Quantifying the $\delta^{15}N$ trophic offset in a cold-water scleractinian coral (CWC): implications for the CWC diet and coral $\delta^{15}N$ as the marine N cycle proxy

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Abstract. The nitrogen (N) isotope composition ($\delta^{15}N$) of cold-water corals is a promising proxy for reconstructing past ocean N cycling, as a strong correlation was found between the $\delta^{15}N$ of the organic nitrogen preserved in coral skeletons and the $\delta^{15}N$ of particulate organic matter exported from the surface ocean. However, a large offset of 8-9 ‰ between the $\delta^{15}N$ recorded by the coral and that of exported particulate organic matter remains unexplained. The 8-9 ‰ offset may signal a higher trophic level of coral dietary sources, an unusually large trophic isotope effect or a biosynthetic $\delta^{15}N$ offset between the coral's soft tissue and skeletal organic matter, or some combinations of these factors. To understand the origin of the offset and further validate the proxy, we investigated the trophic ecology of the asymbiotic scleractinian cold water coral *Balanophyllia elegans*, both in a laboratory setting and in its natural habitat. A long-term incubation experiment of *B. elegans* fed on an isotopically controlled diet yielded a canonical trophic isotope effect of 3.0 ± 0.1‰ between coral soft tissue and the *Artemia* prey. The trophic isotope effect was not detectably influenced by sustained food limitation. A long N turnover of coral soft tissue, expressed as an e-folding time, of 291 ± 15 days in the well-fed incubations indicates that coral skeleton $\delta^{15}N$ is not likely to track subannual (e.g. seasonal) variability of diet $\delta^{15}N$. Specimens of *B. elegans* from the subtidal zone near San Juan Channel (WA, USA) revealed a modest difference between soft tissue and skeletal $\delta^{15}N$ of 1.2 ± 0.6 ‰. The $\delta^{15}N$ of the coral soft tissue was 12.0 ± 0.6 ‰, which was ~6 ‰ higher than that of suspended organic material that was comprised dominantly of phytoplankton – suggesting that phytoplankton is not the primary component of *B. elegans'* diet. An analysis of size-fractionated net tow material suggests that *B. elegans* fed predominantly on a size class of zooplankton ≥ 500 μm, implicating a two-level trophic transfer between phytoplankton material and coral tissue. These results point to a feeding strategy that may result in an influence of regional food web structure on the cold-water coral $\delta^{15}N$. This factor should be taken into consideration when applying the proxy to paleoceanographic studies of ocean N cycling.

1

1 Introduction

Interactions between ocean circulation and nutrient cycling modulate the marine biological carbon pump, the consequent partitioning of CO_2 between atmosphere and ocean, and thus influence planetary climate on centennial to millennial time scales (Sigman and Boyle 2000). The marine nitrogen (N) cycle is highly sensitive to these interactions, such that knowledge of modern and ancient ocean N cycling can help illuminate drivers of past climate and contextualize modern global change (*e.g.*, Altabet et al., 1994; Francois et al., 1997; Robinson and Sigman 2008; Sigman et al., 1999; Kast et al. 2019).

The main tool to investigate the oceanic N cycle history is the nitrogen (N) isotope composition (*i.e.*, the $^{15}N/^{14}N$ ratio) of particulate organic nitrogen (PON) exported from the euphotic zone and preserved in various paleo-archives, including bulk sedimentary N in anoxic sediments (*reviewed by* Robinson et al. 2023). Hereafter, we express the $^{15}N/^{14}N$ ratio in delta notation, where $\delta^{15}N$ (‰ *vs.* air) = $[[(^{15}N/^{14}N_{sample})/(^{15}N/^{14}N_{air})] – 1]*1000$. The $\delta^{15}N$-PON recorded in paleo-oceanographic archives reflects both regional N cycling processes and the balance of global ocean N source and sink terms (Sigman and Fripiat 2019; Brandes and Devol 2002). In regions of the ocean where nitrate is quantitatively consumed, the annually integrated $\delta^{15}N$-PON exported from the surface reflects the isotopic composition of thermocline nitrate (Altabet et al. 1991). The latter is influenced by the circulation history of nitrate (*e.g.*, Marconi et al., 2015), by regional N_2 fixation (*e.g.*, Casciotti et al. 2008; Knapp et al. 2008) and by water column denitrification (*e.g.*, Pride et al., 1999; De Pol-Holz et al., 2007). In regions with incomplete consumption of surface nitrate, such as Southern Ocean, the isotopic discrimination imparted during nitrate assimilation is reflected in the $\delta^{15}N$-PON, which can be used to reconstruct the degree of surface nitrate consumption in the past (*e.g.,* Sigman et al., 1999; Francois et al. 1997).

Accurate interpretation of the N cycle's paleo-history relies on the presumption that the $\delta^{15}N$-PON preserved in various palaeoceanographic archives is impervious to organic matter diagenesis. Bulk sedimentary $\delta^{15}N$ measurements are thus generally inadequate in this respect, subject to post-depositional processes (Robinson et al. 2012) – barring fast-accumulating organic-rich anoxic sediments with negligible contribution from terrestrial sources (*e.g.*, Altabet et al., 2002; Ganeshram and Pedersen, 1998). To circumvent this limitation, several "biological" archives of the $\delta^{15}N$-PON have been developed that are deemed resistant to diagenetic alteration. These include the organic matter in in diatom frustules and foraminifera tests (*e.g.*, Ren et al., 2009; Robinson and Sigman, 2008) and the organic matter in proteinaceous corals (*e.g.*, Sherwood et al. 2009; Williams and Grottoli 2010). Recently, the $\delta^{15}N$ of organic N enclosed within the aragonite mineral lattice of asymbiotic scleractinian (stony) cold-water corals (CWCs) has been found to reflect the $\delta^{15}N$-PON exported from the surface

138 ocean (Wang et al., 2014), offering an exciting new archive of marine N cycling (Wang et al. 2017; Li et al.,

139 2020, Studer et al., 2018; Chen et al. 2023). A robust cold-water coral archive of δ^{15}N-PON can complement the

140 existing suite of nitrogen proxies by reducing the potential biases inevitable for almost any individual proxy,

141 allowing for a broader geographic and temporal reconstruction, and increasing resolution of the proxy record.

142 Foremost, as with foraminifera and diatom shells, organic material trapped within the coral's original aragonite

143 mineral lattice is largely protected from diagenetic alteration (Drake et al. 2021), and compromised areas can be

144 avoided by inspecting the skeletons for contamination and recrystallization (*e.g.*, borings) using microscopic

145 techniques (Gothmann et al. 2015). CWCs have a broad geographic distribution, being present in all ocean basins

146 from the surface to 5000 m (Freiwald, 2002). CWCs also offer the potential to generate high-resolution records

147 extending relatively far back in time, and corals have continuous skeletal accretion that records ocean conditions

148 at the time of growth, so the analysis of multiple individuals provides enhanced temporal resolution of long-time

149 record (Robinson et al., 2014; Hines et al. 2015). Unlike sediments containing microfossils (e.g. diatoms and

150 foraminifera) CWC skeletons are not subject to bioturbation and absolute ages of this paleoarchive can be

151 determined with decadal precision on the time scales of glacial-interglacial climate variability through U-Th

152 series dating (Cheng et al., 2000; Goodfriend et al. 1992, Robinson et al., 2014, Li et al., 2020). Remarkably,

153 individual coral samples can archive multiple seawater properties, such that a single CWC specimen can

154 potentially be used to reconstruct deep (*e.g.*, Δ^{14}C, pH, temperature, and circulation proxies such as Ba/Ca

155 and ϵNd) and surface ocean conditions (δ^{15}N) at a precisely-known time (U-Th dating), making CWC unique as a

156 paleoceanographic archive (Robinson et al., 2014; Thiagarajan et al., 2014; Rae et al. 2018).

157 Yet an outstanding concern about the fidelity of the δ^{15}N of coral-bound organic N is a reported 8 - 9 ‰

158 offset between coral-bound δ^{15}N and the corresponding δ^{15}N-PON exported to regions of coral growth (Wang et

159 al. 2014). The magnitude of this offset substantially exceeds the 3 - 3.5 ‰ expected for a single trophic transfer

160 (Minagawa and Wada 1984), assuming CWC feed predominantly on algal material exported from the surface

161 ocean. Wang et al. (2014) explained the magnitude of the offset by arguing that CWCs feed on the more

162 abundant pool of surface-derived suspended organic material (SPOM), as the δ^{15}N SPOM at depth is typically

163 ~4-5‰ higher than that of sinking PON (Altabet 1988, Saino and Hattori, 1987). While CWCs are considered

164 generalists with regard to diet (e.g., Mortensen, 2001; Freiwald, 2002; Carlier et al., 2009, Maier et al. 2023), a

165 number of studies suggest that many species of CWC subsist predominantly on metazoan zooplankton prey (e.g.,

166 Naumann et al. 2011; Kiriakoulakis et al. 2005; Purser et al. 2010; Tsounis et al. 2010). A zooplankton diet

167 should result in an approximate two-level or more trophic transfer between surface PON and coral tissue (*e.g.*,

194 Sherwood et al. 2008), closer to the observed 8-9 ‰ offset, potentially rendering coral-bound δ^{15}N sensitive to

195 spatial and temporal differences in trophic-level food web structure. An alternative explanation for the offset is

196 that there is a large biosynthetic offset between the δ^{15}N of the CWC polyp and its skeletal tissue (Horn et al.

197 2011; Muscatine et al. 2005), assuming that CWCs' diet derives directly from sinking algal material from the

198 surface ocean. Otherwise, there could be an atypically large N isotope fractionation associated with the trophic-

199 level transfer between the coral diet and its tissue (>3-3.5‰), possibly borne out of intermittent starvation periods

200 (Doi et al., 2017), which is then passed on to the organic matrix within the coral skeleton. The gap in our

201 understanding of how corals record the δ^{15}N-PON exported form the surface ocean raises questions regarding the

202 consistency of the offset in space and time, and whether it is likely to differ among CWC species or due to intra-

203 specific variations in diet.

204   Due to the challenges of accessing deep-ocean environments, the trophic ecology of cold-water corals is

205 sparsely documented, yet is fundamental to understanding the role of CWCs in cold-water reef ecosystems and to

206 defining their utility as paleoceanographic archives of N cycling. The nature of the δ^{15}N offset between CWC

207 skeletal material and exported PON must be explained in order to further validate and potentially improve the use

208 of δ^{15}N of CWC skeletons as a proxy to reconstruct the history of exported PON and to further understand the

209 role of CWCs in benthic ecosystems. To this end, we studied *Balanophyllia elegans,* an asymbiotic scleractinian

210 cold-water coral found along the west coast of North America that grows as individual polyps (Fadlallah, 1983).

