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# Feces as food: The nutritional value of urchin feces and implications for benthic food webs

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ABSTRACT

Algal subsidies are important to basal consumers of the deep benthos where there is little to no primary productivity. Algal detritus such as pieces of kelp that sink into deep habitats can be an important direct nutritional subsidy, but sea urchin feces may provide an additional, indirect energetic link from shallow-water macroalgae to benthic community members that are too small to handle and consume large detritus directly. Urchins digest macroalgae inefficiently, creating the potential for two key trophic consequences to the benthic food webs they live in. First, urchins act as marine 'shredders' creating smaller detrital particles from larger drift; second, the poor digestion may enable microbes to enrich the food value of both the digesta within the urchin guts, and the egesta (feces) after it leaves the gut. We quantified the relative nutritional value of algae and of feces of red and green sea urchins (*Mesocentrotus franciscanus* and *Strongylocentrotus droebachiensis)* fed on monodiets of various algae in laboratory experiments. We then conducted feeding experiments with an epibenthic copepod (*Tigriopus californicus*) to evaluate consequences to a model consumer of different diets including feces. We also quantified assimilation efficiencies of red urchins fed a diet of bull kelp (*Nereocystis luetkeana*). In many cases, key indicators of nutritional value (especially calories and protein content) of algal material increased after being consumed and egested by urchins, and urchin feces "aged" in seawater generally became even more calorie-rich. Benthic copepods raised on diets of urchin feces derived from kelp had faster population growth than those raised on chopped fresh kelp tissue. It is likely that microbiota inside urchin guts are driving these counter-intuitive results. The creation of nutritious feces could add to the importance of urchins as a link to benthic communities that rely heavily on detritus for their success.

## 1. Introduction

Because photosynthetic primary productivity in the ocean is limited to shallow waters, heterotrophs in deep subtidal habitats rely on the transfer of food from the photic zone. Macrophytes from productive nearshore environments may thus provide an important trophic subsidy to deep subtidal food webs, in the form of small particles (Wernberg and Filbee-Dexter, 2018) or larger pieces of sinking detritus (Britton-Simmons et al., 2009). In shallow benthic algal communities, only about 10% of algal biomass is consumed directly by herbivores (Mann, 1988), with the rest entering food webs in the form of detritus (Duggins et al., 1989; Krumhansl and Scheibling, 2012), and dissolved organic matter (Newell et al., 1980). Consumers may be abundant in the deep subtidal zone despite the lack of local vegetative growth because of the abundance of detritus that is transported there by hydrodynamic forces (Britton-Simmons et al., 2009). This detritus may be entire plants or large fragments (drift algae); when transported from shallow subtidal source habitats to deep sink habitats, these constitute a large flux of organic matter. The food value of kelp detritus varies with species and season but in some cases is high, with elevated levels of nitrogen (Britton-Simmons et al., 2009; Mann, 1988; Orr et al., 2005); Norderhaug et al. 2003 and low levels of defensive chemicals (Duggins and Eckman, 1997).

Drift algal detritus is common in the deeper habitats adjacent to

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kelp forests (Filbee-Dexter et al., 2018; Harrold et al., 1998), where accumulations often fuel secondary production (reviewed in Krumhansl and Scheibling, 2012). For example, Britton-Simmons et al. (2012) found drift macrophytes in 97% of observations within a 60-km$^2$ section of sea floor deeper than 30 m in the San Juan Archipelago, and most of the biomass came from kelps. Such sinking macrophyte detritus may be an important direct food source to consumers (Dethier et al., 2014; Duggins et al., 2016), but it may also enter food webs by an indirect pathway, as sea urchin feces. Urchin feces may represent an important energetic link between algal subsidies and benthic communities, both shallow and deep (Mamelona and Pelletier, 2005). Feces are "compact aggregations of organic matter" (Sauchyn and Scheibling, 2009a; Wotton and Malmqvist, 2001) that are readily transported by currents (Wernberg and Filbee-Dexter, 2018). The small size of undigested kelp particles contained within urchin fecal pellets also might make them more available to small consumers than are larger pieces of macrophyte detritus. Fecal material deposited in deep subtidal environments (Sauchyn and Scheibling, 2009a) may be another important pathway for organic matter export from shallow subtidal ecosystems (Taghon et al., 1984; Yoon et al., 1996).

Stronglylocentrotid sea urchins are very abundant herbivores in temperate reef ecosystems along the western coast of North America from Alaska to Baja California (McCauley and Carey, 1967) as well as in the North Atlantic and elsewhere (Ebert et al., 2018). While common in shallow, algal-dominated subtidal zones, they are plentiful as deep as 110 m (Britton-Simmons et al., 2012). In the San Juan Archipelago, Washington, both large red urchins (*Mesocentrotus franciscanus*) and several smaller species (*Strongylocentrotus droebachiensis* and *S. pallidus*) are present in deep subtidal environments (where attached macroalgae are absent), where they consume mostly drift algae captured from the water column (Britton-Simmons et al., 2009; Duggins, 1981). For example, in 2018, *M. franciscanus* was observed from a submersible at a depth of 284 m off the west side of San Juan Island in close proximity (< 5 m) to algal detritus (Galloway and Lowe, unpublished data). Urchins are often patchily distributed, found where they can maximize access to drift algae. Adult red urchins are known to move very little locally, and the abundance of drift algae appears to allow this sedentary behavior (Britton-Simmons et al., 2012; Lowe et al., 2015; Parnell et al., 2017). However, green urchins are mobile over these deep habitats and seem to converge on accumulated drift algae resulting in large aggregations (as seen in ROV images: Britton-Simmons et al., 2012).

