



Carbon and nitrogen assimilation in the Bering Sea clams *Nuculana radiata* and *Macoma moesta*

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ABSTRACT

We analyzed bulk carbon and nitrogen stable isotope values ($\delta^{13}\text{C}$ and $\delta^{15}\text{N}$) of the benthic clams *Nuculana radiata* and *Macoma moesta* from the Bering Sea during controlled feeding experiments (spring of 2009 and 2010) using isotopically labeled sea ice algae. The aim was to determine the ability of these clam species to assimilate carbon and nitrogen from sea ice algae. Specimens were collected in the Bering Sea and placed into jars without sediment (2009, *N. radiata* only) or into natural sediment cores (2010, both species). The clams were offered isotopically enriched (both C and N) or non-enriched algal feeds for time periods of 42 (2009) and 18 d (2010). Isotopic assimilation rates for carbon and nitrogen were calculated using the change in the isotope ratios of the clams over the experimental time. *N. radiata* in the jar experiments had slow isotopic assimilation rates (0.01 to 0.23% d^{-1}), with solvent-extractable organic matter/lipids taking up both of the isotope markers fastest and muscle tissue the slowest. Lipids may thus be particularly suitable to track the immediate ingestion of sea ice algal production in benthic consumers. *M. moesta* showed 30% higher isotopic assimilation compared to *N. radiata* in sediment cores, likely reflecting the different feeding behaviors of these two species. Based on our results, *N. radiata* is likely better able to utilize food sources buried in the sediment and may be more competitive over the sediment surface feeding *M. moesta* under conditions of reduced ice algal production in the northern Bering Sea.

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1. Introduction

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 continue to be strongly influenced by climatic changes (Grebmeier et al., 2006a). Specific to the Bering Sea ecosystem, Hunt et al. (2002, 2011) suggested two alternate ecosystem states, where most of the organic matter produced by algae growing in sea ice and phytoplankton is either consumed in the pelagic or the benthic realm, depending on climate conditions. This change in energy flow could influence the abundance and distribution of the commercially and ecologically important fauna in the Bering Sea (Coyle et al., 2007; Grebmeier, 2012; Jin et al., 2007) and may be comparable to ecosystem responses proposed for other sub-arctic seas (Bluhm and Gradinger, 2008; Mueter et al., 2009; Tamelander et al., 2006).

Species-specific consumer dependence on sea ice-derived (e.g., ice algal) production is largely unknown and could be significantly impacted by climate-induced changes in seasonally ice-covered regions of the Arctic (Kaufman et al., 2008; Lovvorn et al., 2005). Additionally, the response of benthic feeders to changes in sympagic (ice-associated) and pelagic primary production has complicated the interpretations of food web connectivity in the Bering Sea and other similar seasonally ice-covered systems (Dunton et al., 1989; McCormick-Ray et al., 2011; McMahan et al., 2006). Carbon and nitrogen stable isotope ratios have been used as natural and/or experimental markers of feeding ecology and food web structure (Iken et al., 2010; Martinez del Rio et al., 2009; Peterson and Fry, 1987). The often higher $\delta^{13}\text{C}$ values of sea ice algae compared to phytoplankton were used to track consumer-specific feeding and overall trophic level connectivity and energy flow based on two distinct food sources (Budge et al., 2008; Gradinger et al., 2009; Hobson and Welch, 1992; Søreide et al., 2006). Challenges when applying this approach are the temporal changes in a) availability and b) isotopic characteristics of the potential endmembers, which require estimates of assimilation rates (e.g. Kaufman et al., 2008).

Our objective was to determine the response of two benthic bivalves, *Nuculana radiata* (Krause, 1885) and *Macoma moesta* (Deshayes, 1855), to a sudden switch in the stable isotopic composition of their food source. These clams were selected as they represent 20–85% of the

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benthic biomass (~20–700 clams m²) reported in the northern Bering Sea and are of importance to higher trophic consumers in the northern Bering Sea (Grebmeier, 2012; Lovvorn et al., 2009). Additionally, both clam species represent different feeding strategies, with *N. radiata* burrowing shallow and gathering subsurface sediments with labial palps while *Macoma* spp. burrow deeper and extend siphons to the surface to vacuum surface sediments (Richman and Lovvorn, 2003). Both species may thus be important consumers of the seasonally abundant primary production reaching the benthos in the Arctic (Lovvorn et al., 2005). We aimed to track the assimilation of carbon and nitrogen from ice algae by these clams using stable isotopically labeled (carbon and nitrogen) ice algal feed stocks. We aimed to answer: 1) How fast will the isotopic ratios change in various body components of *N. radiata* and *M. moesta* following input of isotopically enriched algal food? and 2) Is the response different between these two species?

2. Materials and methods

2.1. Algal food culture

Two isotopically distinct algal food stocks were prepared from a mixed ice algal (mainly *Nitzschia frigida* and a minor microflagellate component) culture established from sea ice samples in the northern Bering Sea (59°53'26"N, 171°08'43"W) in 2008. The ice algal cultures were incubated at 5 °C with Guillard's F/2 marine nutrient solution for 3 months. Each weekly harvest (4L) from the mass culture was split and separated into two food stocks. Half of the harvest was designated as the enriched food stock and was further incubated for 24 h with the addition of 1 mL NaH¹³CO₃ and 0.4 mL Na¹⁵NO₃ spiked seawater solutions per 1 L ice algal culture (3.4710 g of 98% NaH¹³CO₃ in 100 mL distilled water, 2.0585 g of 98+% Na¹⁵NO₃ in 100 mL distilled water, Cambridge Isotope Laboratories, Inc.). Immediately after collection, the remaining culture harvest, designated as the non-enriched food stock, was centrifuged for 5 min at 4000 rpm to concentrate algal cells, which were stored frozen at –20 °C (same for the enriched food stock after the additional 24 h incubation). The weekly algal harvests from each of these isotopically distinct algal food stocks were then homogenized and stored frozen for their separate use in the feeding experiments (described below). Samples from the two food stocks were filtered onto pre-combusted Whatman GF/F filters for isotopic and chlorophyll *a* (henceforth: chl) analyses prior to each experiment. For stable isotope analysis, filters were freeze dried at –80 °C and measured as described below (samples were not acid fumed). Chl concentrations (µg L⁻¹, following methods by Arar and Collins, 1997) were used to define food rations used during the experiments.

2.2. 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 (see Supplementary data). Subsequent feeding experiments were conducted in environmentally controlled incubators aboard the USCGC *Healy* in March–April 2009 and aboard the USCGC *Polar Sea* in March 2010.

In 2009, sediments from 22 replicate van Veen benthic grabs (0.1 m²) were sieved over a 1 mm mesh to collect 215 live adult *N. radiata* (21.3 ± 0.2 mm length). All *N. radiata* were allowed to clear their guts for 24 h prior to the start of the experiment. An initial (t₀) sample of 15 clams was taken to determine the isotopic baseline and intra-specific variability in isotopic composition. The remaining clams were placed into individual jars (33.2 cm² bottom area) containing 150 mL Instant Ocean seawater (S=32) in the dark at 4.6 ± 0.1 °C. The temperature regime in this and the following core experiment was higher than the ambient bottom temperatures at the collection time (approximately –1.7 to 2 °C during spring) and mimicked more late spring and summer conditions (~4 °C,

Brodeur et al., 2008; Coyle et al., 2007; Stabeno et al., 2010). This was constrained by incubation chamber capabilities, and likely increased the metabolic activity of the clams (Pörtner et al., 1998).

