Spatial Foraging Patterns in Puget Sound Pigeon Guillemot (*Cepphus columba)*: An Investigation Using Stable Isotopes and Community Science

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Introduction

Seabirds can provide valuable information about ocean ecology and food web dynamics, because spatial and temporal variations in their foraging behavior can signal differences in the distribution and abundance of trophic levels beneath them (Becker, Peery, and Beissinger 2007; Sydeman et al. 1997). These variations can be inferred through numerous approaches including the study of stable isotopes (Davies et al. 2009; Sydeman et al. 1997), a technique that uses isotopes extracted from an organisms' tissues as an indicator of their diet and the environment from which their food energy and nutrients were obtained. Stable isotope analysis can provide a nuanced understanding of the system in which seabirds forage, and the nature of their interactions with that system, because nitrogen signatures can indicate trophic position and

carbon can be traced through a food web to indicate the transfer and flow of energy in a system (Fredriksen 2003; Quillfeldt et al. 2008; Hobson, Piatt, and Pitocchelli 1994).

This study serves as an example of how seabird isotopes can be used to learn more about local marine ecosystems. Here, I address the ecological question of how breeding Pigeon Guillemot (*Cepphus columba*), a species of small seabird, utilize the environment in a large, fjord-like estuary (Puget Sound, WA) for foraging and how their interactions with their local environment can be spatially variable. Pigeon Guillemot are a species with high potential to provide new insight into the marine ecosystem they interact with, using a stable isotope approach, due to their life history and foraging behavior. Year-round residents of Puget Sound, these birds are known to be both pelagic and epi-benthic foragers that prey on a wide range of fish species within several kilometers of their nest sites (Litzow et al. 2000; Pearson and Hamel 2013; Ewins 1993b). Generally, they nest in cliffs less than 50 m tall in previously excavated burrows (Sanger and Cody 1994), creating colonies that are relatively small and widely dispersed for a seabird species (Sowls, Lensink, and Hatch 1978). This is true for birds in Puget Sound, whereas of 2003, 471 colonies could be found throughout the estuary, consisting of a single active burrow to 37 active burrows (Bishop et al. 2016; Evenson et al. 2003). Previous studies have shown that Pigeon Guillemot feed at a consistent trophic level throughout successive breeding stages, but that prey abundance may influence foraging site selection or prey selection of individual birds or entire colonies (Kuletz 1983; Litzow et al. 2000.; Davies et al. 2009). Black Guillemot, the equivalent species occurring in the Atlantic, display individual foraging site fidelity, which is the tendency to forage consistently in only a small part of the population's range as an individual (Owen et al. 2019). These characteristics of breeding Pigeon Guillemot suggest that these birds generally forage opportunistically within close proximity of their colony, but individually spread their foraging across the habitat. This means that individuals cannot be assumed to be 'ecologically equivalent' (Owen et al. 2019), but that a survey of a whole population may give a representative sampling of foraging conditions over the total area where the population occurs. Thus, to infer differences in foraging conditions, foraging variability may be better studied at a larger spatial (and population) level than at an individual level.

To investigate spatial variability in Puget Sound Pigeon Guillemot foraging, I conducted stable isotope analysis on discarded egg tissues from birds breeding within several oceanographically-

distinct basins. The stable isotopes ($\delta^{15}N$ and $\delta^{13}C$) of egg tissues provide information about the female bird's diet during a short period prior to breeding (Kowalczyk et al. 2014; Polito et al. 2009). Eggs are typically laid between mid-May and mid-June (Ewins 1993a), so isotopes likely reflect Pigeon Guillemot foraging near their colony sites during April to June. Chicks or adults often expel egg fragments from the burrows after hatching, where they can be found below the colony, often on the beach (Guillemot Research Group, pers comms). Collecting this tissue offers a non-disruptive method for obtaining isotope data (Oppel, Powell, and O'Brien 2009) and gives the opportunity for researchers to collaborate with community scientists and beach naturalists who can easily identify this species' eggs and assist in the field sampling. In Puget Sound, a community science group, the Guillemot Research Group, has been monitoring accessible Pigeon Guillemot colonies during the breeding season since 2009 to assess population health and dynamics. Their knowledge of this local population made them an invaluable partner for this study.

Spatial dynamics are important to study, because they can affect how populations are distributed and how they are affected by natural and anthropogenic impacts (Owen et al. 2019). By understanding variations in seabird foraging, processes that contribute to differences in population heath may be identified (Becker and Beissinger 2003). Describing the distinctive foraging patterns of these birds in this region can add value to the ongoing monitoring of Pigeon Guillemot populations in Puget Sound. This study seeks to describe spatial patterns in foraging behavior within the estuary and identify possible causes of variation.

Methods

Study Location

I conducted this study on Whidbey Island and in the South Sound region of Puget Sound, WA. I chose these two locations to coincide with long-term monitoring performed by a community science group[1] who were willing to collaborate with this study. Volunteers on Whidbey Island

[1] The Guillemot Research Group deploys groups of volunteers to monitor previously observed colony sites once a week for 10 weeks every summer. Volunteers are assigned a colony site that they survey each week, prior to 9am, for one hour. During each survey, volunteers 1) locate and map burrows in which Pigeon Guillemot are observed entering or exiting (referred to as active

planned to survey 28 colony sites and volunteers in South Sound planned to survey 40 colony sites during the summer of 2019. These sites were selected based on prior years' bird activity. Surveyed sites were on beaches adjacent to three oceanographically distinct basins, Admiralty Inlet, Whidbey Basin, and South Basin (Fig 1).

