Vol. 509: 213–225, 2014
doi: 10.3354/meps10888

MARINE ECOLOGY PROGRESS SERIES
Mar Ecol Prog Ser

Published August 27

Degradation state of algal diets affects fatty acid composition but not size of red urchin gonads

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ABSTRACT: Kelps in temperate marine ecosystems produce substantial detrital biomass that provides a carbon subsidy to consumers. After detachment, detrital kelp degrades and potentially changes in nutritional quality. Red sea urchins *Mesocentrotus franciscanus* are an abundant consumer of drift algae, but little is known about the effects of kelp degradation on feeding preferences or nutritional value to urchins. We compared the response of gonad index (GI), carbon and nitrogen stable isotope (SI) content, and fatty acid (FA) composition in the gonads of red urchins fed 2 species of fresh or degraded kelp for 17 wk. We found significant effects of kelp species but not kelp degradation state on urchin GI and SI, but also significant interactions between kelp species and degradation state. Urchins with greater gonad growth had $\delta^{13}C$ values more similar to those of their diet than did urchins with low gonad growth. Multivariate FA composition of the kelp diets (both in terms of species and degradation state) and the gonads of urchins fed those diets differed significantly. Several polyunsaturated essential FA (including SDA, EPA, and ARA) and FA summary classes were important for differentiating urchin gonads among treatments, suggesting kelp species-dependent effects of diet degradation. The total concentration of ω-3 and ω-6 FA declined with degradation state in both diets but did not differ in the gonads of urchins fed these diets. Thus, even if diets are depleted in certain FA, urchins are able to maintain concentrations of these FA in their gonads.

KEY WORDS: Fatty acids · Stable isotopes · Gonad index · Food availability · Red urchins · Drift algae

INTRODUCTION

Kelps (Order: Laminariales) are a major component of primary production in temperate nearshore ecosystems. These macroalgae often create large forests that are one of the most productive ecosystems on earth (Mann 1973, Krumhansl & Scheibling 2012a). Relatively little kelp biomass is subject to direct herbivory (Mann 1988); instead, a large amount of biomass detaches from the substrate and becomes drift algae. Through wave action, tidal currents, and storms, drift algae are transported to neighboring ecosystems in both shallow photic and deep aphotic habitats (Duggins et al. 1989, Britton-Simmons et al. 2012, Krumhansl & Scheibling 2012a), where they support substantial secondary production and diversity (Vetter & Dayton 1998). This carbon subsidy from shallow to deep regions constitutes an essential food source for primary consumers and affects many aspects of ecosystem dynamics (Britton-Simmons et al. 2009, Kelly et al. 2012, Lowe et al. 2014).

Some kelps, such as *Agarum fimbriatum*, have high levels of polyphenolic secondary metabolites including phlorotannins (Van Alstyne et al. 1999a), which may act as an herbivore deterrent (Duggins & Eckman 1997, Amsler et al. 1999) and reduce assimilation

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efficiency of certain grazers (Tugwell & Branch 1992, Boettcher and Targett 1993). Loss of phlorotannins as drift algae degrade (Duggins & Eckman 1997) could lead to increased palatability of degraded kelps. However, the rapid decrease of phlorotannins in particulate kelp matter found by Duggins & Eckman (1997) does not appear to extend to phlorotannin content of degrading whole kelp blades (Sosik & Simenstad 2013). Phlorotannin content may also control microbial colonization and subsequent changes to percent nitrogen (Sosik & Simenstad 2013). Detrital algae tend to have increased percent nitrogen (Mann 1988, Krumhansl & Scheibling 2012b) that can enhance the fitness of nitrogen-limited herbivorous urchins (Knip & Scheibling 2007). Drift algal degradation during the passive transport from the photic to the aphotic zone may provide consumers living at different depths with diets in different biochemical and nutritional states (Galloway et al. 2013), potentially influencing deep-water herbivore nutritional condition and population dynamics.

