Using Stable Isotopes to Quantify Ecological Interaction Strengths

by
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A THESIS

submitted to

Oregon State University

University Honors College

in partial fulfillment of
the requirements for the
degree of

Honors Baccalaureate of Science in Biology
(Honors Scholar)

Presented May 19, 2016
Commencement June 2016

AN ABSTRACT OF THE THESIS OF

Isaac D. Shepard for the degree of Honors Baccalaureate of Science in Biology presented on May 19, 2016. Title: Using Stable Isotopes to Quantify Ecological Interaction Strengths

Abstract approved: _____

Mark Novak

Understanding ecological interaction strengths is one of the main objectives of ecology. Recently, two methods for estimating interaction strengths of predator-prey interactions in nature have been proposed: a field observation approach proposed by Novak and Wootton (2008) and stable isotope analysis. The field observation method estimates feeding rates based on feeding observations, handling times and prey abundances. Stable isotope analysis estimates diet proportions using mixing models of ratios of heavy to light carbon and nitrogen isotopes in body tissues of predators and their prey. However, because these two methods are relatively new, few studies have been conducted to understand and contrast their estimates of interaction strengths. I sought to quantify the correlation between the estimates of interaction strength made by the two methods. Both methods were conducted simultaneously in a rocky intertidal community on the Oregon coast with the whelk *Nucella ostrina* as the focal predator and the barnacle *Balanus glandula* and the mussel *Mytilus trossulus* as the two primary prey species. I documented a non-significant and weak correlation between the two method's estimates of interaction strength. I hypothesize that this lack of correspondence between the methods may be explained by abnormal $\delta^{13}C$ enrichment in the whelks compared to their prey, and a lack of variation in observed predator diets between replicate sample populations.

Key Words: Food Webs, Mixing Models, Diet Proportions, Predator-Prey Interactions

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Introduction

Measuring and understanding ecological interaction strengths is a fundamental goal of ecology. Quantifying interactions is important for identifying keystone species and predicting how communities will change with perturbations. Focusing on predator-prey relationships, ecologists have used a wide range of methods to try to quantify the interactions between organisms. Laboratory experiments, allometric approaches, and field studies have been used to try and estimate interaction strengths in nature (Wootton and Emmerson 2005). Unfortunately, estimating interaction strengths is not straightforward. Spatio-temporal factors, indirect effects, competition, and other hard-to-measure variables can all influence interaction strengths and lead to unrealistic assumptions in the methods used to obtain them (Wootton and Emmerson 2005).

Recently, two methods have been proposed for estimating predator-prey interaction strengths in nature. These methods may potentially overcome the logistical issues facing many of the other methods ecologists have used in the past. These two methods are: a field observational method for quantifying per-capita attack rates proposed by Novak and Wootton (2008) and stable isotope analysis, which estimates diet proportions. Both methods are relatively new and have yet to be compared to other methods for estimating interaction strengths. In order to gain a better understanding of how these new methods contrast with each other I conducted them simultaneously in a simple community in the Oregon rocky intertidal. I aimed to quantify the correlation between estimates of interaction strength made by both methods.

The field observation method of Novak and Wootton (2008) estimates interaction strengths by combining feeding observations and estimates of prey-specific handling times and abundances to quantify per-capita attack rates, assuming a Holling's Type II functional response (Novak and Wootton 2008). Additionally this method assumes that surveys of predators and prey were conducted on an appropriate spatial and temporal scale (Novak and Wootton 2008). Aside from the assumptions it makes, this observational method has another potential drawback. It lacks a large body of empirical support. There has only been one study that has given strong supporting evidence to this observational method (Novak 2010).

The other new tool ecologists have for estimating interaction strengths is stable isotope analysis. While only recently emerging as a tool for quantifying interaction strength, stable isotope analysis has been used in a wide range of other ecological applications in the past. Among these are estimation of niche space (Layman et al. 2007) and determination of food-chain length (Post 2002). Relevant to interaction strengths is the proposed use of stable isotopes to infer diet composition (Phillips and Gregg 2003). Through the use of Bayesian mixing models, interaction strength in terms of diet proportion can be estimated (Yeakel et al. 2011).

Mixing models for stable isotope analysis have many associated assumptions that can lead to biased results if they are violated (Moore and Semmens 2008). At a fundamental level stable isotope mixing models assume that the isotope composition of a predator is equal to the weighted average of the isotope compositions of the various prey it consumes (Gannes et al. 1997). Stable isotope mixing models assume that all possible prey sources of a predator are included in the model (Phillips 2012). Another assumption in these models is that the proportional dietary contribution of a food source is the same for both elements in the mixing model (Phillips 2012). When using mixing models ecologists often assume trophic fractionation to be constant (Moore and Semmens 2008). Trophic fractionation is the tendency for high-density isotopes to accumulate at higher trophic levels resulting in an enriched isotopic signature in predators compared to their prey (DeNiro and Epstein 1987). All of these assumptions, while necessary, are at times unrealistic and not always informed by natural data (Moore and Semmens 2008, Phillips et al. 2014).

The validity of the assumptions mixing models make and how often they hold true in nature is still under question (Moore and Semmens 2008, Phillips et al. 2014). No tests have been done to directly compare the results of stable isotope analysis to other methods for quantifying ecological interaction strengths (but see Yeakel et al. 2011). Understanding how stable isotope analysis (SIA) compares to other methods for quantifying interaction strength will allow for better understanding of the conclusions we can draw from SIA.

The observational method proposed by Novak and Wootton in 2008 is a good comparative method to test SIA against due to its realistic approach to interaction strength (Novak and Wootton 2008). By comparing SIA against this method, we will not only be able to learn more about how SIA can estimate interaction strengths, but we will also gain an additional test of the observational method of Novak and Wootton (2008). In an effort to better understand the ability of both these methods to estimate interaction strengths, I directly compared the results of SIA to interaction strengths estimated by the observational method of Novak and Wootton (2008). I conducted this comparison using a simple food web in the Oregon rocky intertidal. Because SIA provides accurate estimates of diet proportions when properly conducted (Yeakel et al. 2011, Phillips et al. 2014), I hypothesize that both SIA and the observational method outlined by Novak and Wootton (2008) will provide equivalent estimates of interaction strength.