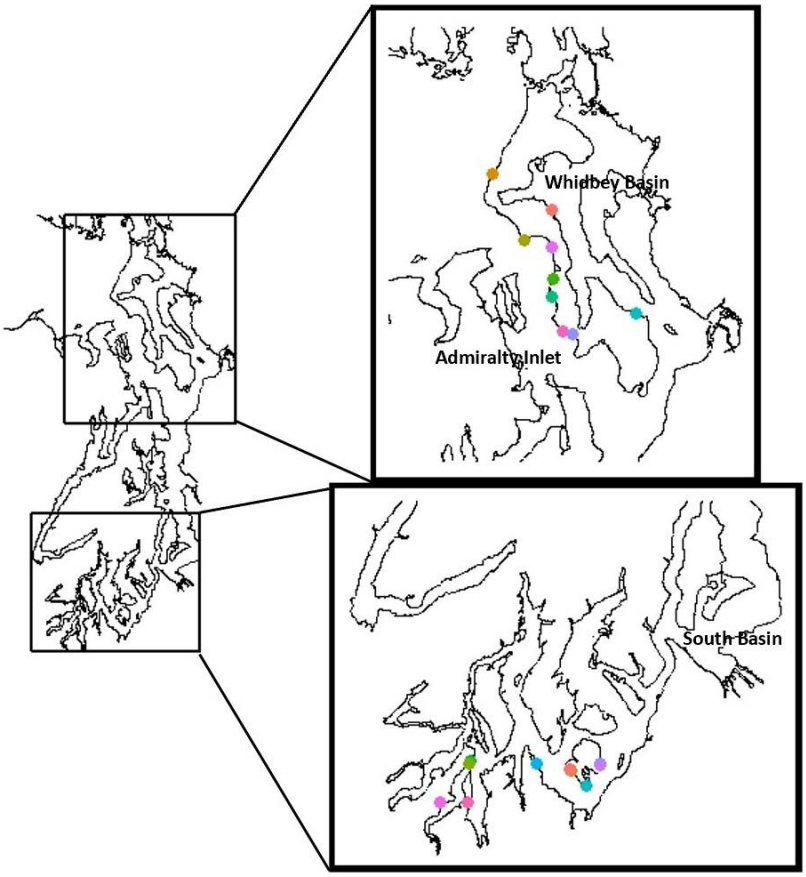


Figure 1. Monitoring sites (n = 22) in Puget Sound, WA where Pigeon Guillemot eggshell and membranes samples were collected by community science volunteers between 6/12/19 and 9/1/19. The top inset shows sites on Whidbey Island, with Admiralty Basin to the west and Whidbey Basin to the east. The bottom inset shows sites in South Sound with the South Sound Basin. Basins are indicated as they are the marine systems where the sampled birds are likely to be deriving their energy and nutrients.

Pigeon Guillemot Isotopes

Sample Collection

From June 12 to September 1, 2019, we collected 34 egg tissue samples from a total of 22 sites (Fig. 1). Volunteers collected discarded egg tissue at the base of burrows when performing their

burrows), 2) record the maximum number of birds present at the colony, 3) count the number of individuals during the beginning, middle and end of each survey, 4) record the number and species of fish delivered to each identified active burrow, and 5) identify the response of the birds to potential disturbances (i.e. eagles, beach walkers, dogs, motorboats, etc.) (Guillemot Research Group, pers comms).

weekly surveys and placed them in labeled whirl packs (location denoted with colony site name and GPS point[2]) (Fig 2). At the end of the breeding season, once birds had fully abandoned their colonies, I returned to several sites where burrows were within arm's reach to check for additional egg tissue samples (Fig 2).

Samples were nearly equally distributed across sites between Whidbey Island (18 samples, 10 sites) and the South Sound region (16 samples, 12 sites), and represented birds likely foraging in Admiralty Inlet (n = 16), Whidbey Basin (n = 2) and South Basin (n = 16). Samples were primarily found below burrows dug into sandy cliffs; however, some were found within the burrows themselves (examples included a rotted pier and jetty rocks). At most sites, only a single sample was collected, however multiple samples (up to 8) were collected at four of the sites.

[2] GPS points were assigned to each sample by several means: volunteers took a picture of the sample before bagging and reported the coordinate info accompanying the picture, volunteers took a GPS point using their phone at the location where they found a sample, or volunteers found a point on an online map (google or apple maps) that represented the average location of the colony site. The researchers assigned only one GPS point to each colony.

Figure 2. Images from the 2019 sampling season (photos by Emily Buckner and Anne Mills). Top left: Volunteers from the Guillemot Research group survey the nearshore of Whidbey Island, counting the number of Pigeon Guillemots in sight. Top right: A volunteer observes an adult delivering prey to a burrow. Bottom left: Me looking for discarded egg tissue in an empty burrow at the end of the breeding season in S Sound. Bottom right: Egg tissue samples, with time and location data provided by collector. Eggshells and eggshell membranes were put together if found attached and fragments were composited if within 5m of each other.

Sample Preservation

Collected egg tissues were kept in a cool and dark environment until they could be transported to a laboratory freezer at NOAA's Northwest Fisheries Science Center. Eggshell fragments and egg membranes were placed together in bags if attached or found within 5m of each other.