In the San Juan Islands Archipelago, USA, red urchins (*Mesocentrotus* [=*Strongylocentrotus*] *franciscanus*) are common from the shallow subtidal to depths well beyond the photosynthetic limit of kelps (>100 m; Britton-Simmons et al. 2012). Urchins in this system feed primarily on drift algae (Britton-Simmons et al. 2009) and can remain sedentary owing to the abundance of drift algae throughout their depth range (Britton-Simmons et al. 2012, Lowe et al. 2014). Urchins (*Mesocentrotus* spp. and *Strongylocentrotus* spp.) show feeding preferences for certain algal species, particularly for bull kelp *Nereocystis luetkeana* (Vadas 1977), but it is likely that these preferences can change with the level of degradation. As algae degrade, the overall biochemistry and palatability to consumers may change (Duggins & Eckman 1997, Britton-Simmons et al. 2009). These degradation-related changes in algae can therefore have considerable effects on the nutritional condition of urchins.

In slow growing invertebrates like urchins (Ebert & Russell 1993), gonad production is a useful metric of nutritional and reproductive condition (e.g. Vadas 1977, Minor & Scheibling 1997, Russell 1998, Meidel & Scheibling 1999, Vadas et al. 2000). Urchin gonad index (GI; the ratio of gonad mass to total mass) is a commonly used metric to evaluate how urchin nutritional condition responds to diet type (Vadas et al. 2000, McBride et al. 2004), food availability (Minor & Scheibling 1997), temperature (McBride et al. 1997), and habitat (Rogers-Bennett et al. 1995, Britton-Simmons et al. 2009, Dodge & Edwards 2012). GI is also closely tied to reproductive output (Fabbrocini & D'Adamo 2010). Red urchin gonads are harvested commercially in the northeast Pacific, worth roughly US$ 500 000 annually in the State of Washington alone[1], making the linkage of dietary resources to urchin productivity broadly important to resource managers and fishers.

Analysis of stable isotope (SI) ratios and fatty acid (FA) signatures provides a valuable indirect approach for tracing food web relationships in aquatic habitats. Analysis of SI ratios of resources and consumers has long been the primary biochemical approach for estimating the importance of different basal resources to aquatic consumers (Peterson & Fry 1987). The relative carbon SI ratio ($\delta^{13}C$) is used as a tracer of different primary producers (e.g. kelp, seagrasses, phytoplankton) due to differences in fractionation by these producers during carbon fixation (Peterson 1999). The relative nitrogen SI ratio ($\delta^{15}N$) can be used to identify trophic level, due to expected enrichment with each additional trophic level (Cabana & Rasmussen 1996, Peterson 1999) and may be a useful indicator of bacterial colonization of drift algae during degradation (Sosik & Simenstad 2013). FA are particularly practical biomarkers for the study of benthic ecosystems because the sources of primary production have distinct FA signatures (Galloway et al. 2012) that can be traced to certain consumers (Kelly & Scheibling 2012). For example, both FA signatures (e.g. proportional composition of >30 FA) and just 'essential' FA profiles (e.g. long-chain [$\geq C_{18}$] ω-3 and ω-6 FA) differentiate macrophytes (macroalgae and seagrasses) at phylum, ordinal, and family levels (Galloway et al. 2012), and these patterns may be clearly transferred to macroalgal consumers (Galloway et al. 2014).

While SI and FA biomarkers are common ecological tools, especially in observational analyses, they are rarely used together in the same experimental study. Together these tools can provide useful information regarding the nutritional quality of kelps available to wild urchins, and determine potential effects of degrading kelp tissue on urchins. Urchin FA have been used to generate hypotheses about wild urchin diets (Hughes et al. 2005, 2006, Barberá et al. 2011), response to differing experimental algal diets (Cook et al. 2000, Kelly et al. 2008), and are linked to gamete production (Carboni et al. 2012). However, previous research has investigated only fresh, non-degraded algal biomass, despite the fact that urchins in the wild are likely eating algal diets in varying states of decay.

