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Degrading detritus: Changes in food quality of aging kelp tissue varies with species

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ABSTRACT

Export of detrital kelp from the photic zone is thought to provide an important trophic subsidy to adjacent food webs. In the San Juan Archipelago, Washington, deep habitats (e.g. >90 m) contain abundant, senescent kelp detritus that presumably is colonized by microbes as it sinks. Such aging algal pieces may be of greater nutritional value than fresh material, and may have reduced levels of polyphenolic compounds, which in living kelps deter both bacterial colonization and herbivory. We report here on a diverse set of experiments designed to test the value to consumers of fresh and aged tissue of two kelps with very different biochemical compositions, *Nereocystis luetkeana* (low polyphenolics) and *Agarum fimbriatum* (high polyphenolics). We tested 1) short-term food preference and assimilation efficiency in adult isopods; 2) growth rates of juvenile isopods over 10 weeks; 3) gonad development in urchins over 18 weeks; 4) population growth of copepods over 4 weeks and subsequent egg-hatching time; and 5) caloric content, fatty acids, and C:N ratios of fresh and aged tissue of these two kelps, with some additional data for a third kelp species. In a food-choice situation, *Nereocystis* is strongly preferred over *Agarum*, and is a superior food by a number of metrics. Aging kelp blades in the dark led to *Nereocystis* degrading much faster than *Agarum*. Overall, aging of the two kelp species tended to have opposite effects in various metrics of their value to consumers, so that in many experiments there was a species × age interaction term. Fresh *Nereocystis* was slightly 'better' than aged in terms of isopod growth and preference, gonad development in urchins, and population growth metrics in copepods, while aged *Agarum* was 'better' than fresh in a number of these metrics at least over the time scales of our studies. Degrading kelps show broad changes in fatty acids that differed among species, but we did not find a general increase in N as has been suggested elsewhere. In combination with several other recent studies, our data suggest that the value of different types of detritus to deep trophic webs is likely to be both species- and time-dependent.

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1. Introduction

Coastal ecosystems are dependent on benthic macroalgae, such as kelps in the order Laminariales, as major primary producers and providers of habitat in the photic zone (Estes and Peterson, 2000; Sano et al., 2001; Witman, 1988). While the role of organic matter production of kelp to the coastal food web is well documented (e.g., Bustamante and Branch, 1996; Duggins et al., 1989; Witman, 1988), only a relatively small amount (approximately 10%) is directly consumed (Mann, 1988). Export of unconsumed organic matter to adjacent food webs is thought to provide an important spatial subsidy (sensu Polis et al., 1997) in the form of detrital algae transported via hydrodynamic forces into subtidal, intertidal, pelagic and terrestrial ecosystems (Britton-Simmons et al., 2009; Hagen et al., 2012; Hyndes et al., 2012; Kelly et al., 2012; Krumhansl and Scheibling, 2012; Mews et al., 2006).

In the San Juan Archipelago (SJA), Washington, attached benthic algal cover is <5% below 25 m depth due to light attenuation (Britton-Simmons et al., 2009). However, most (97%) ROV observations within a 60-km^2 section of sea floor in the SJA noted drift algae, with the majority of biomass contributed by kelps (Britton-Simmons et al., 2012). Transportation time of drift algae from the photic zone to local deep habitats (e.g. >90 m) is unknown and certainly variable, but during this process, non-photosynthesizing, senescent algal detritus presumably becomes colonized by microbes and begins to degrade.

Degraded (hereafter "aged") detrital algae (ranging from large detached pieces of drift to tiny particulate organic matter) may be of greater nutritional value than fresh algal material (e.g., Krumhansl and Scheibling, 2012). These pieces are in various stages of degradation depending on age and water conditions such as turbulence (Mann, 1988), and on levels of polyphenolic compounds; polyphenolics can

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deter bacterial colonization (Goecke et al., 2010; Nagayama, 2002; Sosik and Simenstad, 2013) as well as reduce herbivory and assimilation efficiency (Boettcher and Targett, 1993; Steinberg, 1992; Tugwell and Branch, 1992). The rate of loss of polyphenolics may in turn depend on detrital size (e.g., whole blades vs. particles: Duggins and Eckman, 1997; Levinton et al., 2002; Norderhaug et al., 2003; Sosik and Simenstad, 2013) and species (Pennings et al., 2000). Microbial communities that colonize detritus may alter its ecological roles by increasing nitrogen content (Mann, 1988; Smith and Foreman, 1984), cycling N within communities through the 'feces loop' (Newell and Field, 1983), and increasing survival and growth of epifauna (Norderhaug et al., 2003). Details about microbial roles are just emerging (reviewed by Wahl et al., 2012); they may alter the algal substratum physically and chemically. Biofilms on the surface of marine organisms are usually dominated by bacteria, accompanied to a much lesser extent by diatoms, fungi, and protozoa (Wahl et al., 2012).

Relatively few studies have compared the responses of marine consumers to aged vs. fresh kelp-derived diets (but see Duggins and Eckman, 1997; Levinton et al., 2002; Norderhaug et al., 2003). Pennings et al. (2000) showed that three supralittoral crustacean species generally preferred aged algae (washed up as wrack) over fresh samples. A terrestrial isopod demonstrated preference of aged organic material based on the presence of bacterial species it could most easily digest (Ihnen and Zimmer, 2008). Their findings suggested that detrital material is preferred due to greater nutritional content, but this result is not found consistently (e.g., Pennings et al., 2000).

