CARBON AND NITROGEN ASSIMILATION IN THE CLAMS

NUCULANA RADIATA AND *MACOMA MOESTA* FROM THE BERING SEA

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CARBON AND NITROGEN ASSIMILATION IN THE CLAMS *NUCULANA RADIATA* AND *MACOMA MOESTA* FROM THE BERING SEA

A

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Abstract

The predicted climate-induced reduction in sea ice presence in the Bering Sea could impact benthic trophic interactions; however, species-specific consumer dependence on ice algal production is largely unknown. My objective was to track feeding in the benthic clams, *Nuculana radiata* and *Macoma moesta*, using stable carbon and nitrogen isotopes. *Nuculana radiata* had slow isotopic assimilation rates, with lipids taking up isotope markers fastest and muscle tissue the slowest. Lipids may thus be particularly suitable to track the immediate ingestion of sea ice algal export in benthic consumers. When isotopically enriched food was added to natural sediment cores, *N. radiata* assimilated 60% less of the isotope markers than when feeding on algal food in isolation. Possibly, this difference is related to the ingestion of other, naturally present food sources in the sediment. *Macoma moesta* showed 30% higher isotopic assimilation compared to *N. radiata* in sediment cores. I suggest that differing feeding behaviors between the species provide differential access to the sedimented algal food. Based on these results, *N. radiata* is likely better able to utilize food sources buried in the sediment and may be more competitive over *M. moesta* under conditions of reduced ice algal production in the northern Bering Sea.

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Introduction

Stable carbon and nitrogen isotopic ratios are important natural and/or experimental organic matter tracers in trophic and dietary studies (Peterson and Fry, 1987; Martinez del Rio et al., 2009). In nature, stable isotope ratios, e.g. $\delta^{13}C$ or $\delta^{15}N$, undergo stepwise enrichment from food to consumer because of the preferential loss of the lighter isotopes during respiration or excretion, which is known as trophic fractionation (Gannes et al., 1998). $\delta^{13}C$ undergoes small enrichment steps (0-1‰ per trophic level) and is used as tracer of isotopically distinct carbon sources, i.e. diet components, while the large fractionation of approximately 3-4‰ $\delta^{15}N$ between trophic levels is suited to determine food web structure (Gannes et al., 1997; Vander Zanden and Rasmusson, 2001; Post, 2002). Tracking the proportional contribution of foods to various consumers' diets often assumes steady-state conditions in isotopic contributions (e.g. constant isotopic ratios of the endmembers and no switch in food sources, Hobson and Welch, 1992), which is often not the case in marine environments (e.g. McMahon et al., 2006; Kaufman et al., 2008; Sun et al., 2009). An additional complication is the isotopic discrimination within an organism, which occurs through preferential isotope routing during metabolic processes (Gannes et al., 1998; Tamelander et al., 2006; Budge et al., 2008). Lipids are the biochemical fraction with the highest discriminating factor, making them isotopically lighter than other body components (Mateo et al., 2008). Lipid contribution to body tissues can vary greatly between tissues and season, and thus are often removed from

stable isotope tissue and muscle samples for food web analyses (Tieszen et al., 1983; Post et al., 2007).

Food webs in polar seas have frequently been analyzed using stable isotope approaches (Dunton et al., 1989; Iken et al., 2005, 2010; Søreide et al., 2006; Tamelander et al., 2006). Specifically, the two main primary producer isotopic endmembers in polar systems, phytoplankton and sea ice algae, often have significant differences in $\delta^{13}C$ with sea ice algae being enriched in $\delta^{13}C$ values, which can potentially provide information on consumer-specific feeding and overall trophic level connectivity and energy flow (Hobson and Welch, 1992; Søreide et al., 2006; Tremblay et al., 2006; Gradinger et al., 2009). However, this difference in endmember signature is not constant over time as the $\delta^{13}C$ ratio of ice algae varies temporally and depends, among others, on the physical structure (porosity) of the sea ice and primary productivity levels in sea ice (Kennedy et al., 2002; Papadimitriou et al., 2009). Furthermore, algal productivity within the ice and water column is highly seasonal, which together with the above-mentioned factors indicates non steady-state conditions as typical for polar seas (e.g., Grebmeier et al., 2006a; Aydin and Mueter, 2007).

I investigated the suitability of a stable isotope approach for understanding the complexity of Bering Sea food webs. Specifically, I focused on the response of Bering Sea benthic deposit feeders to the seasonally pulsed input of ice algal organic matter. Therefore, this study aimed to track the feeding of two benthic bivalves, *Nuculana radiata* (Nuculanidae) and *Macoma moesta* (Tellinidae), which are regionally abundant and of high trophic importance in the northern Bering Sea (Grebmeier et al., 1989;

Richman and Lovvorn, 2003; Lovvorn et al., 2009). Understanding trophic connections and energy flow, especially the importance of sea ice-derived organic matter, is of particular importance in the seasonally ice covered Bering Sea, which has been and will be strongly influenced by climatic changes (Grebmeier et al., 2006a). Specific to the Bering Sea ecosystem, Hunt et al. (2002) suggested two alternate ecosystem states, where most of the organic matter produced by ice algae and phytoplankton is either consumed in the pelagic or benthic realm, depending on climate conditions. This change in energy flow influences the commercial, subsistence, and ecologically important megafauna in the Bering Sea (Coyle et al., 2007; Jin et al., 2007; Lovvorn et al., 2009) and may be similar to ecosystem responses proposed for other sub-Arctic seas (Søreide et al., 2006; Tamelander et al., 2006; Bluhm and Gradinger, 2008; Mueter et al., 2009).

The exact response of benthic feeders such as the clams *N. radiata* and *M. moesta* to changes in primary production, specifically of ice algae, has posed a problem for the interpretation of food web connectivity in this and other similar annually ice covered systems (Dunton et al., 1989; Post, 2002; McMahon et al., 2006; Sun et al., 2009; McCormick-Ray et al., 2011). I used stable isotope enriched feeding experiments to provide the first invertebrate assimilation and turnover rate estimates for important benthic feeders in the Bering Sea. Specifically I asked: 1) how fast will the isotopic ratios change in various body components of the clams *N. radiata* and *M. moesta* following input of isotopically enriched organic material, 2) will this response be different for these two species, 3) what is the importance of the sediment environment for the uptake of

sedimented algal production in the two clam species, and 4) what are implications of these observations for the interpretation of field data in the Bering Sea ecosystem context.

Materials and Methods

Algal food culture

Two isotopically distinct algal food stocks were prepared based on a mixed ice algal culture collected from the study region in 2008. The majority of the cells in the culture were identified as the pennate diatom *Nitzschia frigida* (Medlin and Hasle, 1990), with a minor microflagellate component, using a Zeiss Axiovert microscope at 400x magnification. An 8 L batch culture was continuously incubated at 5˚C with a 24 h light supply (9.4 ± 0.2 μmol m^{-2} s^{-1}) over a three-month period in artificial seawater medium (Instant Ocean, S= 32) with weekly additions of 160 ml of Gillard's F/2 marine water enrichment solution as nutrient fertilizer (Sigma G9903 with silicate; Guillard, 1983). Constant aeration was maintained through a Tetra Whisper aquarium air pump. The batch culture was weekly sub-sampled, whereby half of the volume (4 L) was removed and replaced with new seawater and nutrients.

Equal aliquots of the weekly algal harvests were treated as follows to produce two isotopically distinct food stocks (non-enriched and enriched). One 2 L aliquot (for isotopically non-enriched algal food) was centrifuged (4000 rpm, 2647 g, 5 min), rinsed, mixed and decanted three times with artificial seawater, and stored frozen (-20˚C). The other 2 L aliquot (for isotopically enriched algal food) was incubated for an additional 24

h with additions of 1 ml of ^{13}C-enriched solution L^{-1} culture (3.4710 g of 98% ^{13}C sodium bicarbonate in 100 ml distilled water) and 0.4 ml of ^{15}N-enriched solution L^{-1} culture (2.0585 g of 98% ^{15}N sodium nitrate in 100 ml distilled water) to achieve algal enrichment through photosynthetic uptake of the now overly abundant heavy isotopes. The enriched culture was then centrifuged, decanted as described above, and frozen for storage (-20˚C). The cells of *N. frigida* (17.3 ± 0.2 μm length) remained intact in microscopic observation of the final homogenized and frozen food samples; hence, integrity of algal food stocks was likely maintained prior to use in experiments.

At the conclusion of the culturing period, all weekly aliquots of the enriched and non-enriched algal food stocks were homogenized separately to create two isotopically homogenous food sources, which were used in feeding experiments. Isotopic values (see below) and food quality and quantity (C, N, and chlorophyll *a* [μg]) of the two feed stocks were obtained from isotopic and elemental analyses (described below), and fluorometry. Known volumes of the two algal food sources were filtered onto pre-combusted Whatman GF/F filters for isotopic and chlorophyll *a* (henceforth: chl) analyses. For stable isotope analysis, filters were freeze dried at -80˚C and measured as described below (samples were not acid fumed). For chl analysis, samples were extracted with 90% acetone at -20˚C for 24 h, then allowed to warm to room temperature and measured with a TD700 fluorometer (Arar and Collins, 1997). Chl concentrations (μg L^{-1}) were used to define food rations during the experiments (Table 1).

Table 1: Duration and treatments for the 2009 *N. radiata* jar and 2010 *N. radiata* and *M. moesta* sediment core feeding experiments. The algal food represents the calculated food quantity assuming that algal food settled to the bottom of each jar or to the sediment surface of each core. At each clam removal time, 3-6 individuals were removed from the experiment for later stable isotope analysis.

2009 Jar *N. radiata*

Treatment	Duration (d)	Clam Removal Time (d)	Algal Food (μg chl cm^{-2} jar^{-1} $3d^{-1}$)
Enriched Algae	42	6, 12, 20, 28, 35, 42	0.36
Non-Enriched Algae			0.27
Starvation			0

2010 Sediment Core *N. radiata*

Treatment	Duration (d)	Clam Removal Time (d)	Algal Food (μg chl cm^{-2} $core^{-1}$ $18d^{-1}$)
Enriched Algae	18	5, 10, 18	88.76
Non-Enriched Algae			110.14

2010 Sediment Core *M. moesta*

Treatment	Duration (d)	Clam Removal Time (d)	Algal Food (μg chl cm^{-2} $core^{-1}$ $18d^{-1}$)
Enriched Algae	18	5, 10, 18	88.76
Non-Enriched Algae			110.14

Field collections and feeding experiments

All clam and sediment core field collections occurred in 2009 and 2010 at 60-70 m water depth in soft, muddy benthic environments approximately 150 km southwest of St. Lawrence Island in the northern Bering Sea (Figure 1, Appendix). Sampling methods are described below for each technique and experiment. To limit natural isotopic variation among samples, collections were done at similar geographic locations between years while the overlaying water column was covered by first-year sea ice. Subsequent feeding experiments were then conducted aboard the United States Coast Guard Cutter (USCGC) Healy in March-April 2009 and aboard the USCGC Polar Sea in March of 2010 in environmentally controlled incubators.

For feeding experiment 1 in 2009, sediments from 22 replicate van Veen benthic grabs (0.1 m^2) were sieved over 1 mm mesh to collect 350 living adult *Nuculana radiata*. All *N. radiata* were allowed to clear their guts for 24 h prior to the start of the experiment. An initial (t_0) sample of 50 clams was taken after the gut clearance period, of which 15 were used to determine the isotopic baseline and intra-specific variability in isotopic composition. Each of the 300 remaining clams were placed into an individual jars (33.2 cm^2 bottom area) containing 150 ml Instant Ocean seawater (S=32) in the dark at 4.6 ± 0.1°C for the entire experiment. Clams were randomly assigned to three feeding treatments (n= 100 $treatment^{-1}$): enriched algal food, non-enriched algal food, and no food (starvation). Enriched and non-enriched treatments received equal amounts of algal food (~0.3 μg chl jar^{-1} 3 d^{-1}) every 3 days from the respective food stock (Table 1).