211 We investigated the following questions: a) Is there a large offset in δ^{15}N between coral polyp tissue and coral

212 skeletal tissue? b) Is there an unusually large trophic-level offset between coral tissue and coral diet? c) Does *B.*

213 *elegans* feed predominantly on suspended particulate organic matter (SPOM) *in situ*? or d) does *B. elegans* feed

214 predominantly on metazoan zooplankton, resulting in a two-level trophic transfer between coral tissue and N of

215 export? To evaluate question (a), we measured the δ^{15}N of tissue-skeleton pairs of coral samples collected in their

216 natural habitat. To evaluate question (b), we cultured *B. elegans* corals in the laboratory in experiments where

217 both the isotopic composition of food and the frequency of feeding was controlled. To evaluate questions (c) and

218 (d), we also investigated the δ^{15}N of various components of the food web at a field site where *B. elegans* are

219 found plentifully. Our observations offer novel insights on the growth and trophic ecology of *B. elegans*,

220 providing unique new data on the N metabolism of CWC and their feeding ecology. We contextualize our

221 conclusions to inform the use of CWC archives as a paleo-proxy for marine N cycling and ocean

222 biogeochemistry.

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2. Methods

2.1 Collection of live coral specimens

   Individual specimens of the cold-water coral *Balanophyllia elegans* were collected during four sampling campaigns in March and June 2019, and September and November 2020 from the San Juan Channel near the University of Washington's Friday Harbor Laboratory off the coast of Washington State in the Salish Sea (48.5º N, -123.0º W; Figure 1). *B. elegans* is a solitary, asymbiotic cold-water cup coral native to the Pacific Northwest that can be found both in shallow rocky environments and at depths as great as 500 m (Durham and Barnard 1952). The genus *Balanophyllia* is cosmopolitan and fossil samples as old as Eocene in age have been used for paleoenvironmental study (Muhs et al. 1994; Gothmann et al., 2015; Gagnon et al. 2021). *B. elegans's* presence at near surface depths makes it an easy target for culture experiments, and *Balanophyllia sp.* can be found co-occurring with the similar but more widely applied cold-water coral archive, *Desmophyllum dianthus* (Margolin et al. 2014). Therefore, we consider the asymbiotic *Balanophyllia sp.* to be generally representative of other deep cold-water coral species.
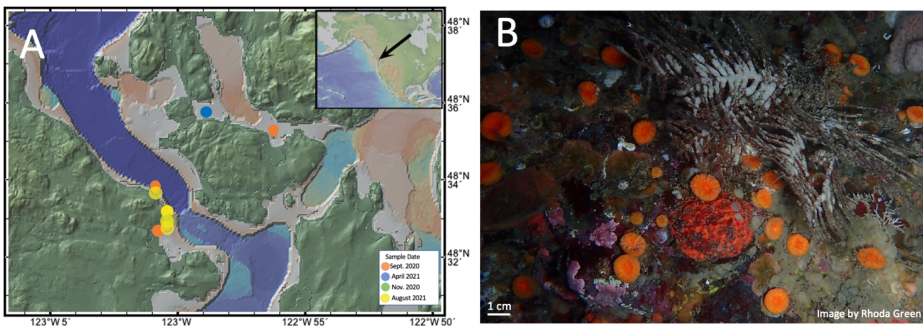
Figure 1. (a) Map of the San Juan Islands indicating the collection site of *B. elegans* specimens and hydrographic measurements (created using http://www.geomapapp.org, Ryan et al. 2009). Inset shows where the San Juan Islands are situated within North America. (b) Image of *B. elegans* from the San Juan Channel near Friday Harbor Labs taken by Rhoda Green.

   B. elegans specimens were collected at 10 to 20 m depth by divers who gently removed the corals from vertical rock walls using blunt-tipped diving knives. Of the live corals collected, a subset was immediately frozen at -18ºC for N isotope ratio analyses of soft tissue and organic matter bound in the coral skeleton matrix. Live specimens were shipped overnight in small bags of seawater on ice to St. Olaf College (Minnesota, USA). Corals

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276 were cleaned by gently scraping the exposed skeleton with dental tools to remove encrusting organisms and

277 placed in incubation bottles with artificial seawater for recovery prior to feeding experiments (described below).

278 2.2 Live coral maintenance

279   Live *B. elegans* corals were maintained in artificial seawater medium prepared from nitrate-free Instant

280 Ocean® Sea Salt. Salts were dissolved in deionized water to a salinity of 28.0 ± 0.25 – akin to the conditions at

281 the collection site (Murray et al., 2015) – and sparged with air to achieve atmospheric equilibrium. The pH of the

282 seawater was measured with a YSI brand 4130 pH probe and adjusted using dilute (0.1 N) hydrochloric acid or

283 sodium hydroxide to 8.14 ± 0.05, slightly higher than *in-situ* conditions to promote skeletal growth. Batch

284 seawater was then allotted to 2 L airtight polypropylene bottles to incubate single coral polyps. Bottles were pre-

285 cleaned with fragrance-free soap and multiple rinses of deionized water. The salinity, pH, and temperature in the

286 incubation bottles were monitored using YSI brand probes (4310(W) conductivity cell and pH probe,

287 respectively) as well as dissolved oxygen concentrations using an optical sensor (FDO 4410; Figure S1); a

288 Multilab 4010-3w was used as the digital meter for the sensors. The bottles containing individual corals were

289 randomly distributed among three recirculating water baths maintained at a constant temperature of 12.5 ± 0.2 ºC,

290 akin to the conditions at the collection site (Murray et al., 2015). Small but quasi-systematic differences of ±

291 0.3˚C were observed among the three recirculating tanks (Figure S2). Corals were sustained on a diet of *Artemia*

292 *salina* nauplii (described below), fed twice a week to ensure maximum growth (Crook et al., 2013). Seawater in

293 the incubation bottles was replaced twice a week after the corals were fed, based on observations indicating that

294 seawater pH in the bottles decreased slightly but significantly by ~0.03 pH units over three days due to coral

295 respiration (statistical analysis was performed with RStudio; Welch two sample t-test; $t(515.07)= 12.8$; p-value <

296 0.01; Figure S3). Dissolved oxygen concentrations remained near atmospheric equilibrium at concentration of 7.5

297 ± 0.3 mg L^{-1} (Figure S1). Nitrate concentrations in the bottles were also monitored from samples taken during

298 each water change, in the freshly prepared seawater and in spent seawater, revealing low variability in NO_3^-

299 concentration of 0.7 ± 0.3 µmol L^{-1} (Figure S4). Nitrate concentrations in the incubations were notably lower than

300 ambient levels at the collection site, where concentration were ~25 µmol L^{-1}, ensuring that the coral's only source

301 of nitrogen was the *Artemia* diet (Murray et al., 2015).
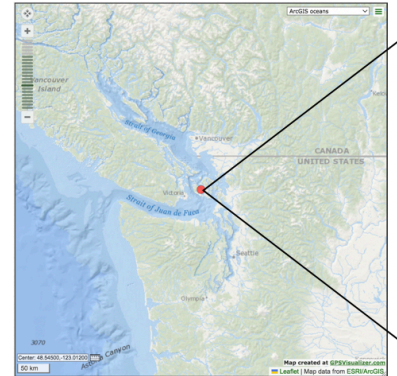
2.3 Coral culture experiments

2.3.1 *Experiment to quantify the trophic isotope effect*

The corals were acclimated to precise incubation conditions for approximately 20 hours before initiating feeding experiments. To assess the $\delta^{15}N$ of coral soft tissue compared to that of its food source, four experimental groups of individual *B. elegans* corals were fed respective diets of *Artemia salina* nauplii with different $\delta^{15}N$ values, twice per week for 530 days (Spero et al., 1993). Unhatched *Artemia salina* sourced from specific geographic locations have widely different $\delta^{15}N$ values, owing to the different N isotope dynamics of the environments from which they were collected, which makes these organisms useful for trophic studies (Spero et al. 1993). Eighteen coral specimens were fed *Artemia* nauplii hatched from cysts from the Great Salt Lake (Reference Code: GSL) with a $\delta^{15}N$ of 17.0 ± 0.3 ‰. Twelve corals were fed hatched nauplii from Lake Ulzhay in Russia (Reference Code: 1816) with a $\delta^{15}N$ of 13.8 ± 0.4 ‰. Twelve corals were fed hatched nauplii from Vinh Chau in Vietnam (Reference Code: 1805) with a $\delta^{15}N$ of 9.9 ± 0.3‰. Twelve corals were fed hatched nauplii from Tibet (Reference Code: 1808) with $\delta^{15}N$ of 6.3 ± 0.2‰. The GSL *Artemia* was procured from Aquatic Foods California Blackworm Co. (Great Salt Lake), whereas all other *Artemia* were obtained from the Artemia Reference Center (Ghent, Belgium). The $\delta^{15}N$ of the diet for each treatment was calculated as the mean value measured from each group of unhatched cysts and hatched nauplii (Table S2 and S3).

Fresh batches of nauplii were hatched from *Artemia* cysts at approximately monthly intervals, filtered into a concentrated suspension, stored frozen at -18ºC, and thawed immediately before feeding to the corals. Due to low hatch rates of the *Artemia* group 1808, corals in that treatment group were fed nauplii harvested from decapsulated *Artemia* cysts from day 151 (November 19, 2019) to 245 (February 22, 2020). The $\delta^{15}N$ of the hatched nauplii ranged from 6.3 ± 0.2 to 17.0 ± 0.3 ‰ (measured by EA-IRMS; Table S2). The $\delta^{15}N$ of the nauplii did not change significantly over prolonged storage of several months in the freezer (ANOVA test; $F(1) = 0.07$, p-value = 0.80; Figure S5). *Artemia* nauplii had a statistically indistinguishable molar C:N ratios among regional groups, averaging 6.0 ± 0.6 (ANOVA test; $F(3) = 0.31$; p-value = 0.82, Table S3). These results show that there was limited variability in the diet of corals due to freezer storage and hatching of multiple individual batches of *Artemia* (Table S2, S3, Figure S5).

Corals were fed their respective nauplii diets by transferring coral individuals from their incubation bottle to a small dish filled with artificial seawater with minimal exposure to air so as not to stress the corals. Each coral was fed 20 µL of thawed nauplii suspension by pipetting the food directly into their oral cavity, making it possible to visually ensure complete consumption and thus minimize variability in feeding rates. Each coral was

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returned to its bottle with a fresh allotment of seawater when its mouth had remained closed for several minutes, signifying that it was finished eating (Figure 2).