Laboratory Analysis

To prepare egg tissue samples for isotope analysis, I followed procedures as described in Emslie and Patterson (2007), Kowalczyak et al. (2014), Oppel et al. (2009), and Polito et al. (2009). For

each sample, I separated the membrane and shell and took an initial weight of each tissue type. After weighing, I cleaned the tissues by spraying them with deionized water and gently wiping/scraping with a laboratory grade cotton swab until the sample tissues were devoid of other material. I then placed cleaned tissues into separate glass vials, one for eggshells and one for membranes. The vials with membrane tissue were placed without caps in a drying oven set at 60° C for 24 hours. The vials with eggshell tissue were placed without caps in a fume hood to air dry for 24 hours. Any eggshell sample that was not dry after 24 hours was placed in the oven for an additional 24 hours at 60° C. I ground all samples into a fine powder by loading dried tissue into metal canisters with ball bearings and then placing them into a SPEX Sample Prep 5100 Mixer Mill for 3 minutes each. Fully homogenized tissue was then returned to glass vials. I weighed 0.25-0.35 mg of each membrane sample into 6mm x 4mm tin capsules and analyzed samples for carbon and nitrogen isotopes using a Thermo Scientific Delta V Advantage IRMS continuous flow stable isotope ratio mass spectrometer.

To remove the calcium carbonate, and allow for the analysis of the organic material in the dried, ground eggshells, I weighted 10 mg of each eggshell sample into new glass vials and added 2, 50 µL aliquots of 10% HCL to each vial (Polito et al. 2009). I created a 10% HCL solution by adding 27.03ml of 37% HCL to 72.97ml LCMS grade water (filtered to 0.2 micros) in a volumetric cylinder. I sonicated the solution for 5 min, inverted several times and then poured into beaker to be pipetted into sample vials. Samples were dried in an oven at 60° C for 48 hours then another 2, 50 µL aliquots of the 10% HCL was added to each vial to check for effervescence and dried for an additional 48 hours at 60° C. This step was repeated for any vial that showed effervescence, until it no longer appeared. All dried eggshell samples were then placed in a -80° C freezer for one hour and then in a vacuum dryer for several more hours to freeze dry. I then weighed 5 mg of each eggshell sample into 6mm x 4mm tin capsules and analyzed for carbon and nitrogen isotopes via Thermo Scientific Delta V Advantage IRMS continuous flow stable isotope ratio mass spectrometer.

Stable isotope abundances are expressed in δ notation in parts per thousand ($^0/_{00}$), according to the equation:

$$\delta X = [(R_{sample}/R_{standard}) - 1] \times 1000$$

Where X is ^{15}N or ^{13}C and R is the ratio $^{15}N/^{14}N$ or $^{13}C/^{12}C$. These values were based on the V-PeeDee Belemnite standard for ^{13}C and atmospheric N_2 for ^{15}N. Sample precision, as indicated by within-run standard deviation of $\delta^{15}N$ and $\delta^{13}C$ reference materials, was less than or equal to 0.17‰. 10% of the run samples (3 out of 38) were tripled to check for consistency and were averaged to make one single point for analysis.

Analyses

Stable isotope values ($\delta^{15}N$ and $\delta^{13}C$) for each Pigeon Guillemot female (as represented by an egg membrane and/or shell sample) were compared across sites, basins, and regions. I used a Bray-Curtis test to calculate a similarity index for each pair of birds, and PERMANOVA to determine whether a significant fraction of similarity was due to region, basin or site.

Egg membranes and shell organics from the same sample were compared to look for isotopic variations between tissue types. This was done by correcting the raw isotopic values with tissue-specific discrimination factors drawn from Polito et al. (2009) (Table 1). Correction is necessary when comparing different tissues as it returns tissue-specific isotopic values to resemble the 'true' values of the individual bird. This is done by accounting for the fractionation of $\delta^{15}N$ and $\delta^{13}C$ that occurs when different egg tissues are created. Corrected isotope values were derived by subtracting the discrimination factors from the raw isotope values.

Table 1. Discrimination factors applied to Pigeon Guillemot raw isotope values to correct for the fractionation of $\delta^{15}N$ and $\delta^{13}C$ that occurs when egg tissues are created. These 'correction coefficients' were drawn from a study on captive Gentoo Penguins feeding on whole fish (Polito et al. 2009) and were determined to be the best available factors in the literature for these tissues and piscivorous seabirds.

Tissue	$\delta^{15}N$	$\delta^{13}C$
Egg Membrane	4.4	2.8
Egg Shell Organic	1.8	1.4

Trophic position of Pigeon Guillemots in different basins was determined using the equation:

$$TL = \lambda + (d15N_{consumer} - d15N_{base})/\Delta$$

where TL is the trophic level of the spatially grouped birds, ƛ is the trophic level of the base (here, 1), $d15N_{consumer}$ is the average $\delta^{15}N$ value of that group, $d15N_{base}$ is the average $\delta^{15}N$ of autotrophs (kelp, eelgrass, epiphytes, algae)(here, 6.24), and Δ is the trophic fractionation factor per level (here, 3.4) (Fredrickson 2003, Post 2002). Autotroph isotope data came from an associated study (in progress) and were collected from northern Puget Sound in the region between Edmonds, WA and Clinton, WA (Chittaro et al. *unpublished*).

Pigeon Guillemot Prey

I analyzed prey delivery information from the Guillemot Research Group's surveys to assess Pigeon Guillemot diet and relative prey selection in different basins. Data represented volunteers' observations of three categories of fish taxa (gunnel, sculpin, and other) delivered by adult birds to burrows for one hour interval per visit to a site. For each site, I averaged prey taxa across all years after accounting for effort and then grouped by basin in which they were located. I used ANOVA to test spatial patterns for significance.

