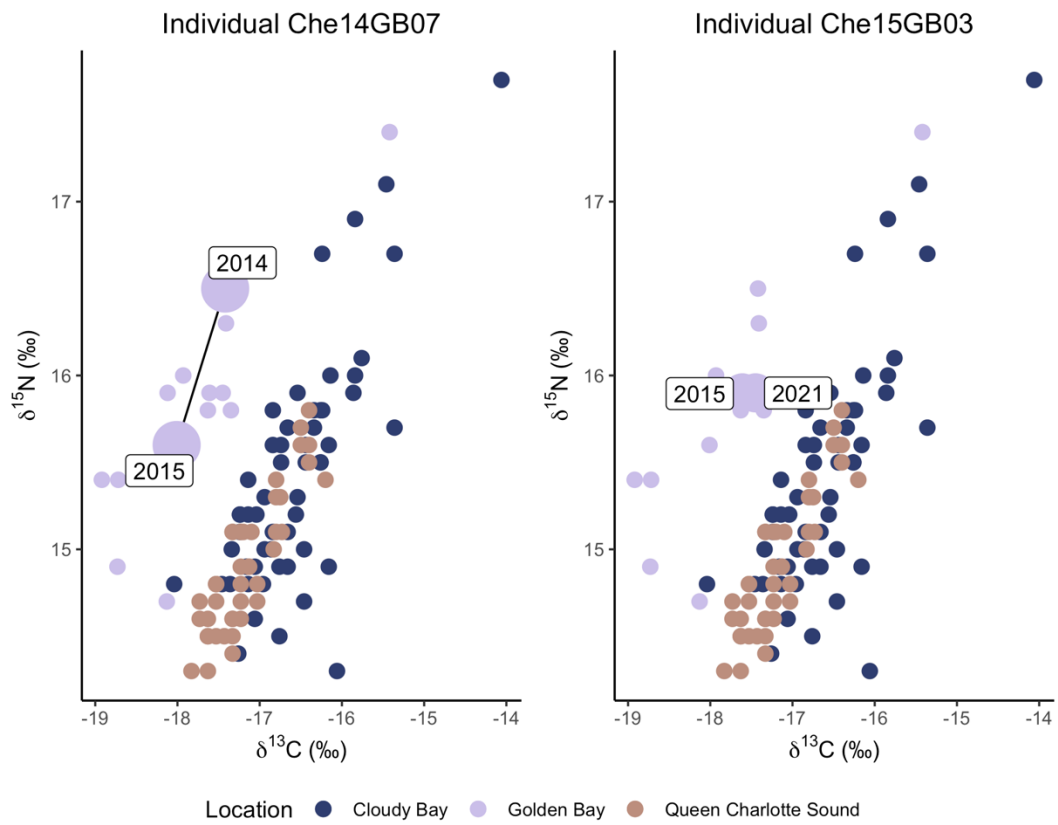


Figure A3: Isotope values ($\delta^{13}\text{C}$ and $\delta^{15}\text{N}$) of genetically identified individuals sampled in Golden Bay in 2014, 2015 and 2021. Samples from the same individual are connected by a black line.