After a shift in the $\delta^{15}N$ of diet, it is expected that coral tissue $\delta^{15}N$ will evolve as a function of time until the composition of tissue reaches an equilibrium in line with the new diet. In order to assess the rate (referred to here as the isotopic turnover time) at which this evolution occurs, individual corals were sacrificed at discrete intervals throughout the experiment. Corals were always sacrificed three days after feeding to ensure that no food remained in the oral cavity. The corals were removed from their bottles and rinsed with artificial seawater. The coral tissue was then separated from the skeleton using a fine stream of compressed air. The tissue and skeleton were frozen at -18ºC and stored separately until processed for isotope ratio analyses.
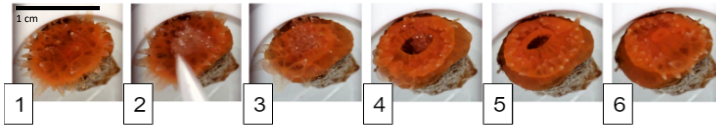
Figure 2. Photo illustration of a coral feeding sequence. Photo 1 shows coral before food is given. Photo 2 shows food being pipetted onto coral mouth. Photos 3 through 6 show the coral feeding as the mouth opens to engulf food and closes when finished, about 15 minutes in total. Corals are ~1 cm in diameter.

2.3.2   Experiment to evaluate the effects of starvation conditions

An additional 522-day feeding experiment was performed to assess the influence of starvation on the $\delta^{15}N$ of the coral soft tissue. Live corals collected during a sampling campaign at the end of November 2020 and shipped live to St. Olaf College were randomly assigned to two treatment groups (starved and not-starved). Corals in the starved treatment were fed at 25% of our normal feeding frequency, or every two weeks, whereas those in the not-starved treatment were fed twice a week. These feeding regimes were chosen based on the work of Crook et al. (2013) and Beauchamp et al. (1989), who assumed feeding every 3 days to represent plentiful food supply and feeding every 21 days (close to our starvation condition) to represent minimal maintenance food supply. Both groups were fed *Artemia* nauplii with a $\delta^{15}N$ of 9.9 ± 0.3 ‰, approximately 3 ‰ lower than the coral tissue of average *B. elegans* collected from Friday Harbor, and thus presumably closest in $\delta^{15}N$ to what the corals is eating in the wild given a canonical trophic isotope effect. Coral incubations and feedings were conducted as described above. Individuals were sacrificed over the course of the 522-day experiment, and tissue samples were frozen at -18ºC until isotope analysis.

405   2.4 Coral preparation for isotope ratio analyses

406   Frozen coral tissue samples (and hatched nauplii) were freeze-dried using a Labconco FreeZone 4.5 and then

407   powdered using a mortar and pestle. The samples were sent to the University of Connecticut, Avery Point

408   (Groton, CT, USA) for isotope ratio analyses.

409   Coral skeletons from specimens collected at Friday Harbor were separated from the coral soft tissue and were

410   rinsed and individually and ultrasonicated two times in Milli-Q™ (MQ) water for 20 minutes each in order to

411   remove any residual seawater. Samples were then individually ultrasonicated in a 1% sodium hypochlorite

412   (bleach) solution for at least two 20-minute intervals with fresh bleach for each new ultrasonication interval until

413   no tissue remained on the skeleton, as assessed visually under a dissection microscope. Individual skeletons were

414   then rinsed and ultrasonicated for 20 minutes in MQ another three times (each time with a new batch of MQ

415   water) in order to remove any bleach residue. Skeleton samples were sent to Pomona College (California, USA)

416   for further processing.

417   It is necessary to isolate organic matter from the coral carbonate matrix in advance of the N isotope

418   measurement methods used here (see Section 2.6 below). Organic material in the skeleton matrix was isolated

419   and oxidized to nitrate following the protocol of Wang et al. (2014). Briefly, bulk samples weighing 50-100 mg

420   were ground into coarse powder, and a fraction between 63 and 200 μm was collected by sieving through two

421   metal sieves. The 10-15 mg of sieved powder was rinsed sequentially with of sodium polyphosphate-sodium

422   bicarbonate buffered dithionite-citrate reagent, then treated with 13.5% sodium hypochlorite overnight on a

423   shaker. Skeletal material was dissolved in 4 N ultrapure hydrochloric acid, then oxidized to nitrate by autoclaving

424   in basic potassium persulfate solution. Standards of glutamine reference material USGS-40 and USGS-41

425   (respective δ^{15}N of 4.52 ‰ vs. air and 47.57 ‰ vs. air) were oxidized in tandem and used to correct for

426   processing blanks. The resulting nitrate samples were sent to the University of Connecticut for nitrate isotope

427   ratio analysis. The long-term averaged reagent blank was 0.4-0.6 μmol L^{-1}, while the typical samples were 10-15

428   μmol L^{-1} (typical amount of nitrogen in skeleton being 2-5 μmole/g of aragonite). Samples were typically run in

429   duplicates with an average reproducibility of ~ ± 0.5 ‰. An internal laboratory standard of ground material of the

430   cold-water colonial scleractinian coral *Lophelia pertusa* had a long-term δ^{15}N value 9.4 ± 0.8 ‰ (n=57)

431   2.5 Hydrographic data

432   To infer the natural food source of the *B. elegans*, we collected samples for analysis of the δ^{15}N of particulate

433   and dissolved N pools in relation to ambient hydrographic variables (temperature and salinity) near Friday

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443 Harbor, WA. Seasonal sampling campaigns were conducted in September and November 2020 and in April,

444 June, and August 2021 (Table S1). In all but the August 2021 campaign, particulate and dissolved N samples

445 were collected by divers at unspecified depths between the surface and the depth of coral collection. Samples

446 were stored frozen in 30 mL HDPE bottles. Surface net tows were performed with a mesh size of 120 µm;

447 materials were stored and shipped frozen and thawed at a later time to be filtered onto pre-combusted GF/F filters

448 (0.7µm nominal pore size) that were stored frozen pending isotope analysis. No hydrographic variables were

449 recorded during the campaigns except in August 2021.

450   During the August 2021 campaign, depth profiles of temperature and salinity from the surface to 35 m were

451 characterized with a CastAway®-CTD (conductivity temperature depth) profiler. Water samples were collected

452 at 5 m intervals between 5 and 30 m using a Van Dorn water sampler. Water was filtered onto pre-combusted

453 glass fiber filters (GF/F; 0.7µm nominal pore size) into pre-cleaned 30 mL HDPE bottles and stored frozen

454 pending analyses of nitrate concentrations and nitrate isotope ratios. The corresponding filters were stored frozen

455 for isotope ratio analysis of suspended particulate organic material (SPOM). Surface (5 m) and deeper (25 m to

456 the surface) net tows were conducted using plankton nets with respective mesh sizes of 150 µm and 80 µm. Net

457 tow material was filtered directly onto a pre-combusted GF/F filters and frozen pending analysis. A portion of the

458 net tow material from the August 2021 campaign was sieved to separate size classes of 80-100 µm, 100-250 µm,

459 $\geq$ 250µm, 250-500 µm, and $\geq$ 500 µm. Material from the respective size classes was filtered onto pre-combusted

460 GF/F filters and frozen until isotope analysis.

461 2.6   Nitrate concentrations and isotope ratio analyses

462   Nitrate concentrations of oxidized coral skeletons and in aqueous samples were measured by reduction to

463 nitric oxide in hot vanadium III solution followed by chemiluminescence detection of nitric oxide (Braman and

464 Hendrix, 1989) on a Teledyne chemiluminescence NOx analyzer Model T200 (Thousand Oaks, CA).

465   The $\delta^{15}N$ and $\delta^{13}C$ of lyophilized coral tissue samples were analyzed at the University of Connecticut on a

466 Costech Elemental Analyzer–Isotope Ratio Mass Spectrometer (Delta V). Approximately 0.75 mg of lyophilized

467 sample (35 µg N) was allotted into tin cups and analyzed in tandem with recognized glutamine reference

468 materials USGS-40 and USGS-41 with respective $\delta^{15}N$ (*vs*. air) of 4.52 ‰ and 47.57 ‰ and $\delta^{13}C$ of -26.39 ‰

469 and 37.63 ‰ (*vs*. PDB). Replicate analyses of (n $\geq$ 2) reference materials yielded an analytical precision of (± 1

470 SD) of 0.3 ‰ for both $\delta^{15}N$ and $\delta^{13}C$.

10

Nitrate N (and O) isotope ratios of aqueous seawater samples and N isotope ratios of the skeleton matrix samples were analyzed at University of Connecticut using the denitrifier method (Casciotti et al., 2002; McIlvin and Casciotti, 2011; Sigman et al., 2001). Nitrate sample solutions were injected at target concentrations of 20 nmol for seawater samples and 7 nmol for skeleton matrix samples. N_2O was extracted, concentrated and purified using a custom-modified Thermo Gas Bench II equipped with a GC Pal autosampler and dual cold traps and analyzed on a Thermo Delta V Advantage continuous flow isotope ratio mass spectrometer (Casciotti et al., 2002; McIlvin and Casciotti, 2011). Individual analyses were referenced to injections of N_2O from a pure gas cylinder and standardized through comparison potassium nitrate reference materials International Atomic Energy Agency nitrate (IAEA-N3) and the isotopic nitrate reference material United States Geological Survey 34 (USGS-34), with respective $\delta^{15}N$ *vs.* air of 4.7 ‰ and -1.8 ‰ vs. air (International Atomic Energy Agency, 1995), and respective $\delta^{18}O$ of 25.61 ‰ and -27.9 ‰ vs. Vienna Standard Mean Ocean Water (VSMOW; Gonfiantini, 1995; Böhlke et al., 2003). To account for bacterial blanks and source linearity, nitrate concentrations of the standard material – diluted in N-free seawater for aqueous seawater samples and air-equilibrated milli-Q water for skeleton matrix samples – were matched to those of samples within batch analyses, and additional bacterial blanks were also measured (Weigand et al., 2016; Zhou et al., 2022). Replicate measurements (n ≥ 2) of all samples yielded an average analytical precision (±1 SD) of 0.3‰ for both $\delta^{15}N$ and $\delta^{18}O$.