We report here on a diverse set of experiments designed to test the value to consumers of fresh and aged tissue of two very different kelps, *Nereocystis luetkeana* and *Agarum fimbriatum* (hereafter *Nereocystis* and *Agarum*). We also summarize recent fatty acid analyses on aging *Nereocystis*, *Agarum*, and *Saccharina subsimplex* (hereafter *Saccharina*). These three species are all very abundant subtidally in the SJA as attached algae and in the drift (Britton-Simmons et al., 2009; Vadas, 1977). *Nereocystis* is often preferred over *Agarum* by herbivores (e.g., Vadas, 1977), probably because of its low polyphenolic content (0.4% by dry mass: Steinberg, 1985), whereas *Agarum* contains among the highest known percentages of total polyphenolics among northeast Pacific brown algae (4.1%: Steinberg, 1985, range of 2–8%: Van Alstyne et al., 1999) and is often avoided by herbivores.

We tested the effects of kelp aging on several consumers with different feeding modes. Isopods in the genus *Idotea* have a close association with macroalgae (Molis et al., 2010), using them for both food and protection (Vesakoski et al., 2008). Aged detrital kelp is likely a readily available food source for such mesograzers, both as shoreline wrack and sunken subtidal detritus. Sea urchins in the SJA consume both living algae (in the photic zone) and drifting detritus (Britton-Simmons et al., 2009; Lowe et al., in press; Vadas, 1977), subsisting primarily on the latter in deep water. Harpacticoid copepods are abundant benthic and epibenthic consumers in many habitat types. In the SJA, *Tigriopus californicus* is a harpacticoid copepod commonly found in high tidepools, where it browses on algae and detritus (Morris et al., 1980); because it is easy to keep in culture and has a short reproductive cycle, it was a model copepod for experimentation.

Many of our experiments were done as short-term undergraduate research projects. While this means that in some cases replicates were few or trials short, in combination the data include diverse species and trophic processes and thus constitute a broad view of the implications of detrital aging.

2. Methods

Blades of *Nereocystis*, *Agarum*, and *Saccharina* were collected from San Juan Channel, WA. Samples were either stored in lighted sea tables and used within a week, or were set aside to age. Blades were stored whole, but for experimental samples we avoided midrib, meristematic and reproductive tissue. Samples were aged 7–30 days in mesh bags or 5-gallon buckets with holes placed in darkened, flow-through seawater tanks, mimicking conditions that detached blades experience as they sink into deep water in nature. Aging intervals varied with species and experiment (see below), because often *Nereocystis* blades degraded into soft scraps within 2 weeks, but *Agarum* stayed intact for over 3 weeks; attempting to age *Nereocystis* for the time required for *Agarum* to visibly degrade (>5 weeks) would cause its complete disintegration. Similar rapid degradation of detrital *Nereocystis* was noted by Mews et al. (2006). Isopods used in three experiments were collected from the intertidal zone on San Juan Island from under cobbles and among beach wrack.

To study feeding preferences of adult isopods for aged and fresh kelp tissue, intertidal isopods *Idotea wosnesenskii* (hereafter *Idotea*) were field-collected and starved for 72 h. Eight individual isopods (4 male and 4 non-brooding females, 0.1–0.4 g each) were placed in each experimental container so that each had a similar mass of isopods. Containers were 1.4 L plastic boxes with 1.5 mm mesh windows on opposite sides to allow water flow, placed in sea tables. In four treatments ($N = 10$ replicates each), *Idotea* were given a choice of either fresh and aged (aged 14–17 days for each species) samples of one kelp species, of fresh samples of both species, or of aged samples of both species. Immediately prior to feeding, kelp blades were cut into 5×7 cm pieces, blotted and weighed. To quantify natural biomass changes during the experimental period, 10 samples of each kelp species/age were placed in identical plastic containers without isopods ($N = 10$). Consumption of each of the blades was measured after visible amounts were consumed (Pennings et al., 2000), 48–120 h. At the end of the trial period, algae were blotted and weighed as before. Consumption was calculated using the formula of Cronin and Hay (1996), correcting for mass change due to natural growth or degradation; Consumption $= T_i * (C_f / C_i) - T_f$, where T represents the feeding treatment, C = the control (averaged over 5 replicates), i = initial mass, and f = final mass. Algae did not grow in the controls in these brief experiments, but degradation was clearly seen in the aged *Nereocystis* (which lost 1–29% of its wet mass). *Agarum* never lost more than 1% of its mass.

Caloric contents of fresh and aged kelp tissue were measured using the micro-assay calorimetry technique of Gosselin and Qian (1999), modified for larger samples. For fresh samples, pieces of *Agarum* and *Nereocystis* were finely chopped with a razor, placed in weigh boats, and oven dried. Aged *Agarum* tissue (aged for 1 month) was treated similarly. Aged *Nereocystis* blades had broken down into soft fragments after one month; sample tissue was collected from the mesh aging-bags via an aquarium net, deposited directly into the weigh boats, and dried. For each of 6 replicates of the 4 treatments, dried samples were powdered in a mortar, and 25–30 mg put into replicate 25 mL test tubes. With samples larger than the 20 μg used by Gosselin and Qian, we increased the amount of potassium dichromate oxidizing solution to 10 mL for each tube. After two incubation periods, 0.5 mL of each sample solution was placed in another tube, and 4 mL of potassium iodide/starch solution added. After 20 min, absorbance of the solution was measured on a DR 5000 spectrophotometer at 575 nm. A calibration curve for the scaled-up method, using dry weights of *Nereocystis* ranging from 0 to 80 mg, had a clear linear relationship of weight with absorbance ($r^2 = 0.99$). Standards were prepared in an identical fashion using 5–50 mg of reagent grade glucose, and tested for caloric content using the same modified chemical technique. The standard curve produced had an r^2 value of 0.92, and was used to determine the amount of glucose present in each sample. We calculated calories from units of glucose following the conversion in Gosselin and Qian (1999). Caloric data were log transformed to meet assumptions of normality.

