

Isotopic evidence of microbial pathways in macroalgal detritus-based coastal
food webs

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Table of contents

| | |
|--|----|
| Introduction | 1 |
| Hypotheses and Objectives..... | 2 |
| Approach..... | 3 |
| Mesocosms | 3 |
| Multiple Stable Isotope Analysis..... | 3 |
| Study Area..... | 4 |
| Chapter One..... | 7 |
| Introduction | 7 |
| Methods..... | 10 |
| Approach..... | 10 |
| Kelp biogeochemistry (MSI, elemental, and phlorotannin)..... | 11 |
| Microbial abundances..... | 11 |
| Samples to test the efficacy of microbe reduction techniques | 11 |
| Statistical analyses | 11 |
| Results..... | 12 |
| Isotope Composition..... | 13 |
| Discussion..... | 14 |
| Ecological consequences..... | 15 |
| Isotopic consequences..... | 16 |
| Applications to Estuarine and Coastal Research..... | 17 |
| Chapter One Figures and Tables..... | 19 |
| Chapter Two..... | 33 |
| Introduction | 33 |
| Methods..... | 33 |
| Results..... | 35 |
| Discussion..... | 35 |
| Recommendations..... | 36 |
| Chapter Two Figures and Tables..... | 38 |
| Isotope triage | 40 |
| Chapter Three | 41 |
| Introduction | 41 |

Methods..... 41

 Consumers 41

 Samples to test the efficacy of microbe reduction techniques 43

 Field data..... 43

 Estimated Trophic Level..... 43

 Statistical Analyses..... 43

Results 44

 Isotopic values 44

 Behavior and growth..... 44

Discussion..... 45

Conclusions 48

Chapter Three Tables and Figures 49

Synthesis 56

References 60

List of Figures

Introduction

| | |
|---|---|
| Fig 1: Existing paradigm of microbial effects on detrital material..... | 5 |
| Fig 2: Conceptual diagram of the topics addressed within this manuscript..... | 5 |
| Fig 3: The San Juan Islands. Sample sites are marked in red | 6 |

Chapter 1

| | |
|--|----|
| Fig 1: Median values of microbial abundance on <i>S. subsimplex</i> blades | 19 |
| Fig 2: Average changes in phlorotannin concentration of the kelps <i>Saccharina subsimplex</i> and <i>Agarum fimbriatum</i> over 5 weeks of decomposition..... | 20 |
| Fig 3: Median values of microbial abundance in <i>Saccharina subsimplex</i> and <i>Agarum fimbriatum</i> during decomposition. | 20 |
| Fig 4: Elemental content of kelp blades at the beginning and end of a five-week decomposition experiment..... | 21 |
| Fig 5: C:N ratio of kelp blades with (unscraped) and without (scraped) biofilm during decomposition.... | 22 |
| Fig 6: <i>Agarum</i> MSI plotted in unconstrained multidimensional space..... | 23 |
| Fig 7: Univariate analysis of $\delta^{34}\text{S}$ in <i>Agarum</i> over time as a function of biofilm presence or absence. | 24 |
| Fig 8: $\delta^{13}\text{C}$, $\delta^{34}\text{S}$, and $\delta^{15}\text{N}$ values in <i>A. fimbriatum</i> at first and fifth week of decomposition..... | 24 |
| Fig 9: <i>Saccharina</i> MSI in unconstrained multidimensional space..... | 25 |
| Fig 10: Univariate analysis of $\delta^{13}\text{C}$ values in <i>S. subsimplex</i> during decomposition..... | 26 |
| Fig 11: Univariate analyses of $\delta^{34}\text{S}$ in <i>S. subsimplex</i> | 27 |
| Fig 12: Univariate analysis of $\delta^{34}\text{S}$ in <i>Saccharina</i> samples as a function of biofilm presence or absence.. | 27 |
| Fig 13: Keeling plot of elemental S in kelp substrate versus $\delta^{34}\text{S}$ | 28 |
| Fig 14: $\delta^{15}\text{N}$ composition in <i>Saccharina</i> over the course of decomposition..... | 29 |
| Fig 15: Univariate analyses of $\delta^{15}\text{N}$ in <i>Saccharina</i> samples..... | 29 |
| Fig 16: Keeling plot of elemental N in kelp substrate versus $\delta^{15}\text{N}$ in kelp and kelp with biofilm. | 30 |
| Fig 17: The loss of elemental carbon and nitrogen from (a) large kelp detritus and (b) kelp particles. | 32 |

Chapter 2

| | |
|---|----|
| Fig 1: Example of model results for one diet in each modelling scenario. | 39 |
| Fig 2: MSI ecological research methods triage | 40 |

Chapter 3

| | |
|---|----|
| Fig 1: Median values of microbial abundance on kelp blades before and after biofilm removal | 49 |
| Fig 2: MSI of <i>S. subsimplex</i> used in feeding trial in multidimensional space..... | 50 |

| | |
|--|----|
| Fig 3: <i>Calliostoma ligatum</i> MSI values in multidimensional space. | 51 |
| Fig 4: Estimated trophic level of three ambient organisms collected in field from 10m and 100m | 53 |
| Fig 5: Topsnails feeding preferences..... | 53 |
| Fig 6: Shrimp feeding preferences. | 54 |
| Fig 7: Shrimp survival by feeding level..... | 54 |
| Fig 8: Estimated trophic level of <i>Calliostoma ligatum</i> by treatment group. | 55 |

Synthesis

| | |
|---|----|
| Fig 1: Updated conceptual model of detrital processes | 59 |
|---|----|

List of Tables

Chapter One

| | |
|---|----|
| Table 1. Average MSI values of <i>S. subsimplex</i> blades with biofilm during decomposition, and the accumulated sediment deposited from the flowing seawater system. | 31 |
| Table 2. Estimated trophic level of <i>A. fimbriatum</i> (a), and <i>S. subsimplex</i> (b) at different states of decomposition | 31 |

Chapter Two

| | |
|---|----|
| Table 1: Effect of each source input scenario on the accuracy of Bayesian mixing model results. | 38 |
| Table 2: Effect of each source input scenario on the accuracy of Bayesian mixing model results in diets where actual contribution of <i>S. subsimplex</i> to consumer diets $\geq 20\%$ | 38 |
| Table 3: Effect of each source input scenario on the accuracy of Bayesian mixing model results in diets where <i>S. subsimplex</i> is the largest contributor to actual consumer diets..... | 39 |

Chapter 3

| | |
|--|----|
| Table 1: MSI values of each snail and food source treatment group. | 51 |
| Table 2: Average $\delta^{15}\text{N}$ values of <i>Calliostoma ligatum</i> by treatment (food source age). | 52 |
| Table 3: $\delta^{15}\text{N}$ values of three ambient organisms collected in the field from 10m and 100m at three sites. | 52 |

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Introduction

Most of the world's food webs depend on photosynthesis as the primary source of carbon. However, in areas where there is no intrinsic photosynthesis the source of carbon is difficult to discern. In areas such as these, carbon must come in the form of subsidies of organic material donated from other ecosystems (Polis et al. 1997), often in the form of detritus. Due to the inaccessibility of these aphotic areas, the type of detrital material, its geographic origin and its relative importance to aphotic food webs are commonly impossible to observe directly. Instead, researchers must rely on tools such as biomarkers to infer what fuels detritus-based food webs.

Multiple Stable Isotope (MSI) analysis has been a popular tool in this regard; in particular, this technique has been employed to investigate sources of carbon in food webs within coastal deep subtidal ecosystems. Through the use of mixing models, MSI data can be used to estimate the probable contribution of various food sources to the diet of a population of consumers. The results of these studies have suggested that kelp detritus provides a critical subsidy to coastal deep subtidal ecosystems (Kaehler et al. 2006, Kaehler 2000, Kelly et al. 2012, Nadon & Himmelman 2006); however, discrepancies in the datasets have generated doubt and speculation about where these discrepancies come from (Nadon & Himmelman 2006, Kaehler 2000, Miller & Page 2012). The role of the microbial loop in detritus-based food webs and its effect on detritus during decomposition has been an especially popular object of speculation.

Several studies have found that microbial decomposition of primary producers will result in an alteration from un-degraded source isotope values, implicitly acknowledging a potential microbial influence (Duggins & Eckman 1997, Kramer et al. 2003, Macko et al. 1982, Couch 1989) though few studies have attempted to measure this phenomenon explicitly (Macko & Estep 1984, Kramer et al. 2003, Norderhaug et al. 2003). In addition, other major changes to algal biochemistry during decomposition have been noted. Pulverized and decomposed macroalgae was found to have decreased phytotoxin concentrations, and increased food quality by way of reduced C:N ratios (Duggins & Eckman 1997, Norderhaug et al. 2006). Taken together, these changes increase the availability of macroalgal detritus as a food source to consumers, while simultaneously improving the quality. However, the mechanism behind these changes can only be speculated upon using currently available data. The significance of each of these chemical changes in regards to the relationship between nutritional benefit and bacterial assemblage is therefore unclear. The abundance and diversity of the detrital bacterial assemblage may play a large role in the chemical processes outlined above, and thus have a direct impact on consumer nutrition. Recent efforts to identify the bacterial assemblage of kelp biofilms (Staufenberger et al. 2008, Bengtsson et al. 2010) have indicated a startling amount of complexity and specificity both within individual blades and over time. Further light must be shed on the intricacies of bacteria-detritus-consumer interactions in order to fully understand their role in coastal marine food webs.

To that end, it is important to note that in ecological studies using MSI analysis, current research typically uses un-degraded primary producers to obtain source isotope signatures, and does not account

for potential microbial alteration of sources within the analysis (Figure 1). In consumers that rely on degraded detritus for carbon, this oversight may result in consumer isotope values that are significantly different from expected values. The extent to which the empirical values stray from assumed values can affect the accuracy of calculated trophic positions (Vander Zanden 2001, McCutchan et al. 2003), which could lead to a fundamental misinterpretation of how disparate ecosystems interact. In detritus-based food webs, the degree of microbial influence on consumers may be significantly higher than in similar studies done in food webs in ecosystems that do not have prominent heterotrophic cycling of organic matter; this influence can act as a confounding factor if ignored. Additional research on how microbes may alter food quality and biogeochemical composition is sorely needed, both to correct existing food web models, and to increase our understanding of bottom-up trophic controls.

A previous study of Deep Subtidal Ecosystem (DSE) food webs downplayed the role of algal detritus as a source of carbon for consumers, citing discrepancies in isotope data between DSE consumers and Shallow Subtidal Ecosystem (SSE) primary producers (Nadon & Himmelman 2006). Instead, Nadon & Himmelman concluded that consumers at these depths must rely on other factors that could not be identified. In this study, this assertion will be revisited. Consumers in the DSE that may feed on degraded algae detritus are likely ingesting large amounts of microbes as well. Previous studies have shown that microbial alteration of algal detritus may have a significant positive effect on the growth of consumers (Norderhaug et al. 2003, Duggins & Eckman 1997), and, moreover, that microbes may have a distinct isotopic signature (Macko & Estep 1984). However, little research has been conducted to reveal how microbes might mediate the availability and isotopic content of organic material in the DSE. To understand these food webs better and to improve our models, I will measure the impact of microbial degradation of detritus on consumers. Due to the inaccessibility of the DSE, and the vast mixing of detrital inputs that may confound the results, this task cannot be completed in the field. Therefore, I will employ a series of controlled laboratory experiments.

Hypotheses and Objectives

I hypothesize that microbial biofilms act as an intermediate trophic level between macroalgae detritus and apparent herbivores in deep subtidal food webs. As a result, I predict that consumers in the DSE may appear to occupy a higher trophic position than their congeners in the SSE. Furthermore, because microbial colonization may result in the isotopic fractionation of source algae during decomposition, I predict that the results of Bayesian mixing model analyses may be highly confounded if microbial influence remains unquantified. Figure 2 illustrates the concepts addressed in this manuscript. I propose to test:

- 1) Whether the microbial assemblage within biofilms utilizes kelp nutrients. I will do this by measuring biogeochemical shifts in decomposing kelp blades of two species. (Chapter 1).
- 2) The importance of including microbial decomposition of macroalgae in food web models. I will do this by performing consumer simulations using a Bayesian mixing model under various scenarios in which microbial effects are included as model inputs to varying degrees (Chapter 2).
- 3) Whether microbial decomposition of macroalgae has cascading trophic effects on higher

consumers. I will do this by comparing isotopic values, calculated trophic level, and fitness between individuals of a single species occupying two different guilds: herbivore and detritivore (Chapter 3).

Approach

Mesocosms

Mesocosm experiments are a popular ecological technique in lieu of or in conjunction with field experiments. They offer the advantage over fieldwork of allowing researchers to tightly control environmental variables which could confound the results of field experiments, such as water quality, food availability, predation rates, and chemical cues. On the other hand, a major criticism of mesocosm work is that this tight regulation of variables may result in a loss of the 'real world' picture and, at worst, be ecologically irrelevant. With this caveat in mind, fractionation experiments use mesocosms that carefully mimic environmental conditions (Gorokhova & Hansson 1999, Macko et al. 1982). For my mesocosm experiments, I have selected ecologically appropriate species for the San Juan Islands. *Saccharina subsimplex* and *Agarum fimbriatum* were chosen to examine algal decomposition, as they have been shown to be the two largest contributors to macroalgal drift in the San Juan Islands (Britton-Simmons 2009), and thus provide the bulk of detrital material available to consumers. To examine cascading trophic effects of algal decomposition, the turban snail *Calliostoma ligatum* and the spot prawn *Pandalus platyceros* were chosen, as these organisms can be found at aphotic depths and preliminary work has found that they utilize algae detritus as a food source.

Multiple Stable Isotope Analysis

Multiple stable isotope (MSI) analysis is a versatile technique with a broad range of applications. It has proven useful across several fields of research, including archeology, paleontology, ecology, and climate change (Fry 2006, Hansson et al. 1997, Hobson 1999). In the field of ecology, MSI analysis has become a popular and effective method for elucidating food web interactions and connectivity among ecosystems. In marine systems, MSI analysis has been used in kelp forests (Page et al. 2008, Fredriksen et al. 2003), seagrass beds (Stephanson et al. 1986), mussel beds (Allan et al. 2010), and the intertidal rocky shore (Bustamante & Branch 1996), among others. It is based upon the premise that stable isotopes such as $\delta^{15}\text{N}$, $\delta^{13}\text{C}$ and $\delta^{34}\text{S}$ can be used as tracers in biogeochemical cycles (Peterson & Fry 1987, Peterson 1999). These 'heavy' isotopes (as opposed to the more conventional 'light' isotopes typically found on the periodic table) are naturally occurring at low concentrations in ambient conditions. The subsequent enrichment or depletion of the ratio of heavy to light isotope species in a system is indicative of the biogeochemical processes that have occurred, such as carbon fixation via photosynthesis, nitrogen fixation via bacterial pathways, and metabolic fractionation in consumers (Fry 2006).

The use of stable isotopes in ecological studies is based upon predictable changes in isotope ratios within a food web. While the transport of carbon (as a metric of energy and production) is often the primary focus, many food web studies use isotopes of multiple elements to increase the resolution of potential carbon sources by creating multiple isotope 'signatures' for consumers and primary producers (Peterson 1999, Fry 2006). Isotopes of carbon, nitrogen and sulfur are often used in ecological research, as each isotope provides its own type of information. Carbon isotope ratios are assumed to change very little ($<+1$ ‰) between trophic levels, and remain close to the ratio at which the carbon was photosynthetically 'fixed' by an autotroph. The type of photosynthesis used to fix the carbon is thus imprinted into its signature (Fry 2006). Nitrogen isotope ratios are assumed to be enriched by an average value of $\sim+3.4$ ‰ between trophic levels, and are therefore used to calculate the number of trophic levels between a consumer and its ultimate source of carbon (Peterson & Fry 1987, Minagawa & Wada 1984, Cabana & Rasmussen 1996, Peterson 1999). Sulfur isotope ratios are assumed to not change between trophic levels, but can indicate whether the sulfur came from oceanic or terrestrial sources. For this reason they are used as another indicator of organic material origin to boost resolution of the model (Fry 2006, Peterson & Fry 1987). By using MSI 'signatures' as inputs into a model, one may deduce the strength of relationships between a consumer and its potential carbon sources. There are caveats, however. All potential sources of carbon must be known and included in the mixing model; failure to include a major source may yield inconclusive results. The accuracy of a given isotope-based food web model therefore hinges on the inclusion of all possible carbon sources and intermediates.

Study Area

The San Juan Islands of northern Washington State (Fig 3) have a complex bathymetry wherein shallow coastal areas with high macroalgae productivity (Shallow Subtidal Ecosystem, SSE) quickly yield to extremely deep channels (>100 m) in which detritus may accumulate (Deep Subtidal Ecosystem, DSE). The DSE can be characterized by low to no primary productivity and high microbial productivity; it is distinct from better studied systems for its potential total dependence on subsidies of organic matter produced in the SSE, as it lies outside the photic zone and experiences no intrinsic primary production. Detrital inputs in the form of drift algae, metabolic wastes and decomposing organic matter are likely the major sources of carbon in the DSE, as previous ROV and SCUBA surveys have identified large volumes of detritus transported far from algal beds in the SSE (Britton-Simmons et al., 2012). SCUBA surveys of unattached drift algae have revealed the major species contributors to be kelps such as *Saccharina subsimplex*, *Agarum fimbriatum*, *Nereocystis luetkeana* and *Alaria marginata* (Britton-Simmons 2009). Preliminary surveys using a ROV have revealed a significant influx of detritus at these depths, as well as large communities of detritivores such as shrimp, urchins, sea cucumbers and scallops.

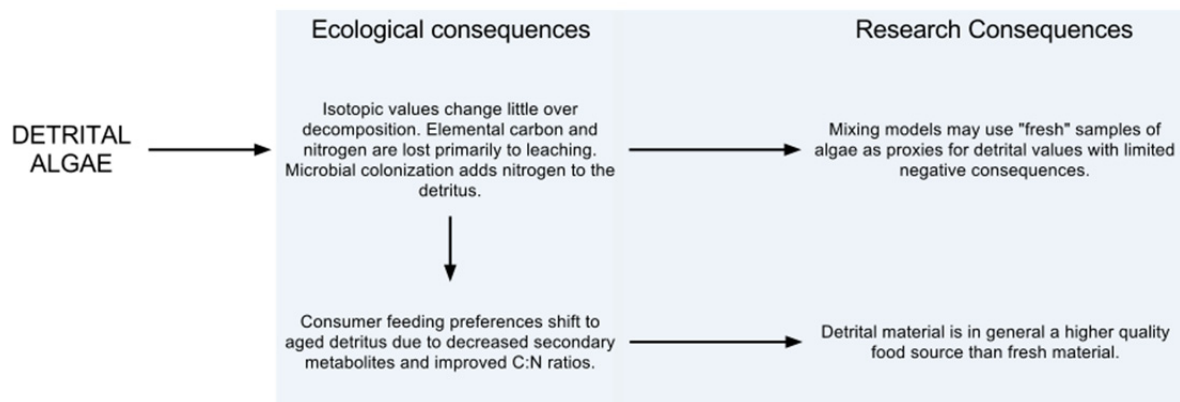


Fig 1: Existing paradigm of microbial effects on detrital material.

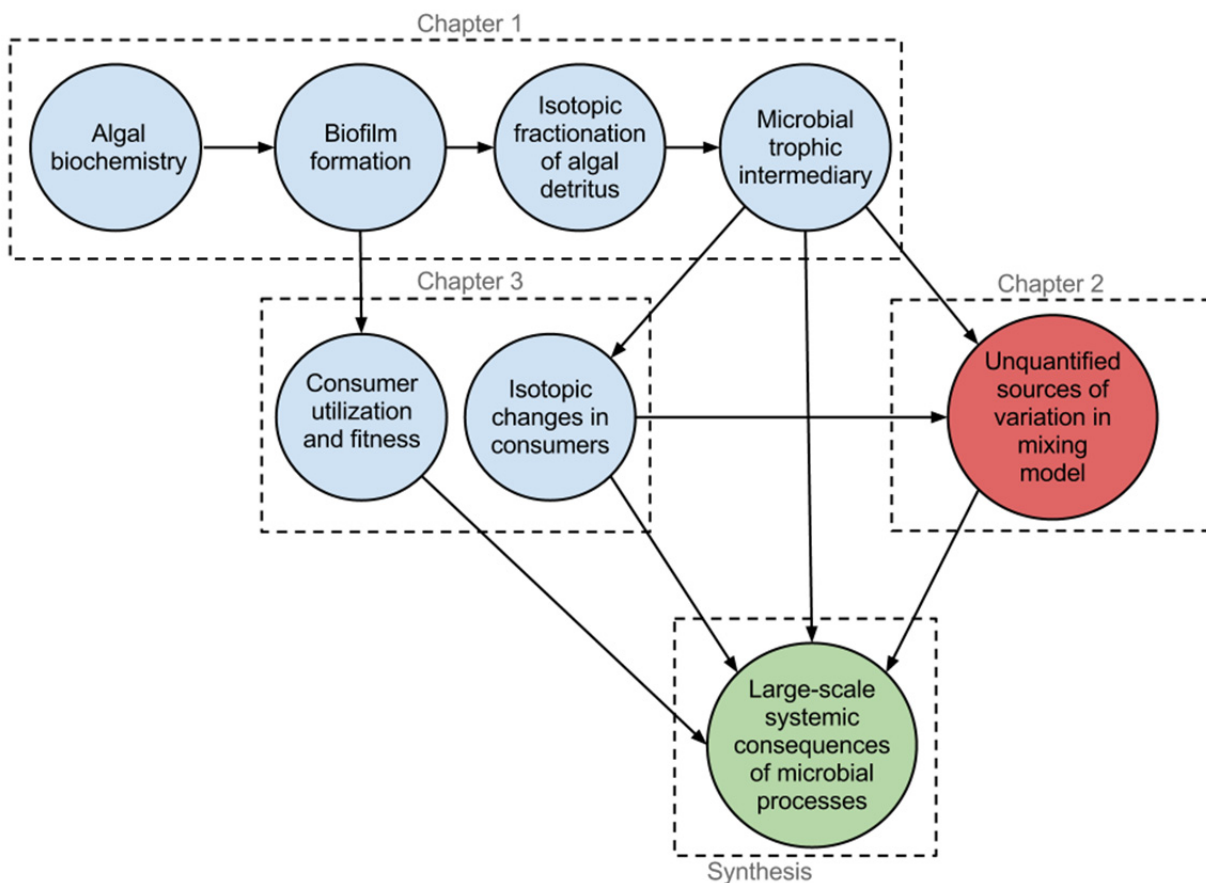


Fig 2: Conceptual diagram of the topics addressed within this manuscript.



Fig 3: The San Juan Islands. Sample sites are marked in red .

Chapter One

Introduction

Deep subtidal coastal food webs are increasingly a source of debate among coastal researchers, specifically in regards to what basal resources support them. While there are certainly exceptions, typically food webs rely on photosynthetic primary producers as the main sources of carbon and energy, and thus rely directly on sunlight. However, in atrophic ecosystems such as the deep subtidal, where there is no intrinsic primary productivity, the ultimate source of carbon within food webs is much more difficult to discern. In these ecosystems, organisms must rely on subsidies of organic matter donated from allochthonous sources (Polis 1997), which arrive via detrital pathways from photic ecosystems. However, due to the inaccessibility of deep subtidal ecosystems the type of detrital material, its geographic origin, and its relative importance to aphotic food webs are commonly impossible to observe directly. As a result, the relative importance of the respective sources of detritus to deep subtidal food webs is poorly understood. To better understand the role of detritus in these food webs, researchers have been relying on biomarkers such as multiple stable isotope (MSI) analysis.