3. Results

3.1 <u>Trophic isotope effect</u>

At the onset of the culture experiment, the soft tissue among all experimental corals had a $\delta^{15}N$ of 11.7 ± 0.5 ‰. Over the course of the experiment, the $\delta^{15}N$ of the tissue increased or decreased in respective treatments depending on the $\delta^{15}N$ of their *Artemia* diet (Figure 3); the tissue $\delta^{15}N$ increased in corals fed diets with $\delta^{15}N$ values of 17.0, 13.8, and 9.9 ‰, whereas the tissue $\delta^{15}N$ decreased for the diet of 6.4 ‰. The $\delta^{15}N$ of soft tissue in all groups trended towards an asymptotic offset relative to the diet $\delta^{15}N$, as expected for an approach to a new equilibrium. However, at day 530, at the end of the experiment, it appeared as though the coral tissue $\delta^{15}N$ had not yet reached a constant offset value, suggesting that the coral tissue had not yet reached an equilibrium with the new diet. Specifically, at the end of the experiment, the coral tissue of the treatment groups reached $\delta^{15}N$ values of 9.4 ± 0.3‰, 12.6 ± 0.5‰, 15.9 ± 0.1 ‰, and 18.1 ± 0.1 ‰ for groups fed the lowest to highest *Artemia* $\delta^{15}N$ values, respectively. The difference between coral soft tissue and diet $\delta^{15}N$ ranged from a minimum of 1.0 ± 0.1‰ to a maximum of 3.0 ± 0.3‰ across the different experimental groups at day 530 (Figure 3).
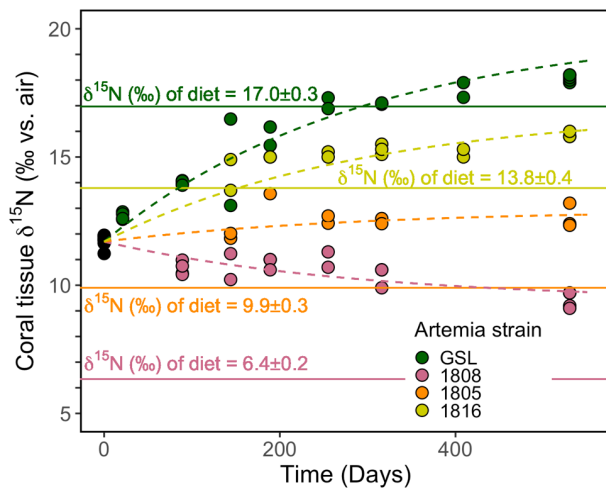

Figure 3. Evolution of the coral soft tissue $\delta^{15}N$ in response to diet $\delta^{15}N$. Colors correspond to the respective *Artemia* strains. Dashed lines are the model output of our simultaneous nonlinear least squares regression fits to the data using Equation 1. Solid lines mark the diet $\delta^{15}N$ ± σ. The mean analytical error on tissue $\delta^{15}N$ analyses was ± 0.2 ‰.

528   Despite the fact that coral tissue had not yet reached an equilibrium with the new coral diet at the end of

529 our experiment, we are able to estimate values of the trophic $\delta^{15}N$ offset for *B. elegans*, ϵ, and the rate of isotopic

530 turnover by fitting the data from our trophic isotope experiment to a nonlinear least-squares regression model

531 corresponding to the isotope mixing relationship shown in Equation 1 below. Equation 1 treats the coral tissue as

532 a single reservoir of N with some initial isotope composition that is evolving to reflect the new diet as a function

533 of time (after Cerling et al. 2007; Ayliffe et al. 2004).

534 $$\delta^{15}N(t) = [\delta^{15}N_{t=0} - \delta^{15}N_{diet} + \epsilon] \cdot e^{-\lambda t} + \delta^{15}N_{diet} + \epsilon. \qquad \text{Equation 1}$$

535 The term $\delta^{15}N_{t=0}$ is the value of the bulk coral tissue at the onset of the experiment, $\delta^{15}N_{diet}$ is that of the corals'

536 new *Artemia* diet (i.e. what it is fed during the experiment), t is the number of days since the start of the

537 experiment, ϵ is the difference between the $\delta^{15}N$ of the diet and tissue at equilibrium (i.e. once the isotopic

538 composition of inputs to the system equals the isotope composition of outputs), and λ describes the specific rate

539 at which new N is incorporated into the coral tissue (days^{-1}). We use this model to calculate the e-folding time of

540 the system, which is defined as $1/\lambda$ (days) and represents the time at which ~63% of the original N reservoir in

541 coral tissue has been replaced with new N from the experimental coral diet.

542   To more confidently calculate ϵ and λ for each individual experimental group, we generate 4 equations,

543 (one for each experimental group of the form given in Eq. 1 but with different values of $\delta^{15}N_{diet}$) and fit them

544 simultaneously using least-squares regression. From this fit, we are able to obtain estimates for both ϵ and λ in *B.*

545 *elegans*. An inherent assumption of this approach is that all experimental groups have the same e-folding time

546 and the same trophic isotope effect. We note here that we refer to the e-folding time as the 'turnover rate' of N in

547 corals throughout the rest of this text (e.g., Tanaka et al. 2018). Our model fit yielded a trophic isotope effect, ϵ,

548 of 3.0 ‰ with a standard error of 0.1 ‰ between coral tissue and diet. The turnover rate of N (i.e. e-folding time,

549 $1/\lambda$) was 291 days with a standard error of 15 days. The four individual model equations generated by our

550 nonlinear least squares regression are presented as the dashed lines in Figure 3.

551 ### 3.2 Effect of starvation

552   At the onset of the starvation trial, the coral tissue had an average $\delta^{15}N$ of 11.5 ± 0.1 ‰. At the end of the

553 522-day experiment, the starved group (N=15 coral individuals) had an average $\delta^{15}N$ of 12.4 ± 0.4 ‰ and the

554 frequently fed group (N=15) with a $\delta^{15}N$ of 12.7 ± 0.1 ‰ (Figure 4). The starved group was +2.5 ± 0.4 ‰

13

635   compared to its diet, statistically indistinguishable from that of the frequently fed group of +2.8 ± 0.1 ‰ higher
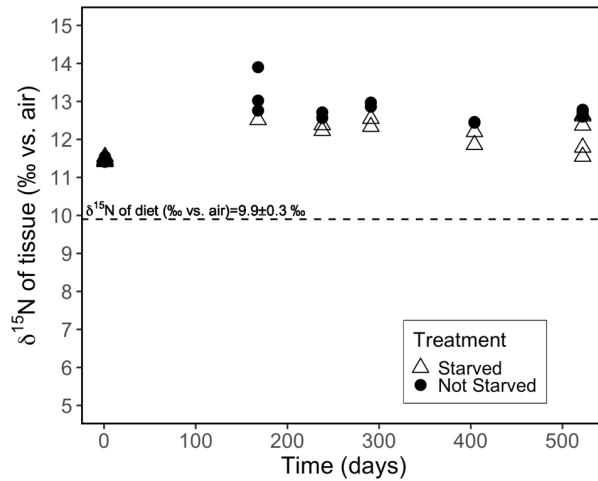
636   than the diet (p-value = 0.059, pairwise t-test).

Figure 4. Evolution of the δ^{15}N of individual coral polyps fed *Artemia* nauplii (δ^{15}N 9.9 ‰) twice weekly (not starved) *vs.* every two weeks (starved). The analytical error associated with individual tissue δ^{15}N measurements was ± 0.2 ‰.

637   3.3 δ^{15}N comparison of field specimen polyp tissue and skeleton

638   The δ^{15}N of the soft tissue from individual *B. elegans* specimens collected live near Friday Harbor ranged

639   between 11.2 to 13.1 ‰, averaging 12.0 ± 0.6 ‰ (Figure 5a). The soft tissue δ^{15}N differed among coral groups

640   collected during different sampling campaigns, with higher values in spring (March 2019 and April 2021)

641   compared to summer and fall (June 2019, September and November 2020; ANOVA F(4) = 40.39; p-value ≤ 0.01,

642   post-hoc pairwise t-test; p-value < 0.05). The average δ^{15}N of corresponding skeletal tissue was 13.5 ± 0.7 ‰ and

643   did not differ discernibly among sampling campaigns (ANOVA F(2) = 0.916; p-value = 0.431). The average

649 difference between skeleton and soft tissue δ^{15}N (Δδ^{15}N) among coral individuals for which both soft tissue and

650 skeleton was measured was 1.2 ± 0.6 ‰ (Figure 5b).
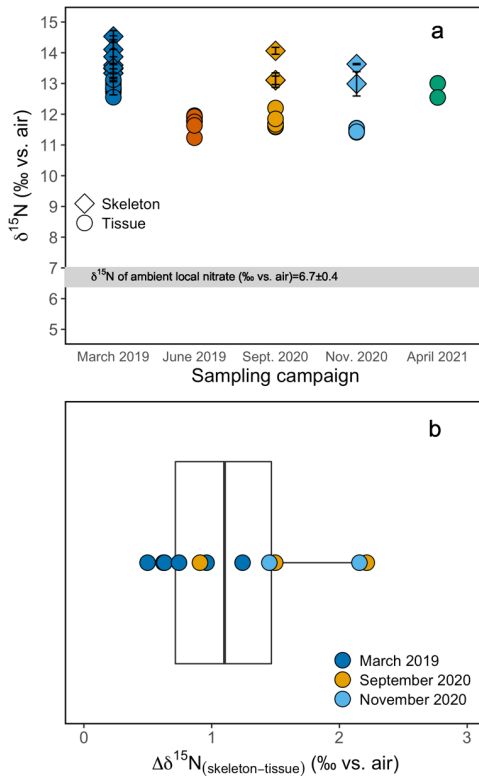
Figure 5. (a) Tissue and skeleton δ^{15}N measurements from *B. elegans* individuals collected during different sampling campaigns. Errors on skeleton data are based on replicate analyses of samples from individual polyps. (b) Boxplot of the difference between tissue and skeleton of individual *B. elegans* corals. The boxplot shows the mean, first and third quartile, maxima, and minima. Individual data points are overlaid on the plot. Colors correspond to respective sampling campaigns.

651 3.4 Regional hydrography and N isotope ratios of nitrate and plankton material

652 Hydrographic profiles recorded at stations near Friday Harbor in August 2021 showed characteristic density

653 structures that were sensitive to tidal phase (Figure 6 a,b; Banas et al. 1999). Profiles collected during flood tide

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665 (collected between 11:40 and 14:20 on August 2, 2021) were relatively well-mixed (salinity 30, temperature

666 11.8˚C), with fresher and warmer water restricted to the near surface (≤ 5 m), whereas ebb-tide profiles (collected

667 at 9:00 on August 2, 2023) showed a progressive decrease in salinity from 30 to 27 and a corresponding increase

668 in temperature from 11.8˚C at 35 m to 14.5˚C at the surface.