To infer prey availability for Pigeon Guillemot between basins, fish abundance data were acquired from quarterly beach seining conducted by WDFW in South Sound and NOAA in the Strait of Juan de Fuca. Given a lack of fish abundance data from Admiralty inlet, I determined that the Strait was the most closely representative basin oceanographically. I analyzed data collected during the spring from 2015 to 2019, because this time period most closely represents the fish available to Pigeon Guillemot while they are gestating. I grouped all fish species into 'gunnel' (n = 5), 'sculpin' (n = 15), or 'other' (n = 80). I included all non-gunnel and sculpin species in the 'other' category, with the exception of jellyfish species (which were removed), as Pigeon Guillemot have been recorded eating a large diversity of species, including shrimp, crabs, flatfish, and juveniles of fish such as salmon and rockfish (Ewins 1993a; Kuletz 1998; Mills et al. 2007). I calculated the average abundance of these three taxa groups found in the two basins over the 5-year sampling period.

I combined these two data sets to infer Pigeon Guillemot prey availability and selectivity, comparing the proportion of different fish taxa in birds' diet with the proportion of fish taxa in the basin where they were assumed to be foraging. Data were normalized using the R package 'vegan' (Oksanen et al. 2019). The difference in proportions was then calculated for each basin

by subtracting the average proportion of each taxon group in the environment from that of the birds' diet.

Results

Spatial Variation in Isotopic Signature

For most samples, both membrane and shell organic tissues were analyzed. However, for a few samples only one of the two tissue types were useable. I analyzed 26 eggshells and 32 egg membranes for $\delta^{15}N$ and $\delta^{13}C$. Tissue types showed similar isotopic patterns across the spatial scales studied. Samples from sites adjacent to the South Sound Basin were relatively enriched in C and N than samples from sites adjacent to Admiralty Inlet Basin (Fig 3). Shell tissues were consistently more enriched in $\delta^{15}N$ and $\delta^{13}C$ than the corresponding membrane tissue (Fig 4).

I used a Bray-Curtis test for dissimilarity to test the significance of spatial patterns in membrane isotopic values (Table 2). For this analysis I chose membrane tissue over egg shell because of the larger sample size and higher confidence in the accuracy of analytical results for membrane tissues (there were more steps in the egg shell sample preparation procedure, which may have increased the chance of error). The region where samples were collected and the basin adjacent to sites where samples were collected were both significant variables ($p = 0.001$ and $p = 0.006$ respectively). Within the South Sound region, some variance between samples could be attributed to the site where they were collected ($p = 0.044$), however no significant relationship at the site level could be detected within the Whidbey region, possibly due to the small sample size.

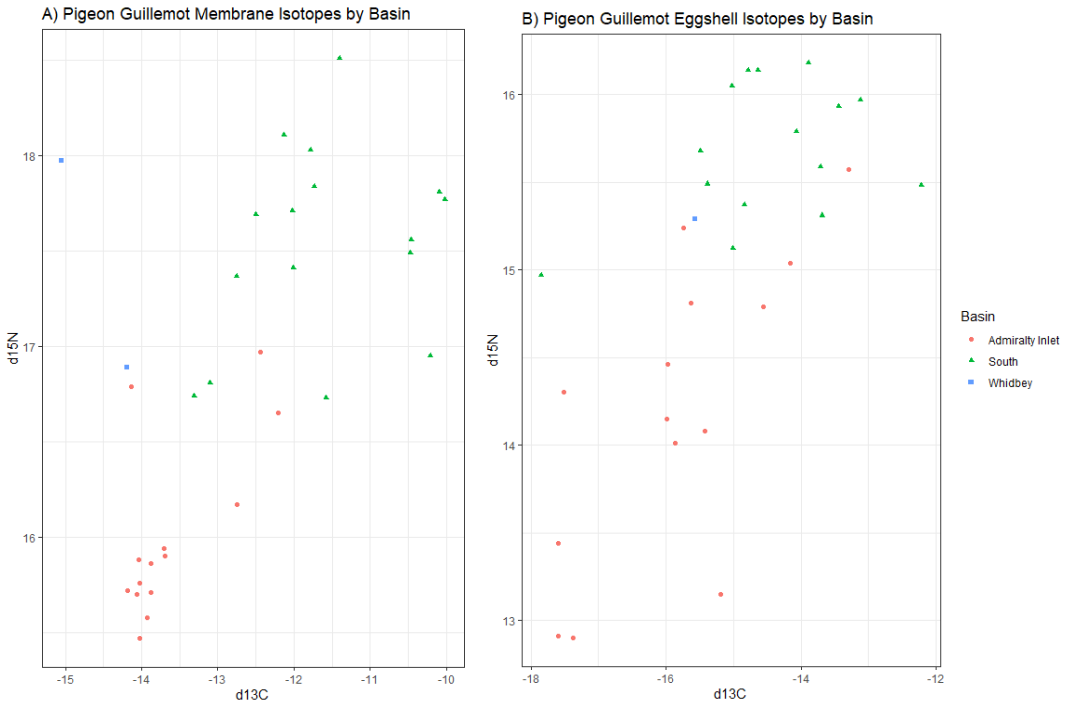


Figure 3. Ratio of raw $\delta^{15}N$ and $\delta^{13}C$ values for Puget Sound Pigeon Guillemot egg membrane (a) and eggshell (b) identified by the basin in which the samples were collected. Samples from South Basin were more enriched in $\delta^{15}N$ and $\delta^{13}C$ than samples from Admiralty Inlet. Samples from Whidbey Basin were more enriched in $\delta^{15}N$, but similar in their $\delta^{13}C$ enrichment to samples from Admiralty Inlet. Isotopes derived from membrane tissue showed more variability amongst samples from South Basin sites while isotopes derived from shell tissue showed more variability amongst samples from Admiralty Inlet.