To study assimilation efficiency in isopods fed fresh and aged kelp, male *Idotea* were field-collected, placed individually in clear plastic beakers with airstones, and kept in a temperature controlled room at ~14 °C on a 16:8 h light:dark cycle. Mesh screens in each beaker created a platform to hold isopods and algal pieces, but allowed isopod feces to

fall to the bottom of the beaker and avoid being broken up by the animal. For each treatment, isopods were given a blotted and weighed (~3 g) piece of algae: fresh or aged *Nereocystis*, or fresh or aged *Agarum*. Algae were either freshly collected or had been aged for one week before being fed to isopods for 3 days, after which we removed the uneaten remnants and freeze-dried them to obtain dry weights. Fecal matter was collected and frozen daily for 7 days from the start of the experiment (i.e., allowing animals to clear their guts of the algae they ate over 3 days). Dry weights of initial algae were calculated from regressions ($r^2 > 0.98$) of wet and oven-dried pieces of each kelp species. To control for changes in algal weight, ~3 g pieces of algae (N = 5 for each treatment) were kept in the cold room in plastic containers over the course of the treatment period; experimental algal weights were corrected by the mean weight change for these samples. Accumulated feces from the 7-day experiments were thawed and filtered using preweighed 0.22 μm filters. The samples were then weighed (wet), freeze dried, and weighed again (dry). We ran 8 replicates of each of the four treatments, although a total of 5 replicates were discarded (4 from aged *Nereocystis* treatments, 1 from fresh *Nereocystis*) because the animals died midway through the trial, or no fecal matter was produced. Assimilation efficiency was calculated as $(I - F) / I * 100$, where I is the dry weight of ingested food, and F is the dry weight of fecal matter produced (Catalan et al., 2008; Romero et al., 2006).

To study growth rates of newly-hatched isopods raised on aged and fresh kelp, brooding females of *Idotea* were field-collected and held in tanks until juveniles (ca. 3 mm length) were released and swimming freely, at which point they were placed in experimental tanks with airstones and kept in a cold room at 8 °C on a 16:8 h light:dark cycle. The two feeding treatments were aged and fresh *Nereocystis*; *Agarum* was not used, as we suspected that providing it as the sole food to newly hatched isopods might result in no growth or high mortality because of the concentrated polyphenolics: see Introduction. Kelp aging time varied from 14 to 21 days, until blades showed visible degradation while still retaining structural integrity. Tanks were cleaned and food replaced (one 4 × 3 cm piece) every 3 days. Each replicate tank (N = 3 per treatment) contained 41 juvenile animals from two broods, distributed equally between all six tanks. At day 0, an average (of N > 10 replicates) individual length (tip of head to tip of pleotelson) was measured for each brood. After 10 weeks all surviving juveniles (175 total) were measured, and mm grown calculated from the average starting size for each brood.

Long-term gonad development as well stable isotope and fatty acid signatures of urchins fed fresh and aged kelps was studied by Raymond et al. (in press); results are summarized briefly here. The gonad index is commonly used to evaluate how urchin nutritional condition responds to diet type (e.g., McBride et al., 2004). Medium-sized (mean test size 98 mm; N = 12 per treatment) red urchins *Mesocentrotus* (=*Strongylocentrotus*) *franciscanus* were held for 18 weeks in cages suspended in the water column and fed (ad libitum) one of four kelp diets: fresh or aged *Agarum* or *Nereocystis*. Aged *Nereocystis* was aged for one week so that relatively intact blades could be offered to the urchins, and *Agarum* for three weeks. At the end of the feeding trial all urchins were dissected and measured for total wet mass, test diameter, and wet gonad mass (blotted-damp weight). Gonad index was calculated as the ratio of gonad wet mass to total wet mass and reported here as a percentage.

The effect of aging kelp diets on population growth of the copepod *T. californicus* was studied in the lab. Copepods were collected from two high tidepools on the FHL property and stored in glass jars with no additional food at room temperature for 2 days. We placed 30 gravid females into each of 12 jars of filtered sea water containing one 2 × 2 cm piece of one of the following diets, for 3 replicates of each of 4 treatments: fresh (unwashed) *Nereocystis* or *Agarum*, or 1-week aged *Nereocystis* or *Agarum*. The jars were stored at 15 °C in a 16:8 h light: dark cycle. After 7 days, each piece of algae was replaced; this process was repeated for 4 weeks, then the total population and gravid female abundances were counted. At the end of this experiment, ten gravid females from each replicate were individually placed into small vials of filtered sea water. The egg sac of each female was monitored every 8 h over the span of 96 h to determine hatching time.