The current debate has centered largely on what sources these detritus subsidies are derived from: phytoplankton or marine macroalgae (Duggins et al. 1989, Kaehler et al. 2006, Kaehler 2000, Kelly et al. 2012, Nadon & Himmelman 2006, Miller & Page 2012). Several studies have been conducted either in support of or in opposition to the idea that marine macroalgae play a critical role in deep subtidal food webs, an idea sometimes referred to as 'the kelp detritus hypothesis' (Miller & Page 2012). The results of several studies have suggested that kelp detritus provides a critical subsidy to coastal deep subtidal ecosystems (Kaehler et al. 2006, Kaehler 2000, Kelly et al. 2012, Nadon & Himmelman 2006); however, discrepancies in the MSI datasets have generated doubt regarding the kelp hypothesis and speculation about where these discrepancies come from (Nadon & Himmelman 2006, Kaehler 2000, Miller & Page 2012). The microbial communities that colonize detritus have been especially popular objects of such speculation, despite a lack of quantitative data targeted specifically at microbial dynamics.

Investigation of the microbial loop has focused primarily on its role in dissolved organic matter (DOM) and particulate organic matter (POM) cycling. In this view, refractory material such as dissolved organic carbon (DOC) is utilized by bacteria, which form aggregates large enough to be consumed by zooplankton and filter feeders, thus introducing previously inaccessible carbon into the food web (Fenchel 2008). Though the microbial loop has been well established in DOM and POM, little work has been done on microbial utilization of large fragmentary algal detritus. Though speculation on the ecological role and isotopic effect of the microbial loop on detritus has been common, studies to quantify these effects have been sparse.

Ancillary data from prior research support the idea that microbial communities may significantly affect the biogeochemistry of detrital kelp. Studies by Norderhaug et al. 2006 and Duggins & Eckman 1997 focused in part on two common biochemical metrics in pulverized kelps over decomposition:

phlorotannin content and C:N ratio. Phlorotannins are a well-studied class of algal secondary metabolites known to act as deterrents to both herbivory and microbial colonization (Nagayama 2002, Goecke et al. 2010, Dubois & Iken 2012, Johnson & Mann 1986, Van Alstyne 1999, Targett & Arnold 1998, Hay 1996). C:N ratio is a common measure of food quality where lower ratios reflect higher quality foods, which be achieved either by removing carbon or adding nitrogen. The results of the two kelp particulate aging studies were similar. Decreased phlorotannin levels in detrital kelp particulate matter suggested that secondary metabolites were rapidly lost in detrital material (Norderhaug et al. 2006, Duggins & Eckman 1997). Decreased C:N ratios in this same material suggested that once released from phlorotannin controls, detrital material was rapidly colonized by microbes, which possibly augmented the nitrogen content of the detritus with microbial nitrogen obtained from dissolved organic nitrogen (Norderhaug et al. 2006, Duggins & Eckman 1997). These findings have been used as evidence for the idea that aged algae is of higher nutritional value to consumers than fresh algae due to increased microbial activity. In addition, several studies have also found isotopic differences between fresh and aged detrital algae (Krumhansl & Scheibling 2012, Hill & McQuaid 2009, Duggins & Eckman 1997, Kramer et al. 2003, Macko et al. 1982, Couch 1989), implicitly acknowledging a potential microbial influence. However, few studies have attempted to measure this phenomenon explicitly (Macko & Estep 1984, Kramer et al. 2003, Norderhaug et al. 2003). Despite these significant findings, little work has been done to explicitly measure the isotopic effects of microbial decomposition on $\delta^{13}\text{C}$, $\delta^{15}\text{N}$ or $\delta^{34}\text{S}$.

Given this significant knowledge gap, it is therefore notable that in studies investigating detritus-based food webs using MSI mixing analysis, current research typically does not account for potential microbial alteration of food sources during decomposition. Instead, the methods are based on the assumption that fresh source materials collected in the photic zone are isotopically similar to the detrital materials that are ultimately utilized by deep subtidal consumers, and that microbes do not represent a significant source of dietary carbon to deep subtidal food webs. Given the extent of decomposition a fragment of detritus may experience on route from photic to aphotic ecosystems, these assumptions raise major concerns about the validity of conclusions drawn from methods that overlook microbial dynamics. Proper interpretation of MSI data collected to address the kelp hypothesis in deep subtidal food webs may therefore hinge on the validity of the underlying assumptions regarding microbial dynamics. Based on these assumptions, three hypotheses may be made regarding the isotopic effects of microbes on algae detritus: (1) microbes have no effect; (2) microbes are an additional food item that settle onto kelp blades out of the water column, and that may need to be quantified in MSI analysis; and, (3) microbes may represent an intermediate trophic level between detritus and apparent detritivores that must be addressed in MSI analysis.

In this chapter, I intend to test whether microbial dynamics have any measurable effect on the biogeochemical composition of kelp detritus, including MSI, phlorotannin, and elemental content, as well as microbial abundance. In addition, I will use MSI analysis to determine the mechanism of the effect—whether microbes represent either a novel food source or an intermediate trophic level. Based on the results of prior studies (Duggins & Eckman 1997, Norderhaug et al. 2003), I expect decomposing kelp blades to cease producing secondary metabolites, which may play a role in anti-herbivory and anti-

microbial defenses, as well as provide more nuanced controls on microbial biofilm development (Goecke et al. 2010, Hay 1996). I then expect the microbial biofilm, once released from these controls, to rapidly accumulate. The three hypothesis regarding microbial effects may then be evaluated:

(1) If microbial dynamics have no effect on kelp detritus biogeochemistry, I expect little change in MSI values during decomposition even as microbial abundance increases. As a consequence, methods based on current assumptions will be validated.

(2) If microbes represent a novel food source that have settled from the water column, I expect unpredictable changes in the isotopic signatures of the detritus during decomposition, as the algae source mixes with an isotopically unquantified microbial source. In addition, I expect the shift in detrital algae values to have some relationship to the isotopic values of POM settling out of the water column. As a consequence, methods based on current assumptions may be inappropriate due to a failure to include all potential food sources in MSI measurements.

(3) if microbes represent an intermediate trophic level, I expect a predictable shift in detritus isotopic values resembling empirically determined trophic shifts found in other consumers—changes of ~0-1‰ in $\delta^{13}\text{C}$ or $\delta^{34}\text{S}$ values, and a shift of ~3‰ in $\delta^{15}\text{N}$. I expect this trophic shift to be the consequence of microbial utilization of kelp nutrients via enzymatic breakdown of the kelp blade (Smith et al. 1992, Martinez et al. 1996). I expect kinetic isotope effects (KIE) of the enzymatic reactions and utilization to result in the microbial biofilm occupying a trophic step above the basal level. The resulting kelp-microbe “biocomplex” will thus shift in $\delta^{15}\text{N}$ during decomposition. As a consequence, methods based on current assumptions may be inappropriate, as detritus-based consumers may utilize food with isotopic signatures that are significantly different from expected values that are based on fresh algae samples. The extent to which the empirical values stray from assumed values can affect the accuracy of calculated trophic positions (Vander Zanden 2001, McCutchan et al. 2003), as well as the accuracy of multiple source mixing models (Dethier et al, unpublished) which could lead to a fundamental misinterpretation of how disparate ecosystems interact.

Microbial effects at microscopic scales may have major consequences on macroscopic systems, as changes in the basal level may echo up through the detrital food web. In the context of isotope-based methodology, unquantified sources of error resulting from frequently overlooked microbial dynamics may significantly affect researchers’ conclusions and interpretations of food web processes. In the context of ecological consequences, the mediating role of microbes on food availability and quality in deep subtidal ecosystems may be a critical, though poorly understood, layer of understanding. In this chapter, I will conduct a targeted investigation of the MSI methodological and ecological consequences of microbial effects in detritus-based food webs. Targeted quantitative data from such an investigation is sorely needed, both to validate or correct existing food web models, and to increase our understanding of bottom-up trophic controls.

Methods

Approach

In this chapter, the hypotheses outlined above were explicitly tested using MSI analysis to examine isotopic changes in decomposing kelps. In addition, phlorotannin concentration, elemental composition, and microbial abundances were also monitored. Phlorotannin concentration was monitored due to its ability to deter both herbivory and microbial colonization (Nagayama 2002, Goecke et al. 2010, Dubois & Iken 2012, Johnson & Mann 1986, Van Alstyne 1999, Targett & Arnold 1998, Hay 1996). Elemental composition was monitored because of prior research which suggested that microbial dynamics may improve the nutritional quality of food items by altering carbon and nitrogen content (Duggins & Eckman 1997, Norderhaug et al. 1997). Microbial abundance was monitored to critically link changes in kelp biogeochemistry to changes in microbial dynamics. Changes in these biogeochemical properties were investigated over the course of decomposition in two species of kelp, *Saccharina subsimplex* and *Agarum fimbriatum*. This was done in the absence of sunlight to most closely resemble the conditions of the deep subtidal ecosystem, where kelp fragments cannot remain viable due to lack of photosynthesis. Such a fundamental shift from photic to aphotic environmental conditions was expected to induce senescence in the kelp fragments, and precipitate a dynamic effect on both the biochemistry of the kelp blade and the associated microbial community. To test whether changes in the isotopic values of the kelp during decomposition could be attributed to the addition of a novel source settling out of the water column, the accumulated material that had settled in the tanks from the flowing seawater system was collected at the completion of the experiment for MSI analysis.

This study was conducted in July and August 2011 in the San Juan Islands of Washington State. Blades of the prevalent shallow water macroalgae (kelps) of *Saccharina subsimplex* and *Agarum fimbriatum* were collected from the field, and cleaned of any visible epibionts by gentle scrubbing. These species were used because past research has found them to be the top two contributors to drift macroalgae within the San Juan Islands, with *Saccharina* comprising 37% and *Agarum* comprising 11% of the total drift algae available to consumers (Britton-Simmons et al., 2009). These two species also conveniently provide a comparison for phlorotannin concentrations, with *Agarum* having relatively high content compared to *Saccharina* (Van Alstyne 1999).

I maintained the blades of each species in the lab in complete darkness at ambient seawater temperatures for five weeks. The blades were allowed to decompose in a flow-through seawater system to encourage the accumulation of natural microbial populations; however, each blade was retained in separate containers so that the blades could not contaminate each other. Samples (5x5cm approx.) were dissected from approximately the same location on each blade to reduce variability and collected weekly. Once collected, the samples were frozen and stored for later analysis. Prior to analysis, each sample was sub-sampled by cutting samples into two. One sub-sample was processed for analysis normally, while the other sub-sample was scraped thoroughly with a razor blade and rinsed to remove any microbial biofilm that may have developed over the course of the experiment (Bengtsson et al. 2010). The efficacy of this removal procedure was verified with microscopy using a DAPI stain.

Kelp biogeochemistry (MSI, elemental, and phlorotannin)

Experimental samples were processed for multiple stable isotope (MSI) and phlorotannin analysis. All multiple stable isotope analyses were conducted according to methods employed in previous studies (Howe & Simenstad 2007, 2010, Page et al. 2008). Briefly, algae tissues were cleaned of epibionts via manual removal and rinsing with de-ionized water. The samples were freeze dried and ground to a fine powder using a modified dental mill (Howe & Simenstad 2007). Samples were weighed using a microbalance and enclosed in tin capsules for analysis at Washington State University's Stable Isotope Core lab using a DeltaPlus XP Isotope Ratio Mass Spectrometer. Phlorotannin analysis was conducted using a Folin assay, and was expressed as the percent composition of hydroxylated aromatic compounds (HAC) in wet kelp mass (Van Alstyne et al. 1999).

Microbial abundances

Algae samples were vigorously palpated for 1 minute in 10mL of 0.22 μ m filtered seawater. Samples that were too large for 10ml were subsampled for a smaller mass. All samples were weighed to calculate the number of microbes per unit wet mass. At the end of the minute, the effluent was poured into a sterile 15ml falcon tube. Formalin was added to the effluent to bring it to a concentration of approximately 2%. Effluent samples were refrigerated, while solid samples were re-frozen.

0.5ml of each sample was diluted in 5ml of 0.22 μ m filtered seawater, and 10 μ l of acridine orange was added to each. Each dilution was quickly added to filter columns, and filtered onto a 0.22 μ m polycarbonate filter. DAPI was added to each filter, enough to cover the surface, and allowed to incubate for 10 minutes before being removed via filtration. The filters were then removed and mounted on slides.

Samples to test the efficacy of microbe reduction techniques

Samples were aged in tanks in complete darkness for five weeks before freezing with the biofilms intact. Three subsamples were first processed for DAPI staining as described above. Subsamples from the same samples were then scraped thoroughly with a razor blade, rinsed in 0.22 μ m filtered seawater, and placed in a new bag. The staining process described in the previous section was then repeated.

Statistical analyses

The absolute value of all multiple isotope data were log transformed to improve normality in the data set. The transformed data were converted into Euclidean distance prior to analyses. Data points that were more than three standard deviations from the mean were removed as outliers. To examine overall patterns in the multivariate isotope data set, the data was first plotted in unconstrained multidimensional space. PERMANOVA was conducted using the package Vegan (Oksanen 2012) in the statistical program R (R-project.org). Each analysis was run for 2000 permutations. An RDA analysis was then conducted on the multivariate isotope data, in order to determine whether the constraining

variables (kelp age, biofilm presence) explained a significant amount of the overall variation seen in the data. Univariate methods (such as ANOVA) were used to compare group means in phlorotannin and elemental composition data sets, as well as to compare group means for each isotope biomarker. ANOVA and post-hoc analyses were conducted using SPSS statistical software (release 13.0).

Due to extremely patchy distribution in some samples, median values were used to evaluate microbial densities rather than mean values. This reduced the influence of outlier values to better approximate the true central tendency of the dataset.

Results

The validity of the method used to reduce microbial abundances was verified by microscopy. Manual scraping of the biofilm reduced median microbial densities by a range of 49-78% (Fig 1). Samples that had low initial microbial abundances experienced comparatively less reduction via scraping (49%), while a larger effect was seen in samples with large initial populations (78%). The final abundance after scraping was similar in all three samples, likely due to the presence of shallow bullations which were difficult to thoroughly scrape. While microbial populations were not eliminated completely, the reduction was deemed sufficient to proceed with the method.

Phlorotannin concentrations appeared to be unaffected by decomposition, and were species dependent. Average phlorotannin concentrations expressed as percent wet mass HAC did not significantly change for either species of kelp over the course of the experiment. As a result, *A. fimbriatum* phlorotannin concentrations remained significantly higher than *S. subsimplex* (Fig 2, $p=0.009$). This sustained species specific phlorotannin concentration was reflected in the median microbial abundances within the biofilm of the respective kelps blades. During decomposition, microbial abundances seemed to be sensitive to the algae species on which they develop. Microbial densities were 3.5 times higher in five-week old aged blades of *S. subsimplex* than in one-week old blades (Fig 3). Median microbial densities in *A. fimbriatum* did not display any clear trends with time (Fig 3). Microbial distribution in *Agarum* samples was extremely clumpy, resulting in high standard deviation values.

Changes in elemental composition during decomposition appeared to be somewhat species specific and related to the biofilm. Elemental carbon and nitrogen significantly decreased in both species of kelps over the course of decomposition (Fig 4a, b; $p=0.011$ and $p=0.003$ for *Agarum* carbon and nitrogen respectively; $p<0.0001$ and $p=0.004$ for *Saccharina* carbon and nitrogen respectively). When the biofilm remained, the C:N ratio did not change over the course of decomposition for either species of kelp, indicating that aged kelp did not have a higher nutritional value over fresh kelp (Fig 5a). However, after the biofilm was removed from the kelp substrate, the kelp C:N ratio increased in both species, but only in *S. subsimplex* was the increase statistically significant (Fig 5b, $p=0.005$). Sulfur content increased in both species of kelp (Fig 4c), though again only in *Saccharina* did this increase approach statistical significance ($p = .051$).

Isotope Composition

Agarum multivariate

Multivariate statistical tools were used to examine overall patterns in the multivariate isotope dataset. *A. fimbriatum* data appeared to support the hypothesis that decomposition of the blade has no effect on kelp isotopic values. In non-metric multi-dimensional space, there was no significant isotopic separation of scraped and unscraped *A. fimbriatum* blades due to age (Fig 6, $p = 0.66$). There was, however, a statistically significant separation of groups due to biofilm removal along the $\delta^{34}\text{S}$ axis (Fig 6, $p = 0.04$). However, when the variance in the dataset was constrained in a redundancy analysis (RDA) by explanatory factors (biofilm and age) only 23% of the total variance among samples was accounted for. This suggested that the bulk of the variance among data points was due to other factors beyond age and biofilm presence. Therefore, age and biofilm presence/absence were not significant predictors of isotopic change in *Agarum* ($p = 0.27$).

Agarum univariate

To better understand directionality and variation in the multivariate dataset, the significant finding of biofilm removal was further examined using ANOVA. Blades that had been manually scraped were significantly more depleted in $\delta^{34}\text{S}$ than blades that were not scraped, though this depletion had no relationship to age of the blades. (Fig 7, $p = 0.002$). *Agarum* $\delta^{13}\text{C}$, $\delta^{15}\text{N}$ and $\delta^{34}\text{S}$ compositions did not change significantly after aging (Fig 8a, b, c).

Saccharina multivariate

S. subsimplex data appeared to support the hypothesis that microbial biofilms utilize kelp trophically. In non-metric multi-dimensional space, the effects of both blade age ($p = 0.2299$) and biofilm removal ($P = 0.02999$) on the MSI signal of *S. subsimplex* were significant (Fig 9). Aged blades with the biofilm were distinct from all other groups along the $\delta^{15}\text{N}$ axis, while no clear separation occurred between fresh kelps and aged kelps that had had the biofilm removed. When variance was constrained in a redundancy analysis, the explanatory factors (Age, Biofilm) explain 39% of the total variance among samples, and were significant predictors for isotopic change in *Saccharina* ($p = 0.0418$).

Saccharina univariate

The results for each stable isotope biomarker were further examined using ANOVA to confirm that the experimental results agree with the expectations of the microbial trophic level hypothesis. $\delta^{13}\text{C}$ composition of *S. subsimplex* did not significantly change in *S. subsimplex* with decomposition (Fig 10). $\delta^{34}\text{S}$ of *S. subsimplex* became significantly more depleted with decomposition (Fig 11, $p = 0.001$) apparently as a function of biofilm presence and absence. In Week 1 samples, $\delta^{34}\text{S}$ values were depleted when the kelp surface was scraped (Fig 12). As the samples aged, the blades with the biofilms became significantly more depleted and approached the $\delta^{34}\text{S}$ composition of scraped blades ($p = 0.005$). By Week 5, blades with the biofilm were not significantly different from blades without the biofilm. Blades without the biofilm were not significantly different at any time. When $\delta^{34}\text{S}$ values of blades with and

without biofilms were plotted against elemental sulfur composition over the course of decomposition, no apparent pattern emerges (Fig 13). The $\delta^{34}\text{S}$ depletion seen in Week 1 *S. subsimplex* between scraped and unscraped samples was also similar to that seen in *A. fimbriatum* samples at all time points. $\delta^{15}\text{N}$ became significantly more enriched in *S. subsimplex* over the course of decomposition (Fig 14, $p = 0.027$). Samples with the biofilm became progressively more enriched as decomposition progressed, while samples without the biofilm were not significantly different from each other at any time (Fig 15). $\delta^{15}\text{N}$ of aged blades with the biofilm at Week 5 were significantly more enriched than Week 1 blades (Fig 15, $p = 0.002$). However, aged blades without the biofilm were not significantly different at Week 5 than at Week 1. $\delta^{15}\text{N}$ of aged blades with the biofilm at Week 5 were more enriched than the Week 1 blades without the biofilm by 2.1 ‰ on average.

There was a relationship between the amount of elemental nitrogen in the *S. subsimplex* substrate and $\delta^{15}\text{N}$ enrichment in both the substrate alone and the kelp with biofilm (Fig 16). $\delta^{15}\text{N}$ of blades with biofilm were enriched relative to the kelp substrate alone, and the amount of enrichment relative to the kelp substrate increased as elemental nitrogen decreased (Fig 16). As the elemental nitrogen of the kelp substrate approached zero, the kelp blade and biofilm isotope signature became more reflective of a pure biofilm sample, while the kelp alone isotope signature became reflective of a fully utilized kelp substrate. The intercept of the trendline for kelp with biofilm samples was 12.13 ($R^2 = 0.59$), which represented the isotopic value of a pure biofilm sample that had maximally fractionated its substrate. The intercept of the trendline for kelp without biofilm samples was 9.336 ($R^2 = 0.28$), which represented kelp detritus that had been maximally fractionated. By subtracting the intercepts of both lines, the theoretical fractionation value (Δ) of a pure microbial biofilm relative to its substrate was calculated to be 2.79‰ (SE = 0.6) at any point during decomposition.

Deposited Sediment

The MSI values of sediment deposited from the flowing seawater system over the course of the experiment bore little resemblance to *S. subsimplex* blades at all stages of decomposition (Table 1), suggesting that the changes seen in the MSI values *S. subsimplex* blades with the biofilm retained were not due to particles settling onto the kelp surfaces.

Discussion

The results of my experiment indicate that microbial decomposition may significantly alter the biogeochemical composition of detrital kelp. In addition, several findings represent a departure from the current understanding of algal decomposition, especially in regards to changes in nutritional quality during decomposition. The results indicate that a new paradigm may be needed to incorporate microbial dynamics in detritus-based food webs. These findings may have far-reaching consequences both ecologically and methodologically.

Ecological consequences

Contrary to previous work (Duggins & Eckman 1997, Norderhaug et al. 2003), the results of this study indicate that aged algae are not of higher nutritional quality than fresh algae. C:N ratios did not drop as expected, and in fact the ratio increased with the removal of the biofilm in both species of kelps. As a post hoc explanation for this finding, the apparent disagreement between the current study and prior results may be due to an interaction between mechanical leaching of the kelp blades and microbial utilization. Carbon and nitrogen may be lost equally from the kelp blade during physical leaching of the substrate; however, due to enzymatic activity in the microbial biofilm, nitrogen (in the form of amino acids) may be absorbed preferentially over carbon (in the form of polysaccharides) (Martinez et al. 1996, Smith et al. 1992). Under these conditions, when the biofilm is retained as part of the kelp biocomplex, the majority of the net loss of C and N may be to the environment alone in equal proportions. When the biofilm is separated from its kelp substrate, however, the nitrogen that was preferentially taken up by the biofilm from the kelp may be removed as well (Fig 17a). In this sense, the microbial biofilm may not be improving the nutritional quality of the food, but instead may be acting as a sponge, absorbing organic molecules that would otherwise be lost to the environment. While more research is needed to confirm this post hoc explanation, this scenario resolves the apparent disparity in C:N ratios between the current study which was done on whole blades, and previous work which was done on pulverized blades (Duggins & Eckman 1997, Norderhaug et al. 2003). Increased surface area in pulverized blades may accelerate the physical leaching of carbon and nitrogen, to the point where there may be greater amounts of carbon and nitrogen in the microbial community that develops around each kelp particle than there is within the kelp particle itself (Fig 17b). Thus, the preferential uptake of nitrogen over carbon in the biofilm becomes more apparent.