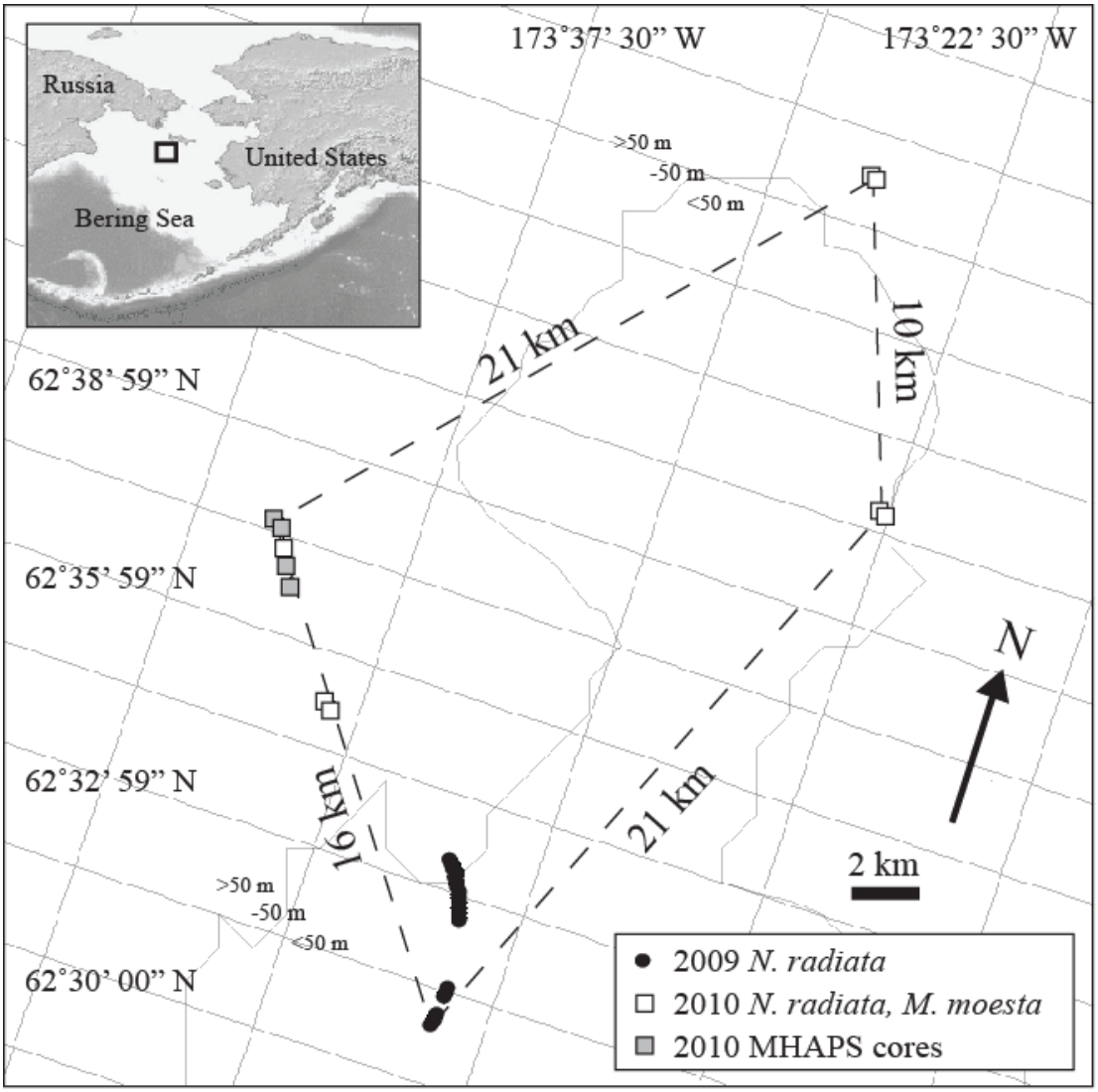

Figure 1: Bering Sea region and sampling sites in 2009 and 2010 with distances (km) between locations (dotted lines). Type of sampling conducted at each location are indicated as black circles= grab sampling in 2009; white squares= grab sampling in 2010; grey squares= MHAPS core sampling in 2010. Sea floor depth is represented by the 50 m contour line.

Aeration was achieved with a complete seawater replacement every three days. From all three treatments, 10 clams were removed on days 6, 12, 20, 28, 35, and 42 and kept frozen at -20˚C where 3-6 clams were then selected for later stable isotope analyses. Not all experimental clams were used for stable isotope analysis as mortality was low (9%) and showed no association with treatment exposure.

Feeding experiments 2 and 3 in 2010 used natural sediment cores with added *N. radiata* (experiment 2) and *Macoma moesta* (experiment 3) for enriched and non-enriched feeding treatments. All clams (n= 44) were obtained from 6 van Veen benthic grabs, while 7 four-barrel, Multiple-"HAPS" (MHAPS) core deployments (modified corer from Kanneworff and Nicolaisen, 1973) were taken to collect undisturbed sediments for incubations (Figure 1). A total of 17 experimental cores were taken from the retrieved MHAPS cores using smaller plexiglas tubes (6.5 cm diameter, 20 cm length) to a sediment depth of 11.7 ± 0.6 cm core^{-1} to accommodate clam burrowing (Richman and Lovvorn, 2003). These core sediments were covered with filtered natural seawater (0.7 μm Whatman GF/F) from the sampling location avoiding sediment re-suspension. Prior to experimental food additions at t_0, a 24 h gut clearance period was given, 5 clams of both species were sampled as initial (t_0) samples, and one *N. radiata* and one *M. moesta* were added to each of the 17 experimental cores and given a 12 h burial period. Enriched (88.8 μg chl cm^{-2} core^{-1}) and non-enriched (110.1 μg chl cm^{-2} core^{-1}) algal food sources were then added as a single-pulse food event to each core at the beginning of the experiment (t_0) (Table 1). The slight difference in chl additions to the two treatments was due to different food stock availability after experiment 1. Core

assignment to experimental treatments was random to limit biases associated with MHAPS cast number, location, and naturally occurring infauna within the cores. After food addition at t_0, cores were incubated in the dark at 3.9 ± 0.1˚C for 18 d. Aeration of the water inside the cores was done using Tetra Whisper aquarium air pumps where each treatment received at least 6 h d^{-1} aeration, with increasing aeration time as cores were removed throughout the experiment. Evaporated seawater from cores due to bubble aeration and open-air exchange across perforated core caps was regularly replaced with filtered, natural seawater. Water salinity (S= 31-36) and dissolved oxygen (8 ± 1% O_2 loss over 18h during non-aeration periods) were monitored with YSI 85/50 sensors. Three enriched and three non-enriched treatment cores were removed from the experiment on days 5, 10 and 18 (note: day 10 of the non-enriched treatment only had two replicates). Sediments were sectioned (1 cm down core), clams removed and noted for burial depth, and both kept frozen at -20˚C until later analysis. All experimental clams survived until sampling occurred and were used for stable isotope analysis. Since one *N. radiata* and one *M. moesta* were added to each core, feeding of the two species may not have been independent.

In all feeding experiments, qualitative behavioral observations were made regularly for at least 30 s on clam movement, siphon and/or foot activity, apparent feeding activity, and shell gape. Rare events such as fecal pellet excretion and reproductive events were observed until completion. In experiment 1, clams were observed a minimum of once per day; core sediments and clams in experiments 2 and 3 were observed every six hours when aeration of the sediments cores was changed.

Laboratory preparation

For all three experiments, clams were thawed and soft tissue removed from the shell and a small part of the foot muscle tissue dissected. Muscle and remaining body samples were dried for 24 h at 60°C and weighed to the nearest 0.1 mg using an Ohaus Adventurer digital scale. A modified Folch et al. (1957) lipid extraction process was used to remove and isolate crude lipid extracts from both clam muscle and body tissues. Briefly, the muscle sample and the remaining whole body were extracted separately in chloroform-methanol (2:1) solution for 24 h with replacement of solvent as needed until the extraction solution remained clear. The resulting crude lipid extracts were dried with continuous-flow N_2 gas, weighed, and stored at -20°C until analysis. Muscle and body samples were dried again, weighed for total dry mass after lipid extraction, and finely ground with a mortar and pestel. In total, three samples were taken from each clam for stable isotope analysis: **muscle** is the lipid-extracted foot muscle tissue dissected from each clam. **Remaining body** is the lipid-extracted, homogenized remainder of all clam soft tissue. These remaining body values were used in mass-balance calculations for lipid-extracted (LEX) body and whole clam values and are not reported separately in this study. The **lipid** portion is the crude lipid extract combined from both the muscle and remaining body samples for each clam.

C and N isotopic analysis

Tissue samples (muscle and remaining body), lipid extracts, and algal filters were analyzed for elemental composition (carbon and nitrogen [μg]) using a Carlo Erba

elemental analyzer (EA) plumbed into a ThermoElectron Delta V Plus continuous-flow isotope ratio mass spectrometer (IRMS) through a Conflo III system interface at the Alaska Stable Isotope Facility (University of Alaska Fairbanks). Stable isotope ratios are expressed in delta notation (below) relative to international standards (Vienna Pee Dee Belemnite for carbon and atmospheric air for nitrogen).

$$\delta R\ (‰) = (R_{sample}/R_{standard} - 1) \cdot 1{,}000 \quad (1)$$

δR is the ^{13}C or ^{15}N of the sample measured in per mil (‰) and R is the $^{13}C/^{12}C$ or $^{15}N/^{14}N$ of the sample or standard. Analytical precision on the algal filter samples was ± 0.5‰ for $\delta^{13}C$ and ± 0.2‰ for $\delta^{15}N$, while clam samples were ± 0.3‰ for $\delta^{13}C$ and ± 0.5‰ for $\delta^{15}N$.

Weighted-means (mass-balanced) isotopic values for each clam were reconstructed in two ways. First, **lipid extracted (LEX) body** values refer to the mass-balanced isotopic values derived from the following lipid-extracted samples: muscle isotope values and mass, as well as lipid-extracted remaining body isotope values and mass:

$$\delta X_{LEX\ Body}\ (‰) = [(\delta R_{muscle} \cdot M_{muscle}) + (\delta R_{remaining\ body} \cdot M_{remaining\ body})] / (M_{muscle} + M_{remaining\ body}) \quad (2)$$

where $\delta X_{LEX\ Body}$ (‰) is the reconstructed $\delta^{13}C$ or $\delta^{15}N$ value, δR is the $\delta^{13}C$ or $\delta^{15}N$ of the muscle or remaining body tissue, and M is the total mass of muscle or remaining body measured to the nearest 0.1 mg.

Second, **whole clam** isotopic values refer to the mass-balance isotopic values derived from lipid-extracted muscle isotope values, lipid-extracted muscle mass, lipid-

extracted remaining body isotope values, lipid-extracted remaining body mass, lipid extract isotope values, and lipid extract mass:

$$\delta X_{\text{Whole Clam}}\,(‰) = [(\delta R_{\text{muscle}} \cdot M_{\text{muscle}}) + (\delta R_{\text{remaining body}} \cdot M_{\text{remaining body}}) + (\delta R_{\text{lipid}} \cdot M_{\text{lipid}})] / (M_{\text{muscle}} + M_{\text{remaining body}} + M_{\text{lipid}}) \quad (3)$$

where $\delta X_{\text{Whole Clam}}$ (‰) is the reconstructed $\delta^{13}C$ or $\delta^{15}N$ value, δR is the $\delta^{13}C$ or $\delta^{15}N$ of the muscle, remaining body tissue, or lipid extracts, and M is the total mass of muscle, remaining body tissue, or lipid extracts measured to the nearest 0.1 mg. Similar mass-balance procedures were used to calculate C:N ratios of LEX body and whole clams, with those ratios taking the place of isotopic values in the equations 2 and 3 above. The body components that constitute whole clam total dry mass were variable in weight for individual clams; however, the average composition of whole clam isotopic samples was approximately 7% muscle, 15% lipid, and 78% remaining body of total dry mass (±3% SD for all components). It should be noted that the true contribution of muscle to the mass-balanced tissue values was likely underestimated, as it was based only on the proportional mass of the sampled foot muscle and did not account for other muscle tissue present in clams, such as siphon, mantle, etc.

Isotopic differences between sample types and mass-balanced body portions result from isotopic routing within an organism, which are referred to as tissue-specific discrimination factors (Bearhop et al., 2002; Martinez del Rio et al., 2009). One-way ANOVA and Tukey HSD (Honestly Significant Differences) Post-hoc multiple comparison tests were used to identify significant differences (α=0.05) in naturally occurring isotope ratios of tissues, lipids, and reconstructed clam values in initial t_0

clams. These and all following statistical analyses were conducted within the SYSTAT Version11 software package.

Isotopic tracer and turnover modeling

A stable isotope mixing model was used to track the isotopic tracer assimilation in enriched treatment clam samples over time (Robinson, 2001; McMahon et al., 2006):

$$X_{tracer}\ (\%) = [(\delta R_{sample} - \delta R_{initial}) / (\delta R_{algal\ tracer} - \delta R_{initial})] \cdot 100 \quad (4)$$

where X_{tracer} is the fraction of the algal isotopic tracer incorporated into the clam samples (%), δR_{sample} is the $\delta^{13}C$ or $\delta^{15}N$ value of the clam sample or mass-balanced sample, $\delta R_{algal\ tracer}$ is the mean enriched algal food $\delta^{13}C$ or $\delta^{15}N$ value, and $\delta R_{initial}$ is the mean initial (t_0) clam $\delta^{13}C$ or $\delta^{15}N$ value of the respective sample type.

Isotopic (‰) or tracer (%) assimilation rates refer to the linear rate of change in tissue isotopic values or tracer incorporation over experimental time, which is assumed to be due to the uptake and assimilation of the enriched algal isotopic values.

$$X_{sample} = X_{assimilation\ rate} \cdot (time) + X_{initial} \quad (5)$$

X_{sample} refers to the isotopic (‰) or tracer (%) value of the experimental sample, $X_{assimilation\ rate}$ is the model-produced isotopic (‰ d^{-1}) or tracer (% d^{-1}) assimilation rate coefficient, time is the experimental day of X_{sample}, and $X_{initial}$ is the initial mean isotopic value from clams sampled from t_0 or percent tracer incorporation set at zero. Muscle, lipid, and mass-balanced isotopic and tracer values in all three experiments were analyzed with general linear regression to measure isotopic assimilation rates over time.

Additionally, for clams within experiments 2 and 3 in 2010 cores an isotopic turnover regression model was applied to isotopic and tracer data (Tieszen et al., 1983; Cerling et al., 2007; Kaufman et al., 2008):

$$\delta R_{time} = \delta R_{final} + (\delta R_{initial} - \delta R_{final}) \cdot e^{(-c \cdot time)} \quad (6)$$

where δR_{time} (‰) is the muscle, lipid, or mass-balanced $\delta^{13}C$ and $\delta^{15}N$ isotopic value at time t (d), δR_{final} (‰) is the estimated asymptotically approached final $\delta^{13}C$ or $\delta^{15}N$ value, $\delta R_{initial}$ is the initial $\delta^{13}C$ or $\delta^{15}N$ value, c (d^{-1}) is the estimated fractional turnover rate constant, and time is the number of experimental days after t_0. For the calculation of tracer (%) instead of isotope (‰) turnover, all δR values in the above equation (5) were replaced with X_{tracer} values from equation (4). In this case, $R_{initial}$ was 0% as no algal tracer had been incorporated. This approach estimated experimental isotopic and tracer turnover, which refers to the complete asymptotic replacement of the clams' initial isotopic or tracer value with that of the altered food source. Only turnover model correlations with $r^2 > 0.30$ were used to calculate half-life time (HL, d), at which 50% of the turnover has occurred toward the estimated asymptote (Cerling et al., 2007);

$$HL = \ln(2) / c \quad (7)$$

where c (d^{-1}) is the fractional turnover rate constant from the non-linear regression model for each sample type. These half-life estimates are experimentally specific to this study, and are different than many literature based half-life estimates, as clams in the core experiments did not necessarily feed directly only on the added enriched feed, but also on other organic material in the core. Furthermore, bacteria and meiofauna might have altered the feed signature within the cores over time. To estimate the food web related

changes in core experiments, I calculated the linear regressions of isotope change versus time across two specific time periods in experiments 2 and 3 (Day 0 – Day 5 and Day 5 – Day 18). This was done to further determine if the earlier (large isotopic increases) and/or later (slow increases) periods of clam isotopic turnover in cores were similar to linear assimilation trends calculated for isolated feeding of *N. radiata*.