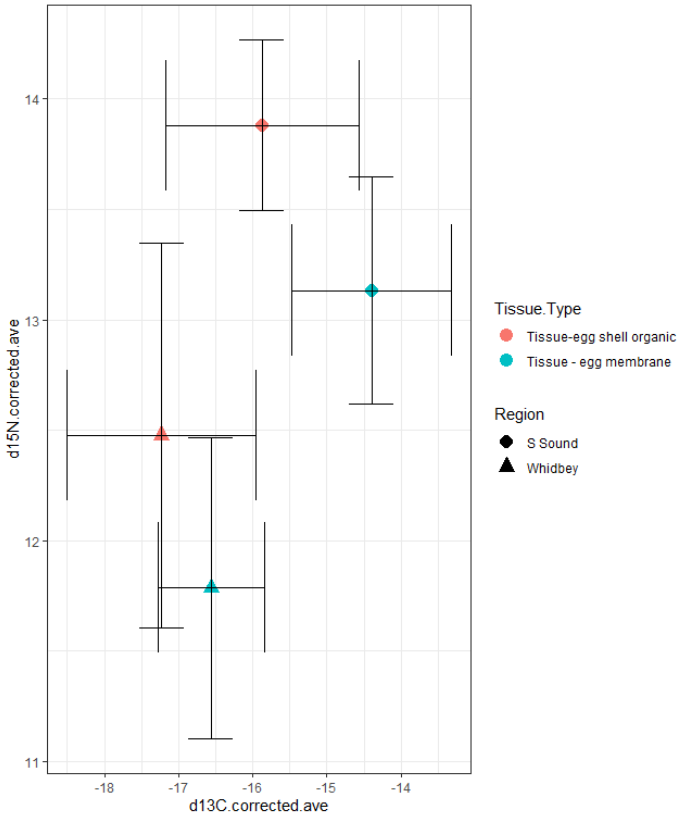


Figure 4. Ratio of the average corrected $\delta^{15}N$ and $\delta^{13}C$ values for Puget Sound Pigeon Guillemot egg shells and membranes (with standard deviation shown with whiskers). All isotopes were corrected by subtracting tissue-specific discrimination factors derived from Polito et al. (2009) from the raw values. Eggshell organics were consistently more enriched in $\delta^{13}C$ and $\delta^{15}N$ than their membrane counterparts.

Table 2. Bray-Curtis statistical test results ascribing significance of variance for spatial patterns observed in Pigeon Guillemot egg membrane isotopic values ($\delta^{15}N$ and $\delta^{13}C$).

Variable	**DF**	**Sum of Sqrs**	**Mean Sqrs**	**F. Model**	**R2**	**P value**
Region	1	52.018	52.018	74.327	0.59116	0.001
Basin	1	5.233	5.233	7.477	0.05947	0.006
All Sites	15	20.945	1.396	1.995	0.23802	0.038
Whidbey Sites	6	3.8305	0.6384	0.9109	0.26110	0.529

S Sound Sites	9	17.1141	1.90157	2.7225	0.8033	0.044

Trophic Position

I examined the difference in isotopic signatures between basins within the context of a simplified Puget Sound food web model (Fig 5). Pigeon Guillemot occupy the highest trophic level but slight variation between birds from different basins was observed, with birds in South Sound and Whidbey Basin occupying a calculated trophic level of ~3.6 and Admiralty Inlet birds a 3.1.