To summarize several of our recent experiments that evaluated how fatty acid (FA) profiles and C:N ratios change when kelp blades age, we combined data from Galloway et al. (2013) [for *Saccharina* and *Agarum*; also see Sosik and Simenstad, (2013)], Raymond et al. (in press) (see above, with *Nereocystis* and *Agarum*), and the juvenile isopod experiment with fresh and aged *Nereocystis*, described above. From each experiment, we froze aged and fresh kelp tissue from 3 to 5 replicate samples of experimental kelps for later extraction and analyses following the methods of Galloway et al. (2013). Aged blades had been aging for 1–5 weeks in the different experiments. Fatty acid methyl esters (FAME) were extracted at FHL from 10 mg of tissue using a modified Folch method. To analyze FAME we used GC-FID (HP 6958, Agilent DB-23 column), and an 85-minute temperature program designed to separate C_{16} and C_{18} monounsaturated FA (MUFA) and polyunsaturated FA (PUFA). We identified peaks using GC-FID and a 40 FA standard (Nu-chek Prep standard 569B), and identified unknown peaks with GC–MS. We reduced the database to the 19 FA reliably identified across the three experiments, normalized them to 100%, and arcsine squareroot-transformed the proportional data. This full dataset for FA of aged and fresh samples of 3 kelps was visualized with an MDS plot, and effects of genus and age on FA analyzed with PERMANOVA tests with both factors fixed. We used SIMPER analysis on raw, percent FA data to identify FA driving the differences between fresh and aged samples. Data were analyzed using multivariate routines in PRIMER v.6.0 (Clarke and Gorley, 2006) with the PERMANOVA + add on (Anderson et al., 2008). C:N ratios were calculated from %C and %N data obtained during stable isotope analyses of kelp tissues by Washington State University's Stable Isotope Core lab.

3. Results

When adult isopods were given choices of pieces of both kelp species, *Nereocystis* was strongly preferred; isopods ate on average 3.5 times as much fresh *Nereocystis* as fresh *Agarum* (paired *t* test, p = 0.0002), and 5 times as much aged *Nereocystis* as aged *Agarum* (p < 0.0001) (data not shown). In experiments where the choice was aged vs. fresh pieces of one species, isopods preferred fresh over aged *Nereocystis* (*t* test, p = 0.011), but showed no preference for fresh or aged *Agarum* (p = 0.42; Fig. 1A).

The caloric contents of fresh and aged *Agarum* tissue and fresh *Nereocystis* tissue were very similar, but the aged *Nereocystis* tissue samples had much higher caloric values (Fig. 1B). In a 2-way ANOVA with both factors fixed, species, age, and their interaction were all significant (p's < 0.001).

Assimilation Efficiency (AE) in adult isopods varied both with kelp species consumed (2-way ANOVA, p = 0.02) and blade age (p = 0.04), with no interaction term (Fig. 1C). Overall, isopods consuming *Nereocystis* showed higher AE than those eating *Agarum*. There was little effect of blade age for *Nereocystis*, while animals fed aged *Agarum* had much higher AE than with fresh *Agarum* (although variances were high among the *Agarum* replicates).

Juvenile isopods fed fresh and aged *Nereocystis* all grew substantially, in 10 weeks approximately doubling in size (from 3 to 6 mm) regardless of treatment. There was no significant tank (block) effect, so we pooled individuals across replicate (N = 3) treatment tanks and treated them as replicates. Isopods in the fresh *Nereocystis* treatments grew slightly but significantly faster than those fed aged kelp (Fig. 1D: *t*-test, p = 0.006).

Urchins developed much larger gonads when fed *Nereocystis* of any age than when fed *Agarum*, and kelp age had much less effect (Fig. 1E). A two-way ANOVA of gonad index showed a clear effect of kelp species (p < 0.001) but a marginal effect of kelp age (p = 0.073),