Jar and sediment core experiment comparisons

Carbon and nitrogen tracer assimilation (from equation 4) was compared between *N. radiata* from the 2009 jar experiments and *N. radiata* from the 2010 sediment core experiments for whole clam values. I assumed that *N. radiata* in the jar experiments only fed on the provided algal food. In contrast, *N. radiata* in core experiments were assumed to have access to both the added algal food and other potential, labile food sources already present in core sediments, which provided an isotopically unknown, second food source. In addition to the comparison between *N. radiata* assimilation in jar and in core experiments, I also compared tracer assimilation for whole clams between *N. radiata* and *M. moesta* in core experiments to determine species-specific nitrogen and carbon assimilation differences under the same environmental and food conditions. Two-sample t-tests ($\alpha = 0.05$) were performed within several sampling time intervals to determine significant differences in tracer uptake. For comparison of experiments 1 and 2, sampling periods compared were day 0, days 5 and 6, days 10 and 12, and days 18 and 20 because sampling days varied slightly between the two experimental set ups. Experiments 2 and 3 time periods were grouped as day 0, day 5, day 10 and day 18 as sampling days matched

for both clam species within the cores. These differences between experimental sampling groups were established in direct comparisons using ^{13}C and ^{15}N tracer assimilation ratios, where *Nuculana radiata* comparisons included $^{13}C_{tracer}$ jars: $^{13}C_{tracer}$ cores and $^{15}N_{tracer}$ jars: $^{15}N_{tracer}$ cores. Species comparisons from the core experiments included $^{13}C_{tracer}$ *M. moesta*: $^{13}C_{tracer}$ *N. radiata* and $^{15}N_{tracer}$ *M. moesta*: $^{15}N_{tracer}$ *N. radiata*. Additional general linear regressions were conducted across these experimental comparisons for slope estimates for overall C and N tracer uptake.

$$N_{tracer} = C_{tracer} \cdot (\text{slope}) + \text{y-intercept} \quad (8)$$

These were used to calculate the relative percent difference in the enriched algal food contribution to the diet for *N. radiata* in the two experimental set-ups, as well as for *N. radiata* and *M. moesta* in the core experiments.

All C and N tracer values in the above whole clam comparisons were recalculated prior to analyses to correct for negative tracer values associated with sediment core *N. radiata* individuals. This was necessary as a result of calculating original tracer values (equation 4) relative to initial, t_0 clam isotopic means. Thus, a few experimental *N. radiata* with low tracer incorporation combined with a relatively low pre-experiment isotopic value showed negative tracer uptake, when in reality, tracer enrichment was similar to those experimental clams above t_0 mean values. Specific corrected values were calculated for all comparative carbon ($^{13}C_{corrected} = {}^{13}C_{original} + 0.126$) and nitrogen ($^{15}N_{corrected} = {}^{15}N_{original} + 0.192$) whole clam tracer values.

Results

Isotopic composition of algal food and initial clams

The enriched algal food had a $\delta^{13}C$ value of 249 ± 17‰ and a $\delta^{15}N$ of 907 ± 67‰ (n= 8 each), while the non-enriched algal food was -24.9 ± 1.5‰ for $\delta^{13}C$ and 3.1 ± 4‰ for $\delta^{15}N$ (n= 8 each). The weight-based C:N ratios were not different between the two algal stocks (Student's T-test, p= 0.61) with an overall ratio of 11.24 ± 1.72. Additionally, weight-based POC:chl ratios for enriched and non-enriched food supplies was 65.6 ± 9.5 (n= 10).

In all initial t_0 *N. radiata* and *M. moesta*, isotopic values of muscle, lipids, mass-balanced whole clams and LEX body ranged from -18.9 to -26.3‰ in $\delta^{13}C$ and 3.6 to 10.1‰ in $\delta^{15}N$; variability within each sample type was less than 1‰ standard deviation, with the exception of $\delta^{15}N$ lipids for both species (1.1 to 2.6‰ SD) (Table 2). Sample-specific isotopic variation existed between 2009 and 2010 *N. radiata* and 2010 *M. moesta* (Tukey HSD, $p \leq 0.05$; Table 2). Initial *N. radiata* in 2009 and 2010 (for experiments 1 and 2, respectively) were significantly different in $\delta^{13}C$ of muscle and LEX body tissue, but did not differ in $\delta^{13}C$ of lipids or whole clams, or in $\delta^{15}N$ of any sample type. *Macoma moesta* initial samples in 2010 were significantly ^{13}C enriched in whole clam and ^{15}N depleted in muscle, LEX body, and whole clam compared to 2010 *N. radiata*. Larger isotopic separation was seen between 2010 *M. moesta* and 2009 *N. radiata*, as $\delta^{13}C$ and $\delta^{15}N$ values of all sample types were significantly different, with the exception of lipids. Despite these species-specific differences, relationships in isotopic composition

Table 2: Initial (t_0) *N. radiata* and *M. moesta* values (n= number of clams). Mean (±SD) of isotopic values from each sample type are presented for $\delta^{13}C$, $\delta^{15}N$, and weight-based C:N ratios. Groups refer to the results of Tukey HSD Post-hoc comparisons ($p< 0.05$) among years and species for the same sample type. Different group numbers were significantly different.

Sample	Initial (t_0) Clams	n	$\delta^{13}C$ ‰ (SD)	$\delta^{13}C$ Groups	$\delta^{15}N$ ‰ (SD)	$\delta^{15}N$ Groups	C:N (SD)	C:N Groups
Muscle	2009 *N. radiata*	14	-19.88 (0.28)	1	9.34 (0.71)	1	3.46 (0.28)	1
	2010 *N. radiata*	5	-19.24 (0.27)	2	10.08 (0.44)	1	3.22 (0.08)	1
	2010 *M. moesta*	5	-19.06 (0.39)	2	8.36 (0.94)	2	3.52 (0.33)	1
Lipid	2009 *N. radiata*	13	-26.34 (0.46)	1	5.29 (1.07)	1	31.39 (19.95)	1
	2010 *N. radiata*	5	-26.17 (0.25)	1	3.66 (1.41)	1	52.16 (12.91)	2
	2010 *M. moesta*	5	-26.19 (0.14)	1	3.61 (2.59)	1	58.12 (19.66)	2
LEX Body	2009 *N. radiata*	14	-19.86 (0.36)	1	9.29 (0.23)	1	3.53 (0.12)	1
	2010 *N. radiata*	5	-19.19 (0.24)	2	9.58 (0.56)	1	3.41 (0.06)	1
	2010 *M. moesta*	5	-18.88 (0.18)	2	8.38 (0.43)	2	3.34 (0.07)	2
Whole Clam	2009 *N. radiata*	13	-20.90 (0.51)	1	8.66 (0.29)	1	4.37 (0.40)	1
	2010 *N. radiata*	5	-20.34 (0.27)	1	8.61 (0.60)	1	4.50 (0.19)	1
	2010 *M. moesta*	5	-19.94 (0.13)	2	7.72 (0.53)	2	4.30 (0.29)	1

among sample types were similar for both species with lipids being the most depleted in both $\delta^{13}C$ and $\delta^{15}N$. Discrimination factors of lipids compared to whole clam values were approximately -6‰ in $\delta^{13}C$ and -4‰ in $\delta^{15}N$ in both species. Similarly, whole clam values tended to be depleted by approximately -1‰ in $\delta^{13}C$ and $\delta^{15}N$ compared to muscle and LEX body tissues, while the latter two were similar in C and N isotopic values in both species. C:N mass ratios for both species ranged from 3.2-4.5 in muscle and mass-balanced samples, while C:N ratios in lipids were highly variable and much higher (31.4-58.1, Table 2). High C:N ratios in lipids was attributed to low nitrogen content in lipid fraction samples (range N% = 0.5-5%), as lipid C:N ratios exponentially decreased with greater N content ($C:N_{lipid} = 62.2 \cdot N\%_{mass}^{-1.018}$, $r^2 = 0.95$). Nitrogen content was not found to be a definitive driver of lipid $\delta^{15}N$ values or variation.

Given the mean, t_0 isotopic compositions of clam samples and the high isotope values of the enriched food source, it was assumed clams feeding on the enriched algal food could increased their $\delta^{13}C$ by ~270‰ and their $\delta^{15}N$ by ~900‰ (Vander Zanden and Rasmussen, 2001; Post, 2002). Exact enrichment toward the enriched algal food isotopic values is clam sample type dependent based on their respective discrimination factors and differing assimilation/turnover rates. In contrast, clams feeding on the non-enriched food source were expected to have minor isotopic changes towards the food source with expected changes mostly within the variability of the isotopic measurements of the non-enriched food source.

Nuculana radiata *isotope assimilation: jar experiments*

Within the enriched feeding treatment of *N. radiata* in experiment 1, nearly all linear regressions of isotope assimilation over the 42 d experimental time revealed significant increases in isotope ratios over time, with the exception of muscle $\delta^{13}C$ (Figure 2A-D, Figure 3A-D, Table 3). Muscle tissue had no significant change in $\delta^{13}C$ and a relatively small, though significant, increase in $\delta^{15}N$ (4‰) over 42 d at 0.09‰ d^{-1} (Figure 2A and 3A). Lipids enriched the most by nearly 3‰ in $\delta^{13}C$ and 10‰ in $\delta^{15}N$ over 42 d (Figure 2B, 3B) with assimilation rates of 0.05‰ d^{-1} in $\delta^{13}C$ and 0.23‰ d^{-1} in $\delta^{15}N$ (Table 3). LEX body and whole clams had similar total increases (+2‰ in $\delta^{13}C$, +7‰ in $\delta^{15}N$; Figure 2C-D, Figure 3C-D) as well as similar assimilation rates (0.05‰ $\delta^{13}C$ d^{-1}, 0.17‰ $\delta^{15}N$ d^{-1}). The separate linear regressions of *N. radiata* for the time periods days 0-6 and days 6-20 showed high initial tracer incorporation (days 0-6) followed by lower assimilation rates during the later period for muscle, LEX body and whole clam samples (Table 3). Conversely, lipid fractions showed early depletion in isotopic and tracer values, then a rapid increase in C and N assimilation rates (Table 3). Overall, the maximum increases in all clam sample isotopic values at the end of the experiment were far below the enriched algal food values (250‰ in $\delta^{13}C$ and 900‰ in $\delta^{15}N$).

While isotopic assimilation rates differed for the various sample types in *N. radiata*, ^{13}C and ^{15}N assimilation was more similar for all samples when standardized as tracer enrichment (Figure 4A-D and Figure 4E-H; Table 3). Tracer assimilation rates in sample types for both isotopes increased as: muscle < LEX body ≤ whole clam < lipids (Table 3). Whole clam tracer assimilation rates were 0.02% d^{-1} in both carbon and

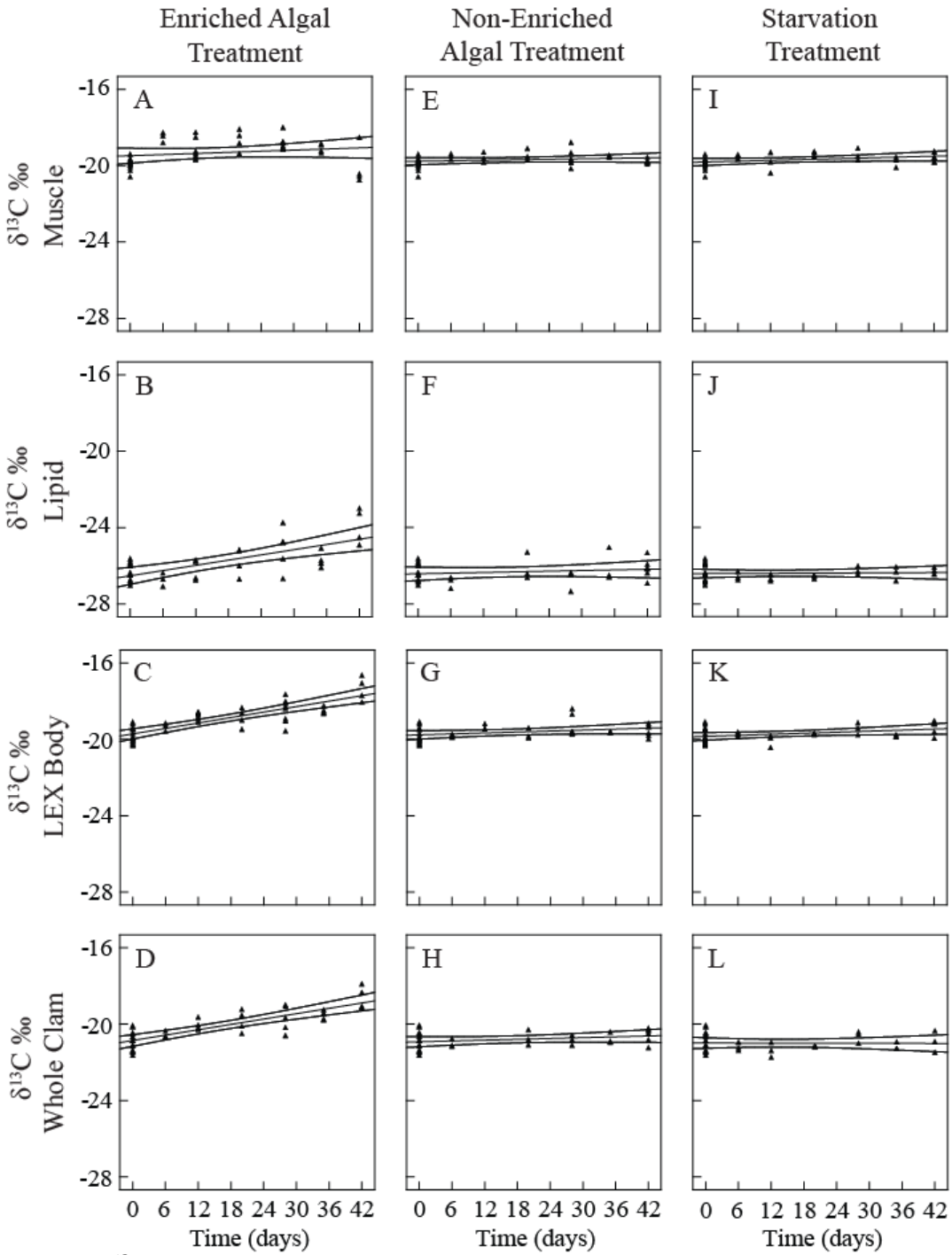

Figure 2: $\delta^{13}C$ isotopic assimilation in the 2009 jar experiment where *N. radiata* was offered enriched food (A-D), non-enriched food (E-H), or starved (I-L). Lines are mean linear regressions with 95% confidence.