Methods

Overview

Both the observational method and SIA were carried out at the same site within the same time frame to limit spatio-temporal effects. The observational method consisted of surveys of prey densities, surveys of predator feeding events, and calculating handling times. Prey density data were used to assess habitat characterization for survey areas. The prey density, feeding survey, and handling

time data were combined to provide frequency-based estimates of interaction strength. SIA estimates interaction strengths in units of mass. To compare the estimates of interaction strength between the observational method and SIA, I used allometric relationships between body size and the mass of prey to convert the frequency-based estimates of interaction strength from the observational method to be in terms of mass. These transformed feeding rates were used to estimate mass-based diet proportions. To estimate interaction strengths through SIA I collected samples from the field, processed the samples in the lab, and then used mixing models on the isotope data to infer mass-based diet proportions.

Site Description

I conducted my study in the rocky intertidal community at Yachats, Oregon (44.32, -124.10). Yachats is characterized by high abundances of marine invertebrates including the predatory whelk *Nucella ostrina*. *Nucella ostrina* is known to feed on a wide range of intertidal invertebrates. Primary among these are the mussel *Mytilus trossulus* and the barnacle *Balanus glandula* (Wieters and Navarrete 1998). I focused my survey and sampling efforts on these three species. Other known prey species include the snail *Littorina sitkana*, the limpet *Lottia asmi*, and the gooseneck barnacle *Pollicipes polymerus* (personal observation).

I selected 8 permanent 2.25 m^2 patches from a set of 18 patches (identified by letter names), which were part of a larger project, to survey and sample. Patches were selected to represent a gradient of mussel and barnacle densities. Patches E, C, and G had greater than 60% *M. trossulus* coverage and were designated as 'mussel patches'. Patches F, AE, and AG had greater than 60% *B. glandula* coverage and were designated as 'barnacle patches'. Patches BB and BC had approximately equal coverage of *M. trossulus* and *B. glandula* and were designated as 'mixed patches'. Nine permanent 0.0875 m^2 quadrats were randomly placed inside each of the eight patches and were used for prey density surveys.

Observational Method

Surveys of Patches

During 2014, PI Novak and I conducted 4 surveys of the 8 permanent patches at Yachats from May to September. In each survey we collected two types of data: prey abundance, and feeding observations. For prey abundance surveys I took photos of each quadrat in a patch. Back at the lab, I counted individuals of all invertebrate species in photos of three quadrats per patch to estimate prey density in each patch. Photos were processed using the ImageJ software (Rasband, National Institute of Health 2016). PI Novak conducted feeding surveys to determine the proportion of whelks feeding on each prey species. All whelks, feeding or not were measured at the longest length of their shell to the nearest millimeter. Prey being consumed were also measured. For barnacles, we measured the aperture width at its widest point. All other prey species were measured at the longest length or height of their shell.

Handling Time

Handling time is the amount of time it takes a predator to consume one prey item of a particular species. Novak (2010) used lab experiments in a community in the New Zealand rocky intertidal to obtain relationships for estimating handling time based on prey size, predator size, and temperature. Using the regression coefficients for similar species from the Novak (2010) study I used prey size, predator size, and temperature data from the Oregon coast to estimate handling times. Specifically I used regression coefficients from the New Zealand species *Haustrum scobina*, *Xenostrobus pluex*, and *Chamaesipho spp* for *N. ostrina*, *M. trossulus*, and *B. glandula* respectively. Size data were collected during the feeding surveys in the field. I gathered temperature data with HOBO TidbiT Water Temperature Data Loggers (Onset Computer, Pocasset, Massachusetts, USA) placed at the field site. I used data from these sensors to find the average temperature from May to September 2014, averaged over air and water.

Diet Proportions

Data collected from the field surveys and the estimated handling times were used to calculate patch-specific feeding rates for each of *N. ostrina's* prey species. Feeding rates (*F*) were calculated as

$$F_i = \frac{\alpha_i}{\alpha_0} \times \frac{1}{h_i}, \quad (1)$$

(Wolf et al. 2015) where α_i is the number of predators observed feeding on species *i*, α_0 is the number predators observed not feeding, and h_i is the species-specific handling time of the predator on prey species *i.* Equation 1 gives feeding rates in units of number of prey per predator per unit time. To transform this into a proportion I used body size/mass relationships to convert the feeding rates given by Equation 1 to units of grams of prey per predator per unit time for each patch.

To establish allometric relationships between body size and dry tissue mass I haphazardly collected 195 specimens of *M. trossulus* and 78 specimens *B. glandula* of varying sizes. Samples were collected during July of 2013 and July of 2015 from Yachats, OR. For mussels, I measured the longest body length while for barnacles I measured the widest aperture width. Next I scraped out the tissue from the invertebrates and placed it in a drying oven for 24 hours at 60 °C. I then took the mass of the dry tissue. With these data I was able to estimate the relationship between body size and dry tissue mass for both species using regression techniques.

I determined the average mass of dry prey tissue consumed by whelks in each patch using the body size/mass relationships and prey size data gathered during the field surveys. Interaction strengths in units of mass and comparable to SIA were estimated for each patch by dividing the grams of prey consumed per predator per unit time for *B. glandula* or *M. trossulus* in a specific patch by the total grams of prey per predator per unit time of that patch.

Stable Isotope Analysis

Sample Collection

During August of 2014 I collected 20 *Mytilus trossulus* (18.9 to 26.9 mm shell length) and 20 *Balanus glandula* (3.6 to 6.3 mm aperture width) from in and around the 8 patches I had been surveying throughout the summer. These specimens were brought back to the lab and stored in the freezer at -19 °C until processing. In September 2014, I collected 3 *Nucella ostrina* from each of the 8 patches for a total of 24 whelks of lengths 12.2 to 19.3 mm. The one-month difference in collection time was to allow for the isotopes in the whelks to turnover so they would more faithfully reflect their diet from the whole summer. Whelk specimens were also placed in the freezer at -19 °C until they could be processed.