669   Nitrate concentrations were nearly uniform with depth during flood tide (~20 μmol L^{-1}), decreasing slightly at

670 5 m, whereas during ebb tide nitrate concentrations decreased progressively from 20 to 10 μmol L^{-1} between 30

671 and 10 m (Figure 6c). Nitrate concentrations in samples collected during the other sampling campaigns ranged

672 from 12 to 32 μmol L^{-1}, and appeared generally higher at stations visited during the September and November

673 2020 campaigns compared to those in April and August 2021 (Figure S6).

674   Depth profiles collected in August 2021 revealed uniform nitrate δ^{15}N values of ~7 ‰ at 30 m among

675 profiles. In well-mixed profiles, nitrate δ^{15}N increased slightly to 7.5 ‰ above 10 m. In stratified profile, nitrate

676 δ^{15}N increased progressively to 8.2 ‰ at 10 m (Figure 6d). Among all sampling campaigns, the δ^{15}N of nitrate

677 ranged from 6.1 ‰ to 8.2 ‰, with median values of 6.8 ± 0.4 ‰ (Figure 7a). The relationship between nitrate

678 δ^{15}N and nitrate concentration in August 2021 was fit to a closed-system Rayleigh distillation model (Mariotti et

679 al. 1981), suggesting a nitrate assimilation isotope effect of 1.5 ± 0.1 ‰ (Figure 8).
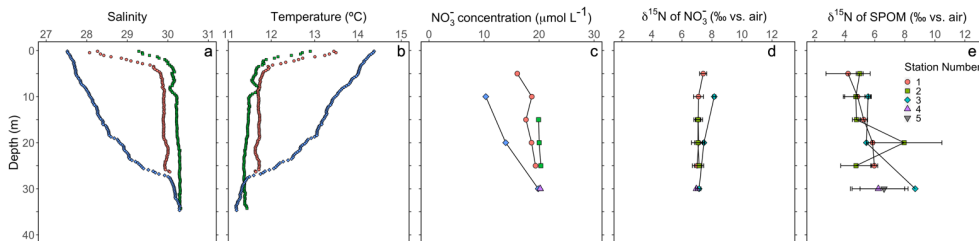
Figure 6. Depth profiles during the August 2021 sampling campaign of (a) salinity, (b) temperature, (c) nitrate concentration, (d) the δ^{15}N of nitrate for analytical replicates and (e) the δ^{15}N of SPOM of replicate samples (n ≥ 2). Green and red symbols correspond to flood tide (collected between 11:00am and 2:00pm on August 2, 2021), blue symbols correspond to ebb tide (collected at 9:00am on August 3, 2021).

680   The δ^{15}N of SPOM collected at depths above 35 m near Friday Harbor during the different sampling

681 campaigns ranged from 1.6 to 11.7 ‰, averaging 5.7 ± 1.7 ‰ (Figure 7b). Values were lowest for the four

682 samples collected in April (4.4 ± 0.4 ‰), and highest for the four samples collected in September and November

683 (6.2 ± 2.6 ‰), although these trends may be an artifact of the low data density in April (n = 4) and Sept./Nov. (n

684 = 5) relative to August 2021 (n = 29), at which time the observed range of δ^{15}N subsumed that in the other two

692 campaigns. Values did not differ coherently with depth in August 2021, although any potential depth structure

693 was obscured by the large variability among sample replicates (Figure 6e).
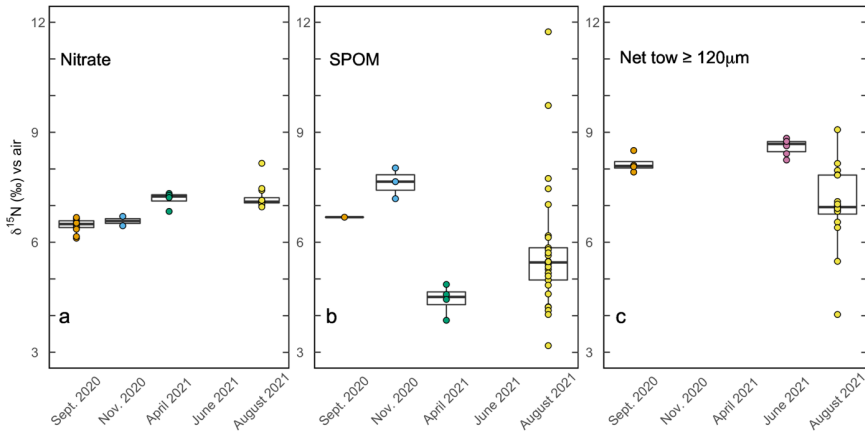
Figure 7. Boxplots of aqueous and particulate N pools at respective sampling times. (a) The δ^{15}N of nitrate from samples above 30 m collected during respective sampling campaigns. (b) The δ^{15}N of suspended particulate organic matter (SPOM) at sites near Friday Harbor during respective sampling campaigns. (c) The δ^{15}N of net tows ($\geq$ 120 μm mesh size) conducted during respective sampling campaigns.

694 The δ^{15}N of material collected in net tows (120 μm mesh size) during sampling campaigns in September 2020,

695 and June 2021 ranged between 7.9 to 8.8 ‰ (Figure 7c). Material collected in net tows of 80 μm and 150 μm

696 mesh size in August 2021 and separated by size class post-collection revealed a coherent δ^{15}N increase with size

697 class (Figure 7c; Figure 9). The $\geq$ 80 μm size class had a mean δ^{15}N of 6.0 ± 0.3 ‰ whereas that $\geq$ 500 μm had an

698 average δ^{15}N of 8.0 ± 0.8 ‰, which was significantly greater than the δ^{15}N of the other size classes (ANOVA, p-

699 value <0.05).

4. Discussion

701   This study of *B. elegans* provides novel constraints on the trophic ecology of scleractinian CWCs. Foremost,

702 our observations of *B. elegans* collectively suggest that the relatively large global δ^{15}N offset of 8-9 ‰ between

703 CWC skeletal tissue and the δ^{15}N of PON exported from the surface ocean is neither explained by a large

704 difference between tissue and skeleton δ^{15}N, nor by an unusually large trophic isotope effect. Further, controlled

17

705 feeding experiments yielded direct estimates of the trophic isotope effect and the corresponding N turnover rate

706 of *B. elegans* soft tissue. Examination of soft tissue δ^{15}N of wild specimens in relation to regional hydrography

707 and food web components near Friday Harbor leads us to conclude that *B. elegans* feeds predominantly metazoan

708 zooplankton prey, implicating more than one trophic transfer between exported PON and coral soft tissue. We

709 contextualize our findings to existing studies of CWC trophic ecology and discuss the implications of considering

710 a two-level trophic transfer for paleo-reconstructions of ocean N cycling using *B. elegans* and CWCs more

711 generally.
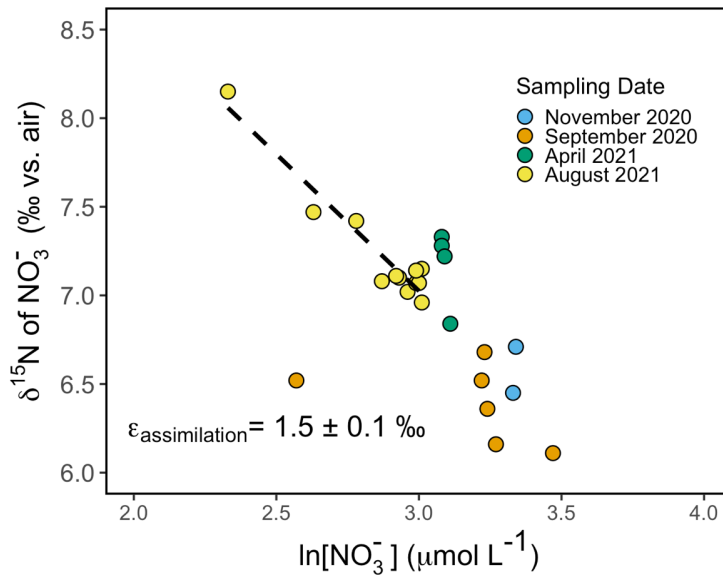
Figure 8. Rayleigh plot of nitrate δ^{15}N vs. ln of nitrate concentration for samples collected from the surface to 40 m around Friday Harbor. The isotope effect of $\sim$1.5 $\pm$ 0.1 ‰ corresponds to the slope of the best fit linear regression line for the August 2021 data, $\delta^{15}N_{NO3} = 11.7 - 1.5 \ln [NO_3^-]$.

712 4.1 Culture experiments revealed a normal trophic isotope effect

713 We investigated whether the large difference in δ^{15}N between PON export from the surface and coral

714 skeleton-bound δ^{15}N (8-9 ‰) observed by Wang et al. (2014) could arise from an unusually large trophic level

715 offset specific to CWCs. The long-term feeding experiment of *B. elegans* polyps revealed a 'normal' trophic

18

719   isotopic offset between coral tissue and diet of ε = +3.0 ± 0.1 ‰. This value conforms to the expected range of

720   +3.4 ± 1.1 ‰ for a single trophic level offset in $\delta^{15}N$ (Minagawa and Wada, 1984).

721
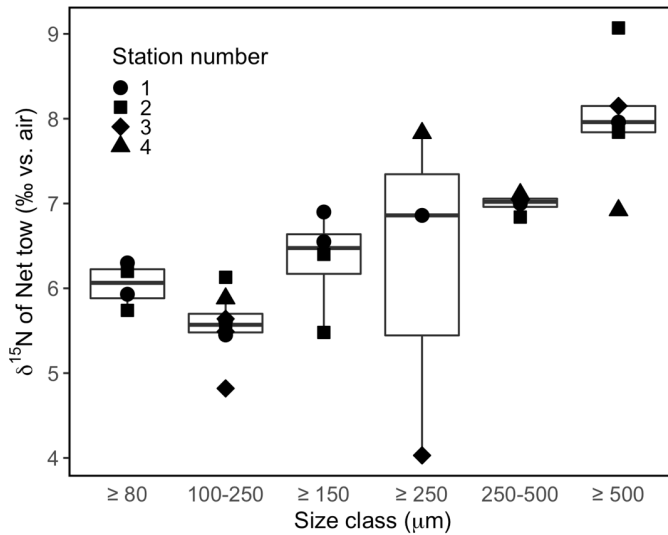
Figure 9. Boxplots of net tow material collected above 30 m in August 2021, separated by size class.