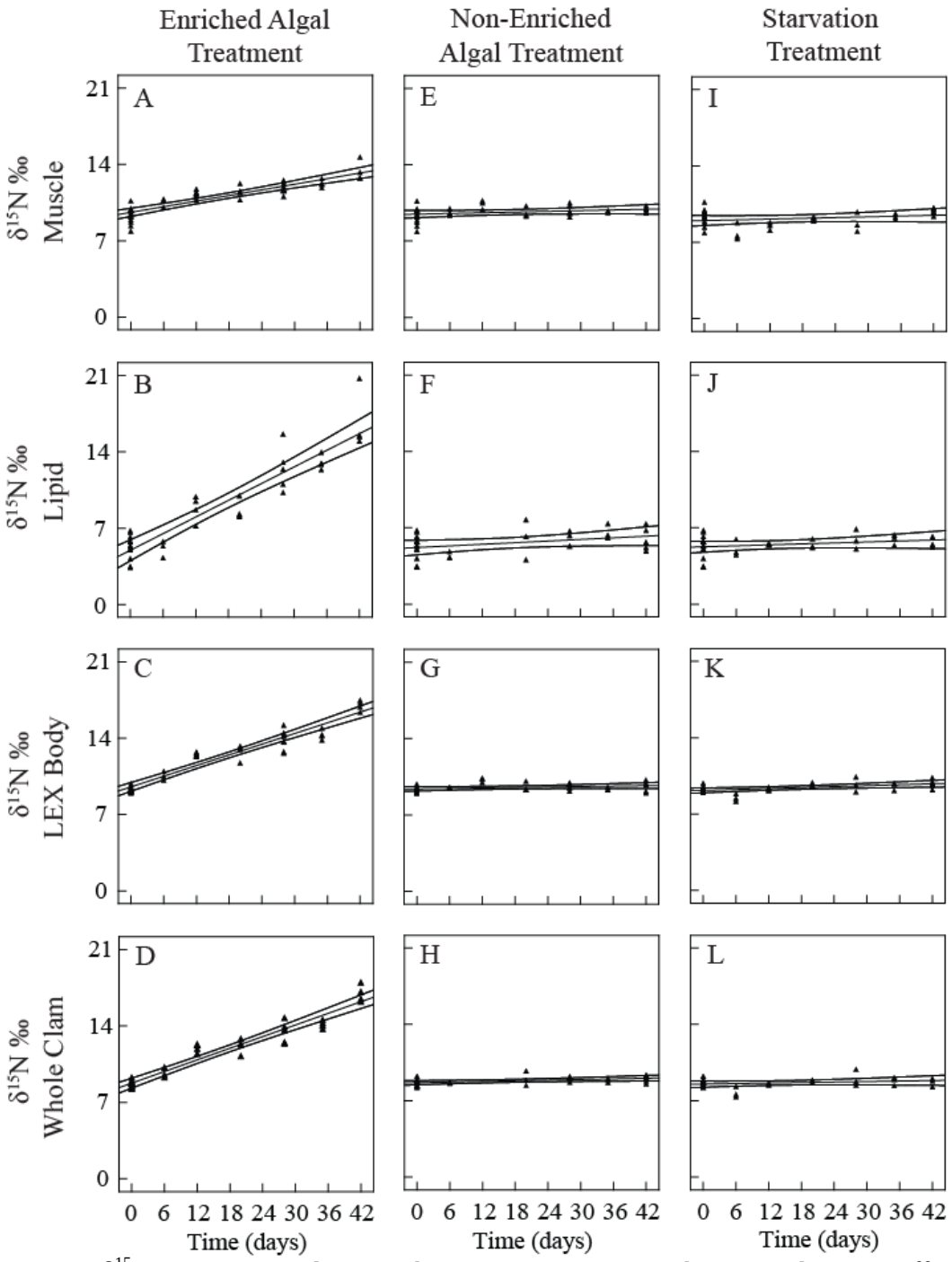

Figure 3: $\delta^{15}N$ isotopic assimilation in the 2009 jar experiment where *N. radiata* was offered enriched food (A-D), non-enriched food (E-H), or starved (I-L). Lines are mean linear regressions with 95% confidence.

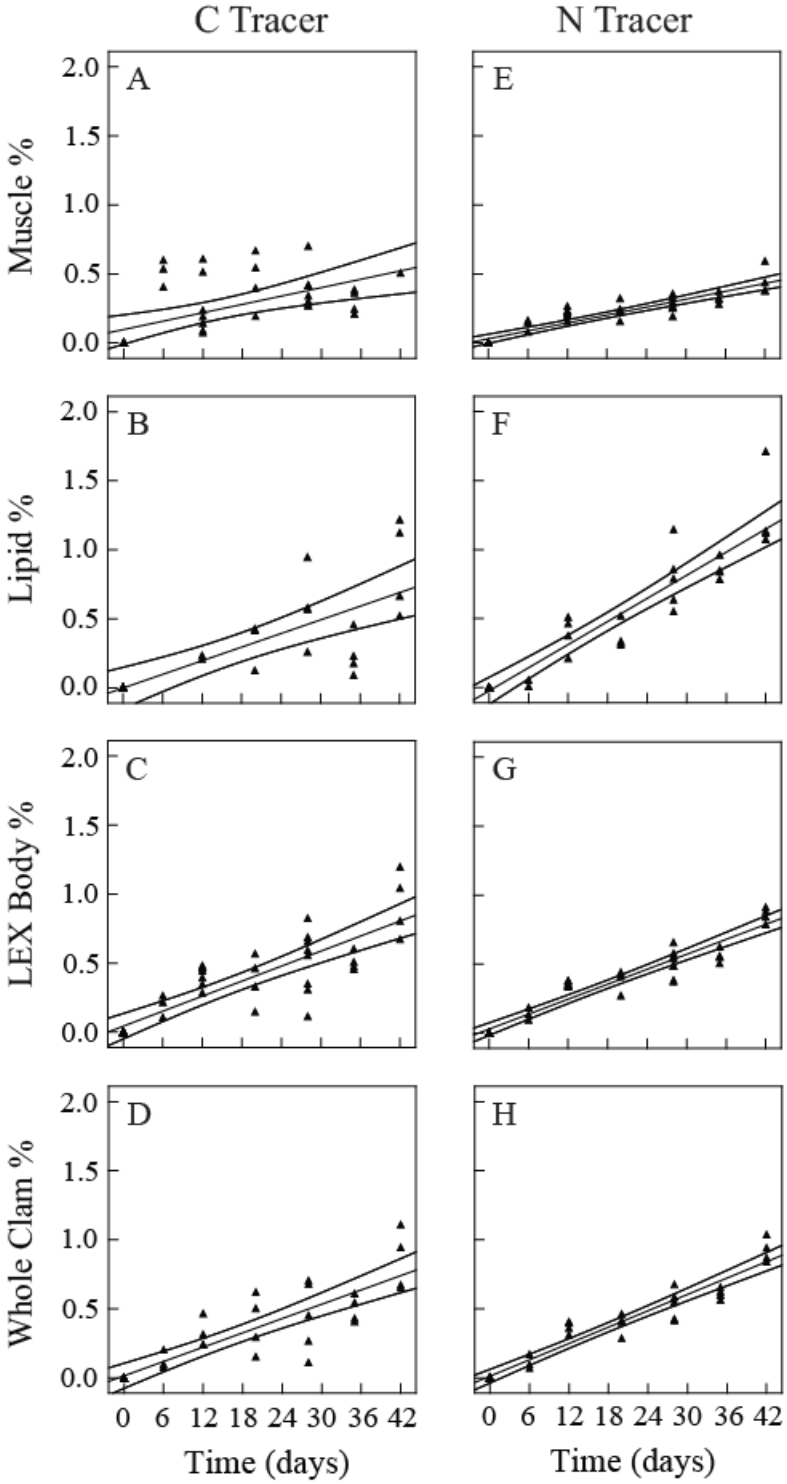

Figure 4: Carbon and nitrogen tracer assimilation in the 2009 jar experiment of *N. radiata* fed with enriched algal food. Lines are mean linear regressions with 95% confidence.

Table 3: Linear regression analyses of the stable isotope and tracer data for *N. radiata* in enriched (E), non-enriched (NE), and starvation (S) 2009 isolated jar treatments. Linear regression statistics are documented with ANOVA, r^2 coefficient, and isotopic/tracer assimilation rates over the entire duration of the experiment (day 0 - day 42), during the early sampling period across all experiments (day 0 - day 6), and over the later remaining comparative sampling period across all experiments (day 6 - day 20). Values represented in bold font are statistically significant ($p < 0.05$).

Jar *N. radiata*			Day 0 - Day 42			Day 0 - Day 6			Day 6 - Day 20		
			ANOVA	Linear Regression		ANOVA	Linear Regression		ANOVA	Linear Regression	
Isotope	Treatment	Sample	p-value	r^2	Assimilation (d^{-1})	p-value	r^2	Assimilation (d^{-1})	p-value	r^2	Assimilation (d^{-1})
$\delta^{13}C$ ‰	E	Muscle	0.18	0.04	0.010	**<0.001**	**0.80**	**0.038**	0.85	0.00	-0.003
	E	Lipids	**<0.001**	**0.49**	**0.046**	0.20	0.11	-0.063	**0.05**	**0.36**	**0.034**
	E	LEX Body	**<0.001**	**0.69**	**0.048**	0.07	0.20	0.079	0.12	0.19	0.016
	E	Whole Clam	**<0.001**	**0.68**	**0.047**	0.28	0.08	0.057	**0.04**	**0.39**	**0.025**
$\delta^{15}N$ ‰	E	Muscle	**<0.001**	**0.79**	**0.086**	**0.02**	**0.33**	**0.032**	**0.02**	**0.40**	**0.033**
	E	Lipids	**<0.001**	**0.87**	**0.225**	0.83	0.00	-0.025	**0.01**	**0.52**	**0.117**
	E	LEX Body	**<0.001**	**0.92**	**0.163**	**<0.001**	**0.79**	**0.210**	**0.001**	**0.64**	**0.075**
	E	Whole Clam	**<0.001**	**0.93**	**0.178**	**<0.001**	**0.62**	**0.167**	**0.002**	**0.67**	**0.086**
$\delta^{13}C$ ‰	NE	Muscle	0.18	0.05	0.004	0.05	0.23	0.010	0.70	0.02	0.003
	NE	Lipids	0.33	0.03	0.006	0.09	0.20	-0.086	0.17	0.41	0.027
	NE	LEX Body	**0.04**	**0.11**	**0.008**	0.94	0.00	-0.003	0.65	0.03	0.004
	NE	Whole Clam	0.10	0.09	0.007	0.72	0.01	-0.019	0.34	0.22	0.010
$\delta^{15}N$ ‰	NE	Muscle	0.07	0.09	0.010	0.50	0.03	0.008	0.99	0.00	0.000
	NE	Lipids	**0.03**	**0.15**	**0.025**	0.21	0.11	-0.139	0.22	0.34	0.056
	NE	LEX Body	0.06	0.10	0.007	0.16	0.12	0.033	0.78	0.01	0.004
	NE	Whole Clam	**0.01**	**0.24**	**0.010**	0.91	0.00	-0.003	0.39	0.19	0.013
$\delta^{13}C$ ‰	S	Muscle	**0.03**	**0.14**	**0.007**	0.06	0.22	0.010	0.60	0.04	0.006
	S	Lipids	0.76	0.00	0.001	0.45	0.04	-0.036	0.90	0.00	-0.001
	S	LEX Body	**0.01**	**0.17**	**0.009**	0.74	0.01	0.013	0.92	0.00	0.001
	S	Whole Clam	0.93	0.00	0.000	0.36	0.06	-0.048	0.91	0.00	0.001
$\delta^{15}N$ ‰	S	Muscle	0.15	0.06	0.011	**0.01**	**0.40**	**-0.040**	**0.01**	**0.61**	**0.045**
	S	Lipids	0.13	0.08	0.015	0.75	0.01	-0.035	0.34	0.13	0.001
	S	LEX Body	**0.001**	**0.28**	**0.014**	**<0.001**	**0.62**	**-0.132**	**<0.001**	**0.85**	**0.043**
	S	Whole Clam	0.15	0.07	0.008	0.00	0.56	-0.150	**0.004**	**0.72**	**0.038**
C Tracer %	E	Muscle	0.16	0.05	0.004	**<0.001**	**0.97**	**0.014**	0.85	0.00	-0.001
	E	Lipids	**<0.001**	**0.52**	**0.017**	**<0.001**	**0.59**	**-0.023**	**0.05**	**0.36**	**0.012**
	E	LEX Body	**<0.001**	**0.74**	**0.018**	**<0.001**	**0.88**	**0.033**	0.12	0.19	0.006
	E	Whole Clam	**<0.001**	**0.77**	**0.017**	**<0.001**	**0.81**	**0.021**	**0.04**	**0.39**	**0.009**
N Tracer %	E	Muscle	**<0.001**	**0.86**	**0.010**	**<0.001**	**0.91**	**0.004**	**0.02**	**0.40**	**0.004**
	E	Lipids	**<0.001**	**0.88**	**0.028**	0.40	0.05	-0.003	**0.01**	**0.52**	**0.013**
	E	LEX Body	**<0.001**	**0.92**	**0.018**	**<0.001**	**0.92**	**0.023**	**0.001**	**0.64**	**0.008**
	E	Whole Clam	**<0.001**	**0.94**	**0.020**	**<0.001**	**0.85**	**0.019**	**0.002**	**0.67**	**0.010**

nitrogen (Table 3), which accrued to a total assimilation of 0.7% of either tracer over the 42 d experimental period (Figure 4D, 4H).