Sea urchins are voracious herbivores that consume large amounts of algal biomass daily, particularly of kelps, transforming it into fecal matter (Miller and Mann, 1973; Sauchyn and Scheibling, 2009a, 2009b; Suskiewicz and Johnson, 2017). As a sea urchin's digestive system is relatively inefficient (Larson et al., 1980; Mamelona and Pelletier, 2005), the fecal matter could be an important source of calories or enriched nutrients for nearby consumers (Sauchyn et al., 2011; Vadas, 1977). A substantial portion of urchin feces is relatively unprocessed vegetative material, often coated in mucus, and available for colonization by the microbial community (Peduzzi and Herndl, 1986; Povero et al., 2003; Yoon et al., 1996). Urchin fecal pellets may gain caloric value as they 'age' because the bacteria on detritus provide an additional source of calories (Fabiano et al., 1994; Mann, 1988), and they can fix dissolved inorganic matter (Povero et al., 2003). Since many benthic suspension and deposit feeders gain nutrients and energy from fecal material (Newell, 1965; Wotton and Malmqvist, 2001; reviewed by Sauchyn and Scheibling, 2009a), this subsidy could be critical to the benthic community. Shallow benthic consumers likely have access to feces from nearby urchins, while deep consumers could get this subsidy either from shallow-water feces transported to deep water, or from deep-water urchins consuming drift algae.

In this study we present data from a variety of experiments exploring how efficiently sea urchins extract calories and other nutritional indicators from their algal foods to determine how much of an energetic impact their fecal matter could potentially have on benthic ecosystems. We measured the assimilation efficiency (= absorption efficiency; Vadas, 1977) of red sea urchins and compared the nutritional value of various algal foods with the feces produced from a diet of those algae for both red and green urchins. To mimic the fate of feces in nature, we also tested how 'aging' feces affects their caloric content. Furthermore, we qualitatively assessed fecal nutritional value to a generalist consumer by comparing population growth rates of a detritivorous copepod grown on particulate algal versus fecal diets.

## 2. Methods

### 2.1. Experimental organisms

We ran all feeding experiments at the Friday Harbor Laboratories (FHL: 48°32.7′N, 123°0.8′W) with adults of the two most common shallow-subtidal species of sea urchins in the San Juan Archipelago, Washington: the large red urchin *Mesocentrotus franciscanus* (hereafter, red urchins), and the smaller green urchin *Strongylocentrotus droebachiensis* (hereafter, green urchins). Both were collected by SCUBA divers from ~10 m depth from south Shaw Island, and held in ambient conditions in flow-through sea tables. Algae used in feeding experiments included five taxa that are regularly available to urchins as attached benthic species or as drift: the canopy-forming kelp *Nereocystis luetkeana (Nereocystis* throughout), collected either by divers directly from kelp forests, from a boat, or as fresh drift kelp from around the FHL dock; the understory kelps *Saccharina latissima* and *Agarum fimbriatum,* collected by divers from the same sites as the sea urchins; the red alga *Pyropia* sp. (*Pyropia* throughout), from rocks in front of FHL at ca. +1.5 m; and the green alga *Ulva* sp. (*Ulva* throughout) from rocks in front of FHL at ca. +1 m. Because of the importance of aging to algal food quality (Dethier et al., 2014), only fresh algae (collected < 1 week earlier) were used to feed sea urchins. Blades with epiphytes and visible reproductive tissue (e.g., kelp sori) were avoided.

### 2.2. Assimilation efficiency of red urchins

Red urchins were kept in sea tables for 8 days with constant water flow and no food to allow them to clear their guts and to ensure a similar state of starvation in all individuals. We placed 15 urchins individually in modified 10 L buckets held within larger outside tanks (275 L) that were kept totally dark to reduce microalgal growth, and cleaned by siphon every 4–5 days. The modified buckets ('urchin outhouses') had ~ 15 holes (2.5 cm diam) drilled around the wall to allow water flow, with no holes near the bottom. A plastic grate (1 cm mesh) was wedged ~5 cm above the bottom of the bucket, allowed the feces to pass through and fall away from the urchin, while also keeping feces from being re-consumed; below the plastic grate, Nitex mesh (500 μm) collected all sea urchin fecal material. The bucket top was open to water flow. For 13 days (June 25–July 7, 2016), each sea urchin was fed *Nereocystis* ad libitum to ensure that after this period, they were satiated to a similar state and their gut contents consisted of only *Nereocystis* material. Excess kelp was removed and fresh kelp replenished daily. On the last day of excess feeding, each urchin outhouse was sampled to collect feces.

We assessed assimilation efficiency by careful daily tracking of mass of food ingested and egested by the 15 individuals. On July 8, 2016, each urchin was fed ~20 g of *Nereocystis* (blotted wet mass); pilot studies showed that they usually ate less than this in 24 h. The following day, uneaten kelp and fresh feces were collected by removing the sea urchin and plastic grate from the bucket and carefully lifting the Nitex mesh out of the water, retaining all fecal pellets and kelp fragments on the mesh. Very little material was lost. Feces and kelp fragments were weighed wet separately, then feces were dried at 65 °C for ~24 h. We rinsed all equipment and the sea urchin was set up again with fresh kelp, repeating this process daily around noon for 19 days. Wet and dry masses of feces and kelp fragments were each tightly correlated

($r^2$ = 0.96, 0.99 respectively; Supplement Table 1). Assimilation efficiency was calculated as: (dry weight ingested - dry weight egested)/(dry weight ingested) * 100.