Sample Processing

All soft tissue was removed from the shells of the barnacles and mussels. Tissue was treated drop-wise with 0.5 M HCl to remove calcified structures that were too small to remove manually. The HCl was rinsed off drop-wise with deionized H_2O. For whelks, a piece of foot tissue was removed and treated with acid to control for potential acidification effects on the isotope signature (Schlacher and Connolly 2014). Samples were lyophilized for 24 hours then ground into powder with a mortar and pestle. All tools used throughout the process were rinsed with MeOH and deionized H_2O between samples to prevent cross-contamination. Approximately 1 µg was measured out of each sample and wrapped in tin capsules. The Stable Isotope Laboratory at UC Santa Cruz processed samples via mass spectroscopy.

Mixing Model for Diet Proportions

I used the mixing model MixSIAR (Stock and Semmens 2013) to estimate diet proportions from the stable isotope data. Like many other mixing models, MixSIAR assumes that all prey species are included in the model and that trophic fractionation is accounted for. As the true fractionation for *N. ostrina* on *B. glandula* and *M. trossulus* is unknown, I systematically evaluated all combinations of N and C fractionation values between 1.5 and 2.5 at 0.1 intervals to find the best-fitting model (McCutchan et al. 2003). Model performance was assessed using the deviance information criteria or DIC which is based on the posterior distribution of the model (Stock and Semmens 2013). The lower the DIC, the better fitting the model is. The diet proportions derived from the best-fitting mixing model were used as the metric of interaction strength as estimated by SIA.

Second Round of Sampling

After analyzing the isotope data from 2014, I made two observations: 1) *N. ostrina* was abnormally enriched in carbon compared to its two primary prey *M. trossulus* and *B. glandula* and 2) there was a large variation in the $\delta^{13}C$ of *B. glandula*. In order to explain these observations I collected more isotope samples in June of 2015. I collected 24 *B. glandula* differing in size and tidal height: 6 that were approximately 3 mm across at the aperture in the low zone, 6 that were approximately 6 mm across at the aperture in the low zone, and then 6 of each size class from the high

zone. Additionally, I collected 6 *M. trossulus* in the low zone and 6 in the high zone. During this second round of sampling I also collected 6 specimens of several other, less common prey species: *Lottia asmi*, *Littorina sitkana*, and *Pollicipes polymerus*. All samples were stored and processed as before taking into account the different anatomies of the prey species. Samples were analyzed by the stable isotope lab at UC Santa Cruz.

Comparing Methods

I plotted the proportional, patch-specific contributions of *B. glandula* to the diet of *N. ostrina* estimated by both methods against each other. To determine the congruency between the observational method and SIA I looked for a correlation between the estimates of interaction strength made by both methods using the sample correlation coefficient. All analyses were conducted in the statistical software R (R Development Core Team, 2015).

Results

Observational Method

Field Surveys and Handling Times

Four field surveys were conducted between May and September 2014. There were consistently higher abundances of *B. glandula* than *M. trossulus* in all eight patches over all 4 survey periods. The average density of *B. glandula* was estimated to be 27185.05 ± 1128.25 (mean ± SE) individuals per square meter across all patches. I estimated the average density of *Mytilus trossulus* to be 4765 ± 131.62 individuals per square meter across all patches.

Analysis of the density data revealed that the actual relative densities of prey in the patches did not fit the original percent-cover based classifications assigned to each patch (Figure 1). Specifically, not all patches exhibiting a higher percent cover of mussels were dominated by mussels numerically, nor did the mixed patches

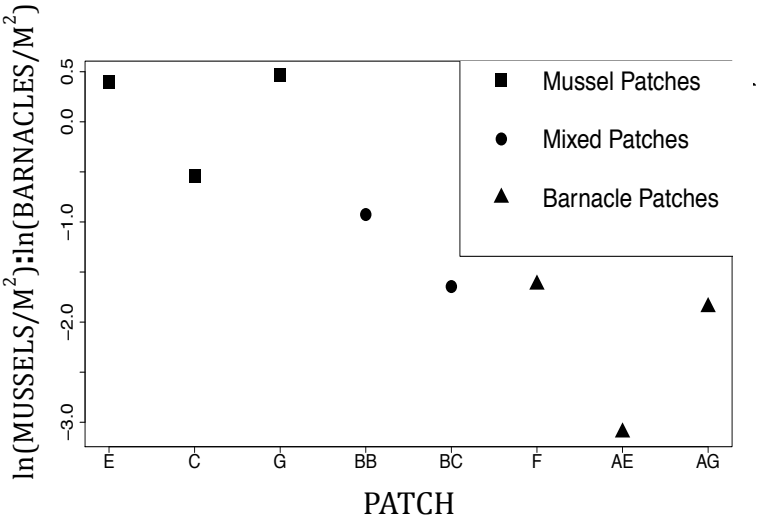

Figure 1. Patch-specific ratios of mussel to barnacle densities. Data were collected from photos of 3 quadrats within each of the patches (n = 8) surveyed at Yachats, OR during the summer of 2014.

exhibit equal numerical densities of both prey species (Figure 1). Due to this inconsistency, diet proportion analyses were conducted on a patch-wise basis rather than by patch type.

Out of 4585 whelks examined in the feeding surveys during the summer of 2014, 569 were observed to be feeding. Of those feeding, 268 whelks were feeding on *B. glandula*, 294 were feeding on *M. trossulus*, and 7 were feeding on other prey species. I excluded the 7 observations of other prey species from my analysis because they made up such a small proportion of the feeding observations. On average, each patch had 7.31% ± 1.14% of whelks feeding on *M. trossulus* and 5.98% ± 1.01% of whelks feeding on *B. glandula*. All patches except C, AE, and AG had a higher proportion of *N. ostrina* feeding on *M. trossulus* than *B. glandula*.

I estimated *N. ostrina's* handling time for *B. glandula* to be 31.61 ± 0.57 hours for *B. glandula* and 57.75 ± 1.31 hours for *M. trossulus*. These values were used in Equation 1 to calculate attack rates for *N. ostrina* on both species in each patch.