Nuculana radiata fed non-enriched algal food showed little isotopic change over the experimental period and most linear regression analyses were not significant (Table 3, Figure 2E-H and Figure 3E-H). The non-enriched algal food isotopic values were similar to those of lipid fractions in both t_0 and experimental clams; thus, no significant changes in *N. radiata* samples were expected, even within the relatively fast assimilating lipid fractions. The *N. radiata* samples from the starvation treatment also showed no statistically significant change in either $\delta^{13}C$ (Figure 2I-L) or $\delta^{15}N$ (Figure 3I-L), except for the small and significant enrichment in $\delta^{13}C$ muscle values and in $\delta^{13}C$ and $\delta^{15}N$ LEX body tissue (Table 3).

Nuculana radiata *isotope turnover and assimilation: sediment core experiments*

Isotopic values of *N. radiata* in enriched sediment core incubations were variable for all sample types for $\delta^{13}C$ and $\delta^{15}N$. Non-linear correlation coefficients indicated little enrichment over 18 d for most sample types and isotopes (Table 4). Two clams may have had a large effect on turnover estimates, one each on days 5 and 10 (Figure 5); however, they were not omitted from the analysis because of overall low sample size, standardized sample preparation techniques, and similarity for other experimental variables (clam size and weight, burrowing depth in cores, lipid content, C:N ratios). Half-life estimates for LEX body and whole clam for $\delta^{13}C$ and C_{tracer} (non-linear regression $r^2 > 0.30$; Table 4)

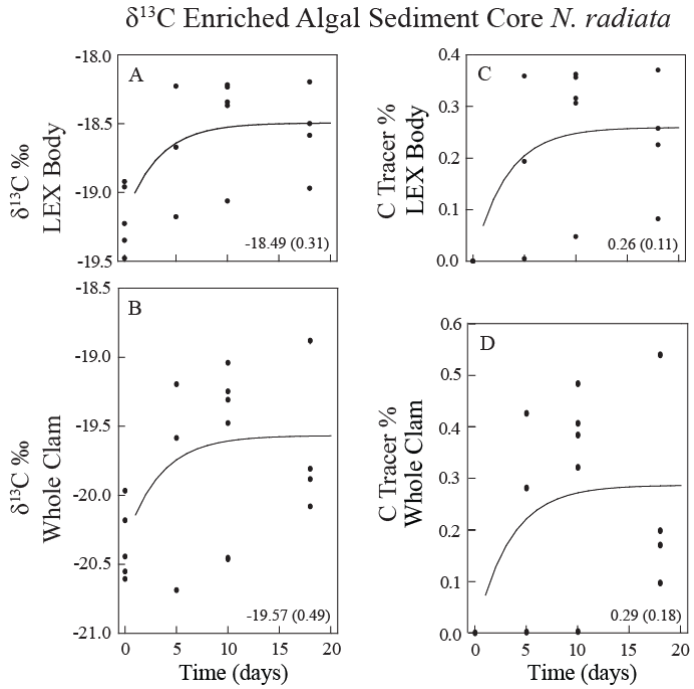

Figure 5: $\delta^{13}C$ isotopic turnover and tracer turnover of 2010 *N. radiata* mass-balanced LEX body and whole body in enriched algal treatments. In each graph, the bottom-right corner number is the estimated turnover asymptote value with standard deviation.

Table 4: Best fit, non-linear turnover regressions for *N. radiata* stable isotope and tracer data in enriched (E) and non-enriched (NE) 2010 sediment core treatments. Non-linear regression statistics include turnover model estimates (r^2), fractional turnover rate constant (c), and half-life (HL). Modeled c and HL estimates were only calculated when regression coefficients exceeded $r^2 \geq 0.30$. Experimentally comparable linear assimilation regression statistics are documented with ANOVA, r^2 coefficient, and isotopic/tracer assimilation rates during the first early sampling period (day 0 - day 5) and the later remaining comparative sampling period (day 5 - day 18). Values represented in bold font are statistically significant ($p < 0.05$).

Core *N. radiata*			Day 0 - Day 18			Day 0 - Day 5			Day 5 - Day 18		
			Best Fit Non-linear Regression			ANOVA	Linear Regression		ANOVA	Linear Regression	
Isotope	Treatment	Sample	r^2	c (d^{-1})	HL (d)	p-value	r^2	Assimilation (d^{-1})	p-value	r^2	Assimilation (d^{-1})
$\delta^{13}C$ ‰	E	Muscle	0.08	·	·	0.71	0.03	0.005	0.71	0.01	0.005
	E	Lipids	0.13	·	·	0.13	0.35	0.134	0.96	0.00	-0.001
	E	LEX Body	0.49	0.311	2.23	0.09	0.40	0.099	0.71	0.02	0.004
	E	Whole Clam	0.34	0.298	2.33	0.20	0.26	0.106	0.78	0.01	0.005
$\delta^{15}N$ ‰	E	Muscle	0.06	·	·	0.55	0.06	-0.007	0.85	0.00	-0.006
	E	Lipids	0.02	·	·	0.37	0.14	0.228	0.34	0.09	-0.054
	E	LEX Body	0.01	·	·	0.98	0.00	-0.002	0.30	0.11	-0.020
	E	Whole Clam	0.04	·	·	0.78	0.02	0.028	0.39	0.08	-0.023
$\delta^{13}C$ ‰	NE	Muscle	0.01	·	·	0.64	0.03	-0.004	0.94	0.00	0.000
	NE	Lipids	0.07	·	·	0.34	0.13	-0.034	0.57	0.03	-0.005
	NE	LEX Body	0.13	·	·	0.29	0.16	-0.031	0.62	0.03	-0.002
	NE	Whole Clam	0.17	·	·	0.46	0.08	-0.027	0.33	0.10	-0.007
$\delta^{15}N$ ‰	NE	Muscle	0.17	·	·	**0.03**	**0.51**	**-0.024**	0.61	0.03	-0.012
	NE	Lipids	0.07	·	·	0.60	0.04	-0.097	0.25	0.13	-0.064
	NE	LEX Body	0.18	·	·	0.06	0.42	-0.220	0.91	0.00	0.002
	NE	Whole Clam	0.33	-0.038	18.2	0.06	0.42	-0.199	0.47	0.05	-0.013
C Tracer %	E	Muscle	0.10	·	·	0.66	0.04	0.002	0.71	0.01	0.002
	E	Lipids	0.31	0.510	1.36	0.10	0.39	0.049	0.96	0.00	0.000
	E	LEX Body	0.54	0.311	2.23	**0.05**	**0.51**	**0.037**	0.71	0.02	0.002
	E	Whole Clam	0.35	0.298	2.33	0.16	0.30	0.039	0.78	0.01	0.002
N Tracer %	E	Muscle	0.00	·	·	0.19	0.27	-0.001	0.85	0.00	-0.001
	E	Lipids	0.03	·	·	0.22	0.24	0.025	0.34	0.09	-0.006
	E	LEX Body	0.00	·	·	0.94	0.00	0.000	0.30	0.11	-0.002
	E	Whole Clam	0.00	·	·	0.64	0.04	0.003	0.39	0.08	-0.003

were just over 2 d, which suggests quick response and uptake of the enriched algal food into clam tissue. Half-life estimates for lipid $\delta^{13}C$ was 1.4 d. The non-linear regression coefficient for lipid C_{tracer} was below 0.30 and no half-life or turnover was calculated for lipid C_{tracer}. For all enriched samples, very low $\delta^{13}C$ asymptote estimates (+1-2‰ above t_0 *N. radiata* values) were seen relative to the enriched algal food $\delta^{13}C$ value (250‰). Linear analyses for experimental days 0-5 and days 5-18 (Table 4) support the conclusion that no significant enrichment was observed.

In the non-enriched algal treatment, *N. radiata* isotopic and tracer turnover during experimental time did not follow the non-linear model in most samples as seen by very low regression coefficients ($r^2 < 0.30$, Table 4), except for whole clam $\delta^{15}N$. In the whole clam samples, isotopic values declined toward the $\delta^{15}N$ (~3‰) of the non-enriched algal food source, with a slow isotopic half-life of 18.2 d, while the slight decrease in isotope values was also seen in both C and N isotope linear regressions from days 0-5 and days 5-18 (Table 4).

Macoma moesta *isotope turnover and assimilation: sediment core experiments*

All *M. moesta* $\delta^{13}C$ samples under the isotopically enriched treatment showed isotopic enrichment over the experimental period with non-linear regression coefficients of $r^2 > 0.30$ (Table 5). $\delta^{13}C$ maximum asymptotic estimates (Figure 6A-D) showed isotopic increases of approximately 1‰ for muscle, 2‰ for lipids, 1.5‰ for LEX body, and 1.5‰ for whole clams compared to t_0 *M. moesta*. Half-life $\delta^{13}C$ estimates were 1.8 d for whole clams, 2.0 d for LEX body, 2.3 d for lipids, and 3.2 d for muscle (Table 5). $\delta^{15}N$

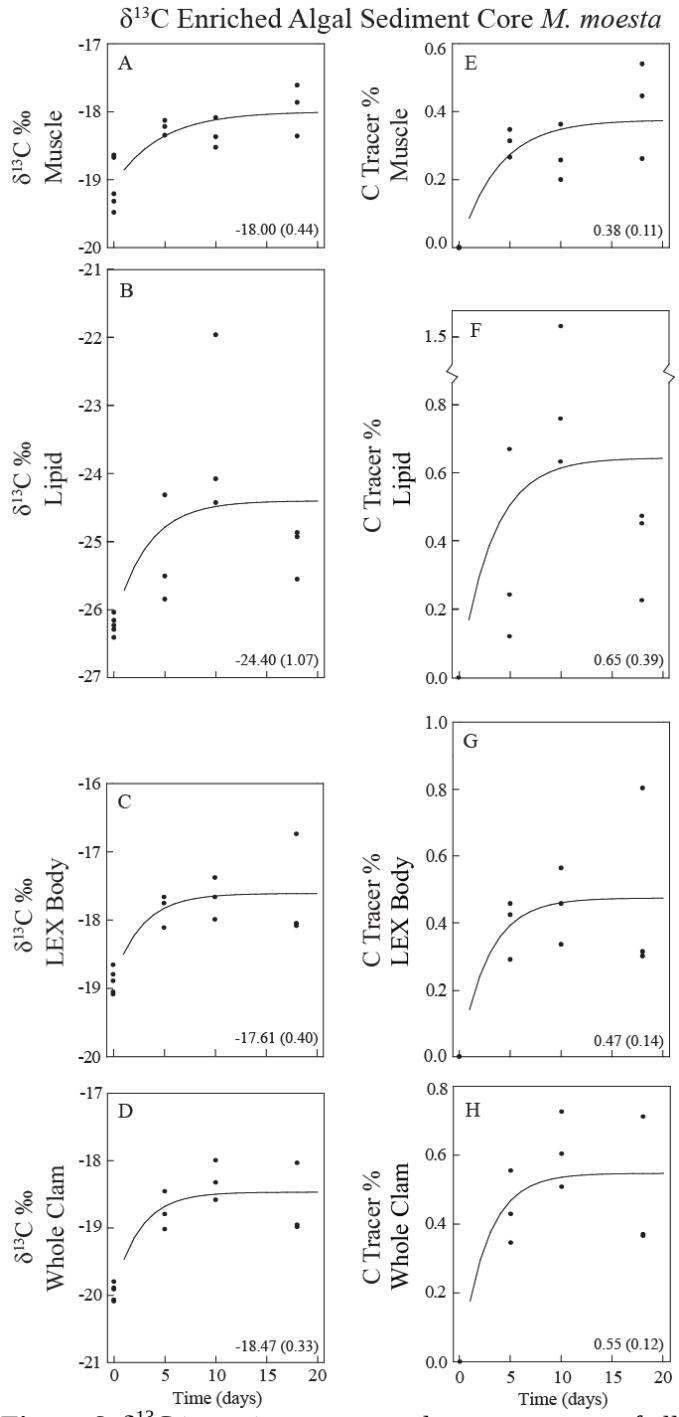

Figure 6: $\delta^{13}C$ isotopic turnover and tracer turnover of all 2010 *M. moesta* sample types in enriched algal treatment. In each graph, the bottom-right corner number is the estimated turnover asymptote value with standard deviation.