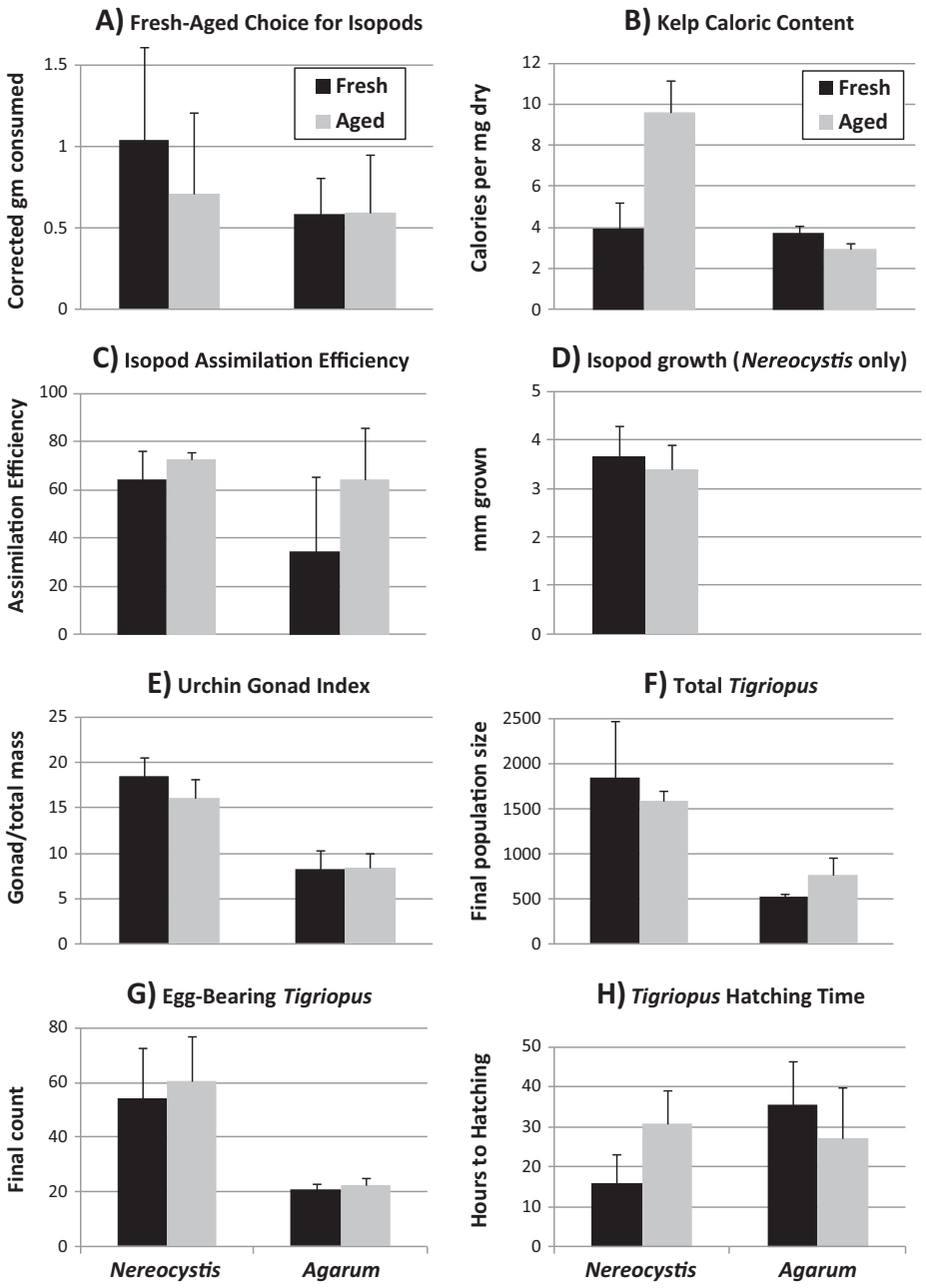

Fig. 1. Results of diverse experiments comparing fresh and aged kelp. All plots show mean values and one s.d.; length of aging of kelp blades varied with experiment (see text). A. Grams of algae consumed by adult isopods given within-species choice of fresh vs. aged blades; data are corrected by mean algal weight change in controls. N = 10 replicates per treatment. Error bar value for Fresh *Nereocystis* (0.58) is truncated to stretch y axis. B. Caloric content per dry mass of blade tissue. N = 6 per treatment. C. Assimilation efficiency of kelp treatments by adult isopods. N for *Nereocystis* Fresh, Aged = 7, 4; for each *Agarum* treatment = 8. D. Growth of juvenile isopods, calculated as total length added over 10 weeks (*Agarum* was not tested; see text). N = 85 fresh, 90 aged. E. Gonad index (reported here as a percentage) of red urchins fed one of 4 diets for 18 weeks. N = 12 urchins per treatment. F. Count of all copepods after 4 weeks in mesocosms with 30 founder individuals. N = 3 containers per treatment. G. Count of only mature female copepods in the *Tigriopus* experiment. N = 3 containers per treatment. H. Average time for eggs to hatch from ten gravid *Tigriopus* females isolated and observed every 8 h.

and a significant interaction ($p = 0.038$); a diet of aged *Nereocystis* resulted in slightly smaller gonads than with fresh *Nereocystis*, but for *Agarum* the opposite effect was seen.

Copepod populations raised on all four diets showed abundances that increased rapidly over 4 weeks from the founding populations of 30 gravid females (Fig. 1F, G). Kelp species had a large effect on population growth for both total copepods and gravid females (2-way ANOVA, $p = 0.0006$ and $= 0.001$, respectively), with both fresh and aged *Nereocystis* diets resulting in far greater copepod abundances than either *Agarum* diet (Fig. 1F). Kelp age did not affect abundance of either total copepods or gravid females ($p > 0.5$ for both comparisons), nor were there significant species × age interactions. Hatching time for gravid females whose entire lives had likely been in the experimental treatments [since generation time under these temperature conditions is ca. 21 days, Powlik and Lewis (1996)] also varied with kelp species but not kelp age ($p = 0.022$ and 0.30, respectively) (Fig. 1H), but for this parameter there was a species × age interaction ($p = 0.001$); animals on the fresh *Nereocystis* diet had eggs that hatched faster than the aged *Nereocystis* diet, but the reverse was true for *Agarum* diets.

As suggested elsewhere (Dethier et al., 2013; Galloway et al., 2012), FA profiles vary significantly with kelp genus (Table 1, Fig. 2); all pairwise comparisons among genera were significant ($p = 0.001$ except *Nereocystis–Saccharina*, $p = 0.011$). A 2-factor PERMANOVA on genus and age with all 'aged' samples considered together regardless of

Table 1
Results of PERMANOVA analyses on fatty acid profiles (19 FA) from algal samples in the urchin and juvenile isopod feeding experiments and from two published datasets. The 3-genera analysis includes *Saccharina*, while the 2-genera analysis does not.