N. radiata jars were randomly assigned to two feeding treatments (n=100 per treatment): a) isotopically enriched and b) non-isotopically enriched algal food. These treatments received equal amounts of algal food (~0.3 µg chl cm⁻² 3 d⁻¹) from the respective food stock every 3 d in combination with fresh seawater replacement (150 mL) for sufficient aeration. Ten clams were removed from each treatment on each of days 6, 12, 20, 28, 35, and 42 and kept frozen at –20 °C. Subsequently, three to six clams from each day and treatment were used for stable isotope analyses (described below). Not all experimental clams were needed for stable isotope analyses as mortality was low (~9%) and showed no association with treatment exposure.

In the 2010 feeding experiments, *N. radiata* (23.6 ± 0.6 mm length) and *M. moesta* (20.5 ± 0.9 mm length) were added to natural sediment cores and used in isotopically enriched and non-isotopically enriched algal treatments. Clams were collected from six van Veen benthic grabs (22 *N. radiata* and 22 *M. moesta*), while seven four-barrel, Multiple-HAPS (MHAPS) core deployments were taken to collect undisturbed sediments for incubations. Seventeen experimental cores were taken as sub-cores 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 experimental cores were covered with filtered (0.7 µm Whatman GF/F) natural seawater from the sampling location avoiding sediment re-suspension. After a 24 h gut clearance period, five clams of both species were randomly chosen as initial (t₀) samples. Of the remaining clams, one individual of each species was randomly added to each of the 17 experimental cores and given a 12 h burial period. Isotopically enriched (88.8 µg chl cm⁻²) or non-isotopically enriched (110.1 µg chl cm⁻²) algal food was then added to each randomly assigned core as a single food pulse at the beginning of the experiment (t₀). After the food addition at t₀ the cores were incubated in the dark at 3.9 ± 0.1 °C for 18 d. Core water was aerated at least 6 h d⁻¹ and any evaporated seawater was replaced with filtered, natural seawater. Water salinity (S=31–36, variation due to evaporation) and dissolved oxygen (8 ± 1% O₂ loss over 18 h during non-aeration periods) were monitored with YSI 85/50 sensors. Three isotopically 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) to retrieve experimental clams and record burial depths. Other, naturally occurring fauna encountered in the 17 cores at the end of the experiment included 7 *N. radiata*, 3 *M. moesta*, 2 *Macoma* spp., 40 *Ennucula tenuis* clams (burial depth of 2.2 ± 0.3 cm), and 4 unidentified polychaetes. Meiofauna and microfauna were not isolated. These non-target species were not analyzed for stable isotope values. The experimental clams were kept frozen at –20 °C for stable isotope analysis. All clams survived until sampling in the core experiment.

2.3. Laboratory preparation

Frozen clams were thawed and the soft tissue was removed from the shell and a small part of the foot muscle tissue was dissected. The muscle and the remaining body tissue were separately dried for 24 h at 60 °C and weighed to the nearest 0.1 mg. The muscle sample and the remaining whole body were extracted separately in a chloroform–methanol (2:1) solution for 24 h (modified after Folch et al., 1957) to isolate lipids from both clam muscle and body tissues. It should be noted that the resulting extract represents the overall solvent-extractable organic matter rather than a purified lipid fraction but as lipids are the likely major component (Folch et al., 1957) we will henceforth refer to these extracts as the lipid fraction. Extracts were dried with continuous-flow of N₂ 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 ground to a fine powder for analysis. Three sample types were analyzed from each clam for their stable isotope composition: *muscle* (lipid-extracted foot muscle tissue from each clam), *remaining body* (lipid-extracted, homogenized remainder of all clam soft tissue) and *lipid* (combined crude lipid extracts from muscle and remaining body samples). Lipid samples were not purified to minimize sample loss and achieve a true representation of mass-balanced whole clams (below).

2.4. C and N isotopic analysis

Tissue samples (muscle n = 123, remaining body n = 123), lipid extracts (n = 123), and algal food filters (n = 16) were analyzed for their elemental composition (carbon and nitrogen [μg]) using a Carlo Erba elemental analyzer (EA) connected to a ThermoElectron Delta V Plus continuous-flow isotope ratio mass spectrometer (IRMS) and routed through a ConFlo III system interface, at the Alaska Stable Isotope Facility (ASIF, University of Alaska Fairbanks). Stable isotope ratios are expressed in delta (δ) notation relative to international standards (Vienna Pee Dee Belemnite for carbon and atmospheric air for nitrogen):

$$\delta X(\text{‰}) = \left(R_{\text{sample}} / R_{\text{standard}} - 1 \right) * 1000. \quad (1)$$

δX is the δ¹³C or δ¹⁵N value of the sample measured in per mil (‰) and R is the ¹³C/¹²C or ¹⁵N/¹⁴N of the sample or standard. Analytical precisions for δ¹⁵N and δ¹³C were tracked using an ASIF bovine peptone standard and sample precisions on the algal filter samples were ±0.5‰ for δ¹³C and ±0.2‰ for δ¹⁵N, while clam samples were ±0.3‰ for δ¹³C and ±0.5‰ for δ¹⁵N.

Weighted-mean (mass-balanced) isotopic values for each clam were reconstructed to create the whole clam isotopic value. *Whole clam* refers to cumulative contribution of all three sample type isotopic values, weighted by sample mass, toward a calculated whole clam isotopic value:

$$\delta X_{\text{Whole Clam}}(\text{‰}) = \left[(\delta_{\text{muscle}} * M_{\text{muscle}}) + (\delta_{\text{remaining body}} * M_{\text{remaining body}}) + (\delta_{\text{lipid}} * M_{\text{lipid}}) \right] / (M_{\text{muscle}} + M_{\text{remaining body}} + M_{\text{lipid}}) \quad (2)$$

where δX_{Whole Clam} (‰) is the reconstructed δ¹³C or δ¹⁵N value, δ is the δ¹³C or δ¹⁵N of the muscle, remaining body tissue, or lipid extracts, and M is the total dry mass of muscle, remaining body tissue, or lipid extracts measured to the nearest 0.1 mg. The average composition of whole clam isotopic samples in both species was similar and 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 tissues present in clams, such as siphon, mantle, etc. Also, the remaining body and muscle samples had similar isotope values and hence, the remaining body values are not presented separately. Similar mass-balance procedures were used to calculate C:N ratios (always weight specific) of whole clams, with those ratios taking the place of isotopic values in Eq. (2).

One-way ANOVA and Tukey HSD post-hoc multiple comparison tests were used to identify significant differences (α = 0.05) in naturally occurring isotope ratios of muscle, lipids, and whole clam values in initial t₀ clams. These and all the following statistical analyses were conducted within the SYSTAT Version11 software package.

2.5. Isotopic tracer assimilation

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

$$X_{\text{tracer}}(\%) = \left[(\delta_{\text{sample}} - \delta_{\text{initial}}) / (\delta_{\text{algal tracer}} - \delta_{\text{initial}}) \right] * 100 \quad (3)$$

where X_{tracer} is the fraction of the algal isotopic tracer incorporated into the clam samples (%), δ_{sample} is the δ¹³C or δ¹⁵N value of the clam sample or mass-balanced sample, δ_{algal tracer} is the mean enriched algal food δ¹³C or δ¹⁵N value, and δ_{initial} is the mean initial (t₀) clam δ¹³C or δ¹⁵N value of the respective sample type.