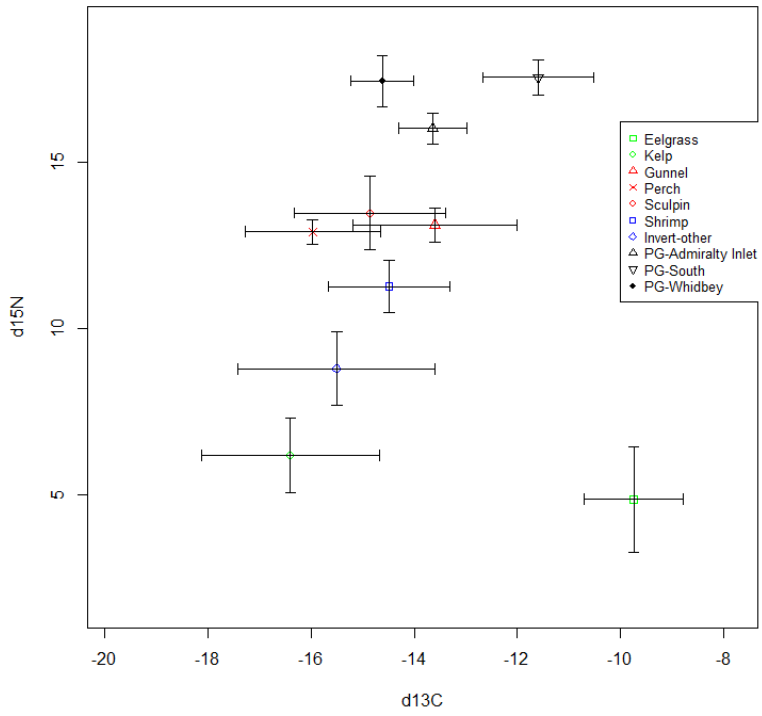
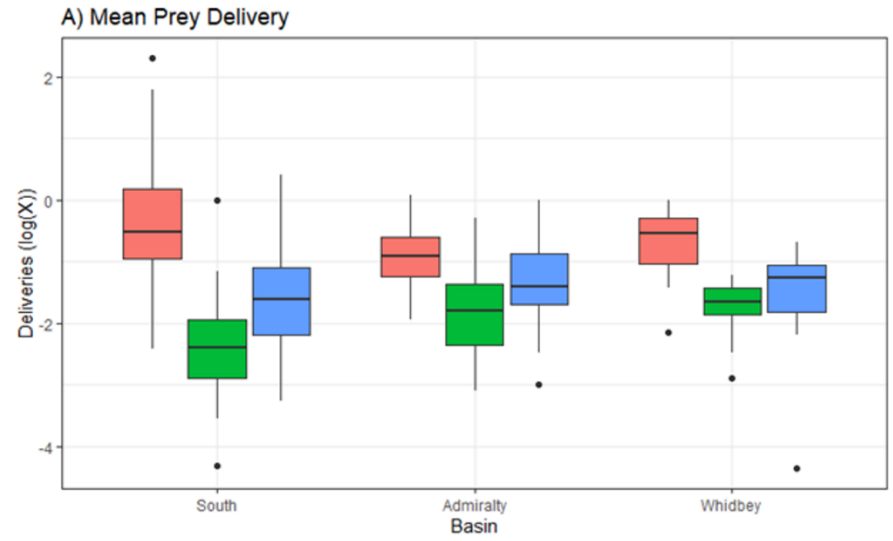
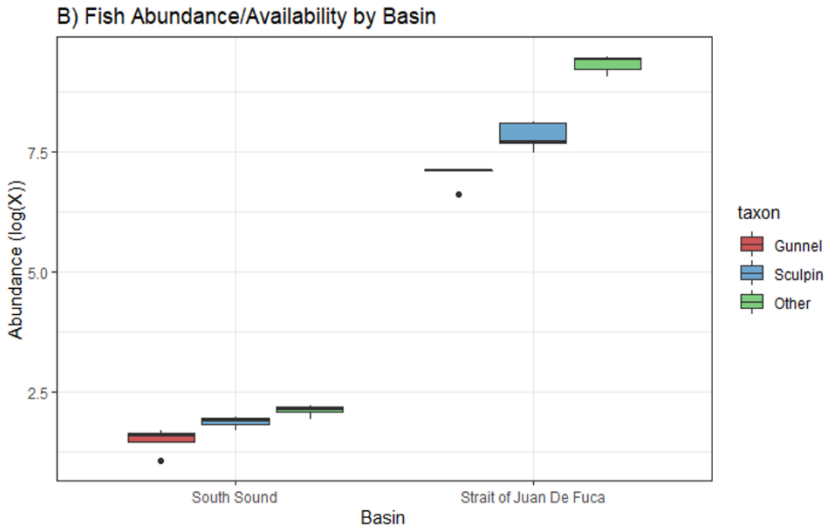


Figure 5. Average stable isotope values (shown as ratio of $\delta^{15}N$ and $\delta^{13}C$) for Pigeon Guillemot egg membranes, sorted by basin, shown in relation to average isotope values for autotrophs, invertebrates, and fish occurring in Puget Sound. The data indicate that Pigeon Guillemot in all three basins feed at the highest trophic level although some variation exists. Non-bird data from Chittaro et al., unpublished.

Prey Selection and Availability in Admiralty Inlet and South Basin

Visual observations of fish delivered to burrows showed similar (not statistically significant for gunnel and sculpin) patterns among the three basins (Fig 6a), suggesting that adults may be actively selecting preferred forage taxa. Birds in all basins were observed delivering gunnel with more frequency, followed by sculpin, then 'other' taxa. A comparison of the relative abundance of those preferred forage taxa in South Basin and the Strait of Juan de Fuca (here, considered the best approximation of assemblages in Admiralty Inlet, for which data were not available) showed variation in potential prey assemblages between basins (Fig 6b). The Strait of Juan de Fuca had a greater abundance for all taxa groups, but both basins showed the same pattern of gunnel being the least abundant taxa group. Comparing prey consumption with what was available showed that Pigeon Guillemot diet is ~33-36% heavier in gunnel than the basin in which they are foraging (Fig 6c, Table 3).


A) Mean Prey Delivery
Deliveries (log(X))
2
0
-2
-4
South
Admiralty
Whidbey
Basin


B) Fish Abundance/Availability by Basin
Abundance (log(X))
7.5
5.0
2.5
South Sound
Strait of Juan De Fuca
Basin
taxon
Gunnel
Sculpin
Other