As further evidence that aged algae is not of higher nutritional quality than fresh algae, phlorotannin content did not decrease in either species of kelp over the course of decomposition. This again is a departure from previous findings (Duggins & Eckman 1997, Norderhaug et al. 2003), and again is likely the result of working with whole blades as opposed pulverized particulate material, which may be more vulnerable to mechanical leaching of compounds due to an increased surface area. The findings in the present study do not suggest that phlorotannins are continually produced in detrital blades, but rather that these compounds are resistant to breakdown. As phlorotannins have been generally considered to act as deterrents to both herbivory and microbial colonization (Nagayama 2002, Goecke et al. 2010, Dubois & Iken 2012, Johnson & Mann 1986, Van Alstyne 1999, Targett & Arnold 1998, Hay 1996), the consistent phlorotannin levels in both species of kelps adds a layer of complexity to the trophic consequences of macroalgae detritus. In genera like *Agarum*, where phlorotannins are high, detritus from these sources may play less of a role in detrital food webs (both as a direct food source and as a source of microbial productivity) than what bulk transport of detritus may imply. Conversely, algae with consistently lower phlorotannins, like *Saccharina* species, may play an outsized role from what bulk transport suggests. This line of reasoning is bolstered by the results which show a dramatic difference in the microbial abundances between the two species after five weeks of decomposition. In

A. fimbriatum, median microbial counts remained low throughout decomposition, whereas in *S. subsimplex* microbial counts increased more than three-fold. The species specific response in microbial abundance may be due to consistently high phlorotannin concentrations in *Agarum* prohibiting microbial development, while concentrations in *Saccharina* are too low to effectively prevent colonization. These findings suggest that the underlying algal biochemistry may be the main determinant of microbial dynamics on decomposing algae.

Isotopic consequences

Phlorotannin concentration (or other bioactive compounds) may control which process dominates the decomposition of detrital kelp— physical degradation or microbial digestion. These two pathways have very different impacts on the biogeochemistry of the detrital kelp, and the kelp species used in this study provide insight into both. For instance, in detrital *Agarum* detritus with or without biofilm, the lack of any significant change in any of the isotopes over the course of decomposition suggests that in the absence of herbivores the breakdown is primarily due to mechanical degradation of the blades into fine particles. Were there a microbial component acting on the kelp, I would have expected kinetic isotope effects to shift the MSI composition of the substrate, and a complementing enrichment in the $\delta^{15}\text{N}$ of the biofilm.

Such changes are exactly what are seen in the decomposition of *S. subsimplex*, suggesting that decomposition in this species is dominated by microbial activity. The kelp substrate becomes increasingly enriched in $\delta^{15}\text{N}$, as $\delta^{14}\text{N}$ is preferentially removed due to the kinetic isotope effects (KIE) that occur during microbial enzymatic activity. At the same time, the biofilm itself is enriched from the substrate by an estimated 2.7ppt (Fig 16), which is within the range of empirically determined trophic enrichment factors in a number of marine organisms (Minagawa & Wada 1984). The trophic enrichment of a consumer from its substrate is due to the preferential loss of $\delta^{14}\text{N}$ after metabolic processes, due to kinetic isotope effects (Fry 2006). Simultaneously, the abundance of microbes in the biofilm increases by a factor of 3.5. The results suggest that the ‘aged *Saccharina*’ available to consumers in the deep subtidal is really a “biocomplex” mixture of enriched kelp substrate plus an even further enriched biofilm. As the biofilm accumulates, the $\delta^{15}\text{N}$ values of the aged kelp biocomplex become progressively more enriched.

The opposite effect is seen in sulfur isotopes in *S. subsimplex*, where $\delta^{34}\text{S}$ of kelp with biofilm becomes depleted with decomposition. This depletion begins to plateau as the values approach that of the scraped kelp substrate. Given that (1) early in the decomposition process scraping the blades results in an immediate depletion in $\delta^{34}\text{S}$, and that (2) the degree of this depletion becomes reduced as decomposition progresses (i.e. kelp with biofilm $\delta^{34}\text{S}$ approaches that of the scraped kelp substrate $\delta^{34}\text{S}$), these results may be evidence of an interaction between sulfur uptake in the microbial biofilm and other abiotic processes occurring in the kelp blades. Interestingly, the amount of depletion seen in scraped versus unscraped samples at Week 1 is similar between *S. subsimplex* and *A. fimbriatum*. However, in *S. subsimplex* this pattern deteriorates as $\delta^{34}\text{S}$ values of unscraped blades approach those of scraped blades during decomposition, whereas decomposition has no effect on the unscraped/scraped

$\delta^{34}\text{S}$ depletion seen in *A. fimbriatum*. The similarity in $\delta^{34}\text{S}$ depletion among both kelps after one week of decomposition suggests that early in the decomposition process, the same processes may be acting on both species of kelps to enrich the surface of the kelps in $\delta^{34}\text{S}$ relative to the inner tissue of the blades. These processes may be compartmentalization of kelp-derived compounds in epidermal tissues (Lüder & Clayton 2004, Shibata et al. 2004, Johnson & Mann 1986) or accretion of seawater sulfate on blade surfaces as it decomposes into humic material (Ferdelman et al. 1990, Swanson et al. 1972). Accretion would explain the apparent increase in elemental sulfur seen in both species of aging kelps. Regardless of the process, microbial colonization and subsequent fractionation may subsume these processes in *S. subsimplex* as decomposition progresses. Trophic fractionation of $\delta^{34}\text{S}$ is generally estimated to be quite low, 0–0.8‰ (Fry 2006). As *S. subsimplex* blades decompose, the microbial biofilm may be depleted as a result of utilization and fractionation of the depleted kelp substrate beneath it. Blades of *A. fimbriatum* may not experience this depletion due to low microbial activity.

An alternative explanation for the results of this experiment could be that apparent isotopic shifts may be caused by particulate organic material settling out of the water-column onto the kelp blades. As evidence that the changes seen in *S. subsimplex* MSI values can be attributed to microbial fractionation rather than mixing of external sources, the results show that $\delta^{13}\text{C}$ did not significantly change over the course of decomposition. Trophic fractionation of $\delta^{13}\text{C}$ is generally estimated to be low— 0–0.9ppt— and so significant changes to $\delta^{13}\text{C}$ would not be expected with microbial fractionation. Were source mixing occurring, $\delta^{13}\text{C}$ values would likely shift to reflect the addition of carbon from other matter. Given that $\delta^{13}\text{C}$ of the accumulated sediment deposited from the (Friday Harbor Labs) seawater system is highly depleted relative to the $\delta^{13}\text{C}$ of the kelps at any time point (Table 1), it is unlikely that the build-up of sediment on kelp surfaces is contributing to the isotopic changes seen. $\delta^{15}\text{N}$ of the sediment is also depleted relative to the $\delta^{15}\text{N}$ of the aged kelp. $\delta^{34}\text{S}$ are very highly depleted relative to that of aged kelp, and this depletion cannot account for the depletion that occurs *after* the kelp blade has been scraped. The results are highly suggestive that the isotopic changes seen in *S. subsimplex* but not in *A. fimbriatum* are the result of differing rates of microbial decomposition of the kelp substrates. The different rates of microbial decomposition in either species of kelp may be related to differing concentrations of phlorotannins.

Applications to Estuarine and Coastal Research

The consequences of the differences seen in the microbial biofilms of the two kelp species extend not only into the biogeochemistry of the kelp detritus biocomplex, but also into the structure of the web upon which it is based. The MSI results suggest that in *S. subsimplex* the microbial biofilm is acting as a trophic level distinct from the kelp substrate, and that as the biofilm accumulates its isotopic signal becomes larger. In fact, based solely on the calculation for estimated trophic level of aged *S. subsimplex*, without prior knowledge one might mistake it as an opportunistic predator of fresh *S. subsimplex* (Table 2; Fry 2006). This is not the case in *A. fimbriatum*, however; low microbe populations resulting from high phlorotannin concentrations are associated with no significant change in the estimated trophic level of the kelp over the course of decomposition. The effect of the microbial biofilm acting as a discreet trophic level has the capacity to cascade up the food web. Without taking the

microbial trophic shift into account an 'herbivore' of one species feeding on algal detritus may appear to occupy a higher trophic level than another of the same species feeding on fresh algae. Likewise, an 'herbivore' of one species in an area where the detrital pool is largely derived from highly chemically-defended species of algae may appear to occupy a lower trophic level than the same species in another area where the detritus pool is derived from algae with lower secondary metabolites. Two consumers feeding on the same piece of detrital algae may appear to occupy different trophic levels depending on the feeding strategy of each, where surface grazers may utilize the biofilm to greater extent than shredders. On a much larger scale, in ecosystems where primary productivity is low relative to microbial production based on allochthonous material, the entire community may appear to shift upwards in trophic structure due to increased microbial activity (Duggins et al., unpublished).

The isotopic shift that occurs during aging in some species of algae but not others adds a layer of complication when attempting to re-construct a food web based on MSI alone. In such studies, algal source samples for use in mixing models are typically taken fresh from the field, and then scrubbed and rinsed to remove epiphytes before processing. However, the results of this study show that the MSI values of fresh, scrubbed algae samples can, at times, be significantly different from the MSI values of the aged algae that are actually available to consumers, especially those in the Deep Subtidal Ecosystem. If aged algae are utilized by these consumers, the MSI values of the consumers would reflect that of the aged algae signal, rather than the fresh algae used in the model. The subsequent model results may therefore be inaccurate if these isotopic shifts are not accounted for, as the model may misinterpret the unquantified variation in the dataset. To compensate for the unquantified variation, the model predictions may be biased towards potential sources that are outliers in their isotopic ratio relative to the sources actually being utilized (Dethier, unpub).

Little is known about actual retention time of individual kelp blades within a detritus pool, or the length of time spent in transport. Previous work in Salt River Canyon in the US Virgin Islands estimated detrital seagrass and algae turnover to be on the order of days to months depending on environmental conditions (Josselyn 1983), but this measure does not distinguish whether removal from the study system is due to transport rather than from decomposition or utilization. Observations in this study indicate that in the absence of herbivory, kelp blades senesce and dissipate after approximately 6-8 weeks of decomposition. A pool of detrital algae is likely a mixture of algae in varying stages of decomposition. Incorporating the complex variability of source algae species composition, age, and microbial community may prove to be a challenging but necessary next step in stable isotope ecology.

Chapter One Figures and Tables

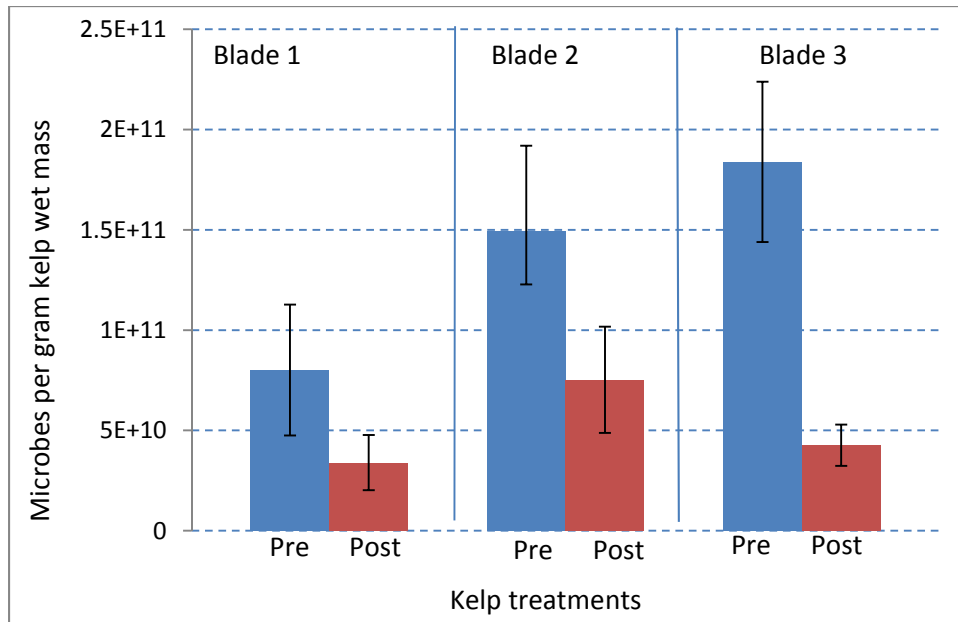


Fig 1: Median values of microbial abundance on *S. subsimplex* blades before (Pre) and after (Post) biofilm removal. Error bars indicate one standard deviation from the median. Scraping reduces median microbial abundance by 49-78%.

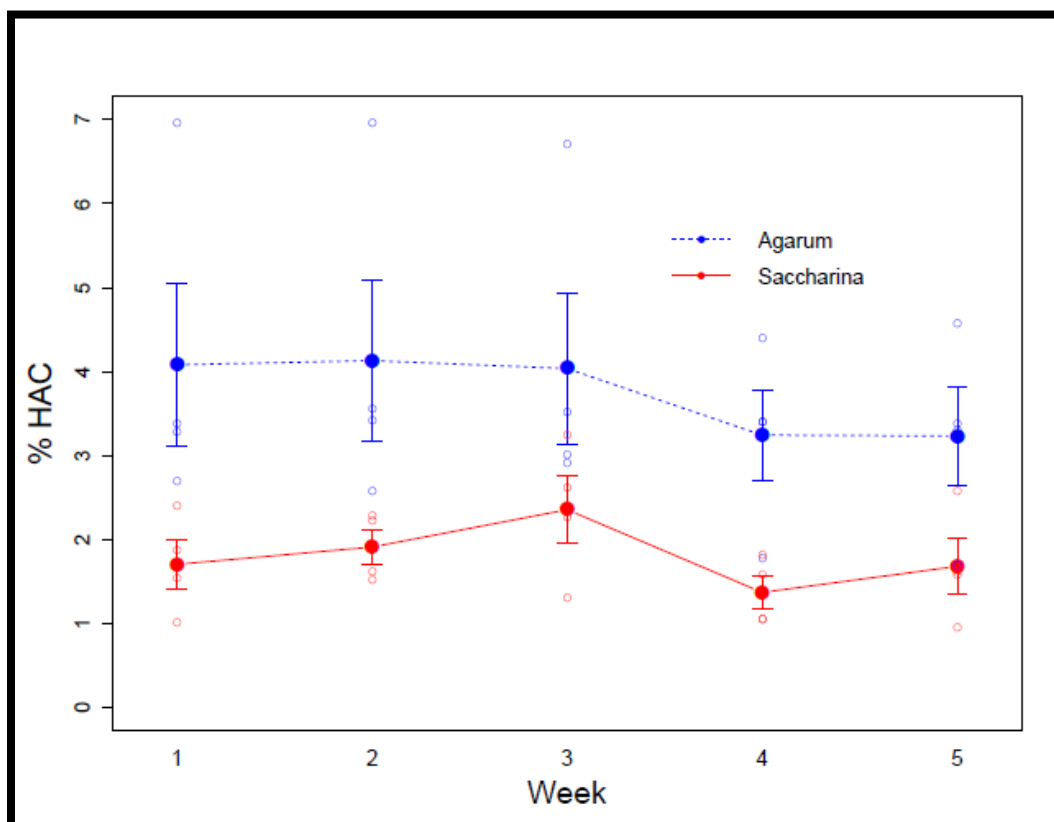


Fig 2: Average changes in phlorotannin concentration, expressed as percent hydroxylated aromatic compounds (HAC), of the kelps *Saccharina subsimplex* and *Agarum fimbriatum* over 5 weeks of decomposition. Error bars are 1 standard deviation from the median.

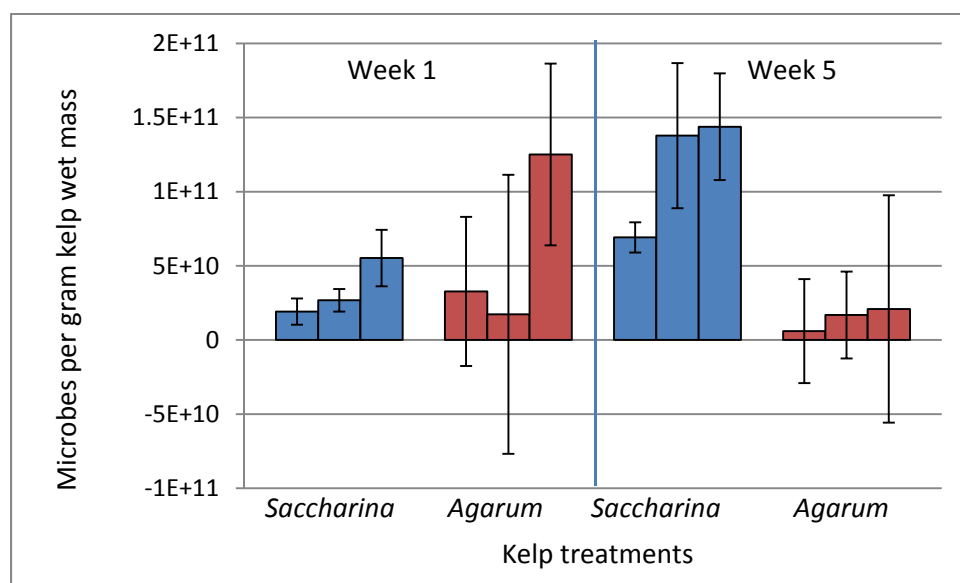


Fig 3: Median values of microbial abundance in *Saccharina subsimplex* and *Agarum fimbriatum* during decomposition. Error bars indicate one standard deviation from the median.

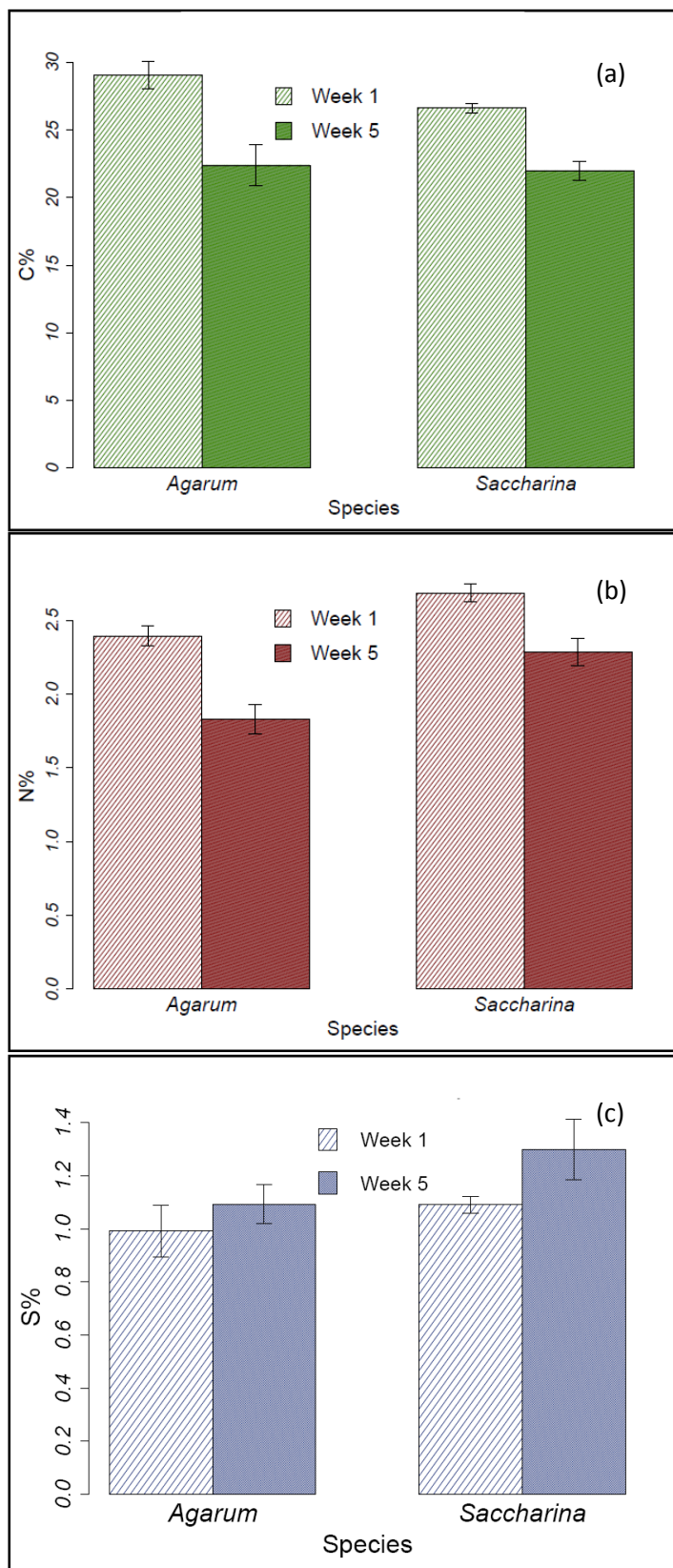


Fig 4: Elemental content of kelp blades at the beginning and end of a five-week decomposition experiment: (a) Carbon; (b) Nitrogen; and (c) Sulfur.

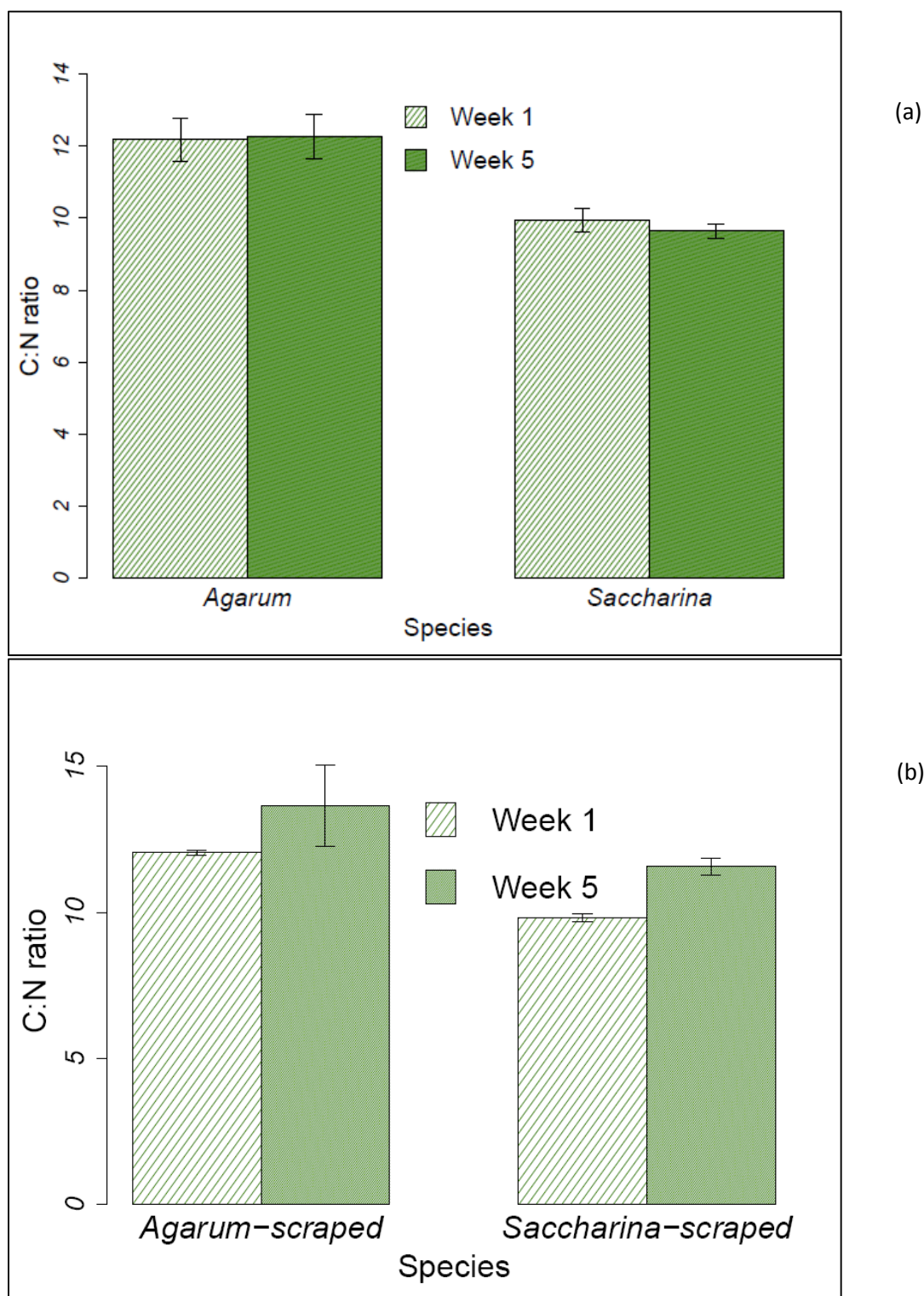


Fig 5: C:N ratio of kelp blades with (unscraped) and without (scraped) biofilm during decomposition. (a) C:N ratio blades with biofilm (b) C:N ratio blades without biofilm.