Feeding Rates and Diet Proportions

Feeding rate estimates were higher for *B. glandula* than *M. trossulus* in every patch except G (Figure 2). The feeding rates for *B. glandula* and *M. trossulus* across all patches were 0.00224 ± 0.0003 prey per predator per hour and 0.00151 ± 0.0002 prey per predator per hour respectively.

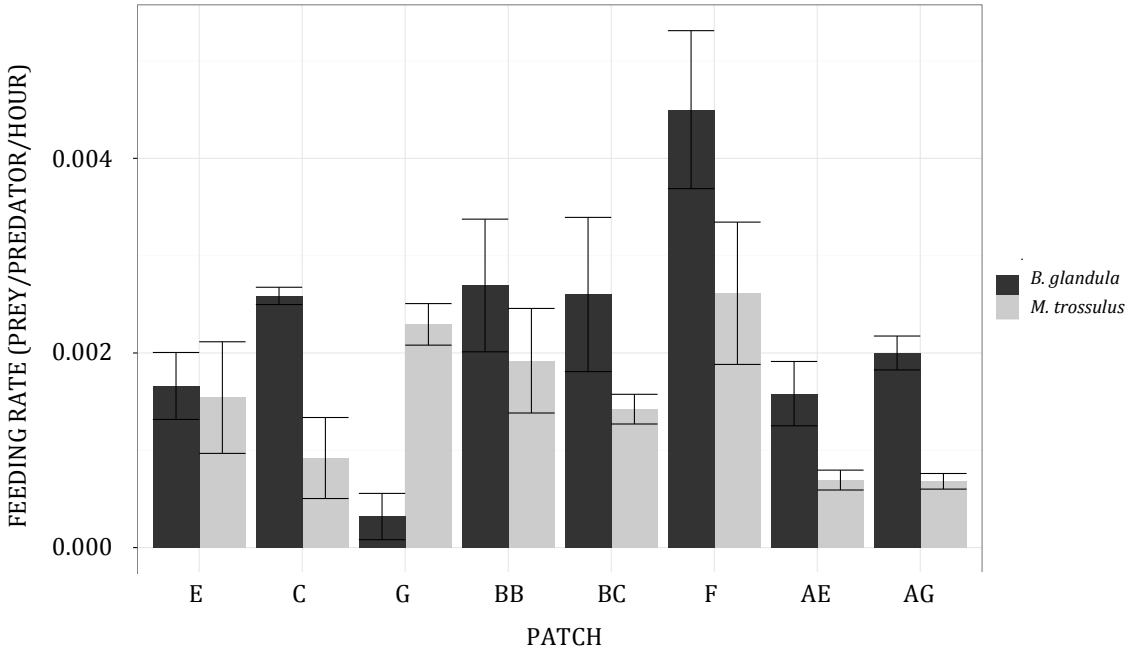

Figure 2. Patch-specific feeding rates for the whelk *Nucella ostrina* on its two primary prey, *Balanus glandula* (in black) and *Mytilus trossulus* (in grey). Feeding rates were estimated from survey data collected at Yachats, OR during 4 separate surveys between May and September, 2014.

The following equations were used to convert frequency-based estimates for the number of consumed by *N. ostrina* in each patch to mass-based estimates (Figure 2):

$$\log(M_b) = 0.84036A - 7.08595 \quad (2)$$

$$\log(M_m) = 0.17797S - 5.49923 \quad (3)$$

Aperture width (A) and shell length (S) were obtained from the feeding surveys *B. glandula* and *M. trossulus* respectively. The average conversion factor for *B. glandula* was 0.0149 ± 0.002 grams per barnacle. For *M. trossulus* I found the average conversion factor to be 0.0953 ± 0.022 grams per mussel. By multiplying the feeding rates and conversion factors, I calculated the proportional contribution of *B. glandula* and *M. trossulus* to the diet of *N. ostrina* in each patch (Figure 3). By mass, *B. glandula* constituted an average of 25.5% of the diet of *N. ostrina.* There were no patches in which *B. glandula* contributed more to *N. ostrina*'s diet than *M. trossulus* (Figure 3).

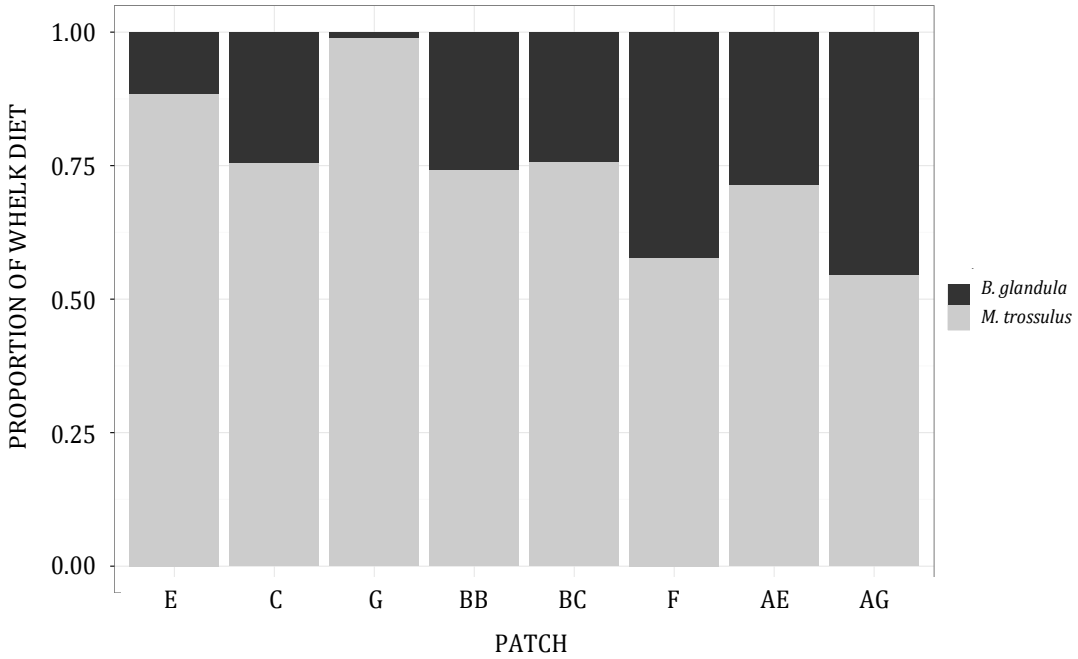

Figure 3. Patch-specific proportional contributions of *Balanus glandula* (black) and *Mytilus trossulus* (grey) to the diet of the whelk *Nucella ostrina* at Yachats, OR. Proportions were estimated from survey data collected during 4 surveys conducted between the months of May and September 2014.