Isotopic (‰) or tracer (%) assimilation rates refer to the linear rate of change (no asymptote observed) in sample isotopic values or tracer over the experimental time, which was assumed to be due to the uptake and assimilation of the enriched algal food.

$$X_{\text{sample}} = X_{\text{assimilation rate}} * (\text{time}) + X_{\text{initial}} \quad (4)$$

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 at t₀ or % tracer incorporation set at zero. Muscle, lipid, and whole clam isotopic and tracer values in *N. radiata* and *M. moesta* were analyzed with general linear regression to measure isotopic assimilation rates over time.

2.6. Jar and sediment core experiment comparisons

Carbon and nitrogen tracer assimilation was compared between *N. radiata* from the jar and those from the sediment core experiments for whole clam values. The small difference in average length measurements between specimens collected in 2009 versus 2010 (21 versus 23 mm) was considered negligible for the results of this comparison as these lengths fall into the same population cohort size as previously documented for this region (Richman and Lovvorn, 2003). We assumed that *N. radiata* in the jars only fed on the provided algal food. In contrast, *N. radiata* in sediment cores 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 additional, isotopically unknown food source. While experimental conditions may also have differed in other aspects, we consider the presence and absence of sediment the major difference and focus here on the relevance of this factor in the comparison of the two experiments. We also compared tracer assimilation for whole clams between *N. radiata* and *M. moesta* within the sediment core experiments to determine species-specific nitrogen and carbon assimilation differences under the same environmental and food conditions. Two-sample t-tests (α = 0.05) were performed for several sampling intervals to determine significant differences in tracer uptake. For comparison of *N. radiata* between jars and sediment cores, sampling periods for the two experiments were matched and compared for days 0, 5/6, 10/12, and 18/20. Sediment core *N. radiata* and *M. moesta* time periods were compared for days 0, 5, 10 and 18. The differences between sampling events were established for C and N tracer assimilation ratios, where *N. radiata* comparisons included C_{tracer jars}:C_{tracer cores} and N_{tracer jars}:N_{tracer cores}. Species comparisons from the core experiments included C_{tracer M. moesta}:C_{tracer N. radiata} and N_{tracer M. moesta}:N_{tracer N. radiata}. Additional general linear regressions were conducted across these experimental comparisons to relate the overall C and N tracer uptake:

$$N_{\text{tracer}} = C_{\text{tracer}} * (\text{slope}) + y\text{-intercept}. \quad (5)$$

These were used to calculate the 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_{tracer} and N_{tracer} values in the above whole clam comparisons were recalculated prior to statistical 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 (Eq. (3)) 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 a negative tracer uptake, when in reality, tracer enrichment was similar to those experimental clams above the t_0 mean values. The corrected values were calculated for all carbon ($C_{\text{CT (corrected tracer)}} = C_{\text{tracer}} + 0.126$) and nitrogen ($N_{\text{CT (corrected tracer)}} = N_{\text{tracer}} + 0.192$) whole clam tracer values.

3. Results

3.1. Isotopic composition of algal food and initial clams

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

The initial t_0 *N. radiata* and *M. moesta* isotopic values of muscle, lipids, and whole clams ranged from -19.1 to -26.3% in $\delta^{13}\text{C}$ values and 3.6 to 10.1% in $\delta^{15}\text{N}$ values; variability (SD) within each sample type was less than 1% , with the exception of the $\delta^{15}\text{N}$ values of lipids for both species (Table 1). Initial sample-specific isotopic variation existed between species and experimental years (Tukey HSD, $p \leq 0.05$; Table 1). Within *N. radiata*, only the initial $\delta^{13}\text{C}$ for muscle tissue was significantly different between the jar and core experiments. For *M. moesta*, initial samples were significantly ^{13}C -enriched in whole clam and ^{15}N -depleted in muscle and whole clam compared to *N. radiata*. Initial lipids had the lowest $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values for both species with discrimination factors compared to whole clam values of approximately -6% in $\delta^{13}\text{C}$ and -4% in $\delta^{15}\text{N}$. Similarly, initial whole clam had $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values that were approximately 1% lower compared to muscle. C:N mass ratios for both species ranged from 3.2 to 4.5 in muscle and whole clam samples, while C:N ratios in lipids were highly variable and much higher (31.4 – 58.1 , Table 1) due to low nitrogen content in lipid fraction samples (range $\text{N}\% = 0.5$ – 5%).

3.2. *N. radiata* jar experiments

N. radiata feeding on non-isotopically enriched food showed no significant isotopic changes in nearly all cases over the experimental period (Table 2). Within the isotopically enriched feeding treatment of *N. radiata* in isolated jars, nearly all isotopic ratios significantly increased over the 42-d experimental time with the exception of muscle $\delta^{13}\text{C}$ values

(Fig. 1A–C, Fig. 2A–C, Table 2). Muscle tissue showed a relatively small, though significant, increase in $\delta^{15}\text{N}$ values (4%) at $0.09\% \text{d}^{-1}$ (Fig. 2A). The $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values of lipids increased the most by nearly 3% and 10% , respectively (Figs. 1B, 2B), with assimilation rates of $0.05\% \text{d}^{-1}$ in $\delta^{13}\text{C}$ and $0.23\% \text{d}^{-1}$ in $\delta^{15}\text{N}$ values (Table 2). Whole clam $\delta^{13}\text{C}$ values increased by 2% , and 7% for $\delta^{15}\text{N}$ values (Figs. 1C, 2C) with assimilation rates of $0.05\% \delta^{13}\text{C} \text{d}^{-1}$ and $0.17\% \delta^{15}\text{N} \text{d}^{-1}$. The separate linear regressions for *N. radiata* feeding on isotopically enriched food in jars for 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 and whole clam samples (Table 2). Conversely, lipid fractions showed an early decrease in isotopic and tracer values, then a rapid increase in assimilation rates (Table 2). Overall, the maximum increases in isotopic values of all clam samples after 42 d were far below the isotopically enriched algal food values ($\delta^{13}\text{C} = 249\%$ and $\delta^{15}\text{N} = 907\%$).

While absolute C and N isotopic assimilation based on delta value increases differed for the various *N. radiata* sample types in the jar experiments, standardized assimilation rates using $^{13}\text{C}_{\text{tracer}}$ and $^{15}\text{N}_{\text{tracer}}$ values showed a similar percent uptake of C and N for each sample type, where: muscle < whole clam < lipids (Fig. 1D–F, Fig. 2D–F, Table 2). Whole clam tracer assimilation rates were $0.02\% \text{d}^{-1}$ in both carbon and nitrogen (Table 2), which accrued to a total assimilation of 0.8% of either tracer over the 42 d experimental period (Figs. 1F, 2F).

3.3. *N. radiata* sediment core experiments

N. radiata in the eight non-enriched treatment cores had an average burial depth of 1.5 ± 0.3 cm ($n=12$). Isotopic values in the whole clam samples declined toward the $\delta^{15}\text{N}$ value of the non-enriched algal food source (-3%) (Table 3). In the isotopically enriched food treatment, *N. radiata* had an average burial depth of 1.7 ± 0.3 cm ($n=12$). Linear regressions for experimental days 0–5 and days 5–18 showed no significant increase in $\delta^{13}\text{C}$ values during either time period (Table 3).