### 2.3. Nutritional value of algae vs. feces

We quantified the nutritional value of algae vs. feces from these experiments with red urchins, and from similar experiments with other algal and urchin species; these studies were done by undergraduate students over several years (Supplement Table 2) using consistent methodology and closely supervised by senior personnel. All studies used the modified buckets described above (for large red urchins) or a similar set of smaller plastic containers (for smaller green urchins). In each experiment, individual urchins (at least N = 5 per urchin and algal species, Supplement Table 2) were fed the test alga for at least a week so that other gut contents would be purged. Gut residence times can vary, but are likely longer than a week only when urchins are starved (Lasker and Giese, 1954). After this period, feces were collected ~every two days from the bottom of each container and frozen. Algal diet samples were also frozen. Feeding and collection continued for up to 2 weeks until sufficient feces were collected from each individual to run chemical analyses.

We ran one additional longer-term experiment feeding monoculture macroalgal diets for 180 days (September 2016–March 2017) to recently settled green urchins (1.95 ± 0.19 mm, mean test diameter ± s.d.) resulting from in vitro breeding at FHL. We allocated 240 juvenile urchins (N = 15 per bin) to replicate (N = 4 per diet treatment) clear, lidless, flow-through plastic bins (20 cm × 20 cm) kept in seawater tables at FHL. Urchins were fed ad libitum on either *Nereocystis, Pyropia*, or *Ulva*; uneaten food was removed several times a week as needed and bins were cleaned regularly to prevent waste accumulation and diatom growth. Feces were collected weekly and immediately stored at −20 °C for later analyses, and algal samples were similarly frozen 3 times over the course of the experiment. When urchins were sacrificed after 6 months (mean test diameter = 19 mm) they had thus been raised entirely on one algal species post-settlement. We collected their small gonads and their gut contents for chemical analyses.

### 2.4. Nutritional content

#### 2.4.1. Calories

We used a general micro assay calorimetry technique of Gosselin and Qian (1999) to assess caloric content of both algal tissues and feces from all experiments. For preparation of reagents, see Gosselin and Qian (1999); new reagents were created every week, as they degrade with time. In the oxidation of the samples, the Gosselin and Qian methods were modified to accommodate larger samples, as we used 20–30 mg of powdered sample (feces or algae, dried and ground to a fine powder using mortar and pestle). Weighed sample and 1 mL of distilled water were added to a 20 mL glass test tube with a plastic lid, and 10 mL of potassium dichromate oxidizing reagent added to each test tube. Tubes were gently swirled to mix, and allowed to cool to room temperature for 5 min. Tubes were incubated at 115 °C for 15 min, then removed from the oven and incubated for an additional 15 min at room temperature. Samples were mixed gently, and 0.5 mL of liquid from each tube was moved to a clean test tube. 4 mL of potassium iodine-starch reagent was added to each new sample tube, and tubes were gently mixed and incubated at room temperature for 20 min. Calorimetric measurements were made without adding RO water. The contents of each tube were transferred to a plastic cuvette, and absorbance was immediately measured at 575 nm with a DR 5000 spectrophotometer.

Glucose standards were prepared using 10–30 mg of reagent grade glucose tested for caloric content using the same technique as the samples. Standard curves generated with glucose always were tight ($r^2$ values > 0.90), but varied among experiments; thus we compare values (e.g., between algae and feces) only within experiments and not among experiments. Some of the samples exceeded the absorbance range of the standard curves and the spectrophotometer's limit of an absorbance of 3.5. For these, we calculated the caloric content using an absorbance of 3.5, so this is a conservative estimate for those samples. Glucose equivalents per sample absorbance value were calculated using each experiment's standard curves. To convert the sample measurements from glucose equivalents to calories, we used the conversion equation of Gosselin and Qian (1999): Calories/mg = (15.7*(glucose equivalents/mg))/4.18.

#### 2.4.2. Carbohydrates and protein

Soluble carbohydrates were extracted from algae and from urchin feces using trichloroacetic acid and quantified using the phenol-sulfuric acid colorimetric method (derived from DuBois et al., 1956). Glucose was used as a standard. Protein was extracted in NaOH and the concentration estimated by the protein-dye binding method (Bradford, 1976). Bovine serum albumin was used as a standard.

#### 2.4.3. C:N and total lipids

C and N content of algal tissues and urchin feces as well as of urchin gonads were quantified from the small green urchins raised for 6 months on mono-diets. Samples were analyzed by the Washington State University Stable Isotope Core Lab. In addition, total lipids were quantified; methods and detailed data are reported in Schram et al. (2018).

#### 2.4.4. Nutritional value of fresh vs. aged feces

To assess how caloric content of feces changed over time, fecal samples from both red and green urchins fed kelp were allowed to degrade under controlled conditions mimicking what feces might encounter in nature. Experiments were run in different seasons but comparisons are only made within-experiment since algal caloric values could vary with season. Fresh feces were collected from individual urchins, and we simulated deep, aphotic conditions by placing them in small opaque plastic bottles submerged in dark, cold sea tables for 7–10 days. Each bottle had a 2.5 cm diam. hole covered with 1-mm Nitex mesh to allow water exchange; no anaerobic conditions were noted at any time. Aged feces were dried at 65 °C for ~24 h and stored in individual vials for chemical analyses.