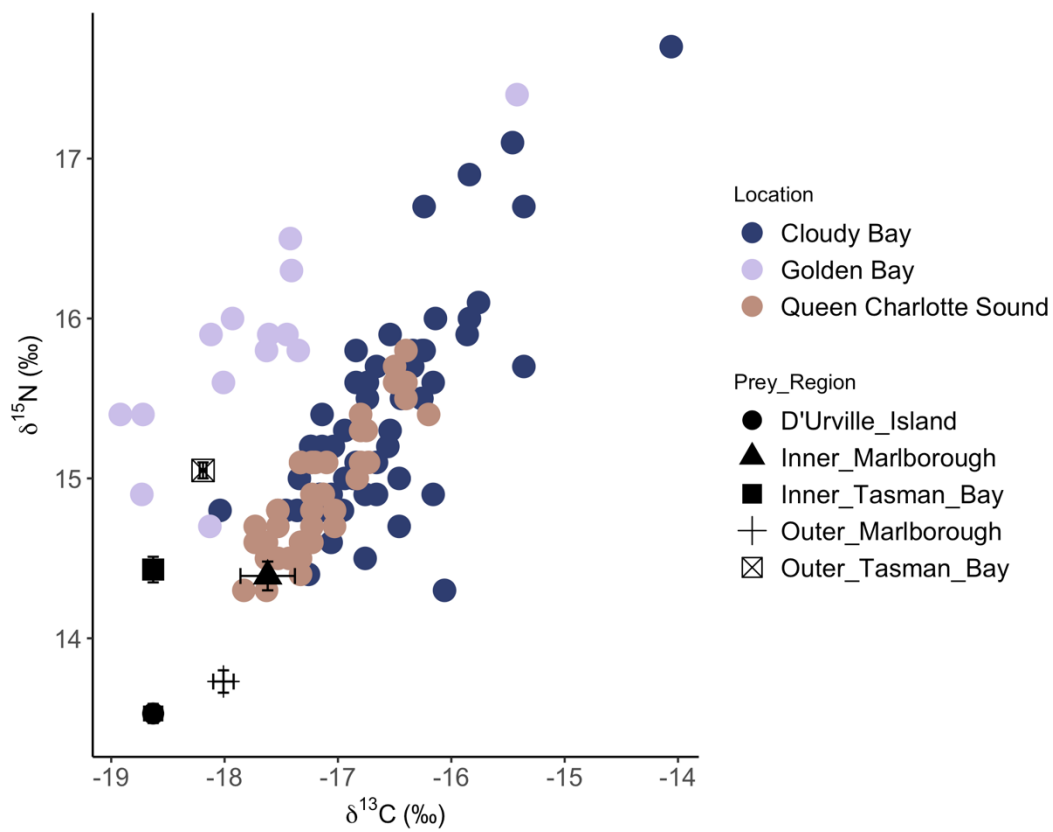


Figure A4: $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values of Hector's dolphin skin biopsy samples from the TOTS compared to published $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values of blue cod (*Parapercis colias*) from the Marlborough Region and Tasman Bay, collected in 2018 (Kolodzey, 2021). Values have not been corrected for trophic enrichment and are not lipid extracted (C:N 3.0 to 3.7). Error bars represent standard error of the mean.

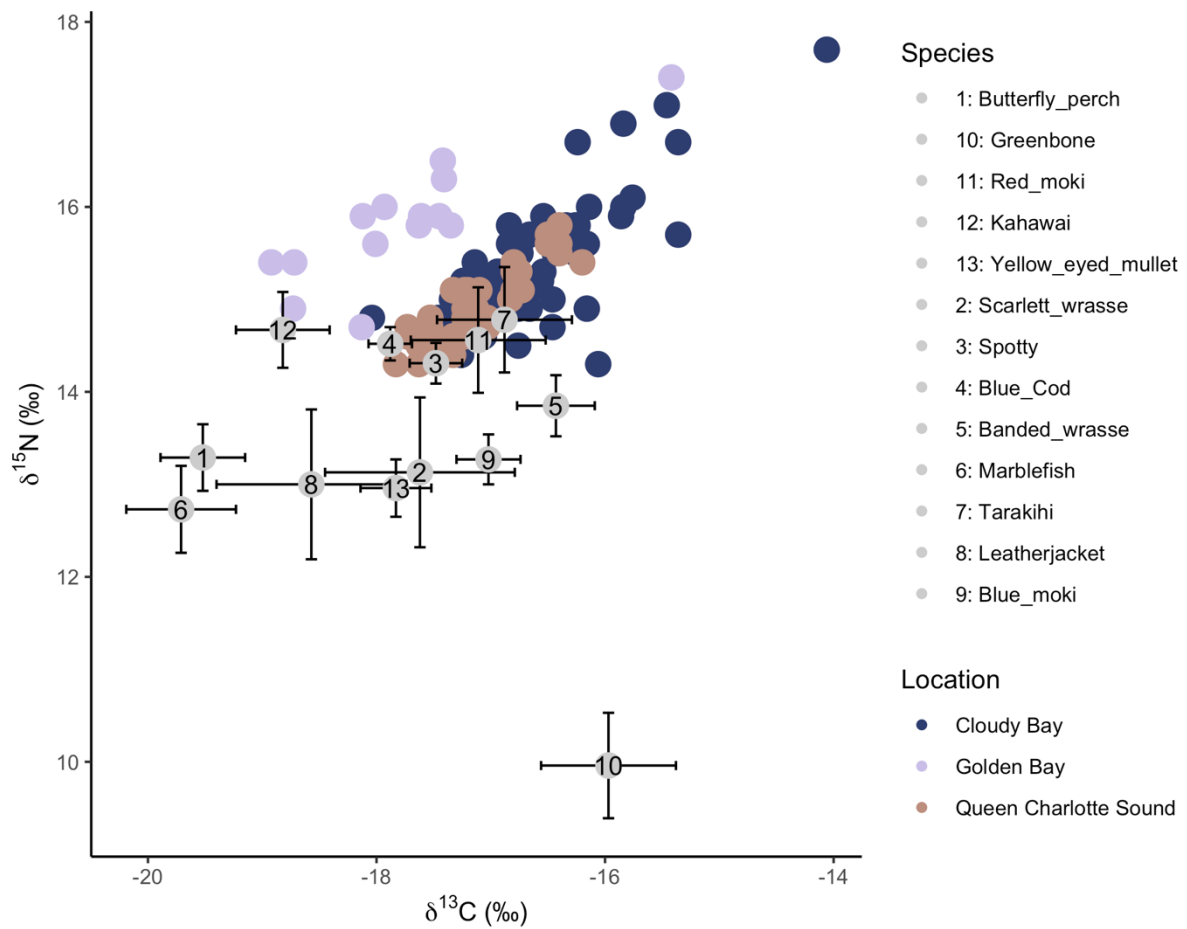


Figure A5: $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values of Hector's dolphin skin biopsy samples from the TOTS compared to published $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values of prey samples from the Marlborough Sounds, collected in 2017/2018 (Udy et al., 2019a). Isotope values of prey shown here have not been lipid extracted or corrected for trophic enrichment.