3.4. *M. moesta* sediment core experiments

M. moesta in eight non-enriched treatment cores had an average burial depth of 3.7 ± 0.5 cm ($n=11$). No significant temporal changes were seen in *M. moesta* isotope ratios when offered the non-isotopically enriched algal food (Table 4). In the nine enriched treatment cores, *M. moesta* had an average burial depth of 3.9 ± 0.8 cm ($n=9$). All *M. moesta* $\delta^{13}\text{C}$ and $^{13}\text{C}_{\text{tracer}}$ values from the isotopically enriched treatment showed significant increases over the first five day experimental period, where significant isotope and tracer increases were observed with maximum increases in $\delta^{13}\text{C}$ values of $0.24\% \text{d}^{-1}$ and C_{tracer} of $0.09\% \text{d}^{-1}$ in whole clam tissues (Table 4). The non-significant regression results from days 5–18 indicate that a plateau had been reached. For $\delta^{15}\text{N}$, significant changes over time were only found for N tracer (%) values for the period days 0–5.

Table 1

Initial (t_0) *N. radiata* and *M. moesta* stable isotope and C:N values (mean \pm SD, n = number of clams). 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	$\delta^{13}\text{C} \%$ (SD)	$\delta^{13}\text{C}$ groups	$\delta^{15}\text{N} \%$ (SD)	$\delta^{15}\text{N}$ groups	C:N (SD)	C:N groups
Muscle	2009 <i>N. radiata</i>	14	-19.88 (0.28)	1	9.34 (0.71)	1	3.46 (0.28)	1
	2010 <i>N. radiata</i>	5	-19.24 (0.27)	2	10.08 (0.44)	1	3.22 (0.08)	1
	2010 <i>M. moesta</i>	5	-19.06 (0.39)	2	8.36 (0.94)	2	3.52 (0.33)	1
Lipid	2009 <i>N. radiata</i>	13	-26.34 (0.46)	1	5.29 (1.07)	1	31.39 (19.95)	1
	2010 <i>N. radiata</i>	5	-26.17 (0.25)	1	3.66 (1.41)	1	52.16 (12.91)	2
	2010 <i>M. moesta</i>	5	-26.19 (0.14)	1	3.61 (2.59)	1	58.12 (19.66)	2
Whole clam	2009 <i>N. radiata</i>	13	-20.90 (0.51)	1	8.66 (0.29)	1	4.37 (0.40)	1
	2010 <i>N. radiata</i>	5	-20.34 (0.27)	1	8.61 (0.60)	1	4.50 (0.19)	1
	2010 <i>M. moesta</i>	5	-19.94 (0.13)	2	7.72 (0.53)	2	4.30 (0.29)	1

Table 2
Linear regression analyses of the stable isotope and tracer data for *N. radiata* in enriched (E) and non-enriched (NE) 2009 jar treatments. Linear regression statistics are documented with ANOVA, r^2 coefficient, and isotopic/tracer assimilation rates over the entire duration of the experiment (days 0–42), during the early sampling period (days 0–6), and the later sampling period (days 6–20). Values represented in bold font are statistically significant ($p \leq 0.05$).

Jar <i>N. radiata</i>			Days 0–42			Days 0–6			Days 6–20			
Isotope	Treatment	Sample	ANOVA		Linear regression		ANOVA		Linear regression		ANOVA	
			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	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	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	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	Whole clam	0.01	0.24	0.010	0.91	0.00	−0.003	0.39	0.19	0.013	
C tracer ‰	E	Muscle	0.16	0.05	0.004	<0.001	0.97	0.014	0.85	0.00	−0.001	
	E	Whole clam	<0.001	0.52	0.017	<0.001	0.59	−0.023	0.05	0.36	0.012	
N tracer ‰	E	Muscle	<0.001	0.77	0.017	<0.001	0.81	0.021	0.04	0.39	0.009	
	E	Lipids	<0.001	0.86	0.010	<0.001	0.91	0.004	0.02	0.40	0.004	
	E	Whole clam	<0.001	0.88	0.028	0.40	0.05	−0.003	0.01	0.52	0.013	
E	Whole clam	<0.001	0.94	0.020	<0.001	0.85	0.019	0.002	0.67	0.010		

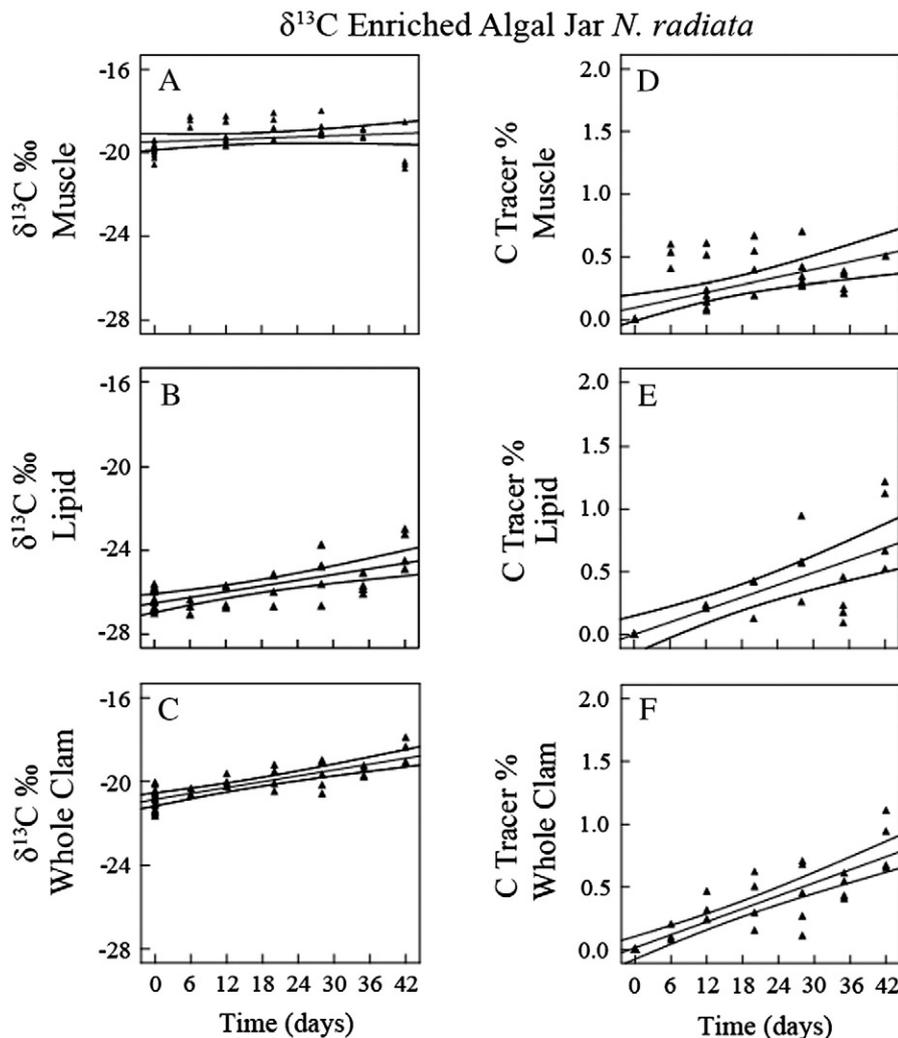


Fig. 1. *Nuculana radiata* $\delta^{13}C$ changes (A–C) and carbon tracer assimilation (D–F) from the isotopically enriched algal treatment in the 2009 jar experiment. Lines are mean linear regressions with 95% confidence.