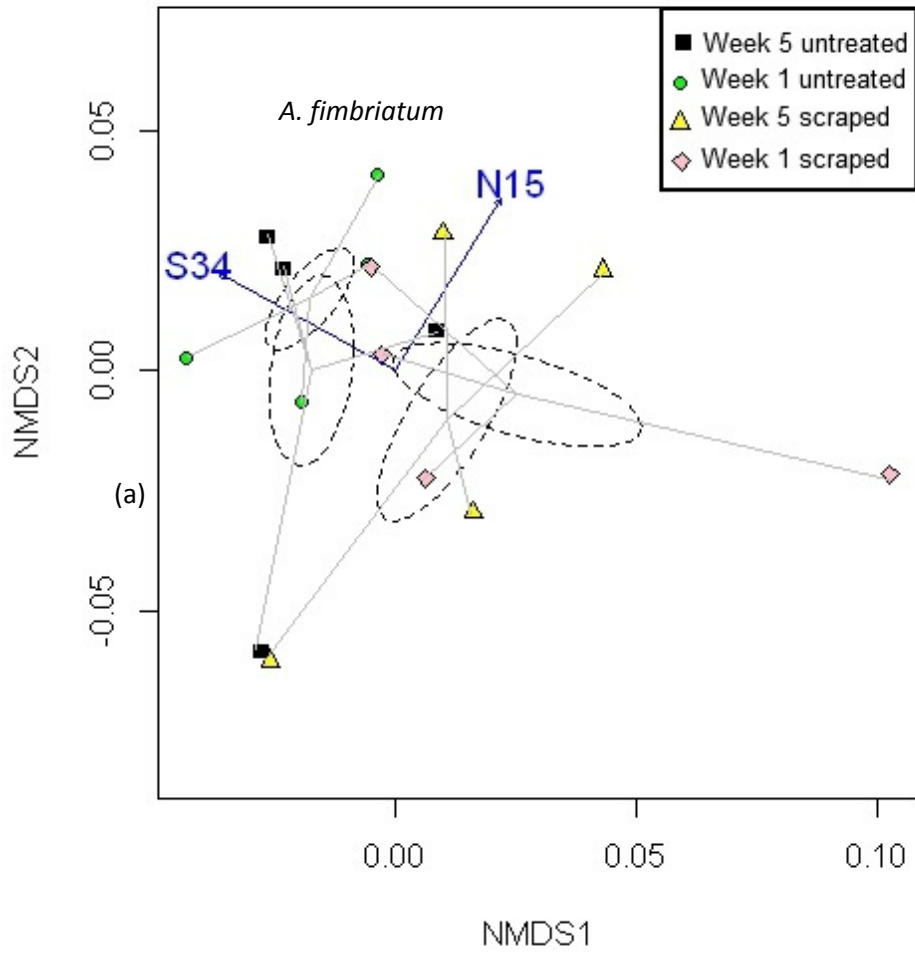


Fig 6: *Agarum* MSI plotted in unconstrained multidimensional space.

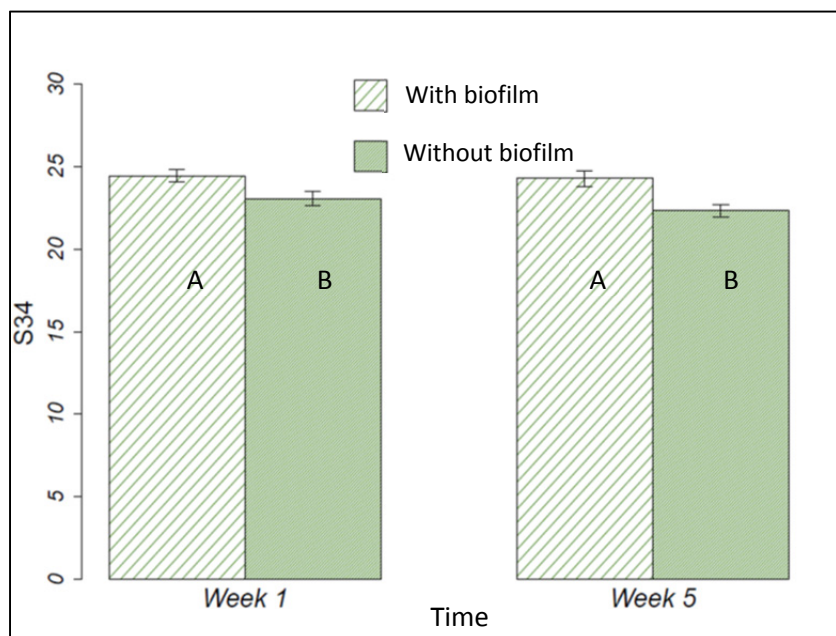


Fig 7: Univariate analysis of $\delta^{34}\text{S}$ in *Agarum* over time as a function of biofilm presence or absence.

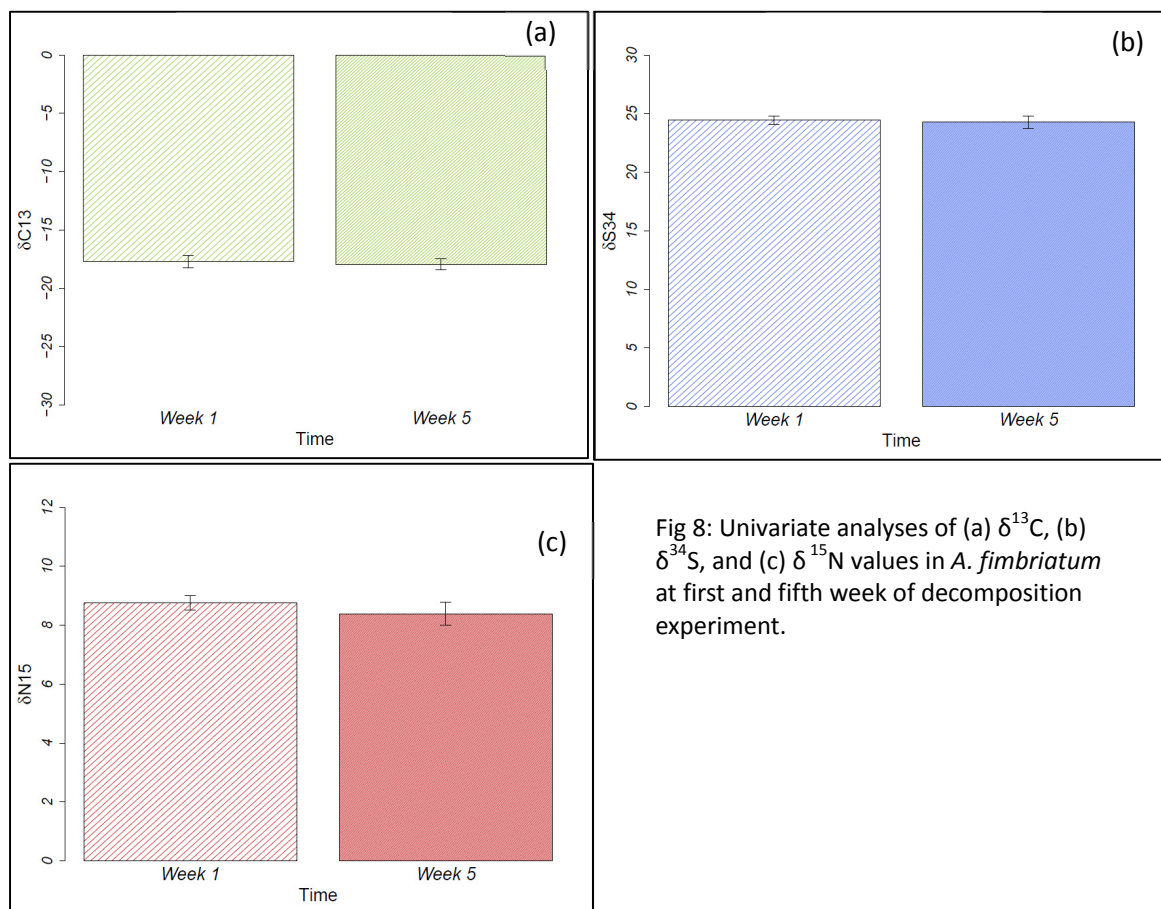


Fig 8: Univariate analyses of (a) $\delta^{13}\text{C}$, (b) $\delta^{34}\text{S}$, and (c) $\delta^{15}\text{N}$ values in *A. fimbriatum* at first and fifth week of decomposition experiment.

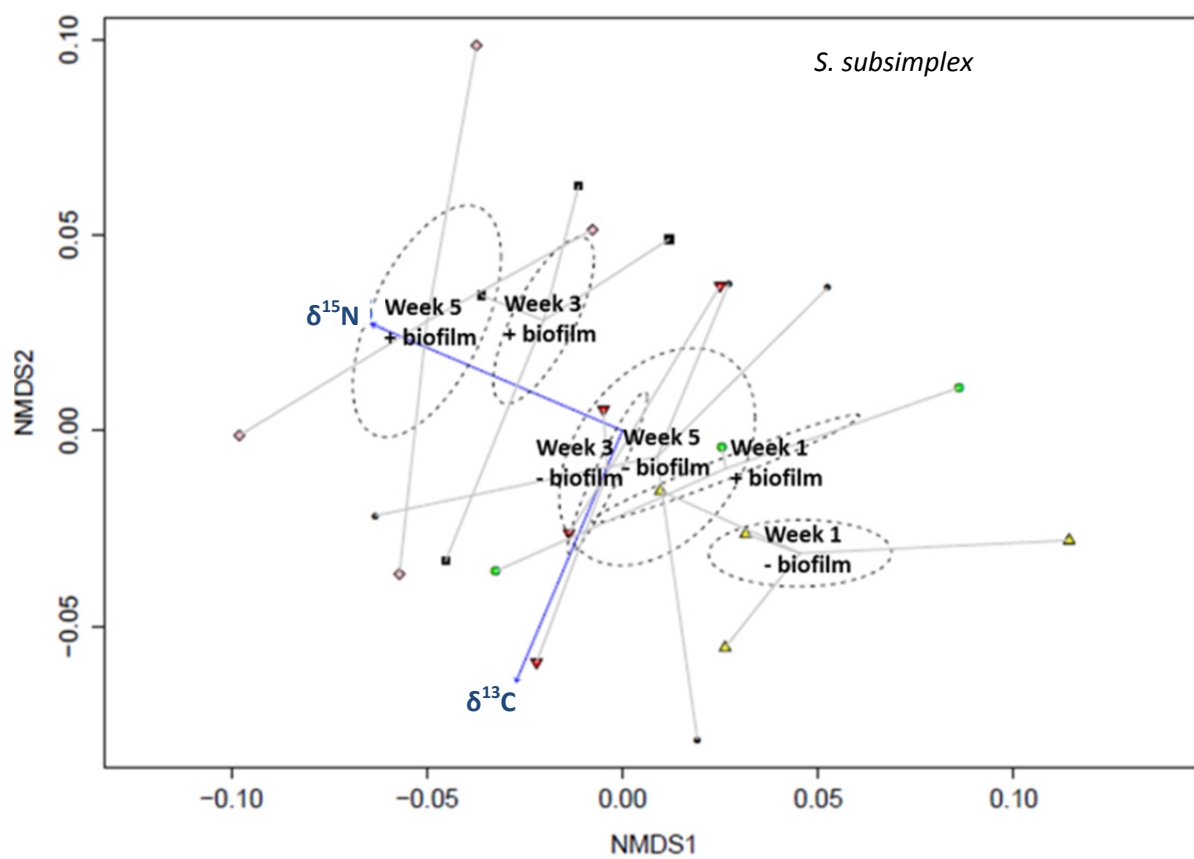


Fig 9: *Saccharina* MSI in unconstrained multidimensional space.

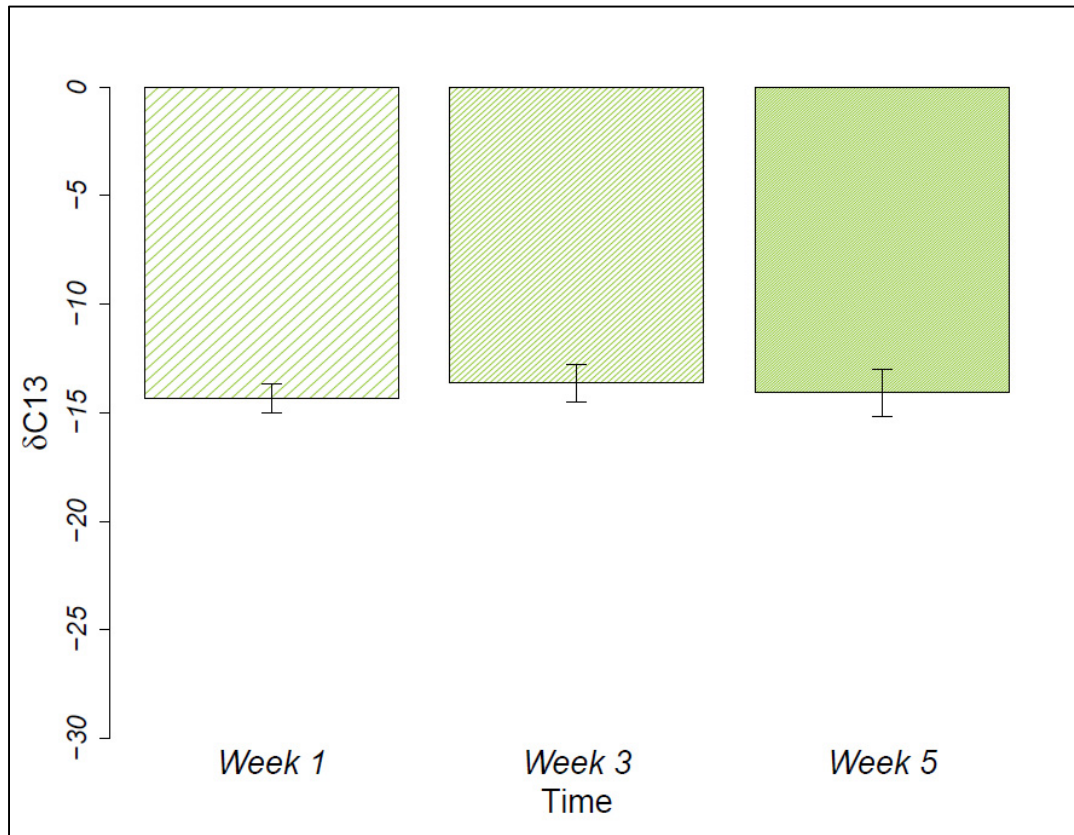


Fig 10: Univariate analysis of $\delta^{13}\text{C}$ values in *S. subsimplex* during decomposition.

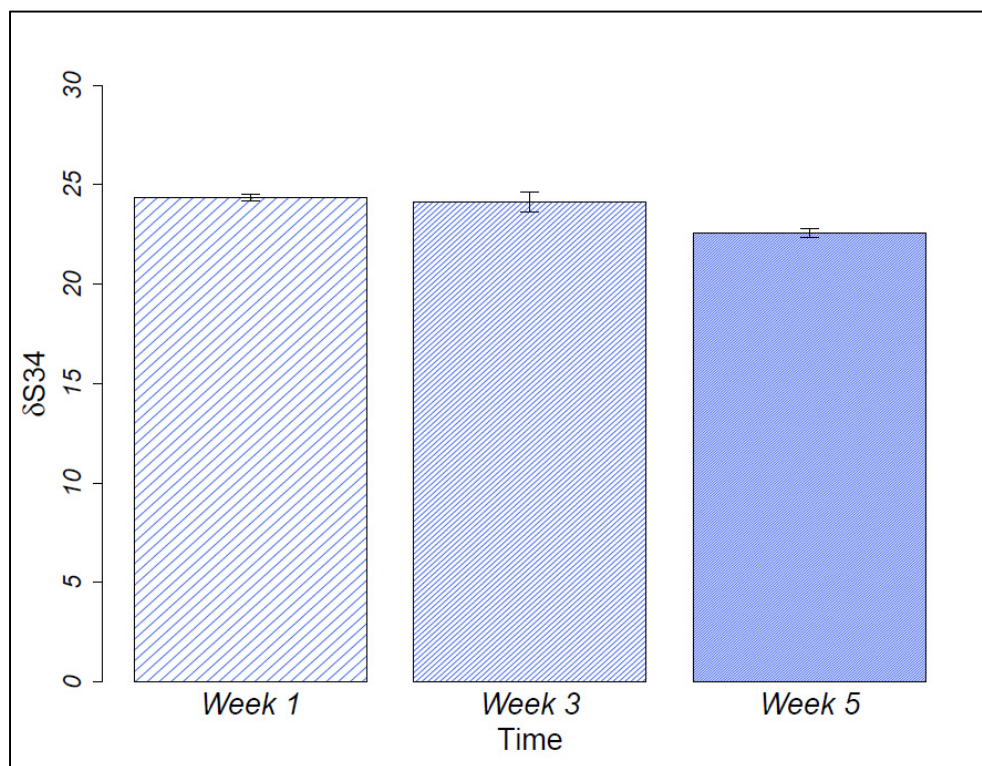


Fig 11: Univariate analyses of $\delta^{34}\text{S}$ in *S. subsimplex*.

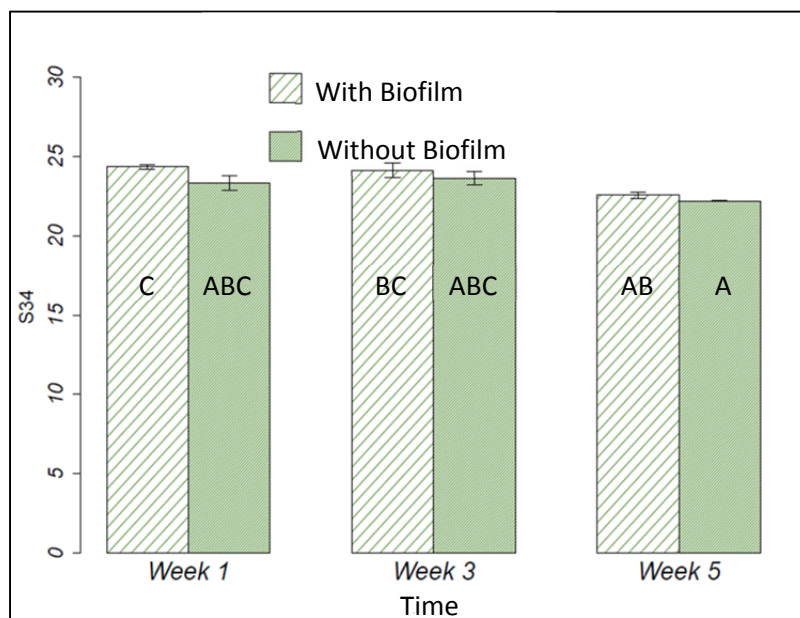


Fig 12: Univariate analysis of $\delta^{34}\text{S}$ in *Saccharina* samples as a function of biofilm presence or absence.

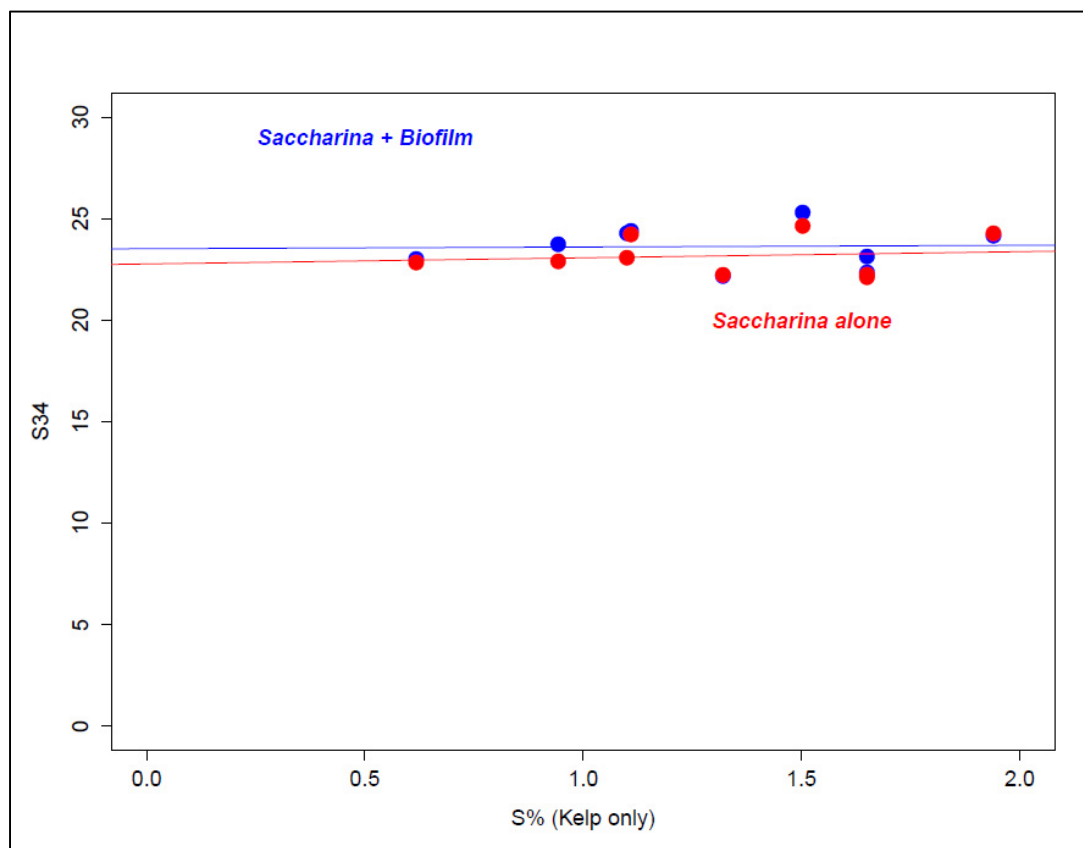


Fig 13: Keeling plot of elemental S in kelp substrate versus $\delta^{34}\text{S}$.