722

723   To support the above conclusion, we assess the assumptions inherent to the isotope mixing model (Eq. 1)

724   used to derive ε and the corresponding nitrogen turnover rate from our culture data. First, the model only

725   accounts for the turnover of a single pool of N, requiring the assumption that all N in the coral polyp tissues

726   equilibrate at the same rate. This notion is unlikely to be wholly accurate, as fluxes of N may vary among tissue

727   types. However, given the relatively low resolution of our sampling over the course of the culture experiments

728   (necessary due to constraints on numbers of total samples) we are unable to extend our model to one with

729   multiple pools (*e.g.* as in Ayliffe et al. 2004). As soft tissues of individual coral polyps were homogenized, we

730   suggest that the $\delta^{15}N$ values and corresponding estimate of ε thus represent the average of soft tissues with

731   potentially different turnover rates. The estimates of ε and N turnover rate further rely on the assumption that the

740 nutritional quality of the respective diets among treatments was equivalent, as trophic isotope effects can be

741 sensitive to food type. Diets low in protein can be associated with greater ε values due to internal recycling of

742 nitrogen (Adams and Sterner, 2000; Webb et al., 1998). For instance, locusts fed a low protein diet were enriched

743 5.1 ‰ from their diet, compared to 2.3‰ for those fed a high protein diet (Webb et al., 1998). Conversely, a

744 compilation of studies of various metazoan consumers raised on controlled diets suggests that high protein diets

745 generally result in higher trophic isotope effects (~3.3 ‰) compared to more herbivorous diets (~2.2 ‰), a

746 dynamic ascribed to higher rates of N excretion to assimilation in consumers fed high protein diets (McCutchan

747 Jr et al., 2003). As noted in Table S3 and in Section 2.3.1, our *Artemia* prey had similar C:N ratios among

748 treatments, in line with our model treatment. Finally, our model assumes that N turnover was dominated by

749 metabolic tissue replacement, rather than net growth, consistent with the observation that adult *B. elegans* growth

750 is slow (Gerrodette 1981).

751   Equation 1 could be invalidated if the corals can access nutritional N sources other than N in *Artemia*, given

752 that the model assumes that *Artemia* are the only source of N to corals in our experiment. Biological N_2 fixation

753 and chemoautotrophy have been detected in association with CWC holobionts, providing some N nutrition to the

754 corals (Middelburg et al., 2016). Our trophic isotope effect estimate was in the range expected for a single trophic

755 transfer, arguably suggesting that N_2 fixation, if occurring, was not a substantial contribution to the corals'

756 nutrition; it would otherwise result in a lower value of ε given a $\delta^{15}N$ contribution of -1 to 0 ‰ (Carpenter et al.

757 1997). That the trophic isotope effect of the poorly fed corals did not differ from that of corals that were well-fed

758 also argues for no sources of N additional to the *Artemia*, as starved corals would presumably increase their

759 reliance on said source. In a related vein, N recycling between the *B. elegans* specimens and potential microbial

760 symbionts (e.g. Middelburg et al. 2016) could also dampen the trophic isotope effect relative to the *Artemia* prey

761 and yield an over-estimate of soft tissue turnover rate for N. The normal trophic isotope effect indicated here

762 suggests a modest role of N retention and recycling by microbial symbionts, in contrast to tropical symbiotic

763 corals wherein bacterial symbionts promote substantial N retention and recycling, and consequently lower trophic

764 isotope effects (Tanaka et al. 2018). Finally, the validity of our estimates could be sensitive to differences in

765 feeding rates, which can influence the rate of N turnover of tissues (Martínez del Rio and Carleton, 2012; Rangel

766 et al., 2019). Corals were fed at identical times among treatments, at a relatively high feeding rate (Crook et al.,

767 2013). However, given the limited number of studies on feeding in *B. elegans*, it is difficult to compare our

768 feeding strategy and that of this species' natural environment. Overall, we consider that the mixing model

784 described by Equation 1 is appropriate to derive the first-order trophic isotope effect and turnover rate of *B.*

785 *elegans.*

786   Changes in metabolism due to underfeeding or prolonged fasting have the potential to increase trophic-level

787 isotope offsets due to increased protein metabolism (Adams and Sterner, 2000). For instance, extensive amino

788 acid recycling in overwintered adult insect larvae was cited to explain trophic isotope effects upward of 10‰

789 (Scrimgeour et al., 1995). A meta-analysis on the effects of starvation on consumer $\delta^{15}N$ revealed that starvation

790 generally led to increased organism $\delta^{15}N$ by an average of 0.5 ‰, up to 4.3 ‰ (Doi et al., 2017). This dynamic

791 was documented for the tropical symbiotic coral *Stylophora phistillata,* where heterotrophically starved corals

792 were enriched in $\delta^{15}N$ by ~0.5 ‰ compared to frequently fed corals (Reynaud et al., 2009). The trophic isotope

793 offset of *B. elegans* soft tissue relative to its diet, ε, was not discernibly influenced by near starvation; that of

794 corals fed once every other week was similar to that of corals fed twice a week – in spite of visible signs of stress

795 among the former, including relatively more sluggish feeding (Figure S7) and thinner soft tissue (data not

796 shown). Deep sea coral reefs are often highly productive environments with high levels of biodiversity,

797 commensurate with a relatively high food supply (Duineveld et al., 2007; 2004; Genin et al., 1986; Roberts et al.,

798 2006; Soetaert et al., 2016; Thiem et al., 2006; Cathalot et al. 2015). Nevertheless, periodicity and spatial

799 heterogeneity in the food supply of CWC reefs implicate periods of lower food density (e.g., Duineveld et al.

800 2007). High currents, downwelling and/or vertically migrating zooplankton temporally boost the export of

801 surface organic matter to the seabed, creating 'feast' conditions, interspersed with 'famine' periods during the

802 non-productive season (Maier et al. 2023). Regardless, our trials suggest that starvation, if pertinent to CWC

803 communities, does not result in greater-than-expected trophic isotope offsets, at least for *B. elegans.*

804 4.2 Turnover rate for *B. elegans*

805   We report the first estimate of the nitrogen turnover for a non-symbiotic cold-water coral of 291 ± 15 days

806 for *B. elegans* soft tissue. This value falls within the range of existing estimates for tropical symbiotic corals.

807 Pulse-chase experiments with ^{15}N-nitrate conducted with fragments of the tropical symbiotic coral *Porites*

808 *cylindrica* yielded a N turnover time of 370 days, and of 210 days for the tropical symbiotic coral *Acropora*

809 *pulcra* (Tanaka et al. 2006; 2018). These relatively long turnover times are attributed to the recycling and

810 retention of N within the coral-symbiont system in nutrient-deplete ecosystems. In comparison, the corresponding

811 carbon turnover in *A. pulcra* was 18 days – compared to 210 days for N – because the system is ultimately N

812 limited (Tanaka et al., 2006). Tanaka et al. (2018) inferred that the N turnover in *P. cylindrica* would be

813 substantially faster than 370 days without symbionts, on the order of 56 days based on estimates of polyp-specific

820 N uptake rates. Nevertheless, the N turnover estimated for the tropical symbiotic coral *Porites lutea* was notably

821 shorter that *A. pulcra and P. cylindrica*, on the order of 87 days (Rangel et al., 2019), implicating different N

822 nutritional strategies among symbiotic coral groups and/or ecosystems. The N turnover for *B. elegans* estimated

823 here is of the same order as but still longer than that for tropical symbiotic corals suggesting that cold-water

824 species have lower metabolic and growth rates compared to tropical symbiotic species, although efficient N

825 recycling has also been documented previously in cold-water corals (Middelburg et al. 2016). The slower

826 turnover of CWCs relative to their symbiotic tropical counterparts may reflect the lower temperatures of the

827 former's habitats (Miller, 1995; Thomas and Crowther 2015).

828 Constraints on N turnover also allow for calibration of the temporal resolution that is achievable with the

829 CWCs δ^{15}N proxy for marine N cycling. Corals are constantly accreting skeleton, such that coral proxies have the

830 potential to provide annual resolution (*e.g.,* Adkins et al. 2004). In theory, a rapid N turnover in CWC could

831 record seasonal changes in regional N dynamics. A turnover time of 291 ± 15 days for N in *B. elegans* soft tissue,

832 however, signifies that the δ^{15}N of coral skeleton is unlikely to provide a faithful record of seasonal differences in

833 the δ^{15}N of the coral diet. Moreover, the turnover of the pool of N that sources the skeletal tissue may be different

834 from that of bulk tissue, and thus decoupled from the soft tissue turnover rate. We suggest that CWCs can likely

835 record changes in their diet on annual or longer timescales, compatible with the ability to date CWC with

836 subdecadal resolution (Adkins et al. 2004).

837 4.3 Soft tissue vs. skeleton δ^{15}N

838 A large biosynthetic δ^{15}N offset between the coral soft tissue and its skeleton could conceivably account for a

839 large δ^{15}N offset between coral skeleton-bound organic matter and N of export that is not explained by single

840 trophic level enrichment of ~3 ‰. However, the mean difference between soft tissue and skeleton-bound δ^{15}N

841 among *B. elegans* specimens collected at Friday Harbor was relatively modest, on the order of +1.2 ‰, ranging

842 between +0.5 and +2.2 ‰. The observed range was dictated primarily by the variability in the δ^{15}N of the coral

843 soft tissue, as skeleton-associated δ^{15}N values were relatively invariant among specimens sampled from different

844 locations and field seasons – likely due to the fact that the amount of skeleton analyzed represented multiple

845 years of growth. The amount of skeleton-bound organic N is small relative to aragonite mass (2-5 μmol N per g

846 of skeleton in our samples), such that homogenization of 50-100 mg aragonite fragments may alias seasonally-

847 driven variability in skeletal δ^{15}N. Soft tissue values in spring were ~1.5 ‰ higher than in summer and fall, such

848 that they appeared to record seasonal changes in diet (Figure 5a). In this regard, the asymptotic nature of the two

849 end-member isotope mixing model (Eq. 1) renders *B. elegans*'s soft tissue sensitive to seasonal changes in prey

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856 δ^{15}N, but not likely to reach isotopic equilibrium on seasonal timescales - given an N turnover of ~291 days, as

857 discussed above. Seasonal variations in the δ^{15}N of the food source of *B. elegans* near Friday Harbor could arise

858 from corresponding differences in the δ^{15}N of nitrate entrained to the surface driven by seasonal hydrographic

859 variability around San Juan archipelago, in the extent of surface nitrate consumption, in food web structure, or

860 from some combination of these. The data density among all but the August 2021 sampling campaign is too

861 sparse to be conclusive in this regard. Otherwise, the observed differences in soft tissue δ^{15}N may result from

862 spatial heterogeneity in food source δ^{15}N among the different collection sites visited for respective campaigns at

863 Friday Harbor.

864   As documented here for *B. elegans*, the δ^{15}N difference between coral tissue and skeleton appears to be

865 modest among various scleractinian coral species. Specimens of the symbiotic tropical coral *Porites lutea* showed

866 a δ^{15}N offset of +1.1 ‰ between skeleton and soft tissue, whereas the symbiotic tropical coral *Favia stelligera*

867 revealed an insignificant offset of -0.1 ‰ (Erler et al., 2015). Similarly, no offset was observed for proteinaceous

868 cold-water corals of the genus *Lepidisis* collected off Tasmania (Sherwood et al., 2009), whereas an offset of -1.9

869 ± 0.8 ‰, was reported for cold-water proteinaceous corals of the genus *Primnoa* from the Gulf of Alaska,

870 *Isadella* from the Central California Margin, and *Kulamanamana* from the North Pacific Subtropical Gyre

871 (McMahon et al., 2018). Conversely, a study of numerous species of both symbiotic and non-symbiotic corals

872 reported a +4 ‰ offset between the skeletal organic matrix and soft tissue among the non-symbiotic corals

873 specifically, but no difference among the symbiotic corals (Muscatine et al., 2005), suggesting that biosynthetic

874 offsets may occur for certain CWC species or conditions.