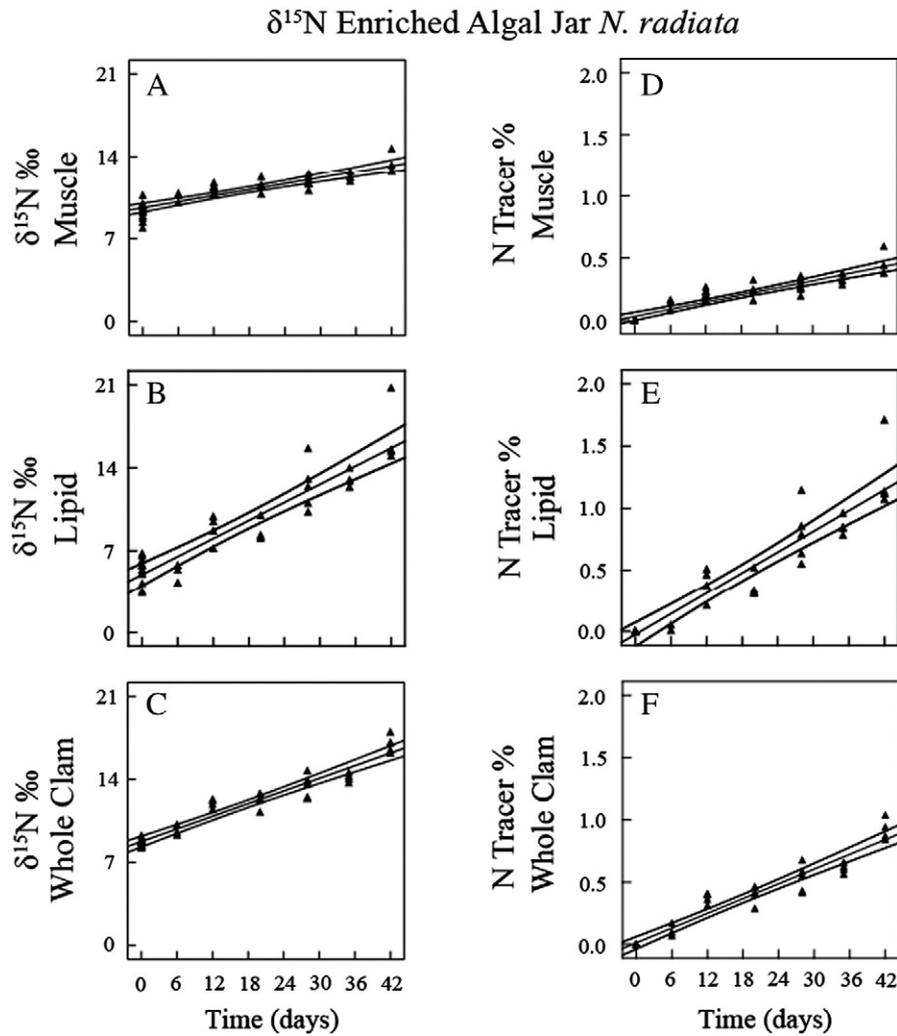


Fig. 2. *Nuculana radiata* $\delta^{15}\text{N}$ changes (A–C) and nitrogen tracer assimilation (D–F) from the isotopically enriched algal treatment in the 2009 jar experiment. Lines are mean linear regressions with 95% confidence.

Table 3

Linear assimilation regression statistics are documented with ANOVA, r^2 coefficient, and isotopic/tracer assimilation rates during the early sampling period (days 0–5) and the later sampling period (days 5–18). Values represented in bold font are statistically significant ($p < 0.05$).

Core <i>N. radiata</i>			Days 0–5			Days 5–18		
Isotope	Treatment	Sample	ANOVA	Linear regression		ANOVA	Linear regression	
			p-value	r^2	Assimilation (d^{-1})	p-value	r^2	Assimilation (d^{-1})
$\delta^{13}\text{C}$ ‰	E	Muscle	0.71	0.03	0.005	0.71	0.01	0.005
	E	Lipids	0.13	0.35	0.134	0.96	0.00	−0.001
	E	Whole clam	0.20	0.26	0.106	0.78	0.01	0.005
$\delta^{15}\text{N}$ ‰	E	Muscle	0.55	0.06	−0.007	0.85	0.00	−0.006
	E	Lipids	0.37	0.14	0.228	0.34	0.09	−0.054
	E	Whole clam	0.78	0.02	0.028	0.39	0.08	−0.023
$\delta^{13}\text{C}$ ‰	NE	Muscle	0.64	0.03	−0.004	0.94	0.00	0.000
	NE	Lipids	0.34	0.13	−0.034	0.57	0.03	−0.005
	NE	Whole clam	0.46	0.08	−0.027	0.33	0.10	−0.007
$\delta^{15}\text{N}$ ‰	NE	Muscle	0.03	0.51	−0.024	0.61	0.03	−0.012
	NE	Lipids	0.60	0.04	−0.097	0.25	0.13	−0.064
	NE	Whole clam	0.06	0.42	−0.199	0.47	0.05	−0.013
C tracer %	E	Muscle	0.66	0.04	0.002	0.71	0.01	0.002
	E	Lipids	0.10	0.39	0.049	0.96	0.00	0.000
	E	Whole clam	0.16	0.30	0.039	0.78	0.01	0.002
N Tracer %	E	Muscle	0.19	0.27	−0.001	0.85	0.00	−0.001
	E	Lipids	0.22	0.24	0.025	0.34	0.09	−0.006
	E	Whole clam	0.64	0.04	0.003	0.39	0.08	−0.003

Table 4
Linear assimilation regression statistics are documented with ANOVA, r^2 coefficient, and isotopic/tracer assimilation rates during the early sampling period (days 0–5) and over the late sampling period (days 5–18). Values in bold font are statistically significant ($p < 0.05$).

Core <i>M. moesta</i>			Days 0–5			Days 5–18		
Isotope	Treatment	Sample	ANOVA	Linear regression		ANOVA	Linear regression	
			p-value	r^2	Assimilation (d^{-1})	p-value	r^2	Assimilation (d^{-1})
$\delta^{13}C$ ‰	E	Muscle	0.01	0.68	0.033	0.24	0.19	0.011
	E	Lipids	0.03	0.58	0.201	0.92	0.00	0.004
	E	Whole clam	<0.001	0.92	0.240	0.77	0.01	0.004
$\delta^{15}N$ ‰	E	Muscle	0.31	0.17	0.032	0.83	0.01	0.006
	E	Lipids	0.58	0.05	0.206	0.73	0.02	0.026
	E	Whole clam	0.08	0.43	0.218	0.65	0.03	0.015
$\delta^{13}C$ ‰	NE	Muscle	0.56	0.04	0.006	0.77	0.01	0.003
	NE	Lipids	0.65	0.02	−0.013	0.70	0.02	−0.003
	NE	Whole clam	0.03	0.42	0.061	0.55	0.04	−0.005
$\delta^{15}N$ ‰	NE	Muscle	0.61	0.03	0.011	0.93	0.00	−0.001
	NE	Lipids	0.93	0.00	0.022	0.24	0.15	−0.044
	NE	Whole clam	0.48	0.06	−0.047	0.96	0.00	0.001
C tracer ‰	E	Muscle	<0.001	0.98	0.012	0.24	0.19	0.004
	E	Lipids	0.03	0.59	0.073	0.92	0.00	0.001
	E	Whole clam	<0.001	0.94	0.089	0.77	0.01	0.002
N tracer ‰	E	Muscle	0.12	0.35	0.004	0.83	0.01	0.001
	E	Lipids	0.28	0.19	0.023	0.73	0.02	0.003
	E	Whole clam	0.02	0.60	0.024	0.65	0.03	0.002