Table 5: Best fit, non-linear turnover regressions for *M. moesta* stable isotope and tracer data in enriched (E) and non-enriched (NE) 2010 sediment core treatments. Non-linear regression statistics include turnover model estimates (r^2), fractional turnover rate constant (c), and half-life (HL). Modeled c and HL estimates were only calculated when regression coefficients exceeded $r^2 \geq 0.30$. Experimentally comparable linear assimilation regression statistics are documented with ANOVA, r^2 coefficient, and isotopic/tracer assimilation rates during the first comparable sampling period (day 0 - day 5) and over the remaining comparative sampling period (day 5 - day 18). Values in bold font are statistically significant ($p < 0.05$).

Core *M. moesta*			Day 0 - Day 18			Day 0 - Day 5			Day 5 - Day 18		
			Best Fit Non-linear Regression			ANOVA	Linear Regression		ANOVA	Linear Regression	
Isotope	Treatment	Sample	r^2	c (d^{-1})	HL (d)	p-value	r^2	Assimilation (d^{-1})	p-value	r^2	Assimilation (d^{-1})
$\delta^{13}C$ ‰	E	Muscle	0.69	0.220	3.15	**0.01**	**0.68**	**0.033**	0.24	0.19	0.011
	E	Lipids	0.47	0.307	2.26	**0.03**	**0.58**	**0.201**	0.92	0.00	0.004
	E	LEX Body	0.74	0.353	1.96	**<0.001**	**0.90**	**0.211**	0.58	0.05	0.008
	E	Whole Clam	0.84	0.388	1.79	**<0.001**	**0.92**	**0.240**	0.77	0.01	0.004
$\delta^{15}N$ ‰	E	Muscle	0.23	·	·	0.31	0.17	0.032	0.83	0.01	0.006
	E	Lipids	0.18	·	·	0.58	0.05	0.206	0.73	0.02	0.026
	E	LEX Body	0.53	0.099	7.00	**0.04**	**0.54**	**0.204**	0.59	0.04	0.016
	E	Whole Clam	0.54	0.185	3.75	0.08	0.43	0.218	0.65	0.03	0.015
$\delta^{13}C$ ‰	NE	Muscle	0.04	·	·	0.56	0.04	0.006	0.77	0.01	0.003
	NE	Lipids	0.06	·	·	0.65	0.02	-0.013	0.70	0.02	-0.003
	NE	LEX Body	0.11	·	·	0.07	0.32	0.042	0.96	0.00	0.000
	NE	Whole Clam	0.13	·	·	**0.03**	**0.42**	**0.061**	0.55	0.04	-0.005
$\delta^{15}N$ ‰	NE	Muscle	0.02	·	·	0.61	0.03	0.011	0.93	0.00	-0.001
	NE	Lipids	0.06	·	·	0.93	0.00	0.022	0.24	0.15	-0.044
	NE	LEX Body	0.01	·	·	0.20	0.17	-0.066	0.37	0.09	0.011
	NE	Whole Clam	0.03	·	·	0.48	0.06	-0.047	0.96	0.00	0.001
C Tracer %	E	Muscle	0.81	0.262	2.65	**<0.001**	**0.98**	**0.012**	0.24	0.19	0.004
	E	Lipids	0.47	0.307	2.25	**0.03**	**0.59**	**0.073**	0.92	0.00	0.001
	E	LEX Body	0.76	0.353	1.96	**<0.001**	**0.95**	**0.079**	0.58	0.05	0.003
	E	Whole Clam	0.85	0.388	1.79	**<0.001**	**0.94**	**0.089**	0.77	0.01	0.002
N Tracer %	E	Muscle	0.44	0.310	2.23	0.12	0.35	0.004	0.83	0.01	0.001
	E	Lipids	0.25	·	·	0.28	0.19	0.023	0.73	0.02	0.003
	E	LEX Body	0.62	0.256	2.71	**0.01**	**0.67**	**0.023**	0.59	0.04	0.002
	E	Whole Clam	0.60	0.266	2.61	**0.02**	**0.60**	**0.024**	0.65	0.03	0.002

enrichment values for LEX body and whole clams approached asymptotic values 2-3‰ greater than t_0 values, which was low compared to the isotopic values in the enriched algal food. Overall, half-life $\delta^{15}N$ and N_{tracer} values were longer than similar estimates for $\delta^{13}C$ and C_{tracer} in *M. moesta* samples. High enrichment was also reflected in the linear regressions from clam samples in days 0-5 where highly significant isotope and tracer increases were observed with maximum increases in $\delta^{13}C$ of 0.24‰ d^{-1} and C_{tracer} of 0.09% d^{-1} in whole clam tissues (Table 5). Additionally, the non-significant regression results from days 5-18 support that an asymptotic turnover plateau was reached as modeled in my non-linear best fit analyses.

Non-enriched *M. moesta* isotope ratios were variable with low non-linear regression coefficients (Table 5). This was expected due to the similarity of non-enriched food and t_0 clam isotopic values, as was the case for most *N. radiata* non-enriched treatment samples.

Experimental comparisons of whole clam tracer assimilation

Comparisons of C_{tracer} and N_{tracer} assimilation in *N. radiata* mass-balanced whole clams samples were made for those individuals fed enriched algal food in 2009 jar experiments and those offered enriched algal food in 2010 core experiments. Significantly (two-sample t-tests) lower $^{15}N_{tracer}$ assimilation occurred in clams from cores compared to those from jars at days 10/12 (difference of 0.35% ± 0.15, $p= 0.002$) and days 18/20 (difference of 0.44% ± 0.13, $p< 0.001$), while absolute assimilation of $^{13}C_{tracer}$ was similar

between clams in jars and cores (Figure 7A). These patterns also caused higher N_{tracer}:C_{tracer} ratios (see equation 8) in jar compared to core animals with N_{tracer}:C_{tracer} incorporation ratios of 1.08 for *N. radiata* in jars (r^2= 0.92, p< 0.001; Figure 7B) and 0.43 for *N. radiata* in cores (r^2= 0.83, p< 0.001; Figure 7B), revealing a 60% discrepancy in slope estimates.

The comparison of C_{tracer} and N_{tracer} assimilation between *N. radiata* and *M. moesta* whole clams within the 2010 sediment cores (Figure 8A) showed significantly (two-sample t-tests) lower carbon and nitrogen tracer assimilation for *N. radiata* at day 10 ($^{15}N_{tracer}$: p= 0.013, difference of 0.26 ± 0.18%; $^{13}C_{tracer}$: p= 0.035, difference of 0.31 ± 0.27%) and day 18 ($^{15}N_{tracer}$: p= 0.036, difference of 0.21 ± 0.19%). Overall, greater absolute $^{13}C_{tracer}$ and $^{15}N_{tracer}$ uptake by *M. moesta* over *N. radiata* (~2:1) was maintained over the duration of the core incubations. Although individual uptake rates were very different between species (see above), the ratios of N_{tracer}:C_{tracer} varied little over time with similar values for *N. radiata* (0.43, r^2= 0.83, p< 0.001, Figure 8B) and *M. moesta* (0.60, r^2= 0.95, p< 0.001, Figure 8B), revealing only a 28% discrepancy between slope estimates.

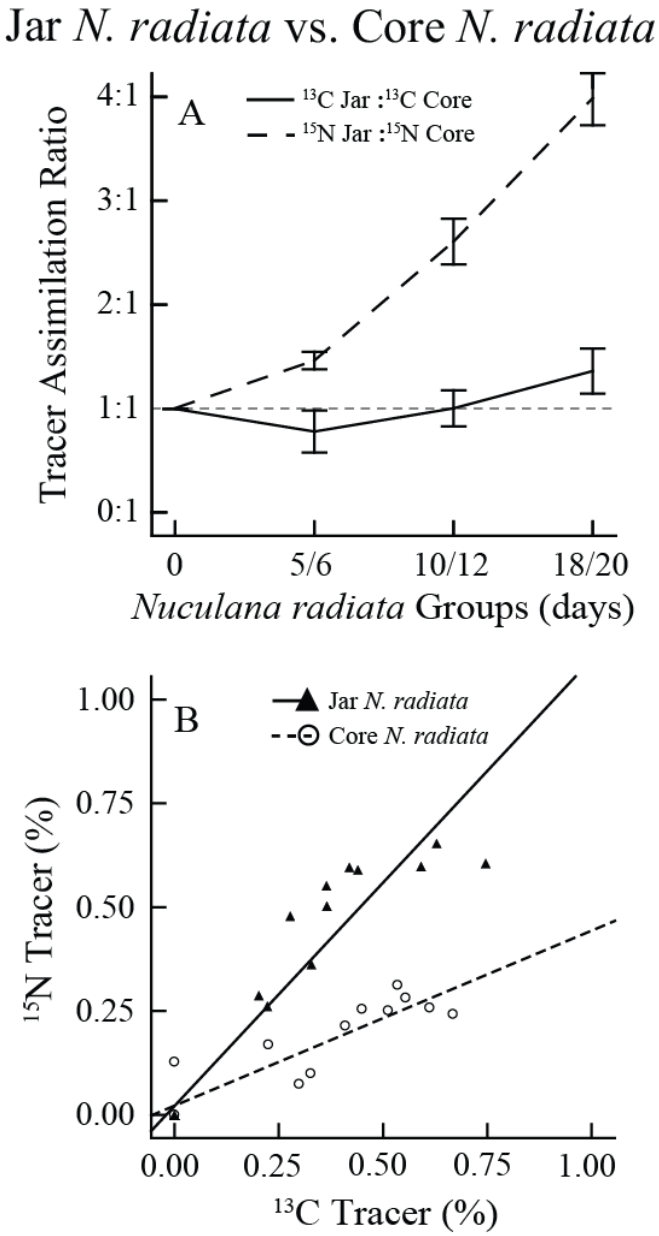

Figure 7: *Nuculana radiata* tracer assimilation in the enriched whole clam tissues from jar and sediment core experiments 1 and 2, respectively. A) Tracer-specific assimilation between jar and core *N. radiata*, which shows similar ^{13}C assimilation across experiments within compared time periods, while jar clam ^{15}N tracer assimilation continually increases over time compared to core clams. B) Absolute tracer assimilation and linear regression of all jar clams (black triangles/solid line) and all core clams (white circles/dashed line) from compared experimental periods (days 0 - 20). Note that all tracer values in inter-experimental comparisons were shifted from original values to correct for negative tracer values associated with sediment core *N. radiata* whole clam samples ($^{13}C = {}^{13}C + 0.126$; $^{15}N = {}^{15}N + 0.192$).

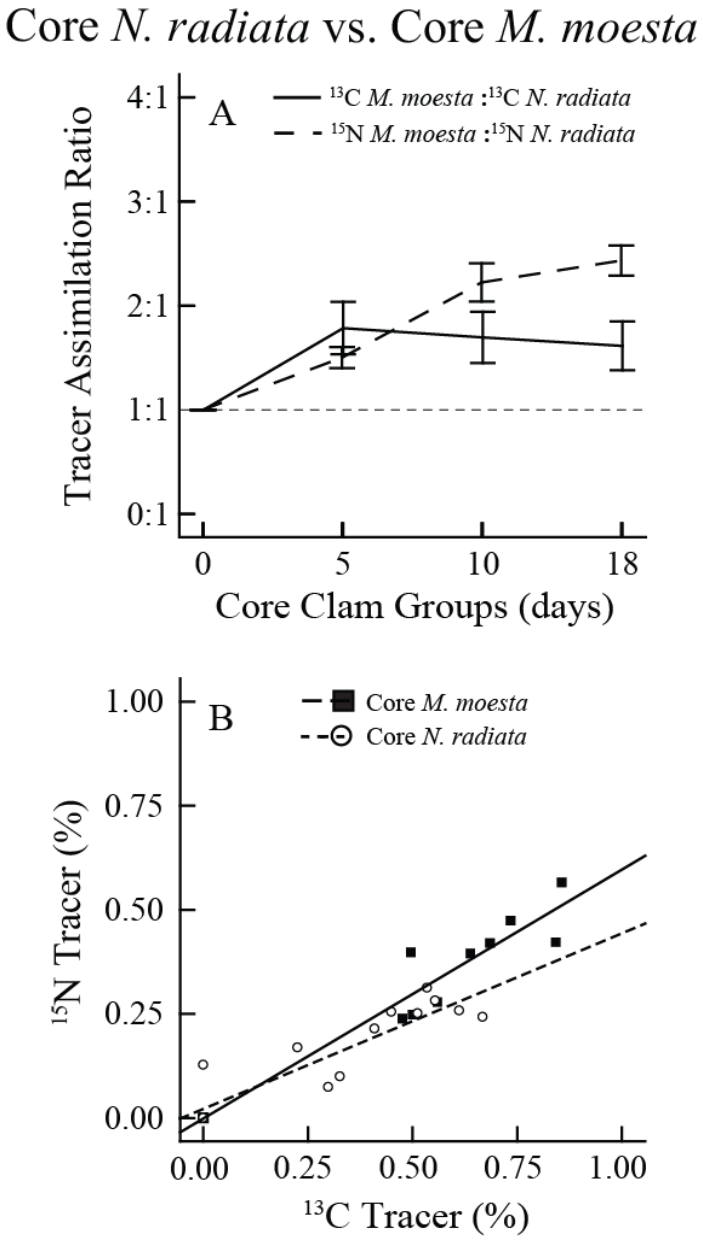

Figure 8: Tracer assimilation in the enriched whole clam tissues from sediment core experiments 2 and 3. A) Tracer-specific assimilation ratios between *M. moesta* and *N. radiata* within the core experiments show overall higher assimilation (~2x) of both ^{13}C and ^{15}N tracers in *M. moesta* over all sampling periods. B) Absolute tracer assimilation and linear regression of all *N. radiata* (white circles/dashed line) and *M. moesta* (black squares/solid line) from experimental days 5, 10, and 18. Note that all tracer values in inter-experimental comparisons were shifted from original values to correct for negative tracer values associated with sediment core *N. radiata* whole clam samples ($^{13}C = {}^{13}C + 0.126$; $^{15}N = {}^{15}N + 0.192$).