Stable Isotope Analysis

Summer 2014 Data

Two *N. ostrina* samples were lost during elemental analysis of the 2014 isotope samples at UC Santa Cruz: one from patch AE and one from patch BB. *B. glandula* had an average $\delta^{13}C$ value of -19.95‰ ± 0.19‰ (mean ± SE) and an average $\delta^{15}N$ value of 9.17‰ ± 0.08‰ (Figure 4). *M. trossulus*'s average $\delta^{13}C$ value was -18.62‰ ± 0.04‰ while average $\delta^{15}N$ was 7.27‰ ± 0.09‰. *N. ostrina* had an average $\delta^{13}C$ value of -16.63‰ ± 0.07‰ and an average $\delta^{15}N$ value of 10.23‰ ± 0.13‰.

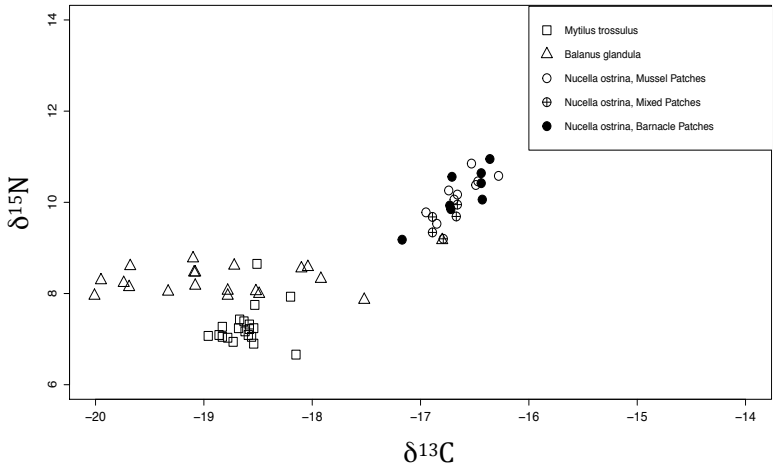

Figure 4. Isospace plot of *N. ostrina* (n = 22), *B. glandula* (n = 20), and *M. trossulus* (n = 20) samples from Yachats, OR. Samples were collected in the summer of 2014.

Summer 2015 Data

The positioning of *N. ostrina* in isospace relative to *B. glandula* and *M. trossulus* suggested that there were additional prey in the whelk's diet that I did not sample (Figure 2). Because of this pattern and the wide spread in *B. glandula*'s $\delta^{13}C$ values (Figure 5) I decided to take more isotope samples in the summer of 2015.

One *M. trossulus* sample was lost during elemental analysis of the summer 2015 isotope

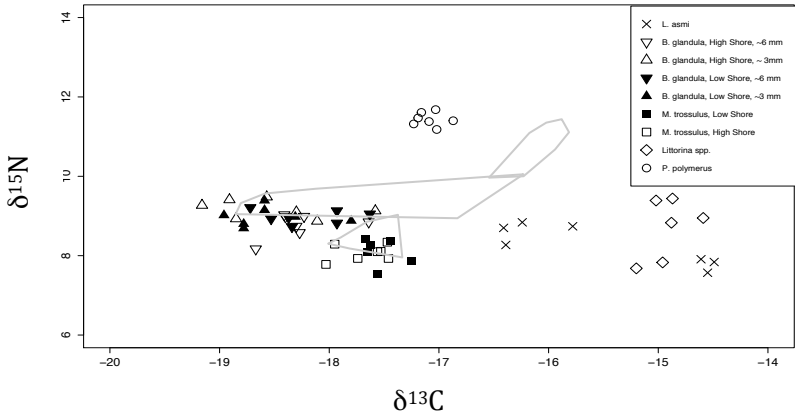

Figure 5. Isospace plot of *Nucella ostrina*'s prey species *L. asmi* (n = 7), *B. glandula* (n = 28), *M. trossulus* (n = 14), *L. sitkana* (n = 6), and *P. polymerus* (n = 7) gathered from Yachats, OR during the summer of 2015. *B. glandula* with apertures approximately 3 or 6 mm wide were sampled from the low and high intertidal zone. Areas outlined in grey represent convex-hull areas from summer 2014 isotope data for (starting at the top) *N. ostrina*, *B. glandula*, and *M. trossulus.*

samples at UC Santa Cruz. *Littorina sitkana* had average $\delta^{13}C$ and $\delta^{15}N$ values of -14.92‰ ± 0.08‰ (mean ± SE) and 8.69‰ ± 0.31‰ respectively. *Lottia asmi*'s average $\delta^{13}C$ and $\delta^{15}N$ values were -15.50‰ ± 0.34‰ and 8.27‰ ± 0.19‰ respectively. *Pollicipes polymerus* had an average $\delta^{13}C$ value of -17.08‰ ± 0.05‰ and an average $\delta^{15}N$ value of 11.43‰ ± 0.06‰. *B. glandula* had average $\delta^{13}C$ and $\delta^{15}N$ values of -18.38‰ ± 0.08‰ and 8.97‰ ± 0.05‰ respectively. These values were significantly different from the $\delta^{13}C$ and $\delta^{15}N$ values for *B. glandula* during the summer of 2014 ($\delta^{13}C$: $t_{26} = -2.15$, $p = 0.041$; $\delta^{15}N$: $t_{35} = -7.31$, $p < 0.01$). *Mytilus trossulus*'s average $\delta^{13}C$ value was -17.61‰ ± 0.06‰ and its average $\delta^{15}N$ value was 8.08‰ ± 0.07‰. Comparison of these values to the $\delta^{13}C$ and $\delta^{15}N$ values of *M. trossulus* from the summer of 2014 showed that they too were significantly different ($\delta^{13}C$: $t_{25} = -13.81$, $p < 0.01$; $\delta^{15}N$: $t_{31} = -6.68$, $p < 0.01$).