3.5. Cross-experiment whole clam tracer assimilation

Corrected C and N tracer assimilation values in *N. radiata* whole clam samples were compared for individuals fed isotopically enriched algal food in both the 2009 jar experiments and the 2010 core experiments. Significantly (two-sample t-tests) lower N_{CT} assimilation occurred in clams from cores compared to those from jars at days 10/12 (difference of $0.35\% \pm 0.15$, $p = 0.002$) and 18/20 (difference of $0.44\% \pm 0.13$, $p < 0.001$), while C_{CT} assimilation was similar between experiments. Thus, higher $N_{CT}:C_{CT}$ assimilation ratios occurred over time for the clams in jars compared to those in cores (Fig. 3A). The $N_{CT}:C_{CT}$ ratios for the two experiments revealed a 60% difference in tracer assimilation by day 18, calculated from the difference in slopes between the two experiments (1.08 for jar experiments, $r^2 = 0.92$, $p < 0.001$; 0.43 for core experiments, $r^2 = 0.83$, $p < 0.001$; Fig. 3B). This was mostly attributed to higher ^{15}N assimilation in the cores.

C_{CT} and N_{CT} assimilation comparisons between *N. radiata* and *M. moesta* whole clams within the sediment cores (Fig. 4A) showed a significantly (two-sample t-tests) lower assimilation of both tracers for *N. radiata* at day 10 (C_{CT} : $p = 0.035$, difference of $0.31 \pm 0.27\%$; N_{CT} : $p = 0.013$, difference of $0.26 \pm 0.18\%$) and day 18 (C_{CT} : $p = 0.191$; N_{CT} : $p = 0.036$, difference of $0.21 \pm 0.19\%$). Overall, a greater tracer uptake by *M. moesta* over *N. radiata* ($\sim 2:1$, Fig. 4A) was evident over the duration of the core incubations. Although tracer assimilation rates were very different between species (Tables 3 and 4), the ratios of $N_{CT}:C_{CT}$ varied little over time with relatively similar values for *N. radiata* (0.43 , $r^2 = 0.83$, $p < 0.001$, Fig. 4B) and *M. moesta* (0.60 , $r^2 = 0.95$, $p < 0.001$, Fig. 4B), revealing only a 28% difference between slope estimates by day 18.

4. Discussion

This study evaluated the changes in stable isotopic carbon and nitrogen ratios of two clam species after feeding on an isotopically enriched (^{13}C and ^{15}N) food source. Assimilation rates for *N. radiata* and *M. moesta* in jar and core experiments were distinctly different for the specific sample types including muscle, lipids, and whole clam within a species and between the two species or experimental conditions. We discuss the implications of these results for using bulk stable isotope analysis to study food web interactions in natural field collections and for future controlled feeding studies of these species using isotopically labeled feeds.

4.1. Experimental food

The algal food source we used was a mixture dominated by the diatom *N. frigida* with a reduced biodiversity compared to natural ice communities (Gradinger, 1999; Poulin et al., 2011). The isotopically enriched algal food considerably exceeded the observed enrichment of natural ice algal isotope ratios in the semi-closed sea ice system (~ 6 – 10% increase in $\delta^{13}C$ and small to negligible in $\delta^{15}N$) (Gradinger et al., 2009; Hobson and Welch, 1992; Søreide et al., 2006; Tamelander et al., 2009). Our enrichment was designed to provide a substantial and unmistakable isotopic food source (Kaufman et al., 2008), while the non-enriched algal food had isotopic values similar to in situ phytoplankton (Gradinger et al., 2009; Hobson and Welch, 1992; Iken et al., 2010). It is important to note that isotopic enrichment did not change the food quality. The weight-based C:N ratio as a measure of food quality was 11.2 in the experimental food, which represents a mid-range quality food source between the high quality of a sinking marginal ice algal bloom occurring late in the spring and a more refractory, open-ocean particulate organic matter in the summer and fall (Falk-Petersen et al., 1998; Leu et al., 2010; Tremblay et al., 2006). Also, the POC:chl ratio of ~ 65 is near the middle of established ranges of 15–180 for ice algae (Fukuchi et al., 1993; 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 (Cooper et al., 2002; Dunton et al., 2005; Fukuchi et al., 1993; Olson and Strom, 2002). 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 typical for a reduced pelagic production period during the late summer and autumn than an ice algal spring bloom. The provided food was, however, not so low as to induce starvation effects. We concurrently conducted starvation experiments (Weems, 2011; results not reported here), in which isotopic values of clams did not change over the experimental time, while clams provided the relatively low food concentrations in the jar experiments had significant increases in isotope values. To ensure sufficient food supply in 2010, sediment core experiments intentionally received algal food concentrations during a single food pulse in excess of ~ 4 – 5 times that of the natural algal bloom export estimates (Cooper et al., 2002; Fukuchi et al., 1993; Grebmeier et al., 1988).

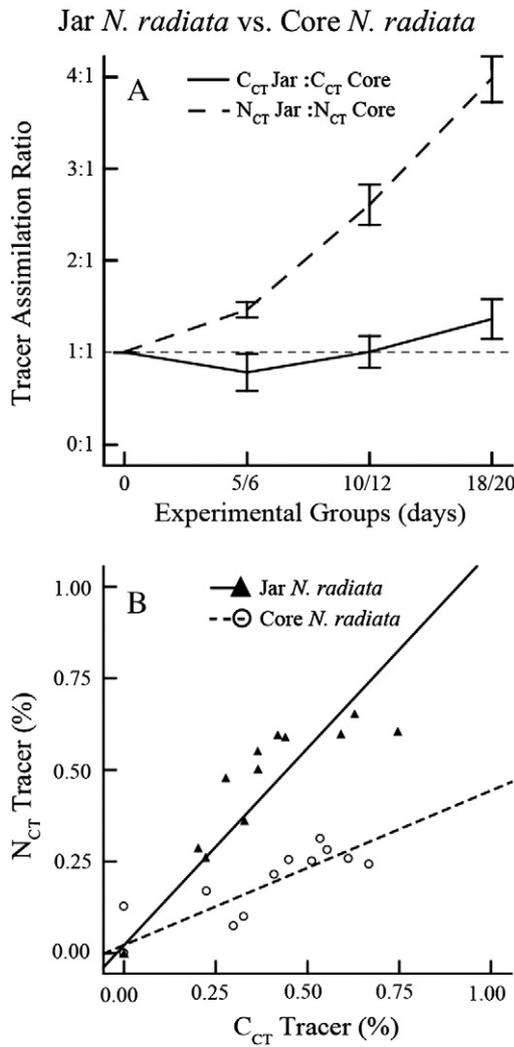


Fig. 3. *Nuculana radiata* tracer assimilation in the enriched whole clam tissues from the 2009 jar and 2010 sediment core experiments, respectively. A) Tracer-specific assimilation between jar and core *N. radiata*, which shows similar C_{CT} assimilation across experiments within compared time periods, while jar clam N_{CT} 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 ($C_{CT} = C_{tracer} + 0.126$; $N_{CT} = N_{tracer} + 0.192$).