#### 2.4.5. Analyses of nutritional data

Because caloric content measurements of fresh algal tissue versus urchin feces varied substantially among experiments, we analyzed the results of each experiment separately using a *t*-test to compare untransformed calories of algal samples and of feces. N's for each experiment are given in Suppl. Table 2. With these multiple *t*-tests, we interpret 'significant' results very conservatively. For other nutritional metrics (carbohydrates, protein, C:N), we ran one-way ANOVAs on untransformed data comparing values of that metric among the algal and feces or other samples tested. For total lipids, algae and feces were sampled in paired fashion for replicate containers of juvenile urchins, so these data are analyzed with paired t-tests for each algal food.

### 2.5. Tigriopus population growth experiments

The intertidal splash pool harpacticoid copepod *Tigriopus californicus* (hereafter, *Tigriopus*) was collected from Cattle Point, San Juan Island and kept in a bucket overnight to acclimate to ambient room temperature (~22 °C). We used *Tigriopus* because it is a robust, widely distributed, and well-studied marine model organism that feeds on detritus and benthic algae (Dethier, 1980; Dethier et al., 2014; Wallace et al., 2014). Harpacticoid copepods like *Tigriopus* are generalist feeders (Powlik et al., 1997) that abundantly colonize detrital kelp piles (Duggins et al., 2016), and an important food source for fish. They therefore represent a reasonable model organism to compare consumer

performance between urchin feces and the fresh algae from which the feces are derived. We filled 16 1 L glass jars with filtered seawater and to each added 20 gravid female *Tigriopus*, all with an orange body and black clutch. Jars were kept on the counter but out of direct sunlight to minimize temperature variation. In the first trial, we set up 4 replicates of each of 4 diet treatments: 1) fresh, chopped *Nereocystis* blade; 2) fresh, chopped *Saccharina* blade; 3) fresh fecal pellets from green urchins fed only *Nereocystis* for at least a week; 4) fecal pellets from green urchins fed only *Saccharina*. In the second trial, we compared fresh, chopped *Ulva* with fresh fecal pellets from green urchins fed only *Ulva* (N = 8 jars per treatment). In each case, algae were chopped with a razor blade into pieces < 5 mm to remove some of the textural differences between foods. Fecal pellets were initially this same approximate size. For both experiments, each jar had a surplus diet of the treatment food. Food and water were refreshed periodically by pouring the jar water through a Nitex filter to catch *Tigriopus* individuals and debris, and washing the filtrate back into the jar with clean seawater. After 4 weeks (first trial) or 6 (second), the jars were drained through a Nitex filter, and copepods were fixed with 95% ethanol. Final *Tigriopus* populations (juveniles plus adults) were counted using a dissecting microscope.

## 3. Results

### 3.1. Assimilation efficiency

Sea urchins consumed kelp readily in the experimental tanks. Red urchins consumed on average 0.62 ± 0.42 s.d. dry g of *Nereocystis* and egested 0.11 ± 0.17 s.d. dry g over a 24-h period (N = 345 samples among the 15 urchins). Sea urchins with a larger diameter test both ingested and egested greater amounts of kelp daily (Supplement Table 1). Based on daily tracking of these masses, red urchins had an average assimilation efficiency of 87% (s.d. = 19, n = 144). However, this value varied substantially among individual urchins and among days, with daily values ranging from 13% to 100%.

### 3.2. Nutritional value of algae vs. feces

#### 3.2.1. Calories

To make it possible to visually compare among our disparate experiments, we present the results of each experiment (N = 14) as the caloric content of feces divided by the average caloric content of algae measured at that time. Values < 1 thus indicate that feces are less calorie-rich than algae. We anticipated that urchins, while inefficient digesters, would be extracting at least some caloric content from their algal food, especially as the assimilation efficiency measurements with red urchins were high. In most (9 of 14) trials, however, we found that the average caloric content of feces (on a per-mass basis) was similar to or higher than that of the algae consumed, for both red and green urchin species (Fig. 1). For red urchins, the mean values of feces from a diet of any of the three kelp species tested were always more calorie-rich than the algae, especially for *Nereocystis* (*Agarum* was not significantly different). Feces from a diet of the one red alga (*Pyropia*) were slightly less calorie-rich than the alga itself, and feces from the green alga *Ulva* were not different from the alga (Fig. 1A). Variance among red urchins was high, as was feeding rate, rate of production of feces, and assimilation efficiency (see low $R^2$ values, Supplement Table 1).

Green urchins were less variable in their rate of food consumption and fecal production, although these parameters were not directly quantified. All ate readily in their containers and produced feces derived from their lab diet within a few days (judged by colour), as opposed to the red urchins which sometimes took weeks to eat and produce feces. Caloric content of green urchin feces was also less variable (Fig. 1B variances; Supplement Table 2). As with red urchins, diets of two different kelps resulted in feces with enhanced caloric content; diets of *Pyropia* and *Ulva* showed reduction or no enhancement of calories (Fig. 1B).