	Contrast	df	SS	p
Genera (all ages)	Genus	2	3.055	0.001
	Residual	37	1.315	
Genera (3) and age	Genus	2	3.039	0.001
	Age	1	0.046	0.224
	Genus × age	2	0.173	0.011
	Residual	34	1.091	
Genera (2) and age	Genus	1	2.623	0.001
	Age	1	0.052	0.215
	Genus × age	1	0.145	0.007
	Residual	28	1.025	

aging period was significant for genus but not age, and there was a significant interaction term (Table 1); this interaction can be seen in Fig. 2, where many of the aged *Agarum* samples are lower on the plot than the fresh *Agarum*, but the opposite is true for *Nereocystis* and *Saccharina*. The length of time of tissue aging (coded in Fig. 2) had no consistent effects. Eliminating *Saccharina* (which was only measured in one experiment) from the analysis results in an even stronger interaction term (Table 1). When each genus is analyzed independently, *Nereocystis* showed a significant aging effect ($p = 0.041$), but *Agarum* did not ($p = 0.082$). The lack of aging effect in *Agarum* stems from the high variation in the FA composition of the fresh samples, which may relate to season of collection (upper points in Fig. 2 from summer collections, lower points from fall); Dethier et al. (2013) also found large variation in FA of *Agarum* collected in different seasons. SIMPER analyses on FA driving the age differences showed that fresh *Nereocystis* tended to have proportionally more 16:0 and 20:5n-3 and less 18:4n-3 and 18:1n-9 than aged; fresh *Agarum* tended to have more 20:4n-6 and 20:5n-3 and less 16-0 and 18:1n-9 than aged.

Key groupings of FA (percentage of total FA summary categories; see Table 2) were analyzed with univariate 2-way ANOVAs (for kelp genus and age). In some cases there was a significant interaction term because *Agarum* FA groups changed from fresh to aged in the opposite direction from how the other two kelps changed (Table 2); *Nereocystis* and *Saccharina* were similar in most parameters. For example, long-chain ($>C_{18}$) PUFA (polyunsaturated FA) decreased from fresh to aged tissue in *Nereocystis* and *Saccharina*, but increased in *Agarum* (Fig. 3). The opposite pattern was seen in both SAFA (saturated FA) and MUFA (monounsaturated FA) (i.e., decreased with age in *Agarum*, increased with age in the other two kelps; data not shown). Omega-3 FA differed strongly among genera, with much less in *Agarum* than in the other two

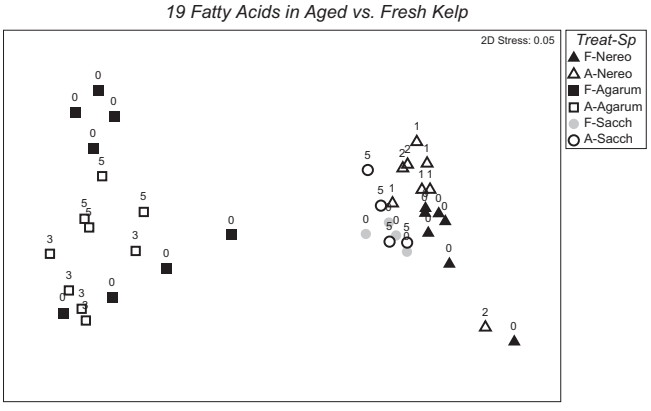

Fig. 2. MDS plot of fatty acids (19 FA, with data normalized to 100% per sample, and arcsine squareroot transformed) from aged and fresh kelps of three species from the experiments where these were measured. Each point = 1 algal sample. Codes on the sample points indicate numbers of weeks the algae were aged. F = fresh, A = aged. Nereo = *Nereocystis*; Sacch = *Saccharina*.

Table 2
Results of 2-way ANOVAs on FA groupings. FA data are normalized arcsine squareroot transformed percentages. 'Genus' includes *Agarum*, *Nereocystis*, and *Saccharina*.

	Factors	df	F	p
SAFA	Genus	2	0.630	0.54
	Aging	1	0.106	0.75
	G × A	2	4.179	0.024*
MUFA	Genus	2	8.45	0.001**
	Aging	1	0.073	0.79
	G × A	2	1.615	0.21
PUFA	Genus	2	13.91	<0.001**
	Aging	1	0.269	0.61
	G × A	2	3.200	0.053*
Omega 3	Genus	2	25.10	<0.001**
	Aging	1	0.092	0.76
	G × A	2	2.026	0.15
Omega 6	Genus	2	1.102	0.34
	Aging	1	0.062	0.81
	G × A	2	2.153	0.13
Omega 3:6 ratio	Genus	2	19.93	<0.001**
	Aging	1	0.147	0.71
	G × A	2	0.252	0.78
"Bacterial" FA	Genus	2	40.55	<0.001**
	Aging	1	5.81	0.021*
	G × A	2	0.497	0.61

* = $p < 0.05$.
** = $p < 0.01$.

kelps, whereas omega-6 FA did not differ among genera; in neither group was there an effect of tissue age (Table 2). The ratio of omega-3 to omega-6 FA, however, was much lower in *Agarum* than the other kelps (Fig. 3). Only two FA previously reported as "bacterial" biomarkers (15:0 and 18:1n-7: Kelly and Scheibling, 2012) were found in all three experiments. The sum of bacterial FA increased with tissue age in all 3 kelps (Table 2), but interestingly these FA were much more abundant in fresh and aged tissue in *Agarum* than in either of the other species (Fig. 3).