4.2. Sample-specific isotope assimilation

Isotopic enrichment during the experimental periods (both jar and core experiments with enriched food) was distinctly different in the different clam sample types. Muscle was the most isotopically stable tissue with little or negligible isotopic enrichment over the course of several weeks. Thus, the muscle may be used as indicator of long-term changes in food quantity and quality but is less suitable to detect short-term responses to diet switches such as sea ice algal export within time frames of days or weeks. Muscle subsampling likely underestimated the true volume of the muscle and its isotopic contribution to whole clam values and did not provide a complete and isolated accounting of all muscle tissues present in 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 this study, lipids consistently had the fastest and highest overall isotopic changes while contributing roughly 15–20% of the whole clam dry weight and isotopic values. Lipids had the lowest $\delta^{13}C$ values across the sample types

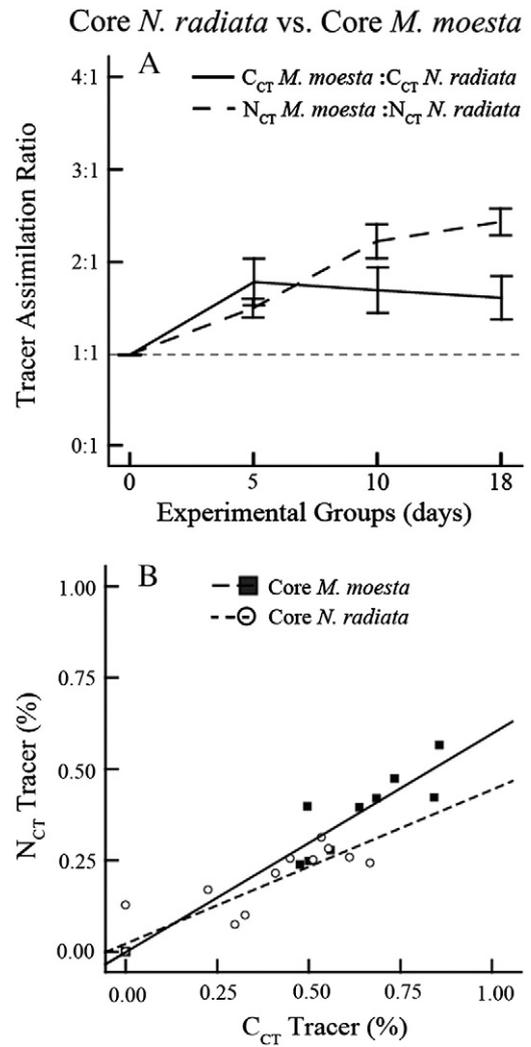


Fig. 4. Tracer assimilation in the isotopically enriched whole clam tissues from the sediment core experiments in 2010. A) Tracer-specific assimilation ratios between *M. moesta* and *N. radiata* within the core experiments show overall higher assimilation ($\sim 2\times$) of both C and N tracers in *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 ($C_{CT} = C_{tracer} + 0.126$; $N_{CT} = N_{tracer} + 0.192$).

because of kinetic isotope effects (DeNiro and Epstein, 1977; Post et al., 2007). The observed increase in the $\delta^{15}N$ values of clam lipids from the jar experiments was unexpected, as lipids generally do not contain a large portion of nitrogen (Elser et al., 1996). We 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 (Mateo et al., 2008; Post et al., 2007). Protein sparing occurred in the mussel *Mytilus edulis* that promoted a 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 (Hawkins and Bayne, 1985). Therefore, the very small pool of labile free amino acids containing nitrogen in the metabolized lipid pool would turn over more rapidly (Hawkins, 1985), causing potentially greater assimilation of the ^{15}N tracer in our enriched treatment clam lipids. The initial t_0 clam high C:N values (31.4–58.1) and low N content in the lipid fractions (0.5–5.0% by mass) identify only a small pool of nitrogen for metabolism in the clams’ lipid fractions. This scenario is likely to overshadow any

unknown amount of contamination from co-extracted proteins, as absolute muscle ^{15}N assimilation was far lower in amplitude than the lipid fractions. Also, previous studies propose that this type of $\delta^{15}\text{N}$ fractionation associated with lipid extraction is minimal (i.e. $<1\%$) (Post et al., 2007; Sotiropoulos et al., 2004). We suggest the use of lipid fraction data to detect short-term changes related to isotopically distinct food sources, such as abrupt or seasonal food switches, where the total lipid isotopic enrichment may be influenced by individual fatty acid compositions of both predator and prey lipid fractions (Budge et al., 2008). For example, McMahan et al. (2006) found that ^{13}C enrichment of a single fatty acid (C20:5(n-3)) in *Macoma calcarea* reached upward of 15% of their ^{13}C enriched food tracer even when the total ^{13}C bulk tissue enrichment was minimal (see discussion below).

Isotopic ratios in bulk tissues of the arctic clams *M. calcarea* and *Liocyma fluctuosa* when offered enriched phytoplankton and ice algal food sources ($\sim 684\%$ $\delta^{13}\text{C}$) increased by $\sim 1\text{--}2\%$ over the 19–30 d in isolation and in core incubations (McMahon et al., 2006). This is about 1.1 to 3.3 times less compared to the whole clam isotopic enrichment we observed in *N. radiata* and *M. moesta* when standardizing for experimental time and food $\delta^{13}\text{C}$ values. Another feeding study by Kaufman et al. (2008) with the arctic amphipod *Onisimus litoralis* in isolated jars yielded half-life estimates of 14 to 30 d in decarbonated whole body samples with asymptotes in isotopic turnover relatively close to their food source values. Since *N. radiata* in our jar experiments did not approach an asymptote for over 42 d, the half-life has to be >21 d and likely much longer, which might be related to the low activity of benthic clams as compared to a highly mobile amphipod.

4.3. Species-specific isotopic tracer assimilation

The ^{15}N assimilation rates for *N. radiata* differed between the incubations conducted in cores and in jars. Part of these differences can be related to the interannual variability in the conditions of the collected specimens, although mean sizes were quite similar for 2009 and 2010. Additional differences between the two experiments relate to the amount of food added and the mode in which it was added (every third day versus a single pulsed addition). Animals in 2009 could feed only on the provided algal feed, while the added food in the 2010 experiment was a) not readily available for the sub-surface feeding specimens and b) might also be consumed by other biota living in the cores including bacteria, meiofauna and other clams. Bacterial response to food influx can be almost immediate (within hours) with changes in bacterial community composition and increases in bacteria-specific fatty acids (Dyda et al., 2009; Sun et al., 2007). This reduces the pool of enriched particulate nitrogen for macrofaunal consumption through bacterial release of DON/DIN (Lovvorn et al., 2005; Sun et al., 2007). The organic matter available in the sediment prior to the food addition would provide additional food for the clams beyond the added material. These fundamental differences between the 2009 and 2010 experiments together explain the lower incorporation of enriched added feed into the clam tissue and suggest that *N. radiata* is not well suited to act as indicator of recent input of freshly-sedimented algal material from the water column or sea ice, at least for organic nitrogen.