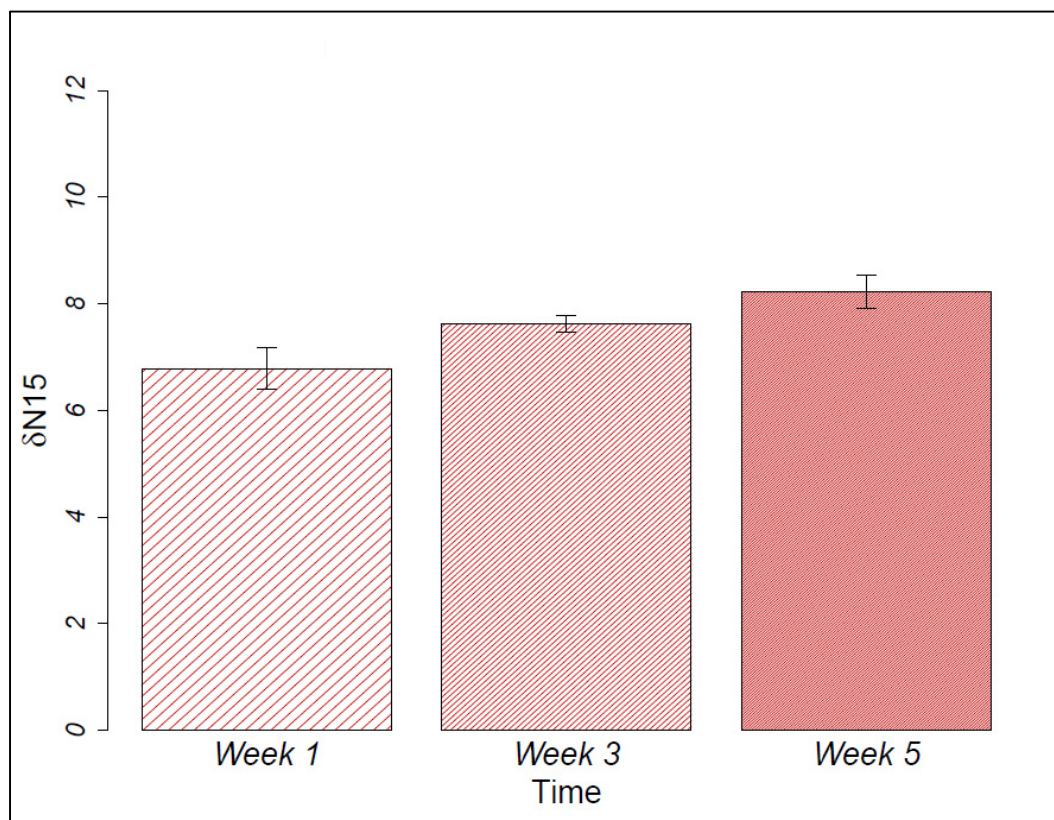


Fig 14: $\delta^{15}\text{N}$ composition in *Saccharina* over the course of decomposition.

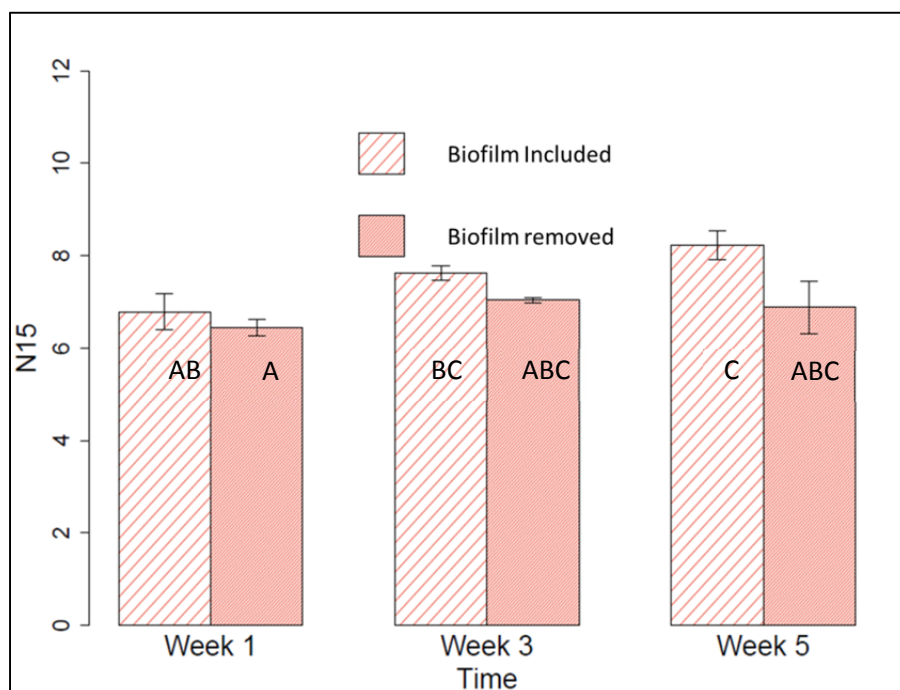


Fig 15: Univariate analyses of $\delta^{15}\text{N}$ in *Saccharina* samples.

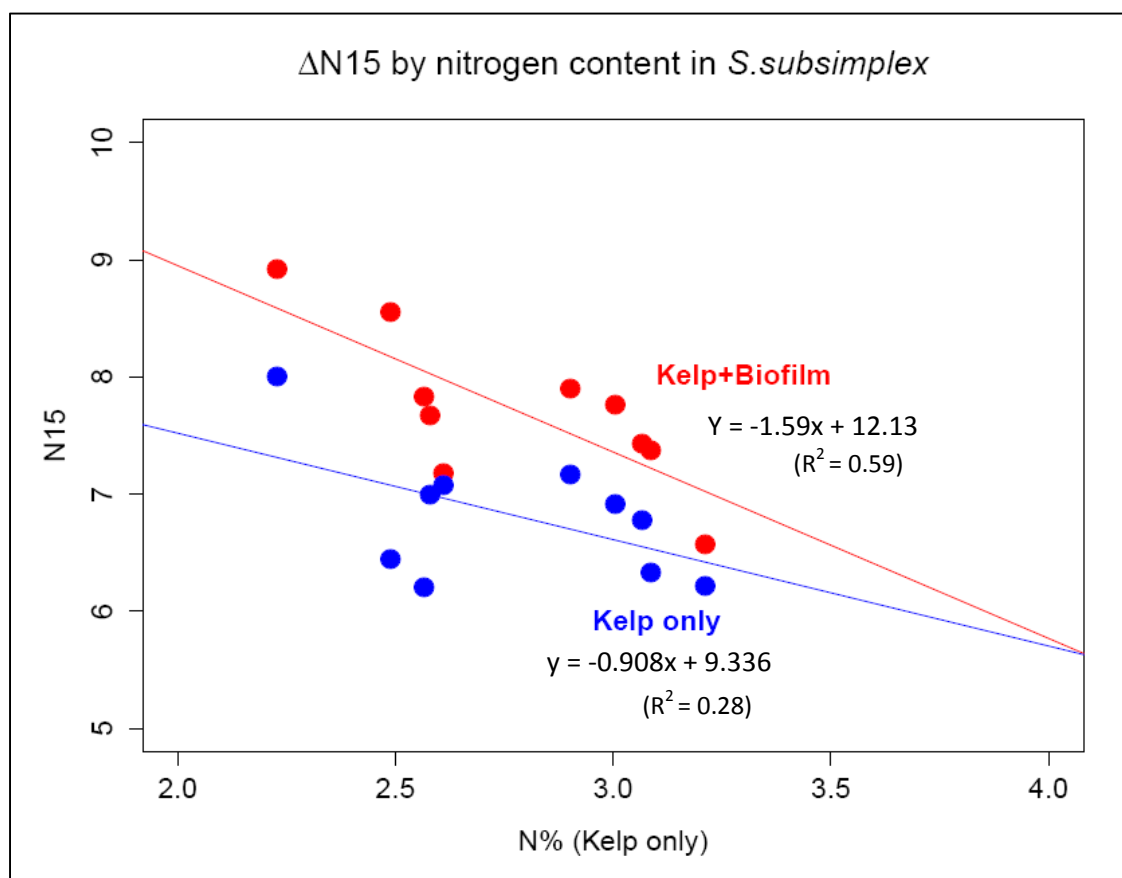


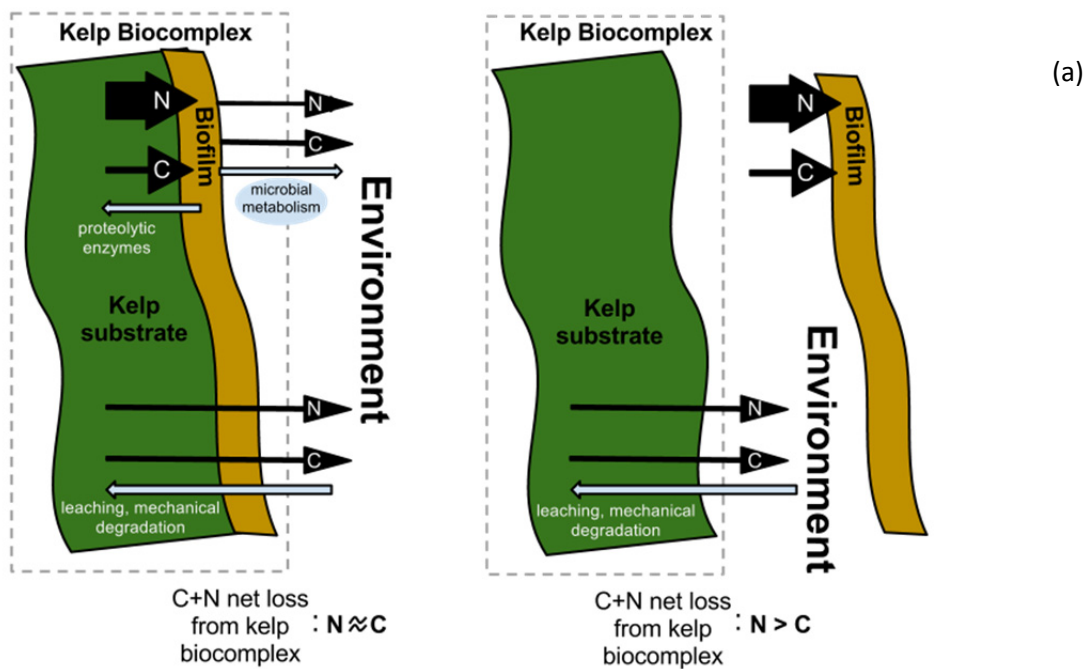
Fig 16: Keeling plot of elemental N in kelp substrate versus $\delta^{15}N$ in kelp and kelp with biofilm.

| Sample | Week | $\delta^{13}\text{C}$ | SD $\delta^{13}\text{C}$ | $\delta^{15}\text{N}$ | SD $\delta^{15}\text{N}$ | $\delta^{34}\text{S}$ | SD $\delta^{34}\text{S}$ |
|-------------------|------|-----------------------|--------------------------|-----------------------|--------------------------|-----------------------|--------------------------|
| <i>Saccharina</i> | 1 | -14.32 | 1.34 | 6.79 | 0.78 | 24.36 | 0.25 |
| <i>Saccharina</i> | 3 | -13.64 | 1.69 | 7.63 | 0.31 | 24.13 | 0.97 |
| <i>Saccharina</i> | 5 | -14.08 | 2.15 | 8.23 | 0.62 | 22.56 | 0.43 |
| <i>Scum</i> | 5 | -19.78 | 0.29 | 6.71 | 0.20 | 15.28 | 0.53 |

Table 1. Average MSI values of *S. subsimplex* blades with biofilm during decomposition, and the accumulated sediment deposited from the flowing seawater system.

| <i>A. fimbriatum</i> | "Basal" comparison | N | Average TL | SD |
|------------------------|------------------------|---|------------|-------|
| Week 1 without biofilm | Week 1 without biofilm | 4 | 1 | 0 |
| Week 1 with biofilm | Week 1 without biofilm | 4 | 0.976 | 0.149 |
| Week 5 with biofilm | Week 1 without biofilm | 4 | 0.849 | 0.327 |
| Week 5 with biofilm | Week 1 with biofilm | 4 | 0.873 | 0.410 |
| <i>S. subsimplex</i> | "Basal" comparison | N | Average TL | SD |
| Week 1 without biofilm | Week 1 without biofilm | 4 | 1 | 0 |
| Week 1 with biofilm | Week 1 without biofilm | 4 | 1.220 | 0.095 |
| Week 3 with biofilm | Week 1 without biofilm | 4 | 1.5 | 0.300 |
| Week 5 with biofilm | Week 1 without biofilm | 4 | 1.699 | 0.160 |
| Week 5 with biofilm | Week 1 with biofilm | 4 | 1.479 | 0.104 |

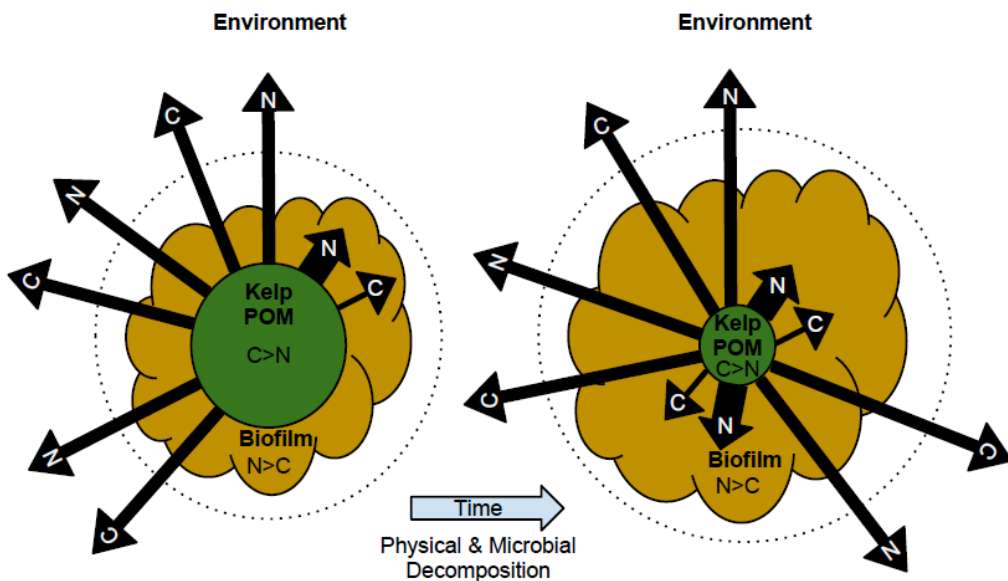
Table 2. Estimated trophic level of *A. fimbriatum* (a), and *S. subsimplex* (b) at different states of decomposition. Calculation for trophic level based on Fry (2006). Week 1 without biofilm is used as the basal substrate for comparison in most scenarios.



(a)

When biofilm is retained, C:N ratio appears to change little over time

When biofilm is removed from aged kelp, the nitrogen that had been taken up by the biofilm is removed as well. C:N appears to increase over time.



(b)

Initially, kelp biomass is high relative to microbial biomass. Apparent C:N of POM biocomplex is reflective of kelp substrate, resulting in high C:N

Increased surface area facilitates leaching and microbial utilization, which reduces biomass of kelp substrate over time, while increasing microbial biomass. Apparent C:N of POM biocomplex becomes reflective of the biofilm, resulting in low C:N

Fig 17: The loss of elemental carbon and nitrogen from (a) large kelp detritus and (b) kelp particles.

Chapter Two

Introduction

In Chapter 1, I found evidence that microbial biofilms can alter the isotopic composition of detrital algae, primarily via increased fractionation of $\delta^{15}\text{N}$. This finding led to the conclusion that in some cases, microbial biofilms may act as an intermediate trophic level between detritus and primary consumers. However, not all algae may develop this biofilm, as the results suggest that biofilm formation is ultimately controlled by the biochemical properties of the algal substrate. This is problematic from the perspective of an isotope ecologist—to ignore the biofilm would be to ignore a potentially large source of unquantified fractionation, yet there is no easy way to include it. There is no single ‘biofilm’ signature that can be included as an end-member, as it is in reality a fractionation process that can apply to many different end-members. Alternatively, universally applying this fractionation to all possible end-members ignores the possibility that some end-members are resistant to microbial colonization, and thus do not change isotopically. Other solutions exist, though they all have their respective drawbacks; and still the question remains: how much can the biofilm really affect model results? In order to arrive at a reasonable solution, I must first examine the scope of the problem.

In this chapter, I will assess the practical implications of microbial fractionation to isotope ecology by simulating different extremes of isotopic fractionation among different algae sources of a hypothetical primary consumer. Specifically, I will evaluate how the inclusion or exclusion of microbial fractionation affects the predictions of a Bayesian mixing model. In these simulations, I will use four different scenarios for ways that microbial fractionation could be applied to the model sources. Based on these results, I will provide recommendations for other researchers on how best to move forward.

Methods

I generated twelve different diets for a theoretical consumer, dubbed “Consumer”, based on macrophyte MSI data combined from Dethier et al. (in review) and the results of the (Chapter 1) kelp aging experiment. In this simulation, “Consumer” consumed only aged algae; however, of the nine species of algae available to it, only *Saccharina* experienced significant microbial alteration. For the purposes of this model, all other algae were isotopically unaffected by decomposition. For each macrophyte species, I calculated the average and standard deviation for each MSI predictor variable. Each diet featured a different macrophyte as the largest component, and alternated between that of a generalist or a specialist consumer. Each diet was composed of 100% known sources. *S. subsimplex* was included in the diets as aged with the biofilm retained; “Consumer” diets were constructed without any fresh *S. subsimplex* signal. The consumer was assigned a particular diet and set of fractionation values for each predictor variable: for C = $0.8\text{‰} \pm 0.09$; for N = $3.4\text{‰} \pm 0.10$; for S = $0.5\text{‰} \pm 0.31$ (Yokoyama 2005, McCutchan et al. 2003). The MSI signature for each “Consumer” was calculated by using the assigned diet to create a weighted average of all potential food items, after which fractionation values

were added. By incorporating macrophyte standard deviations, as well as variation in fractionation rates, a total of nine replicate samples values for “Consumer” were created for each diet.

To investigate the degree to which microbial components of decomposing algae should be incorporated in food web mixing models, I derived theoretical microbial signatures for each species of algae using the estimated microbial fractionation values found in Chapter 1. $\delta^{13}\text{C}$ and $\delta^{34}\text{S}$ values assigned a 0‰ shift from fresh, scrubbed samples, and $\delta^{15}\text{N}$ values were assigned a shift of 2.7‰ (SD=0.6021) from fresh, scrubbed samples. By incorporating the standard deviations of macrophyte MSI signatures, as well as the standard deviations of microbial fractionation rates, I generated an average microbial MSI signature for each species of macrophyte. Applying the shift to fresh scrubbed samples underestimates the maximum $\delta^{15}\text{N}$ value of the theoretical ‘pure’ biofilm achieved near the end of algal decomposition because it does not take into account the simultaneous enrichment that occurs in the algal substrate itself. For this reason, applying a shift to fresh samples provides an estimate of the biofilm during intermediate stages of decomposition. These values were not included in the construction of the “Consumer” diets, but were calculated for use as inputs to the Bayesian model.

The MSI values of “Consumer” were then entered into the Bayesian mixing model, Stable Isotope Analysis in R (SIAR; Parnell et al. 2010). While there are a variety of mixing models available, they all have similar superficial structure. Mixing models require: (1) isotope data of for a consumer or a population of consumers; (2) isotope data for all potential food items (commonly referred to as “source inputs”); and, (3) trophic fractionation values, based either on empirically determined values for a specific consumer, or based on literature values. When run, the model generates probability distributions for the contribution of each food item to the consumer’s diet. The advantage of a Bayesian mixing model over other types is that additional information may be supplied to the model, such as prior information based on gut content analysis, and standard deviations for consumer, source and fractionation values. In addition, while isotope-based food web modeling typically operates under the assumption that all possible food items within a system are known, SIAR allows for the existence of unaccounted food items. Because of these features, Bayesian mixing models such as SIAR are thought to be robust to both quantified and unquantified sources of error (Parnell et al. 2010).

Given this robustness, I wished to evaluate model performance in dealing with unquantified error on par with unquantified microbial MSI shifts. To examine first how well the model performs under ideal versus standard methods, I ran the model under two scenarios for each diet, where the input is: 1) aged algal MSI signatures, and “Consumer” consumes aged algae, resulting in no unquantified error; and, 2) only fresh algal isotope signatures (including *Saccharina*), and “Consumer” consumes aged algae, resulting in the presence of unquantified error in the model. To examine whether model accuracy could be improved without changing standard methods, I ran the model under two additional scenarios for each diet, where sources for the model were: 3) fresh algal MSI signatures, as well as a microbial MSI signature derived from an enrichment estimate of *S. subsimplex*; and 4) Fresh algal MSI signatures, as well as derived microbial signatures for each of the macroalgae species. Comparisons of the accuracy of each model run versus the actual diet were based on the median value of each probability distribution. For these comparisons, median predicted algal contributions were added to the median predicted

contributions of their respective microbial components in order to determine how well the models could predict kelp detritus utilization. The accuracy of each model configuration was evaluated using the Bray-Curtis Similarity index on median predicted versus actual diets.

Results

Mixing model results were most accurate under the ideal conditions of Scenario 1 (Table 1); that is, when the model was given the exact food items actually utilized by the consumer as source inputs. In this instance, the Bray-Curtis similarity index of the median predicted diet versus the actual diet was 72.3%. When standard methods of using only fresh algae as inputs were used (Scenario 2) the Bray-Curtis similarity index dropped by 10.5 percentage points to 61.8%. Interestingly, the average contribution attributed to an 'unknown source' in each diet remained similar between these two scenarios, at approximately 10%. When a microbial signal for *S. subsimplex* was included with fresh algae signatures as model inputs (Scenario 3), the similarity index increased by 7.3 percentage points relative to Scenario 2, and approached that of Scenario 1. However, the average contribution attributed to 'unknown sources' increased modestly to 12.2%. The results from Scenario 4, where calculated microbial signals for all macrophytes were included as inputs, were the least accurate; the similarity values dropped to 59%, and the average contribution attributed to an 'unknown source' increased to 15.6%.

The Bray-Curtis similarity values above were calculated over a range of diets, including many where *S. subsimplex* was not the dominant food source or not a food source at all, despite the fact that *S. subsimplex* is a dominant contributor to macroalgae drift in our study system. To see how the model scenarios perform under more realistic conditions, the model was re-run to be more reflective of the rates of bulk transport of *S. subsimplex*. Differences among scenarios were more pronounced when *S. subsimplex* contributed 20% or more to the total actual diet (Table 2). This effect was even more pronounced when *S. subsimplex* was the largest contributor to the total actual diet (Table 3). In both cases, using only fresh rather than aged algae signals decreased similarity values by 15.35 percentage points and 24.16 percentage points, respectively. *Fucus distichus* was frequently overestimated in Scenario 2, when the actual contribution of *S. subsimplex* was moderately large (Fig 1, for example). In both cases, including a calculated microbial MSI signal for *S. subsimplex* (Scenario 3) increased the accuracy of the model results, so that similarity values approached those seen in Scenario 1. Least accurate results for both cases were from Scenario 4.

Discussion

These results indicate that when investigating food webs in an ecosystem where detrital algae is readily available, using only fresh macrophytes as MSI mixing model source inputs may significantly decrease the accuracy of the predicted diets. To go one step further, in systems where the largest sources of detrital algae are vulnerable to microbial colonization over decomposition, ignoring the microbial shift in MSI signal may even lead to conclusions that are grossly inaccurate (Fig 1). Indeed, given that of the nine possible macrophyte sources only one source was subject to microbial shifts in

MSI, the results may even be an optimistic depiction of the complexity actually seen in the field where multiple sources may be vulnerable to microbial shifts. Despite this simplification, the results are useful as an illustrative example of the problems and choices researchers face in stable isotope ecology.

Not all kelps are vulnerable to microbial colonization, as shown in Chapter 1. The results of that component of my study showed that in two major species of kelp in the San Juan Islands of Washington State—*Saccharina subsimplex* and *Agarum fimbriatum*— one experienced significant microbial colonization and corresponding shifts in MSI values during decomposition while the other did not. Microbial colonization during decomposition is likely controlled by the concentration of secondary metabolites in algal tissues; this is an area of research with room for exploration, as more studies investigate the many ecological roles of these metabolites (Goecke et al. 2010, Engel et al. 2002, 2006). Thus, depending on the biochemical profiles involved, some algal species may experience more microbial alteration of MSI values than others over the course of decomposition. This of course adds not only complexity to the ecological system, but also time and money for the researchers wishing to study it accurately. A form of triage may be required to balance accuracy with time and financial constraints (Fig 2).