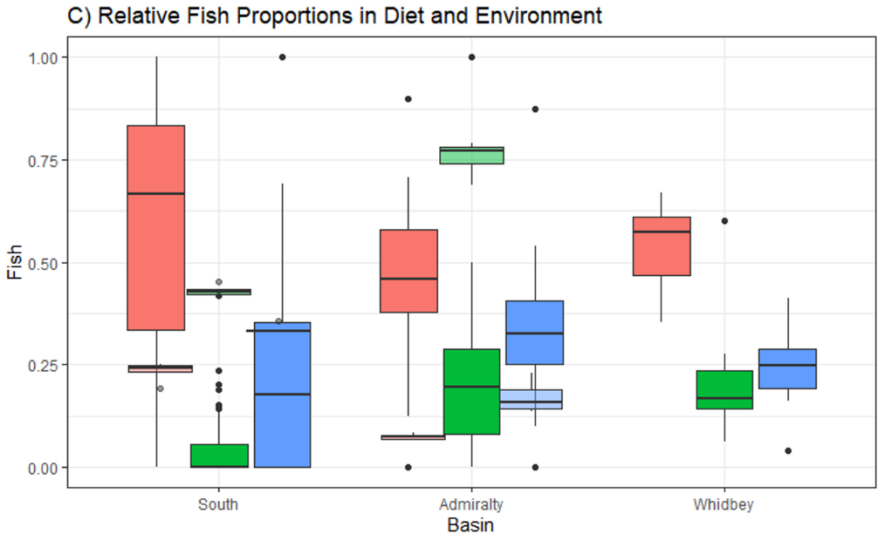

C) Relative Fish Proportions in Diet and Environment
Fish
1.00
0.75
0.50
0.25
0.00
South
Admiralty
Whidbey
Basin

Figure 6. Boxplots showing A) the average number of prey (grouped into gunnel, sculpin, or other taxa) delivered by adult Pigeon Guillemot to their chicks during the breeding season at sites located within three Puget Sound basins. These data came from the Guillemot Research Group and were pooled across all observation years and normalized for effort (defined as number of observation periods made at a site). B) Average fish abundance in two Puget Sound basins. Data come from WDFW and NOAA spring beach seine sampling across the years 2015-2019. 'Gunnel', 'sculpin', and 'other' consisted of 5, 15, and 80 species, respectively, and were chosen because of their prevalence in observations of foraging adult Pigeon Guillemot. C) An overlay of the datasets from A and B, to show average proportion of fish in Pigeon Guillemot diet in contrast with fish abundance in the same basin. Fish abundance data from the Strait of Juan de Fuca was used as a proxy for fish abundance for Admiralty Inlet.

Table 3. Difference in proportion of fish taxa (gunnel, sculpin, other) in Pigeon Guillemot diet and the basin in which they forage**.**

Basin	**Gunnel**	**Sculpin**	**Other**
South	0.333	-0.073	-0.388
Admiralty	0.364	0.185	-0.540

Discussion

Observations from the Guillemot Research Group suggest that Pigeon Guillemot feed on similar proportions and numbers of benthic fish taxa (gunnel and sculpin) across basins, which indicates that this species has a relatively consistent diet across Puget Sound (Fig 6a). However, the isotopic evidence derived from this study shows that the flow of energy and nutrients within the different basins does vary slightly, as it applies to these birds (Fig 3-5). Pigeon Guillemot feeding in the South Sound display a higher enrichment in carbon and nitrogen than birds feeding in Admiralty inlet. These spatial variations are likely explained by two ecological mechanisms. First is that of slight variations in the birds' diet due to spatial differences in prey availability. Second, the underlying biogeochemistry of the basins in which the birds feed is different, which shifts the carbon and nitrogen signatures for all trophic levels. These two hypotheses will be addressed below.

Puget Sound basins do exhibit differences in fish abundance, meaning that potential Pigeon Guillemot prey availability is spatially variable. While we can be relatively confident that the birds are presented with different foraging conditions between basins, their responsiveness to

these conditions via their dietary choices is less evident. Pigeon Guillemot in fact appear to preferentially feed on a single taxa (gunnel) throughout Puget Sound, despite their lower abundance than other prey choices (Fig 6c). Due to this similarity in diet, the isotopic variations between birds cannot be explained by differences in gunnel or sculpin availability but may be due to that unknown 'other' taxa the birds feed on less frequently. It is important to note that these dietary preferences are what breeding adults deliver to chicks during the summer months, thus it is uncertain whether the adults preferentially feed upon them as well, and if they do, whether that preference varies seasonally. Another explanation for isotopic variability, given the observed consistency in the birds' diet, is that the diet of the prey is different between basins. This could be due to variations in the lower trophic levels driven by different environmental conditions.

There are strong linkages between the biotic communities and the abiotic patterns and processes within estuaries (Dethier and Schoch 2003). Factors such as salinity, temperature, sediment type and oxygen influence food web dynamics due to their effect on species' growth, persistence and behavior (Ruesink et al. 2014). They also influence the character of the isotopes assimilated into the tissues of organisms. Carbon signatures in consumers will reflect the primary producers able to persist in local conditions, while nitrogen signatures can be influenced by human-derived nitrogen inputs (Hansson et al. 1997). Puget Sound basins exhibit distinct abiotic conditions. South Sound has a more reduced dissolved oxygen and pH content than the other basins considered in this study, while Whidbey Basin has greater freshwater input, decreasing salinity and increasing sedimentation (Greene et al 2012). Both basins likely have higher levels of human-derived nitrogen than Admiralty inlet due to denser human development in those regions. Estuarine primary consumers, such as oysters, display a similar spatial isotopic shift as seen in Pigeon Guillemots. Oysters occurring in South Sound are more enriched in N than those found in the Central Sound (Ruesink et al. 2014). Decoupling this possible mechanism driving isotopic variation in Pigeon Guillemots from the mechanism of differences in prey availability is challenging, as the biogeochemistry of basins could likely influence the birds both by fundamentally shifting the character of the carbon and nitrogen moving through the food web, but also by influencing lower trophic level abundance and composition (i.e., prey availability). It is probable that both of these mechanisms influenced the pattern observed in bird isotopes.