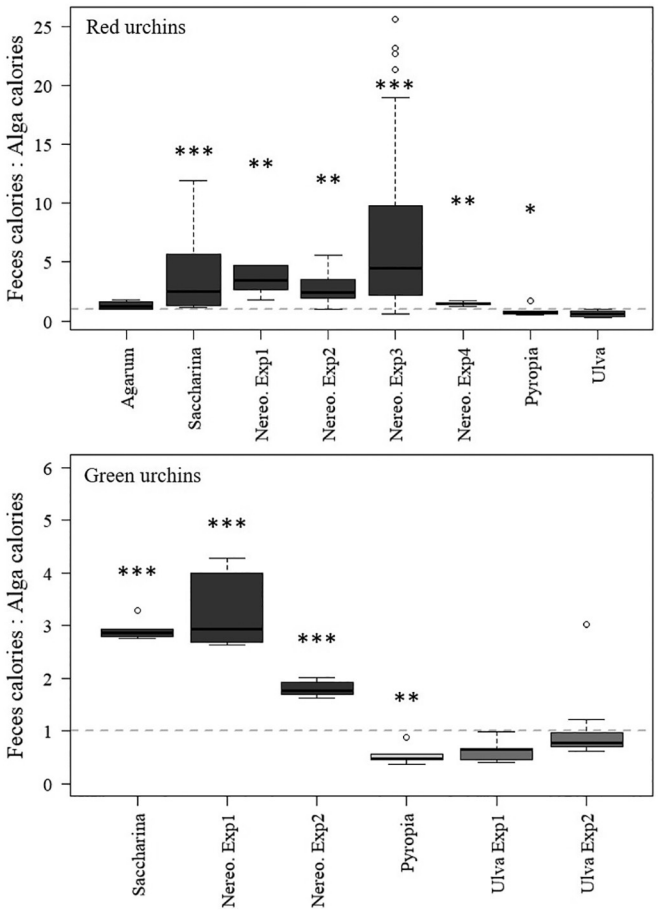


**Fig. 1.** Ratio of calories in fecal material over average caloric content of that alga calculated for each individual experiment. Each bar is from a separate experiment. Boxplots illustrate the median and interquartile range. Whiskers extend to minimum and maximum values excluding outliers (shown as hollow points). Dotted horizontal line at a value of one indicates an equal ratio of feces calories to algal calories. *t*-tests were run for each experiment comparing all per-experiment data (calories of algal samples and calories of feces; for Ns, see Supplemental Table 2) * = p < 0.05; ** = p < 0.01; *** = p < 0.001.

We hypothesized that the feces of individual sea urchins with high assimilation efficiency might have lower caloric value because the sea urchin had extracted more of the calories during digestion. However, there was not a statistically significant relationship between assimilation efficiency of sea urchins and the caloric content of their feces (Supplement Table 1). Sea urchins with larger tests on average had both significantly lower assimilation efficiencies and lower caloric content of fresh feces than smaller sea urchins (Supplement Table 1).

In two separate experiments, fresh feces of red urchins fed *Nereocystis* had lower caloric contents than feces left to age in the dark in seawater (Fig. 2). One difference was not significant due to extremely high variances in the aged fecal calories, but the second experiment had higher aged fecal calories (t-test, p = 0.022). In contrast, feces from red urchins fed *Agarum* and from green urchins fed *Nereocystis* did not change significantly.

#### 3.2.2. Carbohydrates

For *Nereocystis*, algal tissue had relatively low carbohydrate content, and there was a trend towards feces having slightly higher carbohydrates, especially for aged feces and for red urchin feces (ANOVA F = 3.15, df 2.12, p = 0.054; Fig. 3). However, feces from both urchin species fed either *Ulva* or *Pyropia* had significantly lower carbohydrate contents than the algal tissues, which were quite carbohydrate-rich

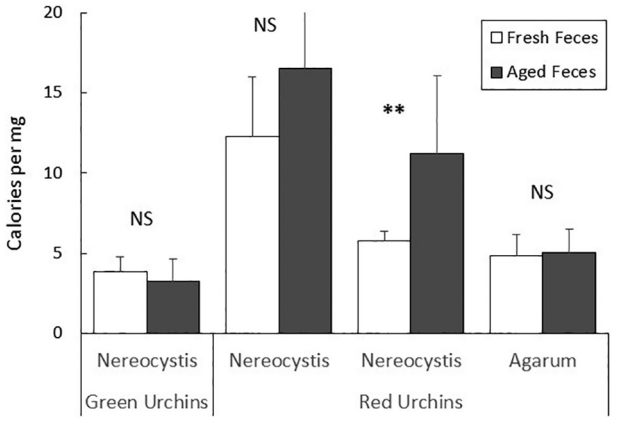


**Fig. 2.** Caloric values (mean and one s.d.) of fresh urchin feces fed one kelp species versus feces left to age in the dark in running seawater for 5–10 days. Cut-off error bar for the second *Nereocystis* Aged value is at 28.8. N values per experiment = 5, 5; 15, 13; 6, 4; 6, 6. N days aging = 7; 7; 10, 10. Statistical results from t-tests per experiment; NS = not significant, ** = p = 0.022.

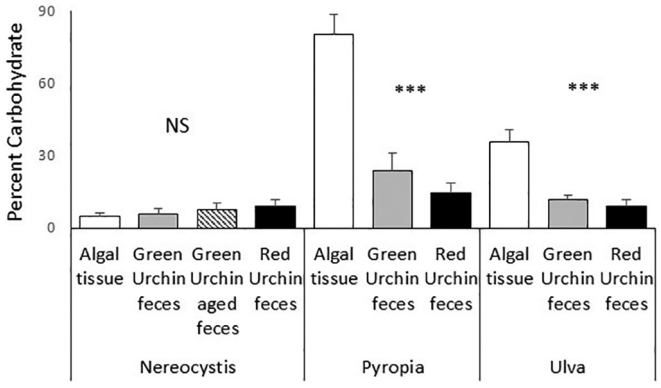


**Fig. 3.** Carbohydrate content of algal tissues and feces from both green and red urchins. N = 5 for each bar, one s.d. shown. NS = not significant; *** = p < 0.001.