I tried to explain the variation in *B. glandula*'s $\delta^{13}C$ values (Figure 4) by sampling varying sizes of *B. glandula* from different tidal heights. However, no relationship was found between barnacle size and $\delta^{13}C$ value or between barnacle tidal height and $\delta^{13}C$ value (Figure 5).

Mixing Models

My systematic search for the best fitting fractionation values yielded two sets of C and N fractionation values that were of interest: ΔC = 1.9‰ ± 0.5‰, ΔN = 1.9‰ ± 0.5‰ and ΔC = 2.0‰ ± 0.5‰, ΔN = 2.5‰ ± 0.5‰ (Figure 6). The first set of fractionation values yielded a model with the lowest DIC (113.314) of all the combinations tested meaning that the first set of fractionation values were the best fitting. *B. glandula* contributed 73.9% of *N. ostrina*'s diet when the mixing model had fractionation values of ΔC = 1.9‰ ± 0.5‰, ΔN = 1.9‰ ± 0.5‰. With the first set of fractionation values, *B. glandula* contributed over 50% of *N. ostrina*'s diet in every patch except for BC (Figure 7). The second set of fractionation values (ΔC = 2.0‰ ± 0.5‰, ΔN = 2.5‰ ± 0.5‰) had a somewhat higher DIC of 122.354 and therefore was not the best fitting model. However, the model with these fractionation values gave very different results from nearly every other combination of values tested. When the fractionation was set ΔC = 2.0‰ ± 0.5‰, ΔN = 2.5‰ ± 0.5‰ *B. glandula* was, on average, 31.9% of *N. ostrina*'s diet. *M. trossulus* made up over 50% of *N. ostrina*'s diet in every patch except AE and C (Figure 7). Despite not yielding the best fitting model, the fact that the second set of fractionation values gave nearly opposite results from nearly every other combination made it worth mentioning. This second set of fractionation values is right on the edge of the range of values I tested. However, testing the combinations of values just beyond the range of reasonable fractionation values revealed a continuing trend of increasing DIC values, which indicates increasingly poor-fitting models. The second combination of fractionation values (ΔC = 2.0‰ ± 0.5‰, ΔN = 2.5‰ ± 0.5‰) had the lowest DIC of the small subset of combinations that gave similar results.

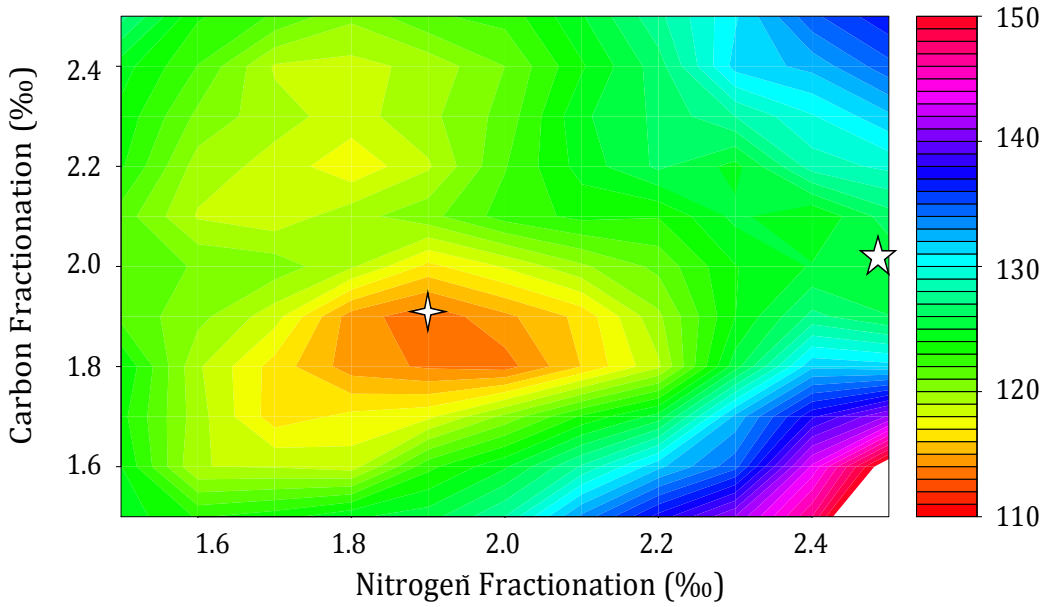

Figure 6. A heat map showing the DIC values from mixing model results using all combinations of N (x-axis) and C (y-axis) fractionation values between 1.5‰ and 2.5‰ at 0.1 intervals. Contours lines and color changes are present at every 1-unit change in the DIC. The four-pointed star (N = 1.9‰, C = 1.9‰) shows the DIC of the best fitting model (DIC = 113.314). The five-pointed star (N = 2.5‰, C = 2.0‰) shows the DIC for the mixing model that gave results more consistent with the observational method (DIC = 122.354). The mixing models were conducted using MixSIAR software on isotope data from *N. ostrina* (n = 22), *B. glandula* (n = 20), and *M. trossulus* (n = 20) samples gathered during the summer of 2014 at Yachats, OR.