Behavioral observations

Nuculana radiata in jars were often seen with a protruded foot scraping or thrashing the bottom of the experimental jars (Figure 9A, B), but no extended siphons or labial palps were observed. No siphon activity or borrows were seen from *N. radiata* in sediment cores, either. However, within the sediment cores, *M. moesta* siphons were frequently out in the water column or in contact with the sediment surface surrounding the siphon hole. Small fecal pellets (~1-2 mm length) of *N. radiata* were only observed in jar experiments throughout the 42 d period in all three treatments, including clams under starvation conditions (Figure 9A). Fecal pellets also were observed around *M. moesta* siphon holes in sediment cores. *Macoma moesta* buried significantly deeper (3.78 ± 0.43 cm; n=23) than *N. radiata* (1.56 ± 0.20 cm; Student's t-test $p < 0.001$; n=27) in sediment cores (Figure 9C). A single *M. moesta* mucus egg sac was extruded into the overlaying water and securely attached to the clam at 3 cm depth on day 8 in one enriched core (Figure 9D).

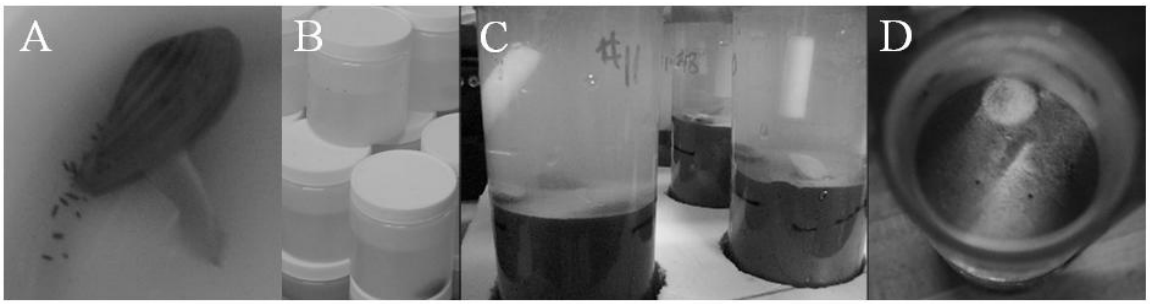

Figure 9: A) 2009 *N. radiata* in experimental jar with extended foot and fecal pellets produced over a period of three days, B) The 250 ml experimental jars used in the 2009 *N. radiata* experiments, C) 2010 *N. radiata* and *M. moesta* added to sediment cores and subsequently burrowing into the sediments, D) 2010 experimental core with egg sac extruded into the water column above the sediments and *M. moesta* siphon holes in the sediments.

Discussion

This study evaluated the response in isotopic carbon and nitrogen ratios of two clam species when offered food enriched in stable isotopes (C and N). Assimilation rates in *Nuculana radiata* and *Macoma moesta* in my jar and core experiments were relatively similar to experiments with other bivalve species (Hawkins, 1985; Raikow and Hamilton, 2001; McMahon et al., 2006). Isotopic enrichment was distinctly different for the specific sample types analyzed, with highest enrichment rates in lipids and lowest rates in muscle tissue. I will discuss the implications of these results for using bulk stable isotope analysis to study food web interactions in natural field collections. Based on the isotopic turnover in *N. radiata* and *M. moesta* during sediment core incubations, I argue their respective importance in the Bering Sea food web and their potential responses to projected decreases in ice algal production due to future loss of sea ice (Hunt et al., 2002; Jin et al., 2007). Lastly, I will compare these results with recent historic clam population fluctuations and the potential impacts on higher trophic level organisms.

Experimental conditions

As with every controlled experimental manipulation, certain limitations in mimicking natural conditions occur and have to be considered in the interpretation of the results. In the 2009 jar experiments I used Instant Ocean seawater because of the ease of controlling salinity and standardized conditions for the duration of the experiment, as well as to avoid adding any possible uncontrolled food sources within the experiment (e.g., bacteria

and dissolved organic carbon still contained in GF/F filtered sea water). Salt deposits were sometimes found on the bottom of the experimental jars, and the dissolution may have affected original salinity (32) within jars over the 3 d water exchange intervals. The physiological consequences of this are unknown; however, overall clam mortality was low. During the 2010 sediment core experiment, I used GF/F (about 0.7 µm pore size) filtered, natural seawater to top off core water as needed. Salinity during the core experiment ranged from 31-36, increasing with evaporation during daily aeration. Bering Sea bottom water salinity is known to fluctuate with seasonal ice presence and water mass flow and advection over the shelf from ~31-34 (Stabeno et al., 2001; Clement et al., 2005); thus, the observed salinity range in both experiments likely did not have negative effects on the clams.

All experiments were conducted within temperature ranges of 3.8-4.5°C, likely increasing clam metabolic rates above those naturally found at the sampling stations at that time. The temperature range used was constrained by the available incubation chambers on the research vessels and also avoided freezing conditions in the jars/cores during the experiments. Typical bottom water temperatures in the region are approximately -1.7 to 2°C during spring and summer (Stabeno et al., 2010). My experimental temperatures were hence more similar to summer bottom water temperatures found on the southeastern Bering Sea shelf (~4°C) during the late spring and summer seasons following low sea ice years (Coyle et al., 2007; Brodeur et al., 2008; Stabeno et al., 2010).

The experimental algal food source was a consortium of the diatom *Nitzschia frigida* and microflagellates. This composition is reduced in diversity compared to natural ice algal communities but does represent its major contributors in terms of biomass (Weissenberger, 1998; Gradinger, 1999; Gradinger, 2009; Poulin et al., 2011). Use and recycling of dissolved carbon and nitrogen sources in the natural, semi-closed sea ice system create natural isotopic enrichment of ice algae (10‰ in $\delta^{13}C$ and small to negligible in $\delta^{15}N$) compared to open system pelagic primary production, specifically at the maximum of the ice algal bloom (Hobson and Welch, 1992; Søreide et al., 2006; Tamelander et al., 2006, 2009; Gradinger, 2009; Gradinger et al., 2009). The isotopically enriched algal food considerably exceeded the naturally occurring isotope ratios, and was designed to provide a substantial and unmistakable enrichment to be traced in assimilation experiments (Kaufman et al., 2008), while the non-enriched algal food had isotopic values similar to *in situ* phytoplankton (Hobson and Welch, 1992; Iken et al., 2005, 2010; Lovvorn et al., 2005; Gradinger et al., 2009). It is important to note that isotopic enrichment obviously does not change food quality and I ensured the cell integrity at least of *N. frigida* was still conserved during the freeze-thaw processes of feed preparation.

Ratios of C:N and POC:chl can be used to assess the quality of a potential food source. The weight-based C:N ratio of 11.2 of the experimental food represents a mid-range quality food source between the high quality molar ratios of a sinking marginal ice algal bloom occurring late in the spring from C:N ratios of 7-10 with decreasing quality toward ratios of 15-20 of more refractory, open-ocean particulate organic matter in the

summer and fall (Falk-Petersen et al., 1998; Tremblay et al., 2006; Leu et al., 2010). Additionally, the POC:chl ratio of ~65 is near the middle of established ranges of 15-180 for ice algae, where the lowest ratios signify a high abundance and quality of diatoms consistently associated with large-cell ice algal blooms under optimal physical and chemical environments (Fukuchi et al., 1993; Juul-Pedersen et al., 2008; Leu et al., 2010). In contrast, pelagic blooms typically have higher POC:chl ratios caused by chemical reworking and recycling in the water column (Falk-Petersen et al., 1998; Juul-Pedersen et al., 2008). Thus, the experimental algal food provided a medium quality food source, similar to that of a diminishing, natural ice algal bloom exported to the benthos (Lalande et al., 2007; Juul-Pedersen et al., 2008; Leu et al., 2010).

Algal food quantity (based on chl) supplied to the experiments was scaled to natural concentrations during sea ice algal release occurring in the northern Bering Sea (Grebmeier et al., 1988; Fukuchi et al., 1993; Cooper et al., 2002; Olson and Strom, 2002; Dunton et al., 2005; Grebmeier et al., 2006b). Unfortunately, the initial chlorophyll measurements of the experimental algal food were faulty, resulting in ~8 times lower than intended food concentrations in jar experiments. Thus, algal food aliquots in jar experiments were likely more similar to quantities from a reduced pelagic production period during the late summer and autumn than an ice algal spring bloom. To ensure sufficient food supply in 2010, sediment core experiments intentionally received algal food concentrations in excess of ~4-5 times that of natural algal bloom export estimates (Grebmeier et al., 1988; Fukuchi et al., 1993; Cooper et al., 2002).

Sample-specific isotope assimilation and turnover

Isotopic enrichment over experimental time (both jar and core experiments with enriched food offered) was distinctly different in the different sample types. Muscle was an isotopically stable tissue with slow metabolic turnover with little or negligible isotopic enrichment over the course of several weeks. This suggests that muscle may be used as indicator of long-term changes in food quantity and quality but not for short-term responses to diet switches like, e.g., sea ice algal export within time frames of days or weeks. The estimated contribution (<10%) of muscle dry mass to total body mass and its weighted contribution into the mass balance calculations of LEX body and whole clams were underestimated. As only a portion of foot muscle tissue was removed for analysis, the remaining body tissue contained unknown portions of muscle tissue (e.g., remaining foot portion, mantle, siphon, etc.), which made an unknown contribution to the isotopic values calculated for LEX body tissues and whole clams.

Lipids in general, and specifically essential, polyunsaturated fatty acids (Nichols, 2003), are important for increased growth and reproduction of arctic marine invertebrates (Sun et al., 2009). In polar seas, phytoplankton and terrestrially-derived matter contain lower concentrations of those essential fatty acids compared to sea ice algae and may be less valuable for the reproductive success of arctic benthic invertebrates (McMahon et al., 2006; Sun et al., 2007, 2009). Lipids contributed roughly 15-20% to total clam dry weight and mass balance fractions, and consistently had among the fastest and highest overall isotopic change (~4‰ in $\delta^{13}C$, >10‰ in $\delta^{15}N$) for N. *radiata* over 42 d in the isotopically enriched food treatments. At the same time, lipids were typically the most

depleted sample type, at least in terms of $\delta^{13}C$, because of kinetic isotope effects (DeNiro and Epstein, 1977; Post et al., 2007). These low lipid isotope values also lead to slightly depleted isotopic values of mass-balanced whole clam samples compared to mass-balanced LEX body values. As pure lipids do not contain a large portion of nitrogen (Elser et al., 1996), the large enrichment in $\delta^{15}N$ in clam lipids was unexpected. I suspect the cause to be related to: 1) potential 'protein sparing' in muscle tissues and higher metabolic use of lipid fractions containing a small but labile nitrogen source (Hawkins, 1985; Hawkins and Bayne, 1985), or 2) contamination by nitrogen-containing amino acids potentially leached from body proteins during the lipid extraction process (Post et al., 2007; Mateo et al., 2008). Hawkins and Bayne (1985) observed protein sparing in the mussel *Mytilus edulis* that promoted higher net conservation of total nitrogen over carbon due to the reduced breakdown of amino acids in muscles in favor of metabolizing carbon laden fat compounds. Therefore, the very small pool of labile free amino acids containing nitrogen in the metabolized lipid pool would turnover more rapidly (Hawkins, 1985), causing potentially greater assimilation of the ^{15}N tracer in my enriched treatment clam lipids. This scenario is thought to overshadow any unknown amount of contamination from co-extracted proteins, as absolute muscle $\delta^{15}N$ assimilation was far lower in amplitude than the lipid fractions, and previous studies propose this type of $\delta^{15}N$ fractionation associated with lipid extraction to be minimal at <1‰ (Sotiropoulos et al., 2004; Post et al., 2007). Assuming that the observed change in $\delta^{15}N$ reflects the true lipid isotopic signal, the relatively quick response in lipid isotopic composition to an enriched food source makes this sample type particularly valuable to detect organism responses to

short-term changes in food sources, such as algal bloom-specific or seasonal food switches. Future efforts could help in resolving the above-mentioned uncertainties by including tracking stable isotope changes of specific individual fatty acids (Budge et al., 2008). For example, enrichment of a single fatty acid (C20:5(n-3)) in *M. calcarea* reached upwards of 15% of the food ^{13}C tracer (McMahon et al., 2006), even when total ^{13}C bulk tissue enrichment was minimal (see discussion in next paragraph). This emphasizes that overall lipid enrichment may depend on the individual fatty acid composition of the lipid fraction.

The turnover constants in isotope tracer of ~0.2-0.5% d^{-1} in isolated sample types and reconstructed body fractions in *N. radiata* and *M. moesta* (Tables 4 and 5) were similar to fractional turnover constants of 0.3-0.4% d^{-1} in muscle and composite tissues in the temperate mussel *M. edulis* (Hawkins, 1985). For all three species, this translates into roughly one complete turnover per year. In a freshwater stream isotopic enrichment experiment (Raikow and Hamilton, 2001) starting with the addition of highly enriched inorganic ammonium (>500‰ $\delta^{15}N$), which caused isotopic increases in various food sources downstream, feeding unionid mussel and fingernail clam $\delta^{15}N$ showed lower enrichment (<1‰) in muscle tissue than digestive gland tissue (up to 8‰; similar to food sources) over 45 days. That represents about 8% tracer uptake in muscle tissue (nearly 100% in digestive gland tissue), which is greater than experimental ^{15}N uptake of 0.5% in *N. radiata* muscle over a similar time frame in this study. Isotopic ratios in bulk tissues of the arctic clams *M. calcarea* and *Liocyma fluctuosa* (McMahon et al., 2006) when offered enriched phytoplankton and ice algal food sources (~684‰ $\delta^{13}C$) increased by ~1-2‰

over 19-30 days in isolated and core incubations. This is about 1.1 to 3.3 times less compared to the LEX body and whole clam enrichment in *N. radiata* and *M. moesta* when standardizing for experimental time and food $\delta^{13}C$ values. Another isolated feeding study by Kaufman et al. (2008) with arctic amphipods in jars yielded half life estimates of 14 to 30 d and asymptotes in isotopic turnover close to their food source values. Since *N. radiata* in my jar experiments did not reach an asymptote in algal food tracer assimilation over 42 d, this indicates that half life has to be at least 21 d. My completely different results from the core experiments (much shorter half life time estimates) and different applied models illustrates that a careful approach is needed when interpreting isotopic turnover and when making cross-experiment comparisons (see also, Martinez del Rio and Anderson-Sprecher, 2008).