C:N ratios did not consistently show declines in aged kelp tissue (Fig. 4). Analyses of *Agarum* and *Saccharina* showed very little difference between fresh and aged tissues (although considerable variation among experiments, perhaps due in part to the variation in duration of aging), and opposite patterns were seen in the two sets of *Nereocystis* tissue comparisons. The *Nereocystis* samples aged for 1 week (from the urchin feeding experiment) showed generally higher C:N than fresh blades, while those aged for 2–3 weeks (juvenile isopod experiment) showed reduced C:N.

4. Discussion

4.1. Food value of Nereocystis vs Agarum

Several consistent patterns emerge from these diverse experiments on the palatability and food quality of fresh versus partly degraded kelps. First, as demonstrated elsewhere and with diverse herbivores, in a food-choice situation *N. luetkeana* is strongly preferred over *A. fimbriatum* (our experiments with isopods; several species of urchins: Vadas, 1977; snails: Steinberg, 1985). The pattern of *Nereocystis* being a "better" food is also seen in several higher-order parameters. *Nereocystis* was significantly superior to *Agarum* in terms of food assimilation efficiency in isopods; assimilation, growth, and gonad development in urchins (this paper and McBride et al., 2004; Vadas, 1977); and population growth in harpacticoid copepods. The ratio of omega-3:omega-6 fatty acids, often used as an indicator of diet quality for consumers (Brett et al., 2009), was also much higher for *Nereocystis* (and *Saccharina*) than for *Agarum*. The consistent differences among kelp species in diverse consumer responses likely relates in part to the low concentration of polyphenolic secondary metabolites in *Nereocystis* and high concentration in *Agarum* (see Introduction). Our limited

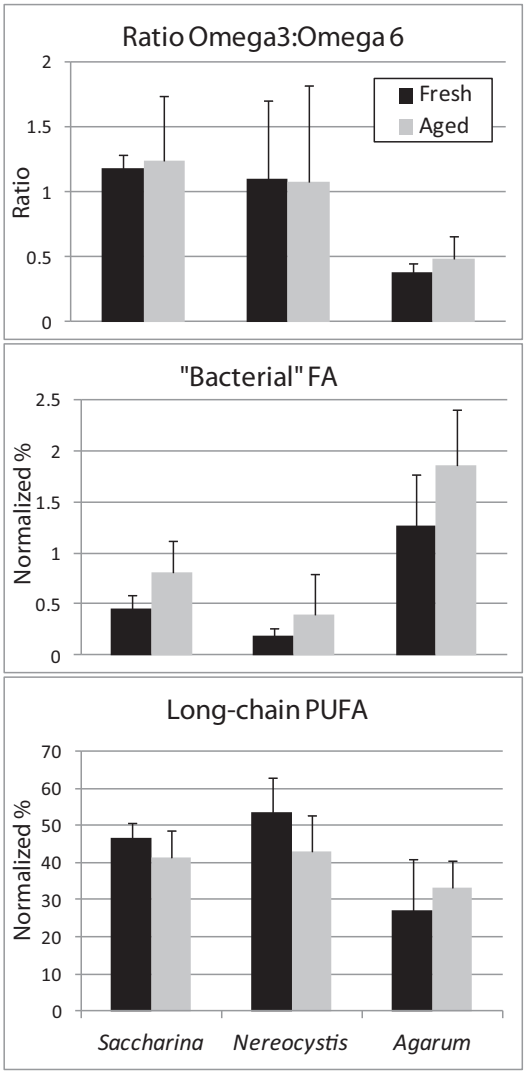

Fig. 3. Differences among fresh and aged kelp (3 species) of three summary categories of fatty acids (FA) quantified. PUFA = polyunsaturated FA. Ns from left to right in plot = 4, 4; 7, 8; 8, 9.

caloric data agree with those measured by Paine and Vadas (1969), showing that *Nereocystis* and *Agarum* have very similar caloric contents, making it unlikely that this metric of food value is a factor determining choice or performance of herbivores.

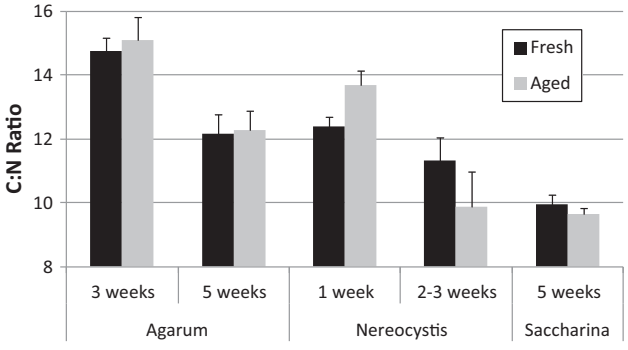

Fig. 4. C:N ratios of kelp blades from three different experiments with different aging periods. Ns from left to right in plot = 5, 5; 4, 4; 5, 5; 3, 3; 4, 4.