The consistency of the diet of Puget Sound Pigeon Guillemot has interesting implications for this population, given the differences in foraging conditions within the different basins where these birds forage and breed. Their reliance on gunnel during breeding suggests a bioenergetic advantage of selecting them as prey (perhaps due to a high lipid content, detectability, or beak-fitting form), and that a decline in gunnel abundance could mean lower reproductive success of the birds. Continued monitoring of breeding birds' diet coupled with fish abundance sampling and bird population dynamics will help in the evaluation of the health of Pigeon Guillemot sub-populations occurring in the Puget Sound. Building upon our understanding of how this species interacts with the marine environment could lead to insight into the adaptability and resilience of this species in a region heavily influenced by anthropogenic factors.

Challenges and Limitations

There were several sources of uncertainty when performing stable isotope analysis to infer trophic dynamics. First, because there are no discrimination factors for Pigeon Guillemot eggshell and membrane tissue available in the literature, I applied factors derived from Gentoo Penguins to my samples. While this species has a close life history to Pigeon Guillemot, the incorrect use of isotopic values of tissues can lead to inaccurate estimations about diet (Polito et al 2009). Using accurate fractionation information permits isotope data from different matrices (e.g. egg shell and egg membrane) to be combined, which augments the data. Thus, for a more accurate interpretation of Pigeon Guillemot isotopes and trophic position, future studies should attempt to validate the discrimination factors for this species (or a closer relative) for multiple tissue forms.

Another limitation of this study was the relatively small sample size. We were only able to collect a single sample from most of our study sites so we could not perform any analysis on foraging variability at a finer spatial scale than the basin level. This study could be easily replicated with volunteers or researchers increasing the sampling effort and scouring the beaches below colonies every day during the weeks when most chicks are hatching (June). These kinds of sample collection adjustments across a diversity of sites in Puget Sound could yield a more robust sample set, from which more patterns in Pigeon Guillemot foraging behavior may emerge. A further extension of this study would be to perform stomach content analysis on the birds and isotopic analysis on lower trophic web organisms throughout Puget Sound basins to build a more

comprehensive food web and successfully conduct a mixing model to identify the relative carbon sources for this species.

The differences between the membrane and eggshell isotopic signatures are also an area that should be explored more. Eggshell tissues were consistently more enriched in $\delta^{15}N$ and $\delta^{13}C$ than their membrane counterparts after discrimination factors were applied, which was inconsistent with Polito et al's observation for $\delta^{13}C$ (2009). The spatial variation also differed slightly between the two tissues. Membrane samples showed a greater isotopic range for South basin and less variation in Admiralty Inlet, while eggshell samples showed a greater isotopic range for Admiralty Inlet and less variation in South Basin. This discrepancy may be a result of the small sample size but could also be due to a difference in tissue turnover rates or timing of egg formation. Oppel, Powell, and O'Brien (2009) suggested that egg membranes and other egg components may reflect different isotope signatures due to different allocations of body reserve and dietary nutrients. Membrane tissue is synthesized toward the end of the egg formation process and likely reflects a different interval of time in a bird's diet (Oppel, Powell, and O'Brien 2009). This process is followed immediately by the synthesis of the calcified shell, however the timing of when organic shell tissue is formed is unclear. The difference in isotopic signatures between these two tissues may be a result of a slight change in diet for female birds between when egg membranes and shell were being formed, but seems more likely to be due to imperfect fractionation information to account for the difference in turnover rates (Hobson 1995). Lastly, the differences we detected between tissues types could be related to inefficiencies in our laboratory methodology during sample preparation, however we have no means to evaluate this possibility at this time. For example, the process for dissolving eggshell carbonate took several iterations and it was challenging to identify when the samples were carbonate-free and thus ready to be run on the mass spectrometer. Although we are confident that eggshells were carbonate-free we had no way to verify to what extent.

Stable Isotope Analysis and Collaborating with Community Scientists

I found that stable isotope analysis of discarded egg tissues was a reasonable approach to conducting research on Pigeon Guillemot foraging dynamics. While previous studies have utilized this methodology for seabird research, this study, to my knowledge, is the first to couple such a non-disruptive data collection method with community science efforts. Pigeon Guillemot

discarded eggs were easily identified and collected by volunteers during monitoring efforts of breeding bird colonies. Protocol trainings, volunteer communications and coordination only required a few hours of my time and was one of the most rewarding and enjoyable aspects of the project. The majority of the data collection time was spent in the lab processing samples, a process that was fairly quick and efficient for the membrane tissues.

This study highlighted how community scientists are valuable partners in local research. Their knowledge, passion and interest in this species made this project about more than just isotopic variations, but about how one species can provide insight about, and connection to, the greater ecosystem. Pigeon Guillemot feeding and breeding in Puget Sound, like so many other studied species of seabird, can provide us insight into the dynamic ecosystem hidden beneath the water. I found that eggshells and membrane isotopes can be used as windows into Puget Sound basin ecology, given that Pigeon Guillemot likely reflect their immediate environment--the near-shore adjacent to where they breed. While these bird's isotopes may not be able to indicate spatial differences in prey abundance, they may be able to point to more underlying, fundamental biogeochemical differences, which can impact the whole system. By combining this information with other data types, such as beach seining and prey delivery observations as shown here, a fuller picture of ecosystem dynamics can be revealed. The challenge is trying to sew together the spatial and temporal patchwork that different forms of data provide. This study can act as a starting point for future studies using similar techniques to build upon our understanding of how this seabird utilizes its environment for foraging, to help identify any future changes to its trophic relationships, and how it may be used as an indicator species for Puget Sound. The more that we know about this species and ecosystem, the better we can inform management and conservation decision making.

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