875 4.4 Implications for components of CWC diet

876 Cold water corals are considered opportunistic feeders, ingesting whatever is available in the water column

877 (Mortensen, 2001; Freiwald, 2002; Duineveld et al. 2004; 2007; Kiriakoulakis et al. 2005; Carlier et al. 2009;

878 Dodds et al. 2009; van Oevelen et al. 2009). They are reported to feed on zooplankton (Kiriakoulakis et al., 2005;

879 Naumann et al., 2011), including microzooplankton (Houlbrèque et al. 2004), on phytoplankton and

880 phytodetritus, including the bacterial fraction of phytodetritus (Maier et al., 2020; Houlbrèque et al. 2004),

881 dissolved organic matter (Mueller et al., 2014; Ferrier 1991, Al-Moghrabi et al. 1993; Hoegh-Guldberg &

882 Williamson 1999; Houlbrèque et al. 2004; Grover et al. 2008), and the CWC holobiont has been observed to

883 display biological N_2 fixation and chemoautotrophy (Middelburg et al. 2016). While it is clear that corals may be

884 able to consume a variety of components within the food web, the soft tissue δ^{15}N of *B. elegans* specimens

885 collected at Friday Harbor averaged 12.0 ‰, signifying that they fed on material with a δ^{15}N of approximately

23

890 9.0 ‰ – accounting for a normal trophic offset relative to their diet (3 ‰) confirmed by our culture experiment

891 results. Here, we seek to determine the primary nutrition source for *B. elegans* at Friday Harbor by comparing the

892 $\delta^{15}N$ of these corals' expected diet with measured $\delta^{15}N$ of different food web components including SPOM and

893 net tow material.

894   We first explore whether the SPOM fraction of the food web was the dominant component of *B. elegans'* diet

895 at Friday Harbor. SPOM is operationally defined as the particulate material retained onto glass fiber filters (GF/F,

896 0.7μm nominal pore size) from filtered aqueous samples. At the ocean surface, including at the stations near

897 Friday Harbor, SPOM is generally dominated by phytoplankton material. At the ocean subsurface, below the

898 euphotic zone, SPOM derives from organic material exiting the ocean surface, but is considered a distinct pool

899 from the ballasted sinking PON collected in sediment traps. The $\delta^{15}N$ of SPOM typically increases with depth,

900 with the steepest gradient across the 100-300 m depth interval, reaching upwards of ~4-5 ‰ in the ocean

901 subsurface, which are higher values than the corresponding sinking particles at abyssal depths due to recycling

902 and remineralization (Altabet, 1988; Casciotti et al., 2008; Saino and Hattori, 1987). Wang et al. (2014) reasoned

903 that because the $\delta^{15}N$ of SPOM is approximately one trophic level lower that of the N preserved in skeletons of

904 the deep-dwelling (deeper than ~ 500 m) CWC *Desmophyllum dianthus,* and because suspended particles are the

905 most abundant form of small particles in the deep ocean, cold-water corals must feed predominantly on SPOM.

906 However, SPOM collected in the upper 30 meters near Friday Harbor was 5.7 ± 1.7 ‰, which is ~ 6 ‰ lower

907 than *B. elegans* soft tissue, a difference greater than expected for a single trophic level. Thus, the SPOM at Friday

908 Harbor was evidently not the predominant food source for *B. elegans* growing in this depth interval.

909   Additionally, it has been suggested that CWCs can assimilate dissolved organic nitrogen (DON) (Gori et al.,

910 2014). We do not have $\delta^{15}N$ DON measurements from our field study. However, we do not expect the potential

911 assimilation of DON to explain the elevated $\delta^{15}N$ of organic tissue that was observed. There are two components

912 of marine DON, refractory and labile (Bronk et al. 2002), which have different $\delta^{15}N$ (Knapp et al. 2018). At

913 Friday Harbor, we don't know the partitioning of the $\delta^{15}N$ between these pools, but even if we did, the labile

914 fraction (which would presumably be the pool available to corals) is expected to converge on the $\delta^{15}N$ value of

915 SPOM (Bronk et al., 2002, Sigman and Fripiat 2019 their Fig. 4; Knapp et al., 2018, Zhang et al., 2020), given

916 that the most recently produced DON is generally most labile. As a result, consumption of DON would not

917 explain the high $\delta^{15}N$ of coral organic tissue.

918   Instead, we suggest that the relatively high $\delta^{15}N$ of ~ 12 ‰ of *B. elegans* soft tissue at Friday Harbor results

919 from these corals deriving nutrition predominantly from larger metazoan zooplankton. Indeed, this is supported

920 by a comparison of the $\delta^{15}N$ coral tissue and the $\delta^{15}N$ of the largest size class of net tow material (≥ 500 μm) of

24

949 8.0 ± 0.8 ‰. This is the only component of the organic matter nitrogen budget that is offset from the coral tissue
950 by ~ 3.5 ‰, consistent with one trophic level transfer. Additionally, the net tow material had a molar C:N ratio of
951 4.4 ± 0.6, compared to 6.5 ± 2.2 for the SPOM (Figure S8), suggesting a dietary preference for metazoan
952 zooplankton would provide higher protein content and nutritional density for these corals (Adams and Sterner,
953 2000).

954   Despite evidence for zooplankton as the main dietary source for *B. elegans* at Friday Harbor, we
955 acknowledge that this feeding strategy may not apply for corals of other species living in habitats that are
956 hundreds to thousands of meters deep. As pointed out in a recent review (Maier et al. 2023), the presence of
957 CWC reefs in the food-limited deep ocean appears paradoxical, and it is not likely that the food available to
958 corals at Friday Harbor looks identical to food available to corals living at >1000 m water depth. Indeed, Maier et
959 al. 2023 suggest that the biodiversity and productivity of CWC reefs in the deep sea are supported by a number of
960 processes such as CWC's ability to consume a range of dietary components (DOM, bacterioplankton, inorganic
961 resources such and inorganic C and ammonium), efficient resource recycling, and their ability to align their
962 feeding strategies and growth with fluctuations in food availability.

963   Maier et al. (2023) and references therein highlight that most deep CWC reefs occur in regions with higher-
964 than-average annual primary productivity, indicating that these CWC reefs are sustained by inputs of high energy
965 to the system, where there is also evidence for the presence of vertically migrating zooplankton. The vertically
966 migrating zooplankton have been found near both relatively shallow (<200 m, Duineveld et al. 2007, Garcia-
967 Herrera et al., 2022) and deep (~1000 m, e.g. Carlier et al. 2009) CWC reefs. Moreover, there are a number of
968 other independent studies that reveal a single trophic level offset between the $\delta^{15}N$ of zooplankton prey and the
969 $\delta^{15}N$ soft tissue of asymbiotic scleractinian corals at specific sites (Duineveld et al., 2004, Sherwood et al. 2005;
970 2008; 2009; Carlier et al., 2009; Hill et al., 2014; Maier et al., 2020). Given the 'normal' trophic level offset
971 reported for CWCs in our laboratory culture experiment, these published observations underscore that
972 zooplankton could be a dominant dietary component of corals other than *B. elegans* as well. Additional evidence
973 from lipid biomarkers corroborates the assertion that deep-dwelling CWC species such *Lophelia pertusa*
974 (recently re-classified as *Desmophyllum pertusum*) and *Madrepora oculata* feed predominantly on metazoan
975 zooplankton (Dodds et al., 2009; Kiriakoulakis et al., 2005; Naumann et al. 2015). Some deep-dwelling CWCs
976 (*Desmophylum pertusum, Madrepora oculata, Dendrophyllia cornigera*) exhibit prey preference for larger
977 zooplankton (Da Ros et al. 2022), suggesting that zooplankton prey are an essential component of their diet.
978 Indeed, an exclusive diet of phytodetritus (Maier et al. 2019) and the exclusion of zooplankton from diet

993 (Naumann et al. 2011) led to decreases in coral metabolism. More fundamentally, the shared traits of tentacles

994 and nematocysts are evidence of a predatory life strategy, indicating that zooplankton are an important food

995 source for corals (Lewis and Price, 1975; Sebens et al., 1996). The coral morphology of *B. elegans* and that of

996 other cold water scleractinian corals is consistent with an adaptation for the capture of prey of a commensurate

997 size (Fautin, 2009). Correspondingly, *D. dianthus* is considered to be a generalized zooplankton predator that can

998 prey on medium to large copepods and euphasiids (Höfer et al., 2018). In contrast, gorgonian corals do not

999 capture naturally occurring zooplankton and have a correspondingly low density of nematocysts (Lasker 1981).

1000 In summary, while our data cannot directly indicate that all CWCs, including the deep-dwelling ones, derive their

1001 primary nutrition from zooplankton, the results of our trophic experiment and field study (when evaluated in the

1002 context of the published literature) suggest that it may be important to consider metazooplankton as a significant

1003 component of CWC diet, and that CWC δ^{15}N is likely to be sensitive to food web dynamics. We discuss the

1004 implications of these suggestions further in the sections below.