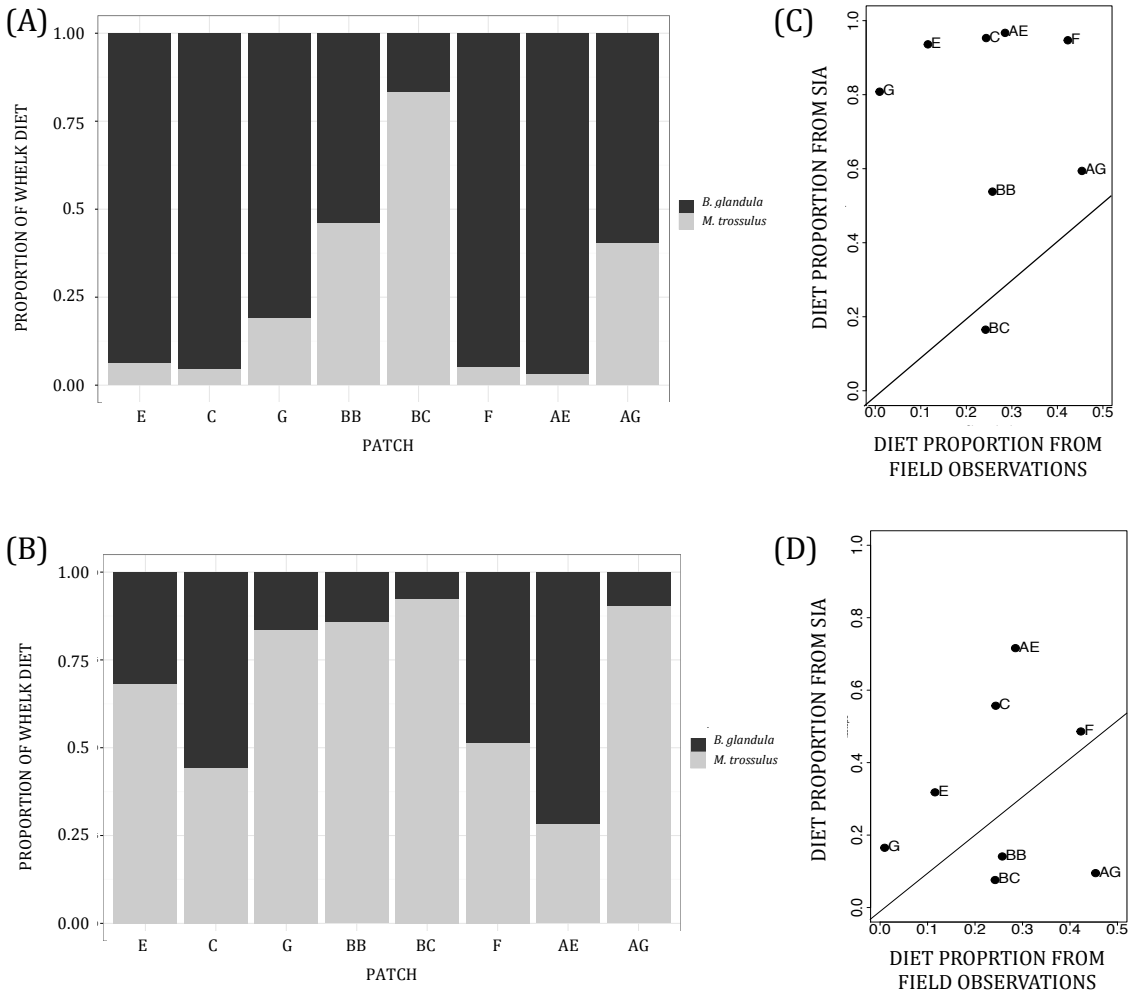

Figure 7. The proportional contributions of *Balanus glandula* (black) and *Mytilus trossulus* (grey) to the diet of *Nucella ostrina* as estimated by MixSIAR when the trophic fractionation was set at ΔN = 1.9‰, ΔC = 1.9‰ (A) and ΔN = 2.5‰, ΔC = 2.0‰ (B). When comparing the estimated proportions *Balanus glandula* contributed to the diet of *Nucella ostrina* between both the model using the first set of fractionation values and the field observation method (C) no strong correlation was detected ($R^2 = 0.018$). Similarly, comparing the proportion *Balanus glandula* contributed to the diet of *Nucella ostrina* from the model using the second set of fractionation values and the field observation method (D) revealed no strong correlation ($R^2 = 0.008$). The black lines on figures C and D represent the 1-to-1 lines that the points would lie around if there were a strong correlation between the two methods.

Method Comparison

There was no strong correlation between the estimates of diet proportion made by the observational method and stable isotope analysis using both sets of fractionation values (Figure 7). However, stable isotope analysis provided more similar estimates of diet proportion to the observational method when the fractionation was set at the second set of values ($\Delta C = 2.0‰ \pm 0.5‰$, $\Delta N = 2.5‰ \pm 0.5‰$).

Discussion

While no correlation was found between the observational method and SIA in this study (Figure 7), the results provided meaningful insights about both methods and their abilities to estimate interaction strengths. Here I will examine the potential mechanisms behind the mismatch of interaction strength estimates from the two methods. Not only will this examination suggest explanations for why the methods did not agree but it will also reveal some of the unique problems and benefits each method has.

One possible factor that could be responsible for the inconsistency between methods is *N. ostrina's* enrichment in carbon isotopes relative to the isotopic signature of its two primary prey. Both trophic fractionation and diet breadth could explain why *N. ostrina* is so enriched in carbon compared to its prey.

N. ostrina was enriched an average of $2.06‰ \pm 0.1‰$ in $\delta^{13}C$ compared to its primary prey (Figure 4). Trophic fractionation of carbon to this degree, while biologically feasible, is uncommon (McCutchan et al. 2003). Typically, trophic fractionation values for carbon in consumers tend to be around 1.3‰ (McCutchan et al. 2003). However, based on the raw isotope data, I cannot determine whether or not the enrichment is caused by *N. ostrina* exhibiting high carbon trophic fractionation or if something else is influencing the carbon values. This uncertainty agrees with the growing body of evidence that using experimentally-determined, species-specific fractionation values in mixing models is the best way to obtain accurate estimates of interaction strengths using SIA (Moore and Semmens 2008, Phillips et al. 2014). The stark contrast in diet proportion estimates made by the two models I used (Figure 7) highlights the fact that even slight changes to the trophic fractionation can cause a model to give very different results.