These results indicate that the microbial component of decomposing organic matter must be taken into consideration when working in detritus-based food webs. However, my findings also demonstrate that it is entirely inappropriate to apply universally applied fractionation estimates. For example, including a calculated *S. subsimplex* microbial signal as a discreet model input in addition to a fresh *S. subsimplex* signal yields a remarkable approximation of the aged *S. subsimplex* actually “consumed” by “Consumer”. However, when this technique is universally applied to all macrophyte sources, the accuracy of the predictions plummet, as the addition of so many sources weakens the model’s ability to distinguish between them. Given that n biomarkers can only definitively resolve $n+1$ sources (Fry 2006), the accuracy of mixing models tend to drop as the number of source inputs increase.

Recommendations

In light of multiple source resolution limitations, targeting the sources most likely to be the largest contributors may be a better option. In the system in which this study was conducted, *S. subsimplex* and *A. fimbriatum* are estimated to be the two largest contributors to the detrital pool available to benthic consumers, at 37% and 11% respectively (Britton-Simmons et al. 2009). By targeting just these two species out of the more than ten potential primary producer sources, the isotopic shifts that occur over decomposition in nearly 50% of the likely detritus biomass available to consumers can be incorporated into the Bayesian mixing model. As the results show, incorporating the fractionation that occurs in the largest source components could add significant explanatory power to the mixing models.

Incorporating the microbial component of decomposing organic matter through estimation alone may also be extremely difficult due to the complexity of the microbial assemblage. Caution must be applied; not all microbes are equal, and not all biofilms are microbial. Different microbial

assemblages may fractionate at different rates (Macko & Estep 1984), and some algal species may inhibit microbial development via the production of secondary metabolites. Recent aging experiments conducted on several different algal species have shown a wide variation in the isotopic changes that occur during decomposition (Hill & McQuaid 2009, Krumhansl & Scheibling 2012). There will not be one single “microbial” MSI signature, as developing research is readily showing that each species of algae and even each part of an algal thallus can have a unique microbial assemblage (Stauffenberger 2008, Bengtsson et al. 2010). Experimental determination of the isotopic shifts that occur during decomposition or *in situ* collection of degraded material for at least the largest contributing algal sources is therefore ideal.

Chapter Two Figures and Tables

| | Model Inputs | | | |
|--|---|--|--|---|
| | <u>Scenario 1</u> | <u>Scenario 2</u> | <u>Scenario 3</u> | <u>Scenario 4</u> |
| | Aged <i>S. subsimplex</i> , 8 other fresh macrophytes (n=9) | Fresh <i>S. subsimplex</i> , 8 other fresh macrophytes (n=9) | Fresh <i>S. subsimplex</i> , <i>S. subsimplex</i> microbial signal, 8 other fresh macrophytes (n=10) | Fresh <i>S. subsimplex</i> , <i>S. subsimplex</i> microbial signal, 8 other fresh macrophytes, 8 microbial signals for each macrophyte (n=18) |
| Bray-Curtis similarity to actual diet | 72.30 | 61.82 | 69.12 | 59.02 |
| Average % attributed to 'unknown source' | 10.70 | 10.21 | 12.2 | 15.64 |
| SD % 'unknown source' | 2.86 | 1.26 | 2.72 | 3.71 |

Table 1: Effect of each source input scenario on the accuracy of Bayesian mixing model results.

| | Model Inputs | | | |
|--|---|--|--|---|
| | <u>Scenario 1</u> | <u>Scenario 2</u> | <u>Scenario 3</u> | <u>Scenario 4</u> |
| | Aged <i>S. subsimplex</i> , 8 other fresh macrophytes (n=9) | Fresh <i>S. subsimplex</i> , 8 other fresh macrophytes (n=9) | Fresh <i>S. subsimplex</i> , <i>S. subsimplex</i> microbial signal, 8 other fresh macrophytes (n=10) | Fresh <i>S. subsimplex</i> , <i>S. subsimplex</i> microbial signal, 8 other fresh macrophytes, 8 microbial signals for each macrophyte (n=18) |
| Bray-Curtis similarity to actual diet | 72.53 | 57.18 | 70.35 | 58.03 |
| Average % attributed to 'unknown source' | 10.2 | 13.1 | 11.06 | 19.59 |
| SD % 'unknown source' | 1.46 | 2.56 | 3.45 | 1.36 |

Table 2: Effect of each source input scenario on the accuracy of Bayesian mixing model results in diets where actual contribution of *S. subsimplex* to consumer diets $\geq 20\%$.

| | Model Inputs | | | |
|--|---|--|--|---|
| | <u>Scenario 1</u> | <u>Scenario 2</u> | <u>Scenario 3</u> | <u>Scenario 4</u> |
| | Aged <i>S. subsimplex</i> , 8 other fresh macrophytes (n=9) | Fresh <i>S. subsimplex</i> , 8 other fresh macrophytes (n=9) | Fresh <i>S. subsimplex</i> , <i>S. subsimplex</i> microbial signal, 8 other fresh macrophytes (n=10) | Fresh <i>S. subsimplex</i> , <i>S. subsimplex</i> microbial signal, 8 other fresh macrophytes, 8 microbial signals for each macrophyte (n=18) |
| Bray-Curtis similarity to actual diet | 74.29 | 50.13 | 69.04 | 55.44 |
| Average % attributed to 'unknown source' | 10.46 | 12.12 | 9.65 | 19.19 |
| SD % 'unknown source' | 1.91 | 3.05 | 4.04 | 1.7 |

Table 3: Effect of each source input scenario on the accuracy of Bayesian mixing model results in diets where *S. subsimplex* is the largest contributor to actual consumer diets.

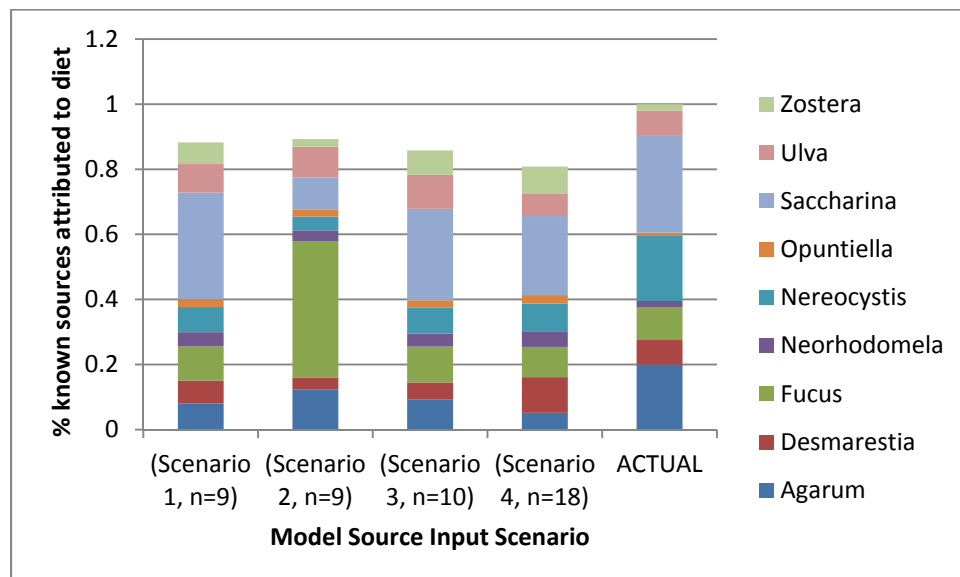


Fig 1: Example of model results for one diet in each modelling scenario.

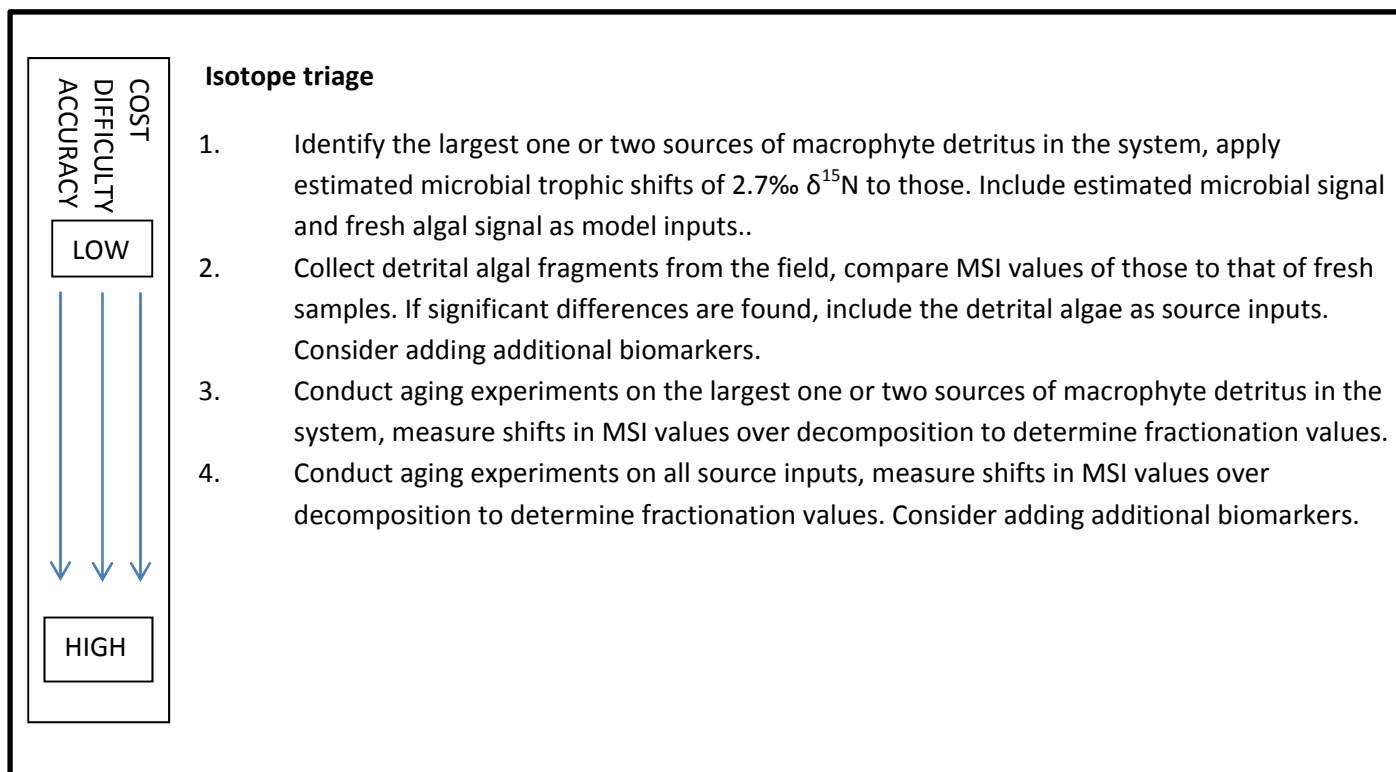


Fig. 2: MSI ecological research methods triage

Chapter Three

Introduction

In this chapter, I will explore the isotopic effects of microbial decomposition of algal detritus on actual rather than theoretical consumers. Much has been speculated about the effects of microbial colonization of detritus on consumer fitness (Duggins & Eckman 1997, Norderhaug et al. 2003). I seek to more explicitly investigate the mechanisms involved in those relationships, in order to further understand the ecological role of microbial dynamics in detrital food webs. More specifically, however, I will measure the effect of algae decomposition on consumer isotopic signatures. Based on Minagawa & Wada (1984), fractionation of $\delta^{15}\text{N}$ values between trophic levels is generally estimated to be about +3.4‰, although Minagawa & Wada (1984) found that number to be highly variable between species. A number of additional studies have found other sources of variability in $\delta^{15}\text{N}$ trophic fractionation, including environmental conditions, feeding strategy, and nutritional stress among others (Cabana & Rasmussen 1996, Vander Zanden & Rasmussen 2001, Hobson et al. 1993). Due to this variability, estimating trophic levels based on $\delta^{15}\text{N}$ requires a sufficiently high signal to noise ratio such that, to be detectable, trophic $\delta^{15}\text{N}$ enrichment at an intermediate trophic step must be large enough to overcome the natural variation in $\delta^{15}\text{N}$ fractionation at the next trophic step.

In a theoretical construct, at least, the hazards of ignoring microbial communities in detritus material can be consequential for isotope ecologists (Chapter 1). However, because of the high costs and difficulty associated with investigating the microbial fractionation of all algal sources, caution demands that I test those hazards in an ecologically realistic manner. To assess the impact of cascading microbial effects on detritus-based food webs I sought to determine whether microbial trophic enrichment is large enough to overcome consumer fractionation variability and thereby become detectable. To eliminate other sources of variability, I conducted this experiment in laboratory mesocosms where diet and environmental conditions could be tightly controlled. In these mesocosm experiments, I sought to simulate 'real-life' conditions; however, there remains the unavoidable potential for experimental artifact. To bolster our laboratory results, I also examined consumer MSI data taken from the field along a gradient of high primary productivity to high microbial productivity, in order to see if there is a corresponding shift in $\delta^{15}\text{N}$ values.

Methods

Consumers

Calliostoma ligatum

In January 2012, 35 individuals of the trochid gastropod *Calliostoma ligatum* (blue topsnail) were collected from Dyes Inlet in Bremerton, WA. They were weighed, and then placed into individual mesocosms held within a single 189L tank with a flowing ambient seawater system. The tank was in an outdoor enclosure during winter conditions, and as a consequence the water temperature within the tank was at least as cold as surface seawater temperatures. Each mesocosm held one organism. The

mesocosms were made of wide-gauge Vexar with styrofoam floats attached at the top; each mesocosm floated just below the surface of the water. Waste material fell through the Vexar to the bottom of the tank, preventing the organisms from encountering previously digested material. Ambient seawater could move freely through the Vexar, reducing the variability of the water quality between mesocosms. In addition, the reduced surface area of the Vexar discouraged the growth of a biofilm that the topsnails could use as an alternative food source.

Saccharina subsimplex blades were collected from Elliott Bay in October 2011; blades to be used in 'Fresh' treatments were frozen, while blades to be used in 'Aged' treatments were held in low light conditions in a single 189L tank in flowing seawater for a minimum of four weeks prior to the start of the experiment. As with the topsnails, the tank was in an outdoor enclosure, and so due to winter conditions the water temperature within the tank was at least as cold as surface seawater temperatures. Blades continued to age as the feeding trial progressed. Fresh blades were prepared by thawing and cutting into small (approximately 2x2cm) pieces. Pieces with visually obvious epibiota were excluded. Fresh scraped blades were prepared by thawing, scraping thoroughly with a razor blade, swabbing with Betadine, and then rinsed and scrubbed in seawater to remove the Betadine before being cut into small pieces. Aged blades were prepared by excluding parts with visually obvious epibiota or senescent material, and cut into small pieces. Aged scraped blades were prepared by removing senescent material before continuing as in fresh scraped blades. Samples of each algae treatment were saved for analysis of microbial abundance.

The mesocosms were numbered 1 through 32, and divided among the four feeding treatments: (1) Fresh *Saccharina* (F); (2) Fresh Scraped *Saccharina* (FS); (3) Aged *Saccharina* (A); and, (4) Aged Scraped *Saccharina* (AS). Organisms were fed twice a week. At each feeding, it was noted whether the previous piece of food had been grazed or not. The previous food item was then removed from the mesocosm, and replaced with a new piece.

At the completion of eight weeks, the topsnails were re-weighed, and sacrificed by freezing. Muscle tissue was then extracted from the foot of each organism, freeze-dried, ground to a powder and sent for multiple stable isotope analysis.

To test whether the presence of residual Betadine affects the palatability of the food items to the organisms, a control was run in which five *Calliostoma* individuals were placed in a single mesocosm with two food items: (1) one aged kelp blade that was scraped, swabbed with Betadine and rinsed; and, (2) another kelp blade that was only scraped and rinsed.

Pandalus platyceros

The above process was replicated using the pandalid shrimp *Pandalus platyceros* (spot prawn). This species was selected based on preliminary observations indicating that these organisms will feed on kelp fragments in laboratory settings, as well as the findings of Britton-Simmons et al. (2012), who found a positive association between *P. platyceros* and detrital kelp piles in the benthos. Prawns were collected from San Juan Island, WA, and fed a diet of algae-based aquarium food for eight weeks to

reduce the variability in their isotopic values. Each individual was kept separate to reduce cannibalism. After eight weeks, the prawns were moved into individual mesocosms and fed Fresh, Aged and Aged Scraped treatments. The feeding preferences of each individual was recorded twice weekly during food changes. Captivity and a prolonged herbivorous diet weakened the prawns such that significant mortality occurred early in the experiment, and the feeding trial was prematurely terminated.

Samples to test the efficacy of microbe reduction techniques

Aged and aged scraped samples were processed for DAPI staining as in Chapter 1.

Field data

MSI values for a variety of consumers collected in Summer 2011 from shallow (10m) and deep (100m) depths at three sites were obtained from Duggins et al. (unpublished data). The sites were located on the north (PTC) and west (Pillar) sides of San Juan Island, and the north side of Skipjack Island (SKP) in Northern Washington State. The consumers include the scavenger snail *Amphissa columbiana*, and the predatory crab *Cancer oregonensis* and the predatory snail *Fusitriton oregonensis*. The $\delta^{15}\text{N}$ values for five individuals of each consumer at each site and depth were then compared across depths.

Estimated Trophic Level

I estimated trophic levels using the formula:

$$\text{Trophic level} = \lambda + (\text{dN15}_{\text{consumer}} - \text{dN15}_{\text{basal source}}) / \Delta_n$$

Where λ represents the trophic position of the basal source, Δ_n represents the enrichment in dN15 per trophic level, and $\text{dN15}_{\text{consumer}}$ and $\text{dN15}_{\text{basal source}}$ represent the measured dN15 values of the consumer and the basal food source, respectively (Post 2002, Fry 2006). Given that primary producers occupy a trophic position of 1, and assuming a dN15 enrichment value of about 3‰ per trophic level, the formula becomes:

$$\text{Trophic Level} = 1 + (\text{dN15}_{\text{consumer}} - \text{dN15}_{\text{basal source}}) / 3$$

where fresh kelp is used as the basal source.

Statistical Analyses

All MSI data were log transformed and converted into Euclidean distance prior to analyses. Data points that were more than three standard deviations from the mean were removed as outliers. PERMANOVA was conducted using the package Vegan (Oksanen 2012) in the statistical program R (R-project.org). Each analysis was run for 2000 permutations. ANOVA and post-hoc analyses were conducted using SPSS statistical software (release 13.0).

Kaplan-Meier survival curves were analyzed with a log-rank Mantel-Cox test. Feeding rates accounted for mortality and missed feeding opportunities (due to loss of blade through cage) by only

including events where food was clearly available for consumption. Feeding rates were calculated as a binary function of daily frequency (i.e. food eaten or not) rather than amount eaten due to the nature of the detrital algae, which is subject to leaching and mechanical degradation. For that reason, loss of mass in aged versus fresh algae would be difficult to definitively attribute to feeding. Positive feeding events were marked only when radula marks were clearly visible on the food item.

Results

Isotopic values

Manual scraping of the biofilm from kelp surfaces followed by treatment with Betadine solution depleted microbial populations to nearly undetectable levels (Fig 1). There was a significant effect of aging on kelp MSI values, especially along the $\delta^{15}\text{N}$ axis; Aged kelps were more enriched in $\delta^{15}\text{N}$ than fresh kelps regardless of biofilm presence or absence (Fig 2, $p=0.003$). Kelps that had the biofilm retained were slightly more enriched than those without regardless of age, but this enrichment was not significant (Table 1). Aged kelps with the biofilm were enriched from fresh kelps without the biofilm by 1.39‰ on average. Aged kelps smelled strongly of sulfur, suggesting the presence of sulfur-reducing bacteria.

The MSI values of *Calliostoma ligatum* treatment levels were separated in multidimensional space along the $\delta^{15}\text{N}$ axis (Fig 3), and PERMANOVA results show that kelp age had a significant effect ($p=0.017$). Topsnails fed Aged kelp were slightly but significantly more enriched in $\delta^{15}\text{N}$ by an average of 0.36‰ than topsnails fed Fresh kelps regardless of the presence or absence of biofilm (Table 2). $\delta^{13}\text{C}$ of the topsnails indicate that the topsnails had not fully integrated their diets (Table 1).

The average $\delta^{15}\text{N}$ values of the scavenger snail *Amphissa columbiana* (wrinkled dove shell) and the predator crab *Cancer oregonensis* (pygmy rock crab) were consistently slightly higher in depths of 100m than in depths of 10m (Table 3). The range of enrichment spanned 0.55-1.88‰ in *Amphissa columbiana*, and 0.30-0.95‰ in *Cancer oregonensis*. There was no consistent pattern of $\delta^{15}\text{N}$ in the predator gastropod *Fusitriton oregonensis* (Oregon hairy triton); at Pillar and PTC sites, the deeper individuals of *F. oregonensis* were on average modestly more enriched than their shallow counterparts, but at SKP the reverse was true. The estimated trophic levels of the three consumers relative to the overall average $\delta^{15}\text{N}$ ratios of nine source macrophytes in the system showed that, on average, deeper individuals occupied a higher trophic level. This pattern was especially strong in *Amphissa columbiana*, where individuals from deeper depths occupied a trophic position more than 1/3 higher than shallow individuals (Fig 4).

Behavior and growth

There were no significant differences in the growth among treatments—consumers in all treatments lost mass. However, there were significant differences in feeding preferences among treatments both for age of the kelp ($p=0.001$) and the biofilm presence or absence ($p=0.036$). The topsnails fed more frequently on aged kelp than on fresh kelp, by nearly 20 percentage points (Fig 5).

Interestingly, regardless of age, when the biofilm was removed feeding preference increased in both treatments by about 10 percentage points. As a result, aged kelp with the biofilm removed had the highest rate of consumption per feeding opportunity, while fresh kelp with the biofilm retained had the lowest. Tukey HSD analysis shows that aged kelp without the biofilm was consumed significantly more frequently than fresh kelp ($p=0.001$) and fresh kelp without biofilm ($p=0.027$), though consumption was not significantly different from aged kelp with the biofilm ($p=0.265$). Visual observations of topsnails in the Betadine control -cage did not reveal any obvious feeding bias in the topsnails as a result of Betadine treatment, suggesting that the Betadine itself did not affect food palatability.

The results of the shrimp feeding trial mirrored that of the topsnail experiment, in which shrimps that were fed aged kelp with the biofilm removed had a significantly higher rate of consumption per feeding opportunity than either aged kelp or fresh kelp with the biofilms retained (Fig 6, $p=0.001$). The fourth treatment of fresh kelp with biofilm removed was not conducted in this experiment, and so is not available for comparison. The shrimps fed aged kelp with the biofilm removed also lived significantly longer than shrimps fed aged ($p=0.044$) or fresh kelps with the biofilm retained (Fig 7, $p=0.049$).

Discussion

Significant changes in the MSI values of the source kelp due to decomposition were tracked by the MSI values of *Calliostoma ligatum*. Though the shifts in the $\delta^{15}\text{N}$ values of the source kelp from Fresh to Aged were small ($\sim 1.3\text{‰}$ on average), this was large enough to overcome the natural variability in the fractionation rates of the topsnails and create slight but significant separation among treatment groups. The amount of time required for full integration of a consumer with its diet is highly variable among species and is affected by a number of factors such as growth (Herzka 2005, Fry & Arnold 1982), temperature (Bosley et al. 2002), and tissue type (Gorokhova & Hansson 1999, Hobson et al. 1993). In comparing the $\delta^{13}\text{C}$ values of the feeding treatments and snails, my results from this study suggest that eight weeks was not sufficient for the slow growing *Calliostoma ligatum* to fully integrate its diet in this experiment. Regardless, the small but significant separation among groups seen after only eight weeks suggests that further separation may be possible when consumers fully integrate their diets.