The sediment core experiments with *N. radiata* and *M. moesta* demonstrated about 30% less isotopic enrichment in *N. radiata* as compared to *M. moesta*. This can be related to the different feeding behaviors of the two species, which has been used to explain natural $\delta^{13}\text{C}$ differences between suspension and deposit feeding bivalves (Crompton et al., 2008). *M. moesta* is a benthic suspension and surface-deposit feeder, utilizing the top surface layer of the sediments upon which algal food (i.e., experimental enriched food source) settles (McCormick-Ray et al., 2011; Sirenko and Gagaev, 2007; own observations). This feeding behavior using an extended siphon allows for a relatively deep distribution within the sediments (in this study: 3.8 ± 0.5 cm, $n=20$), which has been suggested to potentially provide protection from predation (Richman and Lovvorn, 2003). In contrast, *N. radiata* is a sub-surface deposit feeder

using labial palps, mainly feeding on buried food sources in the sediments at the 1–3 cm depth (Richman and Lovvorn, 2003; this study: 1.6 ± 0.2 cm, $n=24$). Only after a certain degree of sediment bioturbation and likely microbial activities, leading to increased isotopic dilution of the labeled food with surrounding sediments, can freshly deposited food enter the shallow 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). Thus, bioturbation-delayed access to a sediment-diluted algal food likely limited the isotopic enrichment in *N. radiata* in our experiments. The most common natural fauna in the cores were the small clam *E. tenuis*, which also is a shallow (2.2 ± 0.3 cm) sub-surface deposit feeder, similar to *N. radiata* (Richman and Lovvorn, 2003, own observations). It is unknown as to what degree *E. tenuis* may have influenced the feeding or isotopic assimilation of our two target species, although its common occurrence in the study region suggests that it may be a player in competitive interactions with the other two species (McCormick-Ray et al., 2011).

Our observations demonstrate that *M. moesta* is likely a good model organism to track fast responses following a food switch (e.g., sedimentation of sea ice algae versus phytoplankton) in the Bering Sea. Even so, the observed isotopic changes in the sediment core experiment for both clam species (max. increase by $\sim 2\%$ for $\delta^{13}\text{C}$) were much lower than would be expected from direct feeding on the highly enriched food. This implies that in nature, the changes in whole clam isotope values following export of isotopically enriched algal food originating from the ice may be very small and difficult to detect within the natural variability. This is supported by the similarity in isotopic values between our enriched and non-enriched core whole clam values and Bering Sea sediment particulate organic matter values of -20 to -22% for $\delta^{13}\text{C}$ values and 7 to 8% for $\delta^{15}\text{N}$ values prior to spring ice algal export events (Cooper et al., 2002). Furthermore, previously published enrichment of sediment organic matter $\delta^{13}\text{C}$ (1.4 to 3.6‰) and negligible changes in $\delta^{15}\text{N}$ values during and after spring ice algal settlement did not, for the most part, translate into significant enrichment in the isotopic values of *M. calcarea*, *N. radiata*, or *E. tenuis* through seasonal (winter versus spring) comparisons (Lovvorn et al., 2005). This makes it more likely that food switches in nature can only be reliably observed using the isotopic composition of individual markers, such as the individual fatty acids (McMahon et al., 2006). We provide some preliminary data (see Supplementary data) to support the use of fatty acid compound-specific isotope analyses of ice algal food stock in future experiments. Our stable carbon isotopic analyses of individual fatty acids from *N. radiata* fed the isotopically enriched and non-enriched food sources in the jar experiment indicate that the highest isotopic enrichment occurred predominantly in the shorter chained ($<C17$) fatty acids (Supplementary data).

4.4. Bering Sea sympagic–pelagic–benthic coupling and clam dynamics

Recent hypotheses such as the oscillating control hypothesis (Hunt et al., 2002, 2011) emphasize major shifts in the Bering Sea ecosystem functioning related to the extent and timing of sea ice retreat and water temperature. Biomass from large, early-season ice algal blooms combined with early phytoplankton blooms is exported to the benthos 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 ($>60^\circ\text{N}$) 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. High primary production export to the benthos during cold year conditions may particularly favor infauna feeding on fresh algal deposits, like *M. moesta* (Coyle et al., 2007; Grebmeier et al., 2006a). *M. moesta*'s greater direct access as a surface

feeder to fresh food sources may provide a competitive advantage over *N. radiata* as their growth and reproduction may be enhanced with access to this abundant and nutritious ice algal food (Richman and Lovvorn, 2003; Sun et al., 2009). It should be noted that other factors such as metabolic differences, reproductive output and success and adaptation to environmental stressors will also determine the competitive outcome between these two species, but these were not measured in this study.

In juxtaposition to high ice algal export conditions, *M. moesta* may be more dramatically affected by projected decreases in seasonal ice algal production with future loss of sea ice while *N. radiata*, and possibly the small sub-surface feeder *E. tenuis* also found in the core sediments, are less behaviorally adapted for direct feeding on fresh export. Pelagic phytoplankton blooms in warmer years are heavily grazed upon by planktonic zooplankton, which have returned to the surface waters from overwintering depths during the late spring (Coyle and Pinchuk, 2002; Hunt et al., 2011). This results in significantly less algal export to the benthos; and thus, bacterial reworking of less abundant food sources in the sediment increases the use of long-term, mostly refractory material by benthic feeders (Grebmeier et al., 2006b). 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), especially for subsurface deposit feeders. If the northern Bering Sea seafloor acted like a food bank for subsurface deposit feeders, it 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, fluctuations in sea ice and oceanographic temperature regimes will influence the abundance of Tellinidae (*Macoma* spp.) and Nuculanidae (*N. radiata*) clam species (Grebmeier, 2012; Lovvorn et al., 2009; McCormick-Ray et al., 2011). While both target species are abundant and important benthic fauna components (Lovvorn et al., 2009), they show fluctuations over time (Grebmeier, 2012). For example, *M. 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 fluctuated inversely, 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 have implications on higher trophic levels that feed upon these and other benthic invertebrates. Diving waterfowl such as eiders (*Somateria* spp.) and scoters (*Melanitta fusca*) feed directly upon these clams (Lovvorn et al., 2003, 2009); thus, changes in clam species abundance may have implications on the feeding energetics of these birds. Additionally, bottom feeding mammals like walrus (*Odobenus rosmarus divergens*), bearded seals (*Erignathus barbatus*), and gray whales (*Eschrichtius robustus*) that mostly feed on an assortment of larger polychaetes (Maldanidae and Nephthyidae), amphipods (Ampeliscidae and Lysianassidae), crabs (*Chionoecetes* spp.) or mollusks (*Mya truncata*, *Serripes groenlandicus*, Gastropoda), often by-catch these small clams and could thus be affected (Bluhm and Gradinger, 2008; Grebmeier, 2012). The changes in abundance of different clam species due to decreased sympagic–benthic coupling with sea ice dynamics 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 (Coyle et al., 2007; Grebmeier, 2012; Hunt et al., 2011; Mueter et al., 2009).

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Appendix A. Supplementary data

Supplementary data to this article can be found online at <http://dx.doi.org/10.1016/j.jembe.2012.06.015>.

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