Species-specific isotopic tracer assimilation

Jar and sediment core experiments examine an organism's isotopic assimilation under different environmental conditions and food compositions. The jar experiments in *N. radiata* provided a single algal food source with no competing biota, compared to algae and sediment sources and biota (e.g., bacteria, meiofauna) in the core experiment. I interpret the significantly lower ^{15}N tracer incorporation in core experiments as a result of the concurrent use of natural (non-enriched) food sources from the sediment and also as potential effects of other biota like sediment bacteria and meiofauna on algal food availability. Much of the slower $\delta^{15}N$ incorporation (~60%) of *N. radiata* in the cores can be explained by the isotopic dilution of the enriched food source with existing, non-

enriched organic matter and bacteria serving as additional food sources within the sediments. A similar response in nature would suggest that *N. radiata* may only be partially reliant on freshly-sedimented algal material from the water column even during episodic direct sedimentation of ice algal or phytoplankton material, at least for organic nitrogen. Lovvorn et al. (2005) suggest two alternative states of nitrogen use in Bering Sea soft sediments, where: 1) a nitrogen-limited environment promotes quick uptake of any new or recycled nitrogenous sources in all biota, or 2) in non-limiting nitrogen conditions, sub-surface feeding macrofauna could prefer a bacterially-derived nitrogen pool rather than feeding directly on exported particulate organic nitrogen. Results suggest that jar experiments could likely represent condition 1, while the core environment is closer to condition 2. Nitrogen limitation conditions in jar clams can be supported by potential muscle protein sparing and higher turnover in lipid fractions, the nearly 1:1 tracer assimilation ratio for ^{13}C and ^{15}N, which was not seen in core clams, and the relatively low quantity of food provided to each jar due to initially faulty algal chl estimates explained above. Whereas, nitrogen-replete conditions and *N. radiata* consumption of mostly sediment food resources could explain the low nitrogen isotopic enrichment in the core clams. Additionally, a rapid degradation and mineralization of newly deposited, labile organic matter of my enriched feeds by bacteria could explain the reduced availability of ^{15}N tracer specifically for the sub-surface feeding *N. radiata* during the core experiments. Bacterial response to food influx can be almost immediate (within hours) with changes in bacterial community composition and increases in bacteria-specific fatty acids (Sun et al., 2007; Dyda et al., 2009) and will reduce the pool

of enriched particulate nitrogen for macrofaunal consumption through release of dissolved organic or inorganic nitrogen (Lovvorn et al., 2005; Sun et al., 2007). Over time scales of several days these processes could lead to $\delta^{15}N$ enrichment of the bacterial biomass (Dyda et al., 2009; Sun et al., 2009) with subsequent enrichment of *N. radiata* feeding on these bacteria. However, the non-significant changes in isotopic and tracer values during experimental days 5-18 in core *N. radiata* do not provide direct support for delayed uptake of potentially enriched bacterial biomass.

The sediment core experiments with *N. radiata* and *M. moesta* demonstrated about 30% less isotopic enrichment in *N. radiata* as compared to *M. moesta* with significantly reduced ^{13}C and ^{15}N incorporation. This may be related to the different feeding behaviors of the two species (e.g., natural $\delta^{13}C$ differences between suspension and deposit feeding bivalves, Crompton et al., 2008). *Macoma moesta* is a benthic suspension and surface-deposit feeder, utilizing the top surface layer of the sediments upon which algal food (i.e., my enriched food source) settles (Sirenko and Gagaev, 2007; McCormick-Ray et al., 2011; own observations). In contrast, *N. radiata* is a shallow sub-surface deposit feeder, mainly feeding on buried food sources deeper in the sediments (Richman and Lovvorn, 2003; own observations) and less on fresh material on the sediment surface. Only after a certain degree of sediment bioturbation and likely microbial activities, leading to increased isotopic dilution, can freshly deposited food enter the sediment layers where *N. radiata* has access to it. In similar sediment core studies over a similar time frame, bioturbation by mobile infauna moved only a small fraction of freshly added food to about 2-3 cm depth (McMahon et al., 2006; Sun et al.,

2007, 2009). This is well within the mean burrowing depth of 1.5 cm I measured for *N. radiata*; thus, bioturbation-delayed access to a sediment-diluted algal food likely limited isotopic enrichment in *N. radiata*.

Bering Sea ice-pelagic-benthic coupling and clam dynamics

Recent hypotheses such as the Oscillating Control Hypothesis (Hunt et al., 2002) emphasize major shifts in Bering Sea ecosystem functioning dependent upon extent and timing of sea ice retreat and water temperature. Biomass from large, early-season ice algal blooms is exported to the benthos combined with early phytoplankton blooms in cold years, while in warm years delayed pelagic phytoplankton blooms in late spring are consumed and recycled mostly through the pelagic food web (Stabeno and Hunt, 2002). Thus, even with predictions of stable total annual primary production under both cold and warm conditions (Jin et al., 2007) and high likelihood of continued northern Bering Sea (>60˚N) cold bottom water temperatures (Stabeno et al., 2010), the shifts in timing, community composition, and magnitude of the spring phytoplankton bloom and altered ice algal availability will likely have a great impact on the entire ecosystem.

Cold year conditions with large ice algal blooms combined with early phytoplankton blooms in the salinity-stratified, marginal ice zone in April (-1.7 to 0˚C, Stabeno et al., 2010) result in high primary production export to the benthos, contributing to overall high benthic biomass. This may particularly favor infauna feeding on fresh algal export, like *M. moesta* (Grebmeier et al., 2006a; Coyle et al., 2007). Furthermore, *M. moesta*'s greater direct access as a surface feeder to abundant fresh food sources may

provide a competitive advantage over *N. radiata* as growth and reproduction may be enhanced with access to this nutritious ice algal food (Richman and Lovvorn, 2003; Sun et al., 2009). In contrast, under warm conditions in the Bering Sea, late thermal stratification of the water column promotes pelagic algal blooms in May or June (Stabeno et al., 2010). Pelagic phytoplankton blooms in warmer years are heavily grazed upon by planktonic zooplankton, which have returned to the surface waters from overwintering depths during this time (Coyle and Pinchuk, 2002; Stabeno and Hunt, 2002). This results in significantly less algal export to the benthos (Grebmeier et al., 2006b). Thus, increased bacterial reworking of less abundant food sources in the sediments increases the use of long-term, mostly refractory food bank material by benthic feeders (Hunt et al., 2002; Mincks et al., 2005). Bioturbation and mixing of the sediments may add to the long-term availability of food sources through a sediment food bank (Levinton, 1972; Mincks et al., 2005; Smith et al., 2008). If the northern Bering Sea seafloor acted like a food bank for subsurface deposit feeders, the sediment food bank could be 'refreshed' through the annual supply of organic material via blooms and pelagic production as found for some other polar environments (Mincks et al., 2005). Lovvorn et al. (2005) suggest that with continued decreases in pack ice extent, the northern Bering Sea might incur a long-term decline in the sediment organic pool; although, a short term sediment food buffer would still be advantageous to those species consuming bacteria and their labile products, such as *N. radiata*.

In the long-term, the above fluctuations in sea ice and oceanographic temperature regimes will influence the abundance of clam species such as *Nuculana* spp. and *Macoma*

spp. (Grebmeier et al., 2006a,b; Lovvorn et al., 2009; McCormick-Ray et al., 2011). For example, *Macoma calcarea* (*M. moesta* and *M. calcarea* have similar feeding strategies) dominated the bivalve communities in the Bering Sea during the 1970-1974 cold period, during which *N. radiata* abundances were low. Subsequently, relative abundances of these two species were inverse, with high *M. calcarea* and low *N. radiata* abundances during cold periods, and reverse patterns during warm periods (Grebmeier et al., 2006b; Lovvorn et al., 2009). Such fluctuations in clam species abundances will likely also have implications on higher trophic levels that feed upon these and other benthic invertebrates. Diving waterfowl such as eiders, scoters, and scaups feed directly upon these clams (Lovvorn et al., 2003; Lovvorn et al., 2009); thus, changes in clam species abundance may have implications on the feeding energetics of these birds. Additionally, bottom feeding mammals like walrus, seals, and grey whales that mostly feed on an assortment of larger polychaetes, crustaceans, or mollusks, often by-catch these small clams and could thus be affected (Bluhm and Gradinger, 2008). The changes in abundance of different clam species due to decreased pelagic-benthic coupling with the loss or degradation of sea ice may thus compromise higher trophic level species distribution with a northward contraction of arctic mammal and bird species and the immigration from the south of more temperate species (Hunt et al., 2002; Grebmeier et al., 2006a; Coyle et al., 2007; Mueter et al., 2009).

Conclusions

Intra- and inter-clam species carbon and nitrogen assimilation from food sources constitute a dynamic system under the influence of several factors. My experiments, through interpretation of the results and some indirect evidence, allude to some potentially important factors that contribute to how much and how fast certain food resources can be assimilated by Bering Sea clams, including: a) food availability, b) adult clam internal isotopic routing, d) adult clam feeding behaviors and adaptations, and d) benthic community interactions including competition and nutrient limitation. Overall, bulk stable isotope analysis established basic, experimental trophic interactions from algal food to clam consumers. My interpretation using only a bulk stable isotope approach could be limited when transferred to a natural Bering Sea sediment environment without the use of auxiliary methods like fatty acid isotope analysis, consumer gut content analysis, and sediment meio- and microfauna identification. However, based on the isotopic turnover and assimilation in *N. radiata* and *M. moesta*, *M. moesta* may be more dramatically affected by projected decreases in seasonal ice algal production with future loss of sea ice. *Nuculana radiata*, which is less behaviorally adapted for direct feeding on fresh export, may outcompete surface deposit feeders as sea ice, ice-algal seeded primary production, and benthic species like *M. moesta* retreat northwards with the receding ice cover.

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Appendix

All Bering Sea sampling events taken in this study for both 2009 and 2010 as represented in Figure 1. Deployment of the van Veen benthic grabs (VV) and the Multiple HAPS core sampler (MHAPS) occurred while on station with event-specific latitude, longitude, and depth as recorded in the BEST Project data archive (www.eol.ucar.edu/projects/best/).

Year	Sampling Event	Station	North Latitude (deg˚min'sec")	West Longitude (deg˚min'sec")	Depth (m)
2009	VV	VNG-3.5	62˚33’29”	173˚32’57”	67
2009	VV	VNG-3.5	62˚33’27”	173˚32’53”	68
2009	VV	VNG-3.5	62˚33’22”	173˚32’44”	68
2009	VV	VNG-3.5	62˚33’19”	173˚32’36”	68
2009	VV	VNG-3.5	62˚33’16”	173˚32’38”	67
2009	VV	VNG-3.5	62˚33’12”	173˚32’30”	67
2009	VV	VNG-3.5	62˚33’11”	173˚32’31”	67
2009	VV	VNG-3.5	62˚33’08”	173˚32’28”	67
2009	VV	VNG-3.5	62˚33’04”	173˚32’24”	67
2009	VV	VNG-3.5	62˚33’01”	173˚32’20”	67
2009	VV	VNG-3.5	62˚33’58”	173˚32’17”	67
2009	VV	VNG-3.5	62˚33’54”	173˚32’13”	67
2009	VV	VNG-3.5	62˚33’48”	173˚32’11”	67
2009	VV	VNG-3.5	62˚33’49”	173˚32’09”	67
2009	VV	VNG-3.5	62˚33’45”	173˚32’05”	67
2009	VV	VNG-3.5	62˚33’40”	173˚32’02”	67
2009	VV	VNG-3.5	62˚32’33”	173˚31’42”	65
2009	VV	VNG-3.5	62˚31’29”	173˚31’42”	65
2009	VV	VNG-3.5	62˚31’25”	173˚31’42”	65

2009	VV	VNG-3.5	62˚31’05”	173˚31’47”	66
2009	VV	VNG-3.5	62˚31’01”	173˚31’48”	66
2009	VV	VNG-3.5	62˚31’59”	173˚31’49”	66
2010	VV	VNG-3.5	62˚35’07”	173˚38’31”	60
2010	VV	VNG-3.5	62˚35’13”	173˚38’43”	60
2010	MHAPS	VNG-3.5	62˚37’48”	173˚41’09”	61
2010	MHAPS	VNG-3.5	62˚37’05”	173˚41’29”	61
2010	MHAPS	VNG-3.5	62˚37’05”	173˚41’29”	61
2010	MHAPS	VNG-3.5	62˚37’20”	173˚41’46”	61
2010	MHAPS	VNG-3.5	62˚38’42”	173˚42’20”	61
2010	MHAPS	VNG-3.5	62˚38’34”	173˚42’05”	61
2010	MHAPS	VNG-3.5	62˚38’42”	173˚42’20”	61
2010	VV	CD-1	62˚41’50”	173˚22’45”	59
2010	VV	CD-1	62˚41’51”	173˚22’48”	59
2010	VV	VNG-4	62˚46’48”	173˚26’41”	61
2010	VV	VNG-4	62˚46’48”	173˚26’45”	61