(ANOVAs, *Ulva* F = 82.15, df 2, 12, p < 0.001; *Pyropia* F = 102.4, df 2,12, p < 0.001; Fig. 3).

### 3.2.3. Protein

Protein content was relatively similar among all samples. There were no significant differences (ANOVA, p = 0.07) in protein content among the *Nereocystis* samples although the algal tissue tended to be lower than any of the fecal samples (Fig. 4). For both *Pyropia* and *Ulva*, however, feces from both urchin species had higher protein content than the algae they were derived from (Fig. 4).

### 3.2.4. C/N ratios and total lipids

We collected C, N, and lipids data from algae, feces and gonad tissue from juvenile green urchins raised on mono-diets. C/N ratios were quite consistent among most samples except for feces derived from *Nereocystis*; these samples were very low in N but similar in C (Supplement Fig. 1), leading to a high C/N ratio (Fig. 5A). Feces and gonads from urchins on *Pyropia* diets showed no differences in C/N, and on *Ulva* diets the feces were relatively low in N although to a lesser extent than from *Nereocystis* diets. Total lipids (Fig. 5B) did not differ significantly between algae and feces for *Nereocystis* or *Ulva*, but increased significantly from alga to feces on a diet of *Pyropia*. Fatty acid changes are shown in detail in Schram et al. (2018).

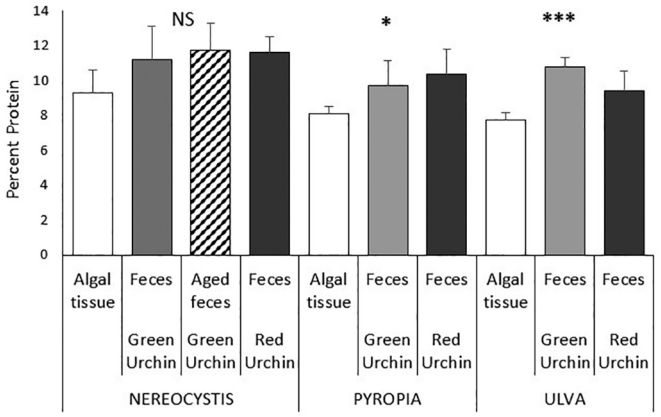


**Fig. 4.** Protein content for all samples. One sd shown. N = 5 for each bar except for *Ulva* tissue (N = 3) and Green urchin aged *Nereocystis* feces (N = 4). ANOVA NS = not significant; * = p < 0.05, *** = p = 0.001.

### 3.3. Tigriopus population growth

*Tigriopus* populations grew rapidly over 4–6 weeks when raised in the lab, completing at least one life cycle with all food types offered. In the first experiment, copepod populations fed chopped kelp blade (*Nereocystis* or *Saccharina*) grew dramatically slower than populations fed feces from green urchins consuming those kelp (Fig. 6), and population growth differed strongly among the 4 treatments (ANOVA $F = 38.77$, df $_{3,11}$, $p < 0.001$). Tukey tests showed that the urchin feces treatments overall were different from the fresh kelp treatments, but the population sizes did not differ between fecal treatments or between fresh kelp species. Population growth parameters (e.g., doubling time) in fecal treatments are on the same order as those of Egloff (1966) who raised copepods on more standard microalgal cultures (Suppl. Table 3). In the second experiment, using *Ulva* (two treatments only), we found the opposite result (Fig. 6); copepod populations fed fresh chopped *Ulva* grew significantly faster than those fed green urchin-*Ulva* feces (t-test p = 0.001).

## 4. Discussion

In a series of independent experiments, we demonstrated that even though sea urchins can have high assimilation efficiency of algal diets, the feces they produce are generally not nutritionally deficient relative to fresh algae, and in some cases actually have higher values of key nutritional indicators than the original algal tissue. Additional experiments suggested that aged feces may have even greater nutritional value. This fecal material thus may be a valuable potential food source for benthic consumers, as we demonstrated for one epibenthic copepod species.

The different metrics of nutritional quality varied in how algae compared with urchin feces. Caloric contents from tissue of two kelps were consistently lower than of feces from both red and green urchin species fed those kelps. This pattern did not hold for the one green (*Ulva*) or red (*Pyropia)* alga that we tested; for each of these, the feces produced had similar or reduced caloric content as the algal diet. Carbohydrates showed a similar pattern, with kelp (*Nereocystis*)-derived feces having slightly increased carbohydrates, but feces derived from *Pyropia* and *Ulva* being far less carbohydrate-rich. Protein content was different than calories or carbohydrates, with feces from all algae and both urchin species having slightly more proteins than the algal tissue. In a separate study (Schram et al., 2018) on fatty acid composition of algae, feces, and tissues from the juvenile urchins raised on single-algal diets, we found that certain long-chain fatty acids declined from algal diet to urchin feces. However, total lipid content increased slightly in most algal-urchin comparisons, and significantly for *Pyropia*. Finally,