1005 4.5 Does coral-bound δ^{15}N reflect surface ocean processes at Friday Harbor?

1006 The effectiveness of coral skeleton-bound δ^{15}N as an archive to reconstruct past ocean N cycling depends on

1007 its ability to record the δ^{15}N of the surface PON export. In turn, the δ^{15}N imparted to the phytoplankton

1008 component of surface particles, from which PON export derives, is highly dependent on surface ocean dynamics

1009 that influence the degree of nitrate consumption and associated isotope fractionation. Here, we describe local

1010 marine N cycling dynamics in order to evaluate whether coral-bound δ^{15}N recorded in the *B. elegans* specimens

1011 reflects local surface ocean processes.

1012 Given complete assimilation of inorganic N pools, the δ^{15}N of phytoplankton material - the dominant

1013 component of SPOM at the surface ocean - converges on the δ^{15}N of the N sources, new nitrate and recycled N

1014 sources (Treibergs et al., 2014; Fawcett et al. 2011). At steady state, the δ^{15}N of the sinking PON flux reflects the

1015 isotope signature of the nitrate upwelled to the surface (Altabet, 1988). Alternatively, given partial nitrate

1016 consumption in the context of a finite pool (Rayleigh dynamic), such as in high-nutrient low-chlorophyll regions

1017 and in upwelling systems, the SPOM δ^{15}N is fractionated relative to the nitrate δ^{15}N as function of the

1018 assimilation isotope effect and the extent of nitrate consumption (Sigman et al., 1999). The δ^{15}N of the sinking

1019 flux then reflects both the δ^{15}N of nitrate upwelled to the surface and the degree of nitrate consumption (Altabet

1020 and François 1994; François et al. 1997). In this section, we discuss whether coral-bound δ^{15}N reflects the δ^{15}N of

1021 nitrate entrained to the surface.

26

Nitrate assimilation at Friday Harbor appeared to be incomplete, potentially implicating the fractionation of N isotopes between nitrate and biomass. Although depleted nitrate concentrations are generally expected at coastal sites during the summer in density stratified water column due to phytoplankton assimilation, nitrate concentrations at Friday Harbor in August of 2021 were upwards of 15 µM at the surface and 20 µM at 30 m depth. Indeed, nitrate in the San Juan Channel is replete year-round, even at the surface, due to vigorous mixing within the channel (Mackas and Harrison, 1997; Murray et al., 2015).

The region experiences tidal mixing, designating it as a well-mixed estuary with minimal density stratification (Banas et al., 1999; Mackas and Harrison, 1997). The tidal influence is clearly identified from the diurnal patterns of vertical hydrographic structure variability with the salinity/temperature gradients changing with the tidal phase (Figure 6a and b). The tidal pumping drives vertical mixing between high nutrient deep water from the Juan de Fuca Strait and fresher surface water from the Strait of Georgia (Banas et al., 1999; Lewis, 1978; Murray et al., 2015; Mackas and Harrison, 1997). Nutrient concentrations in the surface Georgia Strait vary seasonally and are depleted during the summer at the stratified, fresher surface (Del Bel Belluz et al., 2021; Mackas and Harrison, 1997). Our temperature-salinity plot in August 2021 reflects end-member mixing between more saline/colder water from the Juan de Fuca Strait with fresher/warmer surface water from the Georgia Strait (Figure S9; Banas et al., 1999). The influence of Georgia Strait surface water is recognized by the salinity minima originating from the outflow of the Fraser River (Figures S10; Mackas and Harrison, 1997). The nitrate profiles in August 2021, though collected with a lower vertical resolution, do show diurnal variability in vertical gradients similar to salinity/temperature, consistent with the tidal mixing effect (Figure 6c).

The $\delta^{15}N$ of nitrate measured at stations near Friday Harbor also corroborate the mixing of nitrate-rich deeper water with nitrate-deplete surface water from Georgia Strait. The apparent isotope effect for nitrate assimilation in August 2021 was ~1.5 ‰, markedly lower than the canonical value of 5 ‰ associated with nitrate assimilation by surface ocean phytoplankton communities (DiFiore et al., 2006; Sigman et al., 1999; Altabet and François, 1994). A low apparent isotope effect is consistent with two end-member mixing of lower $\delta^{15}N$, nitrate-rich water with highly fractionated (high $\delta^{15}N$), low-nitrate water (Sigman et al., 1999). Highly fractionated nitrate, in turn, likely originated from nutrient-depleted Georgia Strait surface waters entrained into the Channel Islands. The linear relationship between salinity and nitrate concentration in August 2021 further substantiates physical mixing as the dominant control on nitrate concentrations and isotope ratios in San Juan Channel (Figure S10; Mackas and Harrison, 1997). Moreover, the $\delta^{15}N$ of nitrate was relatively uniform with depth, indicating effective vertical mixing of the Georgia Strait and Juan de Fuca Strait water masses. The relatively slight decrease in

nitrate $\delta^{15}N$ with depth suggests a secondary influence of local nitrate assimilation on its concentration and isotope ratios.

The corresponding $\delta^{15}N$ of SPOM at Friday Harbor covered a broad range, from 4.2 ‰ to 8.7 ‰ in August 2021. The depth distribution of SPOM did not mirror the corresponding nitrate $\delta^{15}N$ profile, as could otherwise be expected. At the stratified near-surface (5 m) at station 1, the $\delta^{15}N$ of SPOM averaged 4.2 ‰ compared to 7.4 ‰ for nitrate. In the context of Rayleigh fractionation, this result suggests that particulate material at the surface consisted primarily of the instantaneous product of nitrate assimilation (Mariotti et al., 1981). The lower $\delta^{15}N$ SPOM values could also reflect some degree of reliance on regenerated N species, which would result in $\delta^{15}N$ of SPOM lower than that of incident nitrate (Fawcett et al., 2011; Lourey et al., 2003; Treibergs et al., 2014). Deeper in the water column, the $\delta^{15}N$ of SPOM converged on the $\delta^{15}N$ of incident nitrate, between 6 and 7‰, suggesting that SPOM derived from the complete consumption of an incident nitrate pool (even though nitrate was present at these depths). Phytoplankton at these depths may thus have originated from surface water entrained from the Strait of Georgia – where nitrate was completely utilized. The above dynamics complicate validation of the offset between $\delta^{15}N$ of exported PON and coral-bound $\delta^{15}N$. Yet we find little evidence for nitrate fractionation from partial assimilation on $\delta^{15}N$ of phytoplankton SPOM, which suggests that the $\delta^{15}N$ imparted on local *B. elegans* skeletons should reflect the $\delta^{15}N$ of nitrate entrained to the surface. The ~ 7‰ difference between coral skeleton $\delta^{15}N$ (~13.5‰) and the entrained nitrate (~6.5‰) is similar to the empirical range of 7 - 9‰ reported for other CWC species, (e.g. *D. petusa,* Kiriakoulakis et al., 2005) and *D. dianthus* (Wang et al. 2014) and suggests that *B. elegans* provides a record of the thermocline nitrate $\delta^{15}N$ and surface nutrient dynamics at Friday Harbor.

5. Conclusions and implications for paleo-reconstruction from coral $\delta^{15}N$

We conclude that the solitary scleractinian cold water coral *B. elegans* in Friday Harbor, WA predominantly derives nutrition from metazoan zooplankton prey. While our study was limited to a shallow field site, our isotope feeding experiment, evaluated alongside previously published studies, points to the possibility that deeper-dwelling CWCs could also rely on zooplankton prey as a fundamental component of their diet. SPOM may contribute to these CWCs' diet, but it cannot be presumed to exclusively account for the large offset between $\delta^{15}N$ of PON export and coral skeleton $\delta^{15}N$ documented by Wang et al. (2014). The $\delta^{15}N$ of skeletal material recovered from coral archives is thus likely to be sensitive to local food web dynamics; for a given $\delta^{15}N$ of sinking PON exiting the surface ocean, the $\delta^{15}N$ recorded by CWC may differ among individuals of the same

species feeding on different zooplankton prey, depending on availability. In fact, Wang et al. (2014) did report a "natural variability" of 1-1.5‰ within a single specimen that might have resulted from some variability of the local food web on a short time scale of few years. Some studies have documented an increase in the degree of carnivory of zooplankton with depth (Dodds et al., 2009; Vinogradov, 1962). For instance, Hannides et al. (2013) recorded a 3.5 ‰ increase in zooplankton δ^{15}N from 150 m to 1000 m in the Subtropical North Pacific, with the steepest rate of increase from 100 – 300 m. Koppelmann et al. (2009) reported a similar pattern of zooplankton δ^{15}N through the water column. These findings could explain previous reports of small but resolvable (1-2 ‰) depth-dependencies of coral δ^{15}N (Wang et al. 2014) if corals feed predominantly on zooplankton with depth-dependent degree of carnivory of zooplankton and increasing with depth δ^{15}N. The δ^{15}N recorded in CWC skeletons also tends to differ among species by 1-2‰, as respective species occupy different nutritional niches (Teece et al., 2011). The relationship between CWC species represented in fossil archives to the depth structure of their zooplankton prey warrants further investigation.

Consideration of the possible dependence of coral-bound δ^{15}N on food web dynamics informs the questions that can be competently addressed by this proxy. Although we do not have direct estimates of the δ^{15}N range that can be expected from local food web variability, the scatter around the global compilation of Wang et al. (2014) for coral-bound δ^{15}N of *D. dianthus* relative to the δ^{15}N of PON suggests that this range is modest, on the order of ~1-2 ‰. Given this range, we suggest that the coral-bound δ^{15}N proxy will be most useful for reconstructing larger environmental δ^{15}N signals and where chosen coral samples belong to the same species and are collected at comparable depths as has already been successfully demonstrated by Wang et al. (2017), Studer et al. (2018) and Chen et al. (2023). If used in this way, the broad geographic and temporal coverage afforded by CWCs, the opportunity to measure multiple proxies from individual specimens and the imperviousness of coral-bound δ^{15}N to diagenetic alteration render it a valuable paleo-proxy for reconstructing marine N cycling.

Data Availability Data presented in this paper is available at: https://www.bco-dmo.org/project/893811

Author Contribution JG, AG, and MP conceptualized the research presented in this paper. JM and AG designed and carried out culture experiments. MP and AC prepared coral samples for analysis. JM and VR analyzed samples. JM, AG, JG and KD collected water samples, SPOM, and net tows. KD collected live corals for culture experiments and field studies. JM and JG prepared the manuscript with contributions from all co-authors.

Competing Interests: The authors declare that they have no conflict of interest.

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Acknowledgements

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