This finding is supported by the MSI data of the multiple consumers taken from discreet depths in the field. *Amphissa columbiana* and *Cancer oregonensis* were moderately more enriched on average in deeper depths than in shallower depths across all sites; however in some instances deep individuals were enriched from their shallow counterparts by up to 3.36‰ in *A. columbiana* and up to 2.51‰ in *C. oregonensis*, both occurring at the site Pillar. *Fusitriton oregonensis* was less consistently enriched in deeper depths than shallow, and at one site was on average relatively depleted at depth. The three consumers utilize very different feeding modes, and this may partially account for the differences seen. *A. columbiana* is a scavenger snail known to consume dead algae and carrion (O'Clair & O'Clair 1998), and it feeds by rasping surfaces with radular teeth. This mode of feeding may disproportionately utilize microbial biofilms that develop on the surfaces of decomposing material; not surprisingly, of the three species *A. columbiana* has the highest average $\delta^{15}\text{N}$ enrichment between depths, at 1.07‰. *C. oregonensis* is nominally a predator, but like many crabs it may also opportunistically consume detrital

material (O'Clair & O'Clair 1998), tearing large chunks of material with its mouthparts. This mode of feeding allows the crab to readily access and consume all the different parts of a food source, not just surfaces that have been microbially colonized; the average $\delta^{15}\text{N}$ enrichment of 0.38‰ between shallow and deep individuals may then reflect the crabs increased utilization of whole detritus at depth. *F. oregonensis* is a predatory snail that feeds primarily on tunicates and ascidians, though it has been known to sometimes consume echinoderms and molluscs (O'Clair & O'Clair 1998). Its lack of a consistent $\delta^{15}\text{N}$ shift with depth may then be reflective of its position in a food chain that is somewhat independent of detritus; many tunicates feed primarily on crustacean, echinoderm and mollusc eggs and larvae (O'Clair & O'Clair 1998), rather than detrital POM.

The $\delta^{15}\text{N}$ enrichment seen in response to microbially-degraded food items resulted in a slight upward shift in the estimated trophic levels of the topsnails (Fig 8). Because the topsnails did not fully integrate their diets in this study, that shift cannot be considered to be the final endpoint in the trophic levels of the topsnail treatment groups, but rather a trend seen at the mid-point of integration. Larger shifts may be seen in consumers that do fully integrate their microbially-degraded diet. For example, *Amphissa columbiana* from 100m appears to occupy a higher trophic position than individuals in 10m by over 1/3 of a full trophic level. Given that *A. columbiana* feeds largely off detritus, this shift speaks to an increasing microbial influence in detritus at depth. This general $\delta^{15}\text{N}$ and trophic level shift in some organisms between shallow and deep habitats may be applicable in other environments where there is a gradient in microbial productivity with progressive decomposition. Ecosystems like estuaries and marshes that are detritus rich may see similar $\delta^{15}\text{N}$ enrichments in many consumers. Indeed, previous studies have registered higher $\delta^{15}\text{N}$ in organisms along a number of gradients such as depth (Kelly et al. 2012, Nadon & Himmelman 2006, Lowe et al. unpublished), seasonality (Davenport & Bax 2002, Tallis 2009, Decottignies et al. 2007), and upwelling (Allan et al. 2010); many of these gradients could be interpreted in terms of increased microbial-detrital influence. The apparent increase in trophic level within a single species along these gradients has occasionally been attributed to diet-switching; that is, an apparent herbivore resorting to feeding on animal material at times due to a lack of primary producers (Kelly et al. 2012, Tallis 2009). While this indeed may be true in some cases, future interpretation should seriously consider the time-dependent effect of progressive microbial decomposition of detritus as a cause of this apparent shift.

There are at least two different explanations for the lack of an MSI response in feeding treatments to biofilm removal. This experiment took place in outdoor tanks in Mukilteo, Washington in January and February, and air temperatures surrounding the tanks were frequently near or below freezing. While tank temperatures never dipped below freezing, poor circulation near the bottom of the tanks that held the algae resulted in water temperatures that were colder than the water temperatures in Chapter 1. These colder temperatures may have slowed microbial development while at the same time enhanced the kinetic isotope effects (KIE) of the microbes present; it is documented that colder temperatures increase isotopic fractionation rates (Fry 2006). Given that fact, $\delta^{15}\text{N}$ values of the kelp may have been enriched through the action of a smaller microbial community in this experiment, and as a result, biofilm removal had less of an effect. Another possible explanation may be that the kelps in this

experiment were colonized by a different community of microbes with fractionation rates that differed from those seen in Chapter 1. As evidence, a strong sulfurous smell in the Aged kelp samples suggested the presence of sulfur-reducing bacteria. The slightly (though still insignificantly) different response in $\delta^{13}\text{C}$ and $\delta^{34}\text{S}$ values from those in Chapter 1 also support the idea that a different microbial community was present on the kelp in this experiment.

Frequency of feeding in topsnails was higher when fed aged kelp rather than fresh kelps, despite previous research showing no change in either phlorotannin concentrations or C:N ratios (Chapter 1). The source of this preference is therefore unclear but may be related to biochemical conditioning of the algae blades by the microbial biofilm. Previous work on detrital decomposition in riverine environments found that microbial colonization of leaf litter can mediate its availability to consumers in two ways: 1) by strongly affecting the palatability of the leaf litter to deter consumers; and, 2) by excreting enzymes that break down polysaccharides and soften the detritus, thereby improving its digestibility (Suberkropp 1998). Bacteria have been identified from marine systems that can fill these same functions, either promoting breakdown of algal polysaccharides (Goecke et al. 2010, Chesters et al. 1956, Ramaiah & Chandramohan 1992) or deterring predation with bioactive compounds (Lindquist et al. 2005, Lopanik et al. 2004, Burkepile et al. 2006). If this same pattern of conditioning holds true for microbial activity in marine detritus, it may account for the feeding preferences seen here—Aged kelp is more palatable to consumers than fresh kelp due to increased digestibility from microbial conditioning.

Interestingly, in treatments using both fresh and aged kelp, feeding frequency increased among topsnails when the microbial biofilm was removed. Two potential explanations exist for this pattern. First, scraping off the biofilm may also remove secondary metabolites if they are stored in the outer tissues of the alga; past work in other species of brown kelps has found that in non-reproductive tissues most of the organelles that produce phlorotannin, as well as the highest phlorotannin content, are found in epidermal and outer cortical layers (Shibata et al. 2004, Lüder & Clayton 2004, Tugwell & Branch 1989). If this is also true in *Saccharina subsimplex*, it would be in keeping with the “optimal defense theory”, which predicts that defensive metabolites should be most concentrated in tissues most vulnerable to herbivory (Amsler & Fairhead 2005, Tugwell & Branch 1989). However, in the course of this experiment, visual observation suggested that scraping was not intense enough to remove the epidermal layer, and that medullary tissues remained unexposed. A more provocative explanation is the possibility that the microbial community in the biofilm excretes compounds that are unpalatable to consumers and may be acting as resource competitors to other scavengers at late stages of decomposition. This is in line with the findings of Burkepile et al. (2006), who found compelling evidence of microbial competition with larger scavengers for fish carrion through the production of unpalatable secondary metabolites. If this latter possibility is correct, to our knowledge it would be the first instance of microbe-animal competition for algal detritus. The effects of this competition may be masked from direct observation by the enhanced digestibility of microbially conditioned detritus, resulting in a net preference for aged algae.

There may be significant ecological consequences for this apparent preference for aged algae over fresh algae. The retention time of fragmented blades within deep subtidal detritus pools, as well as

the time it takes for kelp blades to reach these pools, is unknown. A cautious assumption would be that deep subtidal detritus is comprised of algae in various stages of decomposition, ranging from relatively fresh to extremely decomposed. However, the results of this experiment would indicate that opportunistic scavengers may not consume all detrital inputs equally regardless of decomposition stage. Well-conditioned algae may play an out-sized role in detritus-based food webs as a result of this preference, and may therefore magnify the importance of microbial productivity in these food webs as well.

The fitness of consumers feeding on conditioned versus fresh algae cannot be evaluated with the results of this study, and more work should be done in this respect. All snails lost weight regardless of their diet, despite the fact that the snails fed aged kelp ate significantly more frequently. This may be evidence that the snails were stressed by the experimental conditions, or it may simply be the result of measurement error due to windy conditions at the start of the experiment affecting the weighing scale. Shrimp fed aged algae with the biofilm removed lived significantly longer than those fed either fresh kelp or aged kelp with the biofilm retained. However, this conforms to the feeding preferences seen and could be attributed to palatability. Given that spot prawns are unlikely to consume only algae for long periods of time, these spot prawns likely starved to death due to malnutrition, and so conclusions cannot be drawn regarding the food quality of the treatment groups. However, other studies (Duggins & Eckman 1997, Norderhaug et al. 2003) have found positive associations between consumer fitness and the utilization of aged algal detritus. In addition, numerous studies have reported that consumers assimilate microbial biomass with a much higher efficiency than the detritus substrate alone (Yingst 1976, Lopez et al. 1977, Cammen 1980, Fenchel & Jorgeson 1977), indicating that microbial colonization of detritus has a major positive influence on diet quality.

Conclusions

This experiment supports the hypothesis that microbial decomposition of detritus significantly alters not only the isotopic composition of a consumer, but also its feeding behavior. In short, the microbial decomposition pathway may yet prove to be a powerful, though overlooked, mediator of detritus-based food webs. Our capacity to understand and draw conclusions about the connectivity between two ecosystems via allochthonous organic matter may be limited then by our depauperate knowledge of microbial dynamics. While there certainly remain many questions to explore on the subject, future researchers would be wise to consider the community dynamics of unseen microbial nano-consumers when investigating the community dynamics of visually obvious macro-consumers.

Chapter Three Tables and Figures

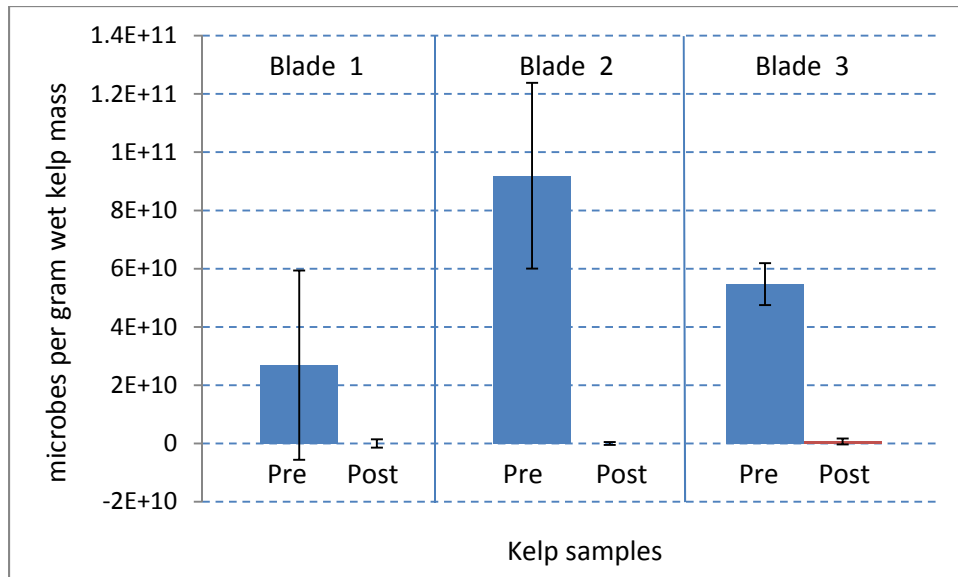


Fig 1: Median values of microbial abundance on kelp blades before and after biofilm removal. Error bars indicate one standard deviation from the median.

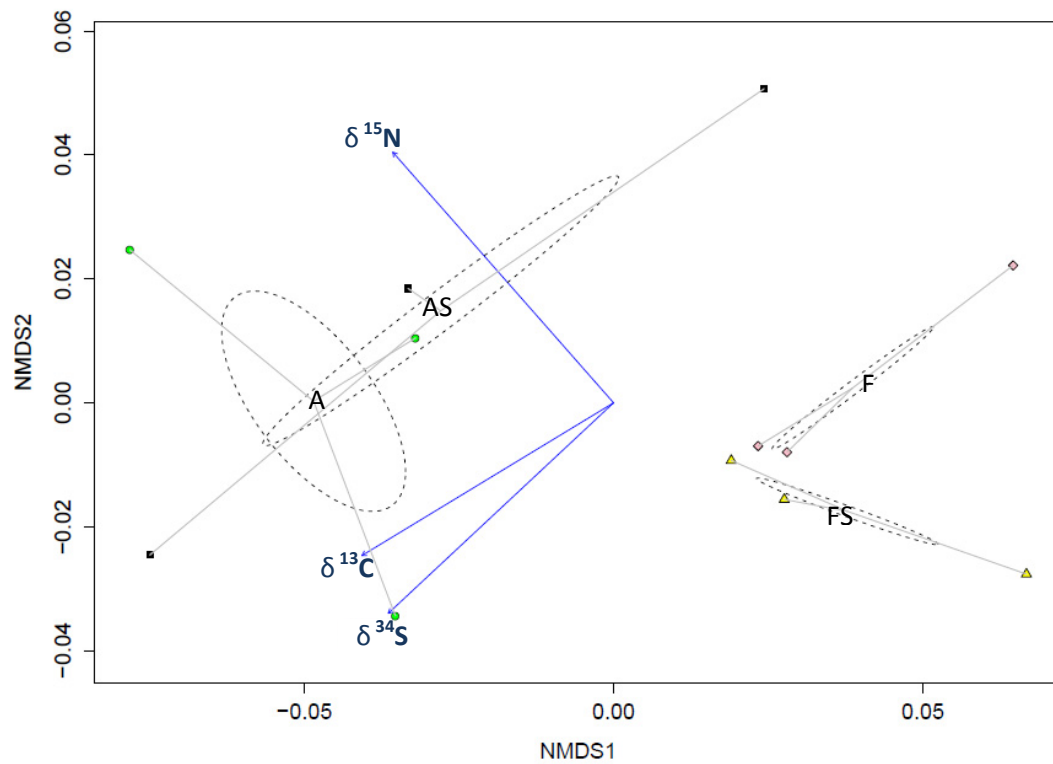


Fig 2: MSI of *S. subsimplex* used in feeding trial in multidimensional space. Ellipses represent the weighted average center of mass for each treatment group. F corresponds to fresh kelp with the biofilm retained, FS corresponds to fresh kelp with the biofilm removed, A corresponds to aged kelp with the biofilm retained, and AS corresponds to kelp with the biofilm removed.

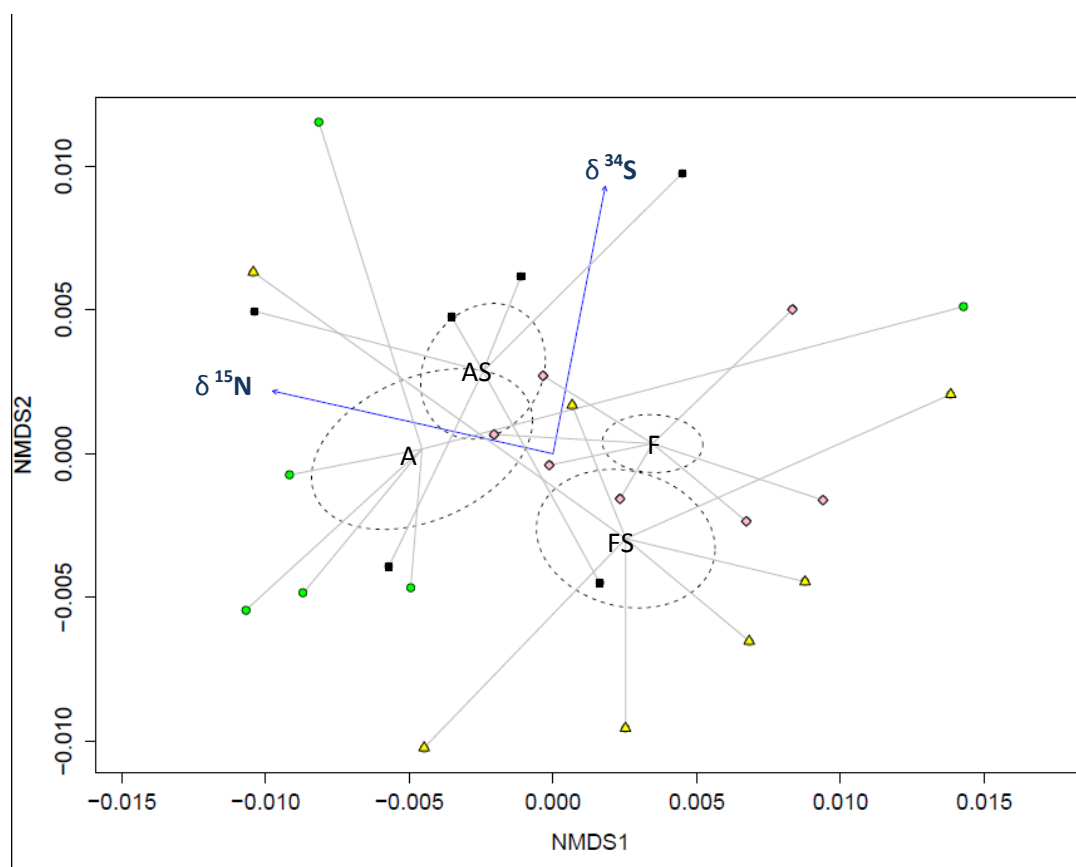


Fig 3: *Calliostoma ligatum* MSI values in multidimensional space. Ellipses represent the weighted average center of mass for each treatment group. F corresponds to snails fed fresh kelp with the biofilm retained, FS corresponds to snails fed fresh kelp with the biofilm removed, A corresponds to snails fed aged kelp with the biofilm retained, and AS corresponds to snails fed kelp with the biofilm removed.

| Treatment Group | | <i>Calliostoma ligatum</i> | | | <i>Saccharina subsimplex</i> | | |
|-----------------------|---------|----------------------------|-----------------------|-----------------------|------------------------------|-----------------------|-----------------------|
| | | $\delta^{13}\text{C}$ | $\delta^{15}\text{N}$ | $\delta^{34}\text{S}$ | $\delta^{13}\text{C}$ | $\delta^{15}\text{N}$ | $\delta^{34}\text{S}$ |
| Aged with biofilm | Average | -17.20 | 13.57 | 20.87 | -13.79 | 7.92 | 22.97 |
| | SD | 0.34 | 0.44 | 0.51 | 0.76 | 0.77 | 0.84 |
| Aged without biofilm | Average | -17.08 | 13.54 | 21.23 | -13.27 | 7.77 | 22.24 |
| | SD | 0.33 | 0.26 | 0.35 | 1.50 | 0.11 | 2.67 |
| Fresh with biofilm | Average | -17.01 | 13.17 | 21.07 | -12.24 | 6.70 | 20.58 |
| | SD | 0.27 | 0.24 | 0.12 | 0.06 | 0.08 | 1.62 |
| Fresh without biofilm | Average | -17.06 | 13.23 | 20.83 | -12.07 | 6.53 | 21.50 |
| | SD | 0.21 | 0.45 | 0.45 | 0.72 | 0.27 | 0.41 |

Table 1: MSI values of each snail and food source treatment group.

| Treatment | Average snail $\delta^{15}\text{N}$ | SD $\delta^{15}\text{N}$ |
|------------|-------------------------------------|--------------------------|
| Aged kelp | 13.56 | 0.35 |
| Fresh kelp | 13.20 | 0.34 |

Table 2: Average $\delta^{15}\text{N}$ values of *Calliostoma ligatum* by treatment (food source age).

| Site | Depth | Species | $\delta^{15}\text{N}$ | SD $\delta^{15}\text{N}$ |
|--------|---------|-------------------------------|-----------------------|--------------------------|
| Pillar | Deep | <i>Amphissa columbiana</i> | 13.25 | 1.07 |
| | Shallow | " | 11.37 | 0.15 |
| PTC | Deep | " | 12.11 | 0.20 |
| | Shallow | " | 11.30 | 0.16 |
| SKP | Deep | " | 12.22 | 0.09 |
| | Shallow | " | 11.67 | 0.70 |
| Pillar | Deep | <i>Cancer oregonensis</i> | 13.86 | 0.64 |
| | Shallow | " | 12.91 | 0.64 |
| PTC | Deep | " | 13.08 | 0.09 |
| | Shallow | " | 12.78 | 0.61 |
| SKP | Deep | " | 12.54 | 0.41 |
| | Shallow | " | 11.88 | 1.75 |
| Pillar | Deep | <i>Fusitriton oregonensis</i> | 12.92 | 0.09 |
| | Shallow | " | 12.46 | 1.23 |
| PTC | Deep | " | 13.13 | 0.25 |
| | Shallow | " | 12.84 | 0.39 |
| SKP | Deep | " | 12.84 | 0.72 |
| | Shallow | " | 13.02 | 0.26 |

Table 3: $\delta^{15}\text{N}$ values of three ambient organisms collected in the field from 10m and 100m at three sites.

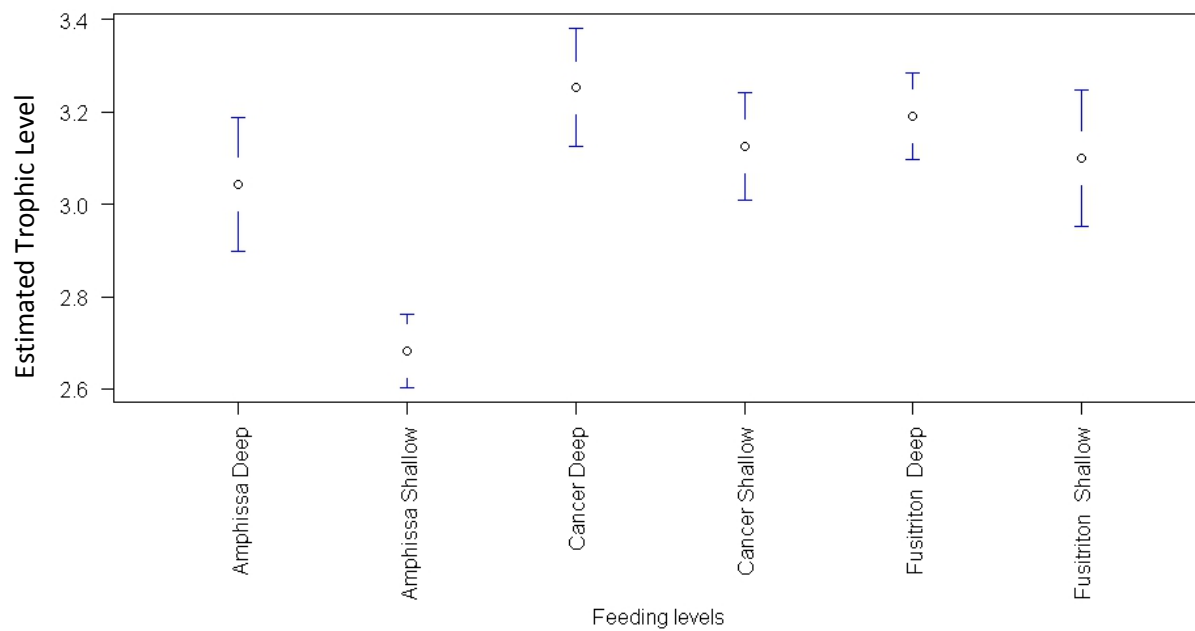


Fig 4: Estimated trophic level of three ambient organisms collected in field from 10m and 100m relative to the overall average $\delta^{15}\text{N}$ of nine different source macrophytes.