4.2. The aging process in Nereocystis vs Agarum

Our observations showed that *Nereocystis* consistently 'rots' faster than *Agarum* under identical conditions representative of dark, subtidal habitats. The rate of decomposition seemed somewhat unpredictable but may have related to temperature and flow, which likely contribute to high variability in degradation state seen in natural kelp detritus. While we did not consistently quantify mass lost, in all trials *Nereocystis* blades became soft and disintegrated within 1–2 weeks, while *Agarum* blades remained crisp and intact for as long as 4 weeks. Correlative evidence suggests that this difference relates to the concentrations of polyphenolic compounds in each. These secondary metabolites are known to inhibit not only herbivory but also colonization of microbial communities in some algae (Goecke et al., 2010; Nagayama, 2002; Targett and Arnold, 2001; Van Alstyne et al., 1999). Sosik and Simenstad (2013) quantified microbial abundances on aging kelps and demonstrated that *Agarum* shows no increase in microbes over 5 weeks, whereas another laminarian kelp, *S. subsimplex*, shows significant microbial colonization. They also demonstrated no reduction in polyphenolic concentrations in intact *Agarum* blades over this time scale, in contrast to studies with pulverized *Agarum* blades which do lose these compounds over weeks (Duggins and Eckman, 1997; Norderhaug et al., 2003). C:N ratios may decrease in parallel to polyphenolics losses (but not in Pennings et al., 2000), which could be interpreted as colonizing microbes augmenting the overall detrital nitrogen content; this would be significant to the nutrition of marine herbivores, which are often nitrogen-limited. No consistent changes in C:N were observed over the aging timescales in this study, but these were relatively short (1–5 weeks) compared with other studies (e.g., 16 weeks in Krumhansl and Scheibling, 2012).

Overall, aging of the two kelp species resulted in a largely consistent pattern in a range of response variables; degradation tended to have opposite effects in various measures of the value of kelp to consumers. Fresh *Nereocystis* was "better" than aged tissue in most tests; it was preferred by adult isopods, led to higher growth in juvenile isopods, slightly better gonad development in urchins, and faster egg-hatching and slightly better total population growth in copepods. Differences in hatching time may relate to how long it takes eggs to develop within the egg sacs on the females, which could relate to the females' nutritional state. Kahan et al. (1988) also found that gravid females are inhibited from depositing their eggs when stressed, e.g. by poor nutrition. In contrast, fresh *Agarum* and aged *Agarum* were not different in isopod preference or urchin gonad development. Aged *Agarum* was superior to fresh tissue in assimilation efficiency by isopods, and slightly better for copepod population growth and egg-hatching time. One mechanism of impact of polyphenolics on consumers is reduction of digestibility (Targett and Arnold, 2001), so that gradual loss of these chemicals with time in aging *Agarum* could contribute to increases in food value. Only two comparisons did not follow these patterns: *Nereocystis* caloric content was much higher in aged than in fresh tissue; this experiment had blades aging longer (1 month) than in most of our experiments (1–3 weeks), so that caloric values were taken of soft fragments that likely contained large populations of unknown microbial colonists. Aging *Agarum* to the same point of degradation (i.e., much longer than 1 month) might have resulted in a similar increase in caloric content. Aged (1 week) *Nereocystis* fragments were also assimilated slightly more efficiently by isopods.

The next critical steps in understanding the process of aging in algal detritus are investigating the fate and timing of detached kelp in the field, and characterizing the microbial communities on these degrading tissues. Yorke et al. (2013) recently found that kelp (*Macrocystis*) detritus broken mechanically into small particles does not create a significant food source for kelp forest suspension feeders. They suggest that larger pieces of kelp detritus may constitute a more important trophic pathway to benthic food webs. While the importance of particulate matter from kelp may vary with system, we agree that aging of large algal

detritus and processing by inefficient herbivores such as urchins may be the most likely pathways by which kelp carbon ultimately reaches deeper-water consumers.

The character and nutritional value of disintegrating kelp versus its attendant biofilm have significant implications for the food quality of detritus as it sinks from the photic zone into deeper waters. A recent review of the ecological role of marine biofilms (Wahl et al., 2012) concluded: "We do not really know what an epibiotic microorganism "does," e.g., which compounds it anabolizes, or which of the compounds transiting between host and environment it catabolizes or transforms to new compounds." There are many factors that contribute to food "value" for consumers. Early studies suggested that detritus has a relatively high concentration of nitrogen, which was inferred to be contributed by the fungi and bacteria that settle on it, but we did not find such a pattern consistently in measured C:N ratios in our relatively short-term experiments. Sosik and Simenstad (2013) suggest that the situation may be more complex, with microbes preferentially absorbing Nitrogen from kelp tissue, while Carbon is physically leached from degrading blades; they found kelps scraped of their microbial films to have significantly higher C:N than unscraped, aged blades. The broad changes in fatty acids we found in degrading kelps (with generally opposite changes in *Agarum* versus the other kelps) could translate into more subtle changes in food value than alteration of C:N ratios (see also Galloway et al., 2013; Raymond et al., in press). If detritus gains calories as it ages, as we found for *Nereocystis* but not *Agarum*, then the value of different types of detritus to deep trophic webs could be both species- and time-dependent. While *Agarum* is clearly slower to acquire a biofilm and degrade, our data suggest that "aged" *Agarum* is better than fresh tissue for consumers in a number of parameters. It is likely that as its secondary metabolites break down (after >4 weeks) and more microbes colonize, this tissue should become more nutritionally accessible. Because *Agarum* is one of the most common kelps in the San Juan Archipelago, gradual changes in its microbial and chemical characteristics could potentially release an important organic subsidy for organisms throughout the nearshore ecosystem.

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