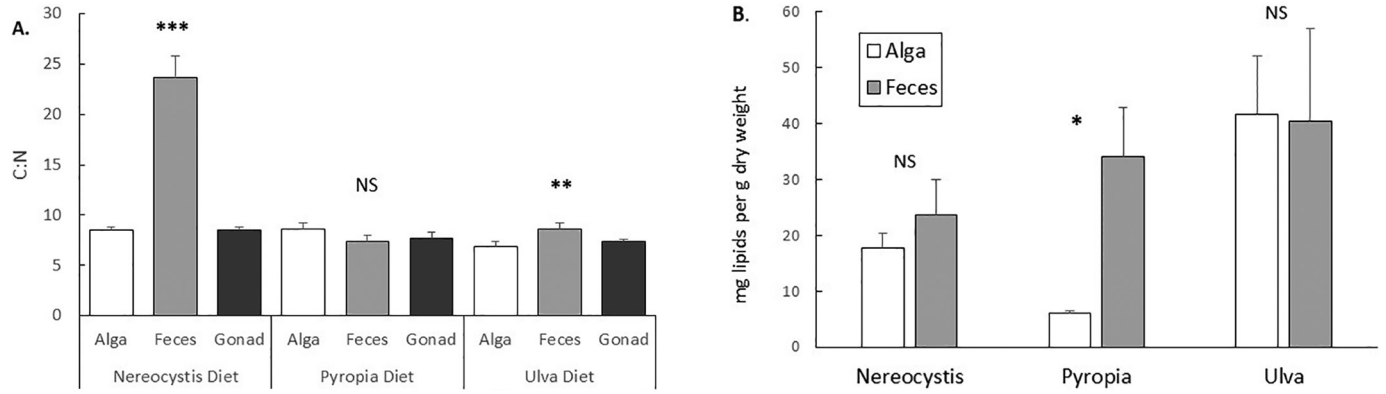


**Fig. 5.** A. C/N ratios of algal tissues, urchin gonads, and urchin feces of juvenile green urchins raised for 6 months on a mono-diet. Mean and one s.d. of N = 3–4 replicates per bar. Raw C and N data are in Supplemental Fig. 2. B. Total lipids from 3 algae consumed by juvenile green urchins in long-term mono-diet experiments, and from egesta from those urchins. Bars are means of 3–4 samples each, and one s.d. Data from Schram et al. (2018). Paired *t*-tests comparing algal tissue and feces for each species: NS = not significant; * p = 0.019, ** = p = 0.005, *** = p < 0.001.

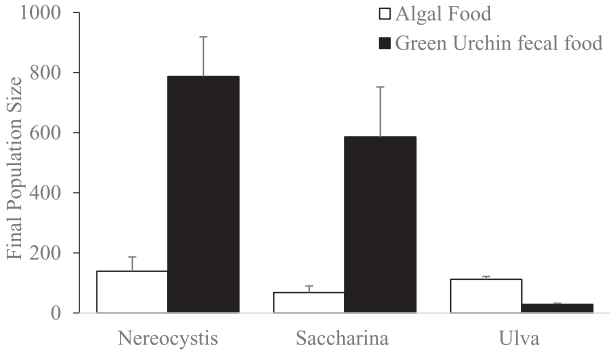


**Fig. 6.** Final population size (starting from 20 gravid females) after 4 weeks on algal vs. fecal diets. Bars are mean and one s.d. of N = 4 jars per treatment for the *Nereocystis* and *Saccharina* experiment, N = 8 for the *Ulva* experiment.

our relatively limited data on C/N content showed that juvenile green urchins remove most of the N from *Nereocystis* during digestion, resulting in high C/N in the feces. This change was much less dramatic for the other two algal species. Overall, it is difficult to find consistent patterns in these nutritional metrics, which change somewhat unpredictably from alga to feces. A next step would be to do complete biochemical analyses to understand the full extent of these changes.

Urchins are surprisingly variable in their rates of algal consumption (over an order of magnitude) among individuals and days, even within one experiment (reviewed by Suskiewicz and Johnson, 2017). Algae avoided in one experiment or trial may be consumed voraciously in another. This unpredictability was evident in most of our experiments and has been noted by other researchers, increasing the difficulty in finding consistent patterns in ecologically important parameters such as food preferences and overall consumption. Timing of food retention in the gut also varies; several sources of data (including our own observations) show that retention time may increase when food supply is limited. Starved urchins may retain their last meal for up to two weeks (Lasker and Giese, 1954).

We found an average assimilation efficiency of 87% for red sea urchins consuming *Nereocystis*, similar to the result of Vadas (1977), who found an 84 to 91% assimilation efficiency for this same food-consumer combination. Data for green urchins from the North Atlantic have found a wide range: 65–87% (Sauchyn and Scheibling, 2009b), 49–71% (Miller and Mann, 1973), or 65–67% (Larson et al., 1980), varying with diet and season. Other studies of sea urchin digestion show that 30 to 50% of algal material consumed is egested as globular, mucus-covered fecal pellets, 1 to 3 mm in diameter (Larson et al., 1980; Miller and Mann, 1973; Sauchyn and Scheibling, 2009a). Our average egestion rate was lower, as is often seen when urchins are feeding on a preferred alga, such as *Nereocystis* (Larson et al., 1980; Mamelona and Pelletier, 2005; Vadas, 1977). Vadas (1968) found *Nereocystis* to be the preferred food (out of 8 species offered) for both red and green urchins but did not test the other two algae in our experiments. Not surprisingly, we found that larger red urchins consumed more kelp and egested more material daily than smaller individuals (also described by Barker et al., 1998; Larson et al., 1980; Mamelona and Pelletier, 2005). This also implies that in nature, aggregations of larger urchins may provide a greater net ecosystem service of both detritus 'shredding' and fecal production compared with smaller urchins. In green urchins, mass-specific consumption rates vary little with urchin size (reviewed by Suskiewicz and Johnson, 2017). The higher metabolic demand of larger urchins may require greater nutrient uptake compared to smaller individuals (Barker et al., 1998), but our data suggest that larger sea urchins had a lower assimilation efficiency than smaller individuals (Suppl. Table 1), as they pass food through their guts more quickly. Our results also suggest that the amount of time kelp stays in the gut before being egested can affect the fecal caloric value. Larger urchins showed a trend of producing feces with lower caloric value than those of smaller urchins, suggesting that algal material held in the gut longer (as is the case with smaller sea urchins) undergoes increased biochemical changes before being egested.