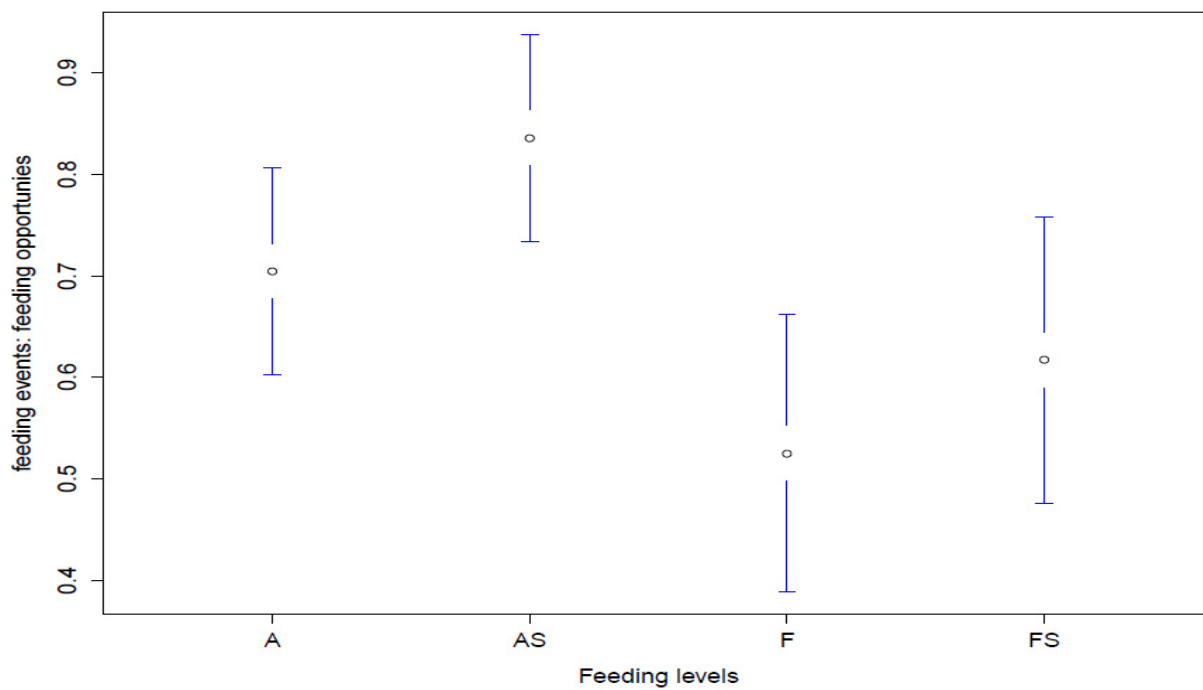


Fig 5: Topsnails feeding preferences.

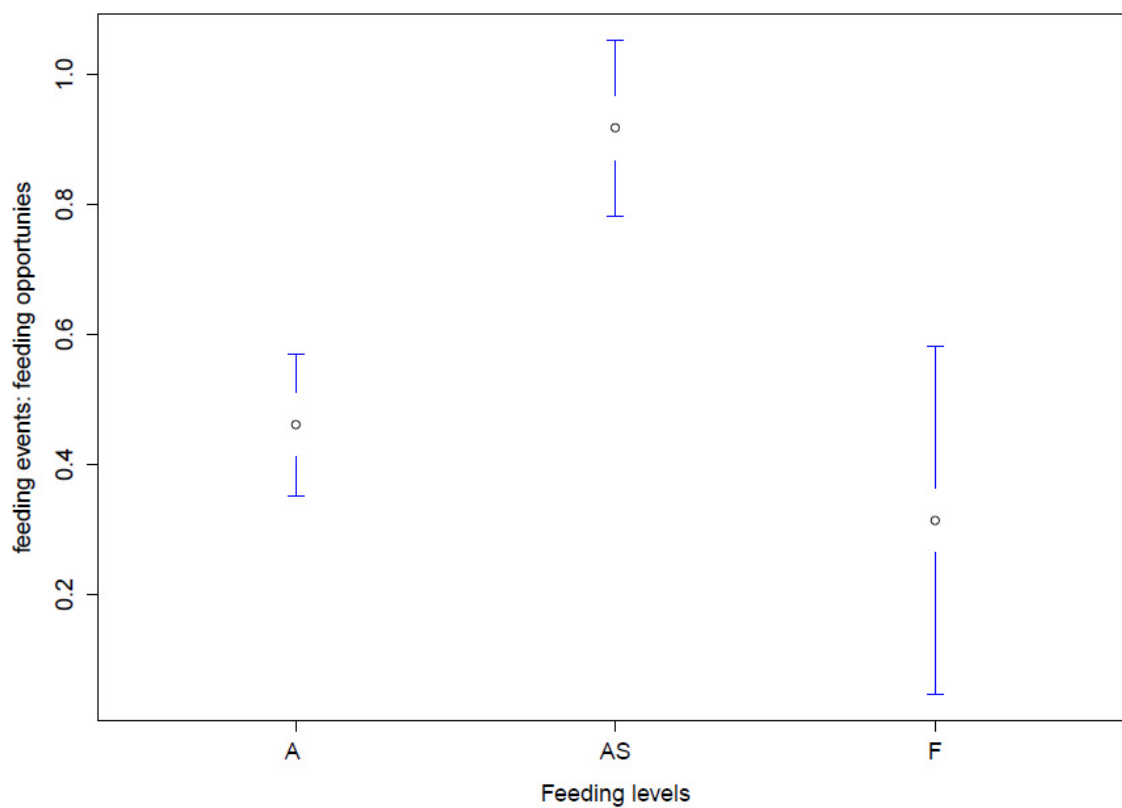


Fig 6: Shrimp feeding preferences.

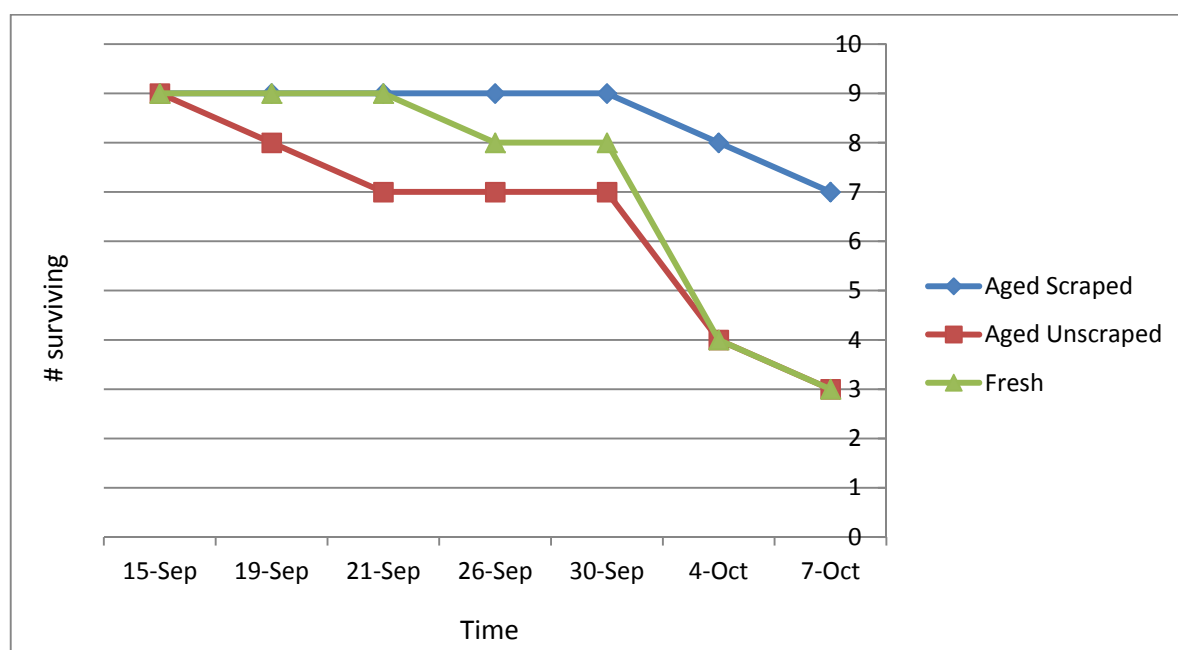


Fig 7: Shrimp survival by feeding level.

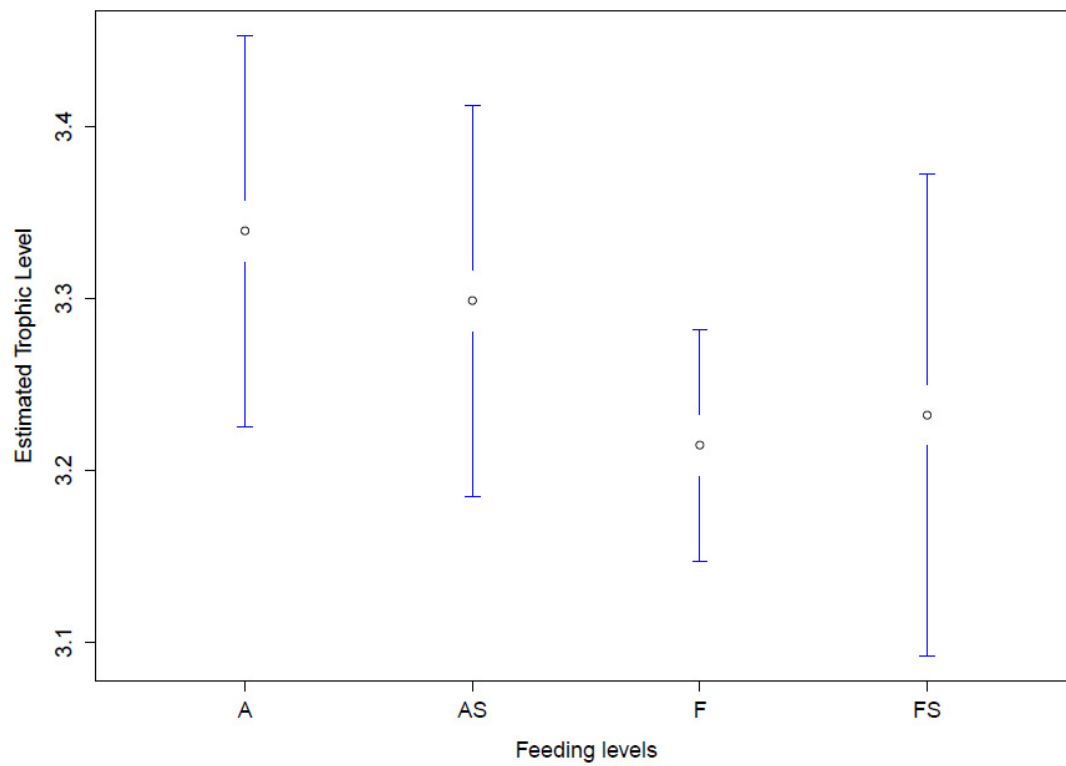


Fig 8: Estimated trophic level of *Calliostoma ligatum* by treatment group. (A) corresponds to snails fed aged kelp with the biofilm retained; (AS) corresponds to snails fed kelp with the biofilm removed; (F) corresponds to snails fed fresh kelp with the biofilm retained; and, (FS) corresponds to snails fed fresh kelp with the biofilm removed.

Synthesis

The results of my experiments indicate that the microbial dynamics associated with kelp detritus decomposition are highly complex, and thus lend complexity to the dynamics of the overall ecosystem. My experiments also suggest that understanding the role of microbial dynamics in detritus-based food webs is critical to researchers' ability to accurately interpret these food webs, especially when interpreting multiple stable isotope analyses. Our current limited quantitative knowledge of microbial dynamics may then be restricting our ability to understand and accurately draw quantitative conclusions about the connectivity between ecosystems and organisms via detritus-based subsidies. While this study cannot be considered an exhaustive exploration of microbial dynamics and its impact on food webs, several important findings from it may be used as a platform for further research which may yet reveal further complexity (Fig 1).

The influence of the underlying algal biochemistry on microbial biofilm development may be considered the root determinant of microbially-derived complexity within a detritus-based food web. While this study examined only phlorotannin concentration within two species of kelps, numerous other anti-microbial bioactive compounds are found within Phaeophytes, as well as in Rhodophytes and Chlorophytes (Goecke et al. 2010). These algal compounds and the mechanism of their effect on microbial activity are the subject of increasing research (Goecke et al. 2010, Hay 1996). In addition, phlorotannins and other bioactive compounds can occur in varying concentrations among: (1) algal species; (2) individuals of the same algal species depending on environmental conditions; and, (3) tissues of the same algae individual depending on grazing pressure and defense strategy (Shibata et al. 2004, Luder & Clayton 2004, Tugwell & Branch 1989, Amsler & Fairhead 2005, Dubois & Iken 2012, Iken & Dubois 2006, Van Alstyne 1999). It is reasonable to imagine then that the presence of anti-microbial compounds within algal material will not always lead to a simple binary of microbial presence or absence, but may yield a gradient of abundance or assemblage.

The complicated interaction among microbial assemblages and algal hosts may have far reaching ecological consequences beyond the biofilm. The degree to which microbes can colonize a piece of detritus will affect the degree to which detritus-based food webs will be microbially influenced in metrics such as consumer feeding behavior, trophic position, and diet quality, among others. In areas where microbes can extensively colonize detritus subsidies, microbes may serve to increase the availability of nutrients within allochthonous material, both by retaining algal nutrients in the biofilm that would otherwise be lost to leaching, and by improving the digestibility of the material via enzymatic breakdown (Smith et al. 1992, Martinez et al. 1996). While microbes may not improve the nutritional quality of the detritus directly through the addition of microbial nitrogen (as is commonly thought), microbes may instead mediate the availability of the nutrients already present in the detritus.

Microbial mediation of detritus appears to spur a strong feeding response in consumers, which fed most frequently when given microbially-conditioned algae. This apparent bias occurred despite persistent levels of phlorotannin concentration, which may deter grazing. C:N ratios also remained static, suggesting that the nutritional quality of the kelp was unaffected. While this experiment

evaluated feeding frequency only on fresh and aged *S. subsimplex*, other research has shown that consumers given a microbe-resistant algae species (*A. fimbriatum*) had no preference between fresh or aged samples (Duggins, unpublished), suggesting that feeding preference is microbially-influenced. If this is indeed the case, detritus that is subject to microbial decomposition may be a larger component in consumer diets than would be assumed by looking at material transport rates alone. For example, in an area where microbe-resistant or fresh algae are the dominant source of detritus within the ecosystem, consumers may rely on relatively minor sources of microbe-vulnerable, aged algal detritus for more of their energy. As a result, detritus-based food webs may be more dependent on the age and availability of specific kinds of algae than simply overall algal transport. As a further consequence, microbes may be utilized by consumers to a much greater degree than what the biochemical composition of available algae would suggest. While this study did not look at the effect of microbial utilization on consumer health and fitness, the results of other studies suggest that the effect may be positive (Norderhaug et al. 2003, Duggins & Eckman 1997).

Despite the higher frequency of consumer feeding on microbially-degraded foods, the results of my study suggested that microbes may compete with other consumers for detritus. Although consumers appear to prefer microbially-conditioned algae, this study found that feeding frequency increased when the biofilm was removed. This bias towards microbially-decomposed but microbe-free food may be due to microbes emitting unpalatable chemical defenses (Lindquist et al. 2005, Lopanik et al. 2004, Burkepile et al. 2006); when the microbial biofilm is removed, the food may have all the benefits of improved digestibility, but less of the unpalatable compounds. It is easy to imagine that microbes in detritus-based food webs are effectively in resource competition with other scavengers for a limited supply of detritus. Unlike other competitors, however, microbes are also at risk of being consumed along with the resource they are competing for, thus creating the need for chemical defense.

In addition to ecological implications, microbial utilization of detrital algae may have far reaching research consequences as well. The results of my research show that researchers who rely on MSI methodology to investigate food web dynamics should take care to consider the community dynamics of microbial nano-consumers, and their effect on MSI data. Significant MSI shifts can occur in detritus sources that are vulnerable to microbial decomposition. As seen in Chapter 2, the effect of this shift on the accuracy of MSI mixing model predictions can be extreme, and researchers who fail to adequately incorporate microbial dynamics into their models of detritus-based food webs may be led to inaccurate conclusions. As a consequence, adjustments in mixing model assumptions and calculations will be required to more accurately incorporate microbial components in MSI mixing models for detritus-based food webs. However, this is no easy task. Using universal estimates of microbial fractionation is not a realistic option for a number of reasons: (1) the complexity of the interaction between algal biochemistry and microbial abundance and assemblage virtually guarantees that MSI shifts will depend on the algal species; (2) the addition of so many end-members to the model can overwhelm its ability to resolve sources unless additional prior information is used; and, (3) detritus pools are likely to be composed of algae in various stages of microbial decomposition, which will affect MSI shift. Researchers are advised to consider conducting experiments to test the algae species used as

model inputs for microbial shifts with decomposition, as well as to consider the condition of the algae detritus available to consumers.

In addition to affecting the accuracy of MSI mixing models, microbial utilization of algal detritus may affect researchers' ability to understand food web hierarchy in detritus-based ecosystems. Rather than acting as a novel food source, the results of my experiments strongly suggest that the microbial biofilm that develops on algae acts as an intermediate trophic level capable of further enriching $\delta^{15}\text{N}$ in organisms that consume the biofilm relative to the source algae $\delta^{15}\text{N}$. As a result, the calculated trophic level of organisms that consume the detritus "biocomplex" of algae and microbes will likely be higher relative to organisms that feed on fresh algae. The $\delta^{15}\text{N}$ values of consumers taken from the field seem to reflect this microbially-sourced trophic shift, as several species of organisms appeared to occupy higher trophic positions with increasing depth. This trophic enrichment may be attributed to diet switching, where an apparent herbivore becomes an opportunistic predator when resources are limited. While diet switching undoubtedly can occur and has been documented, research by Britton-Simmons (2009) suggests that there is a positive relationship between depth and macroalgae detritus in the San Juan Islands, indicating that at these depths organisms are not necessarily algae limited and may not need to resort to diet switching. A possible alternative hypothesis then is that the availability of increasingly microbially-decomposed detritus with increasing depth is a cause of this apparent shift in detritus-based food web hierarchy.

Trophic effects introduced by microbial decomposition may be further influenced by consumer feeding mode. For example, surface-grazing organisms that have greater proportional exposure to the microbial biofilm may have a larger response in trophic level than 'shredder' organisms that consume large detritus fragments. While this study did not explicitly examine feeding mode, the species specific differences in trophic enrichment seen in consumers taken from the field suggest that feeding mode may be a relevant factor that warrants further study.

Knowledge of microbial dynamics may be a major limiting factor on researchers' capacity to understand how coastal detritus-based ecosystems and organisms interact. Thus far, understanding of interactions within these systems has focused largely on observable phenomena— macroscopic dynamics on macroscopic scales. However, coastal researchers must start to shift their focus to the invisible processes that occur on microscopic scales, as these processes can have very real consequences on macroscopic systems—including our ability to understand the world around us and interact effectively with it. By limiting our knowledge of detritus-based food web dynamics, we effectively limit our ability to make informed decisions regarding conservation or restoration of deep subtidal ecosystems. More research is required, but the results of this study indicate that microbial decomposition may prove to be a powerful, though overlooked, mediator of coastal detritus-based food webs.

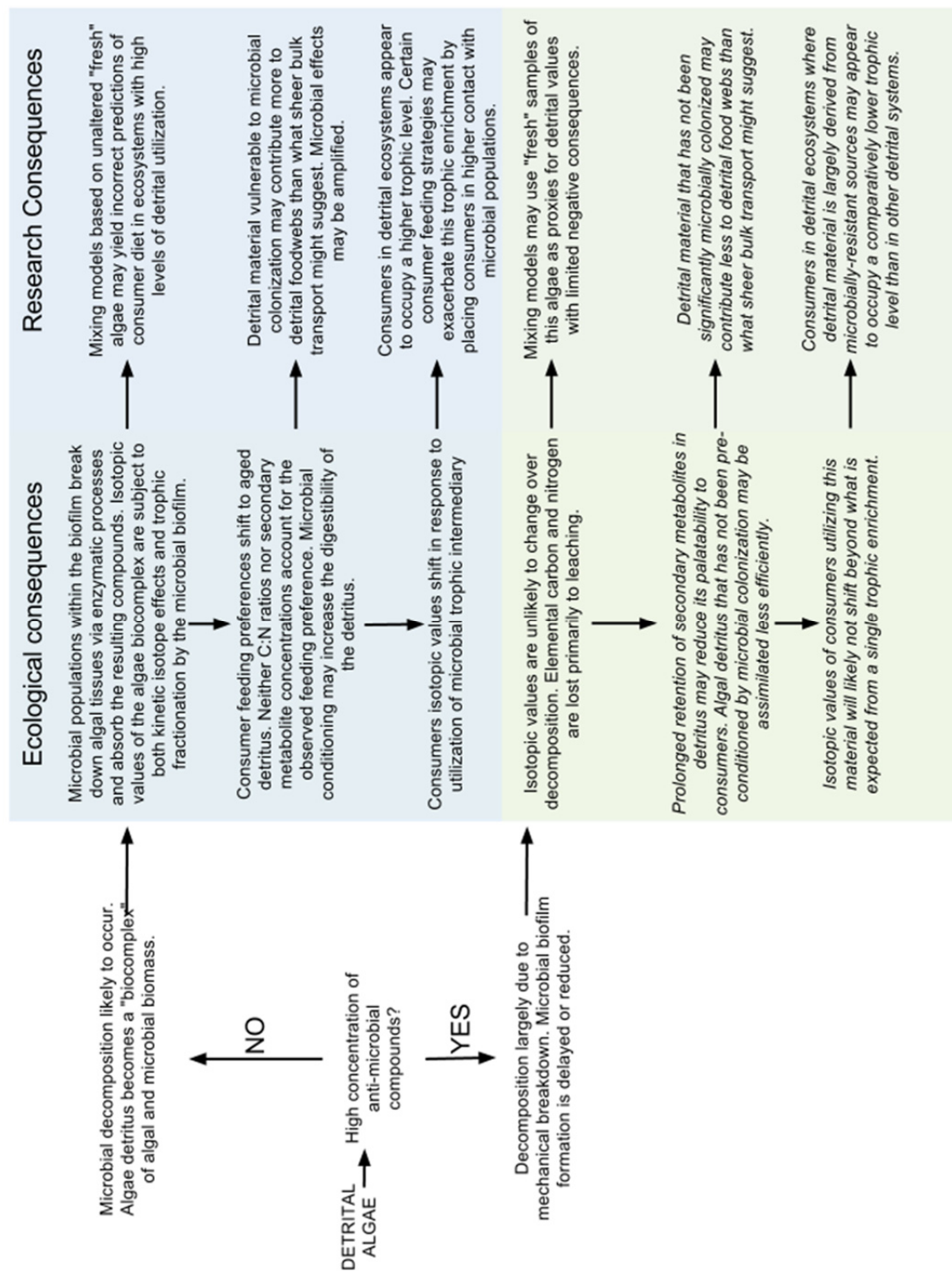


Fig 1: Updated conceptual model of detrital processes, incorporating both microbial mediation of detrital material as well as source algae biochemistry. Sections in italics were not explicitly tested in this research, but inferred from the data as well as prior research.

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