A second way the carbon isotope enrichment in whelks compared to their two primary prey can be explained is an expanded *N. ostrina* diet (Fry 2008). All but seven feeding observations were made on *B. glandula* and *M. trossulus* suggesting that any other prey species constituted an insignificant proportion of *N. ostrina*'s diet. Assuming *N. ostrina* does not fractionate carbon at a high rate, I would expect its carbon value to be nearly the average of its prey species (Fry 2008). This similarity is not what is seen in the isotope data (Figure 4). However, if *N. ostrina* were to consume prey comparatively enriched in carbon then its observed average

$\delta^{13}C$ could be explained (Fry 2008). Isotope data from 2015 revealed that *N. ostrina* has two known prey (*L. asmi* and *Littorina sitkana*), which if consumed in moderate proportions could influence the whelks' carbon value (Figure 5). While no feeding observations were made of *Lottia asmi* or *Littorina sitkana* during the summer of 2014, *N. ostrina* is known to feed on these herbivorous invertebrates (personal observation).

If carbon enrichment in *N. ostrina* is caused by the consumption of *Lottia asmi* and *Littorina sitkana*, there are also some implications for the observational method. In my study feeding surveys were constrained to the marked patches, which were surrounded by mostly-intact mussel beds or a mix of natural patches at various successional stages. Whelks observed within the patches may have been traversing these patches and feeding on different prey in the surrounding habitat. As a result, feeding observations made within the patches might not have been reflective of the overall whelk's overall diet and thus their isotopic signatures. Overall, these potentially missing feeding observations show that the observational method is only as good as a researchers ability to detect feeding events at a scale relevant to the predator they are studying (Novak and Wootton 2008).

Prey species sampled for SIA were selected based on the observations of what *N. ostrina* was eating in the observational method. One of the assumptions of mixing models is that all prey species are included in the model (Phillips 2012). Because no feeding events of *Lottia. asmi* and *Littorina sitkana* were observed, neither prey was collected during the first round of sampling in 2014. While it is impossible to know for certain whether or not *Lottia. asmi* or *Littorina sitkana* were important parts of *N. ostrina's* diet in the summer of 2014, their positioning in isospace is suggestive that they were consumed regularly by *N. ostrina* (Figure 5). If this was the case, then the above assumption was violated and likely played a role in producing inferences of interaction strengths inconsistent with the observational method.

B. glandula showed wide variation in its $\delta^{13}C$ values (Figure 4). It is possible that patch-specific enrichment could be explained by barnacle consumption in whelks if correlations between carbon value and body size or carbon value and tidal height could be established, as with a similar European barnacle species by Craven et al. (2008). However, the variation in carbon was not explained by either factor (Figure 5). Future investigations could seek to understand why there is so much variation in *B. glandula's* $\delta^{13}C$ values and the effects of this variation on stable isotope analysis.

While the carbon enrichment of *N. ostrina* compared to its two primary prey is a reasonable justification for the mismatch between SIA and the observational method, it is important to consider other explanations as well. *N. ostrina* had a very narrow observed diet which could be another reason why there was discord between the two methods estimates of interaction strength. However, *N. ostrina*'s narrow diet is likely a symptom of a larger issue: lack of variation in prey

availability. Because I was trying to compare interaction strengths across areas of varying prey density, the whelks needed to show significant patch-specific diet variation for a correlation between the two methods to be established. Otherwise, random variation within each patch could make it difficult for both methods to arrive at similar enough estimates of interaction strength for each patch such that a correlation could be established between the two methods. That is, the patches I surveyed and sampled were not very different in their prey densities (Figure 1) leaving little room for a signal to emerge from the noise.

In contrast to my study, Yeakel et al. (2011) compared SIA and the observational method across prey species rather than across prey densities. Using a generalist predator with a broad diet consisting of 8 species, they documented a significant correlation between the two methods (Yeakel et al. 2011). Interaction strengths for each species were distinct and, for the most part, significantly different from each other (Yeakel et al. 2011). Their ability to draw a correlation between the two methods makes it clear that magnitude of difference in interaction strength between units being compared may be of vital importance in the comparison of both methods.

A nutritional requirement in whelks might also be making it difficult to detect distinct, patch-specific interaction strengths. There has been evidence that *N. ostrina* shows preference for mussels over barnacles (Wieters and Navarrete 1998). However, this apparent preference could be the result of a nutritional requirement. It could be that whelks require a specific ratio of mussels to barnacles in their diet to meet a nutritional need. If this were the case, whelks would consume prey regardless of the relative densities of prey in each of the patches. Put more simply, a nutritional requirement could cause whelks to interact similarly with mussels and barnacles in all the patches surveyed regardless of prey availability. It would be difficult to detect distinct, patch-specific interaction strengths using both methods if all the whelks were interacting similarly with the organisms in the patches.

Not being able to establish a correlation between these two methods due to narrow predator diets or prey preferences among predators does not invalidate either SIA or the observational method. Further testing using a stronger prey density gradient while simultaneously accounting for prey preferences needs to be conducted in order to understand exactly how these methods compare to one another.

Conclusions

Most likely, all of the factors discussed worked together to cause the mismatch of interaction strength estimates made by both SIA and the observational method. Importantly, the inconsistent results helped point out key issues and areas for future investigation in both methods. While neither method is perfect on its own, they complement each other. The observational method was good at estimating interaction strengths within patches but likely failed to capture *N. ostrina*'s complete diet by not including individual predator's whole foraging range. SIA has potential to

accurately estimate interaction strengths, but there are many details such as trophic fractionation, diet breadth, and scale of study that need to be taken care of in order for estimates to be accurate.

Accurately quantifying and understanding interactions between predators and their prey is one of ecology's primary goals. Using both SIA and the observational method will allow scientists to make the best estimates of interaction strengths.

Acknowledgements

I would like to thank the Oregon State Student URISC and SURE programs for funds used on this project. I would also like to thank the Terry Lab for letting me use their equipment to prepare stable isotope samples. Lastly, I would like to thank Julia Bingham, Stephanie Merhoff, Beatriz Vaca, and Kyle Coblentz of the Novak Lab for their assistance in the field and lab gathering data.

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