Our study adds to a growing literature on the role of feces in aquatic systems, whether these are pellets egested by predators or herbivores, or aggregations packaged by suspension feeders (Wotton and Malmqvist, 2001). Because feces constitute readily transported units of organic matter, their potential trophic importance is substantial, especially from abundant plankton such as copepods (producing marine 'snow'), or benthic consumers such as urchins. Sauchyn and Scheibling (2009b) compared C:N ratios in undigested kelp vs feces; they found that feces had a higher ratio (poorer in N and thus of lower nutritional quality) than clean kelp, similar to what we found with the kelp we tested. Feces derived from the green alga *Codium* and from kelp encrusted with bryozoans had reduced ratios (Sauchyn and Scheibling, 2009b). We found no reduced C:N ratios in algal feces, although the extraction of N from *Ulva* and *Pyropia* was much reduced relative to the kelp we tested.

All these nutritional changes of algal material during digestion are likely due to colonization and activity by echinoid gut bacteria that are important for food decomposition (Prim and Lawrence, 1975). Lewis (1964) considered gut bacteria to be of little significance under normal feeding conditions because material passes through the gut so quickly (e.g. 8–12 h), but Lasker and Giese (1954) found abundant bacteria capable of digesting algae in urchin guts. *Strongylocentrotus droebachiensis* has food-derived bacteria with a cellulolytic ability and a

capacity to synthesize essential amino acids available to the sea urchin (Fong and Mann, 1980). This microbiota may be nutritionally important to the hosts; Guerinot et al. (1977) found that *S. droebachiensis* in kelp beds may receive 8–15% of their daily nitrogen requirements from N2-fixing gut bacteria. Recent studies on the urchin gut microbiome suggest that these bacteria play a role in carbohydrate, amino acid, and lipid metabolism (Hakim et al., 2016); in addition, different communities of microbes exist in urchin Aristotle's lanterns and gut tissues (Hakim et al., 2019), and these microbes also differ from those that enrich the mucus layer of fecal pellets (Hakim et al., 2015). If urchin gut microbiomes are responsible for many of the biochemical differences we observed between food and feces, then parameters such as time of food retention in the gut (which is likely affected by urchin size and food availability) could be important to the nutritional quality of the feces. Moreover, an added complexity that we are not yet accounting for is that the algae consumed come with their own microbiomes (e.g., Lemay et al., 2018). In addition, recently fed urchins are known to 'leak' DOM from their tissues and/or feces, providing an additional energy source for bacteria (Field, 1972).

As seen from the significant increase of caloric content in one of the aged feces treatments, it also appears that growth of microbial populations can continue after the feces have been egested, adding further to the nutritional value. Sauchyn and Scheibling (2009a) performed a detailed study of biochemical changes in green urchin feces with time, temperature, and depth, and found complex patterns of changes in nutritional value (energy content, protein, C:N, etc) as microbial degradation proceeds after egestion. They found fecal "half-life" of 4.1 days, with rapid colonization by bacteria (perhaps promoted by leaking of DOM: Field, 1972) likely followed by protozoa. Bacteria take up dissolved inorganic N from the seawater, lowering the C:N ratio of the detritus or feces they have colonized and thus increasing its nutritional quality. The degraded but enriched feces are still an important food source for organisms in the deep subtidal (below 28–50 m, Sauchyn and Scheibling, 2009b). Aging kelp detritus similarly became more nutritious in several experiments (Dethier et al., 2014).

The high nutritional value of the feces produced by sea urchins could have profound implications to benthic communities, as urchins may play an important role in connecting both attached algal beds and drift algal subsidy to benthic communities throughout different depth zones by transforming a large amount of algae into fecal material (as shown by Sauchyn et al., 2011). In our lab experiments, harpacticoid copepod populations grew faster when fed urchin feces than when fed fresh material from the same species of algae. This observation is consistent with other evidence of improved nutritional value of urchin feces presented in this study. Powlik et al. (1997) noted that *Tigriopus* will feed on "any living substance which promotes the formation of bacteria" (p. 333). For this benthic feeder, at least, fecal pellets contain sufficient essential nutrients to support their entire life cycle. This is especially true because of the small size of the detritus in fecal pellets compared to drift algae; small particles are both available to a different, potentially broader range of consumers and have greater surface area for microbial colonization. Lowe et al. (2015) found higher abundances of small grazers and detritivores under sessile red sea urchins than in surrounding areas in the San Juan Archipelago, particularly at increasing depths. If detritus gains calories as it ages, then gradually falling to deep subtidal environments may substantially increase its caloric value. If algal material begins this increase in caloric value while still in the gut of urchins, urchin fecal matter may prove to be a vital source of nutrients in benthic communities that depend on the input of detritus for their success.

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## Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jembe.2019.03.016.

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