[1]M. Morningstar, pers. comm. Data extracts from license and fish ticket database (LiFT) in October, 2013

In order to assess potential effects of fresh and detrital kelp food sources on benthic consumers, we carried out a feeding experiment with the abundant red sea urchin. We measured GI, $\delta^{13}C$ and $\delta^{15}N$, and FA in urchins fed 2 kelp taxa, one a chemically defended, less preferred species (*Agarum fimbriatum*, hereafter *Agarum*) and one minimally defended, highly preferred species (*Nereocystis luetkeana*, hereafter *Nereocystis*), each at 2 levels (fresh and degraded) for 17 wk. These kelp species are highly abundant in our study system and are common drift algae in both shallow and deep water (Vadas 1977). On average these species also exhibit very different morphology and phlorotannin content, both of which affect their degradation process and the feeding preference by urchins. We hypothesized that degradation would have a greater effect on urchin preference and consumption for *Agarum* than *Nereocystis*, and therefore increase the nutritional value of this kelp species. We also compared results from the feeding experiment with periodic field sampling of wild urchins as a temporal experimental reference.

MATERIALS AND METHODS

Experimental design

Feeding trials took place at University of Washington's Friday Harbor Laboratories (FHL). To assess the effect of kelp degradation we fed urchins 4 different kelp diets: fresh or degraded *Agarum*, or fresh or degraded *Nereocystis* (hereafter referred to as Fresh-*Agarum*, Degr-*Agarum*, Fresh-*Nereocystis*, and Degr-*Nereocystis* treatments). Experimental urchins were collected on 4 August 2012 in 5 to 12 m depth near Jones Island, Washington (48° 36′ 38″ N, 123° 2′ 51″ W), using SCUBA. Urchin test diameter ranged from 75.0 to 133.5 mm with an average of 98.4 mm. Urchins were transported to FHL and randomly assigned to treatments. The experimental array consisted of 16 plastic cages measuring 69 cm long × 47 cm wide × 30 cm high with a solid bottom, a lattice of 3 cm holes on all vertical sides, and 1.5 cm Vexar mesh on top. These cages were suspended at a depth of 6 m from the FHL breakwater. The cages were randomly assigned to 1 of the 4 diet treatments, and 6 urchins were placed in each cage. We collected 12 additional urchins from the same location on 6 August 2012 (hereafter Wild-Start) and 4 December 2012 (hereafter Wild-End) to use as a reference group. These reference, or 'wild diet' urchins were immediately sacrificed, GI was determined, and gonad material for FA and SI samples was taken.

Diets

The 2 species of kelp were collected weekly: *Nereocystis luetkeana* at the surface from San Juan Channel (48° 32′ 58″ N, 122° 59′ 28″ W) and *Agarum fimbriatum* between 5 and 12 m depth near Brown Island, Washington (48° 32′ 14″ N, 122° 59′ 56″ W). We avoided reproductive sori and any obviously degraded regions of kelp blades to control for potential within-thallus biomarker variability. Since we collected kelps from August to December, their growth rate was expected to change. Therefore, tissue was collected from the region proximal to the meristem to avoid older, degraded or epiphyte-fouled tissue and to standardize the diets within kelp species over the duration of the feeding trial. Algae were stored in flow-through seawater tanks for approximately 1 h before being assigned to either fresh diet or degraded diet treatments. To replicate the degradation conditions experienced by kelp blades being transported to the deep subtidal, we placed kelp blades in blacked out, flow-through seawater tanks. *Nereocystis* was degraded for 1 wk and *Agarum* for 3 wk. This differential time of degradation for the 2 species was prompted by previous experience in degrading kelps (Sosik & Simenstad 2013). *Nereocystis* dissolves if degraded this way for >1 wk (W. Raymond pers. obs.), rendering it unusable as a food source for urchins. The thallus of *Agarum* degraded much more slowly and remained intact during the 3 wk degradation period.

Feeding

The feeding trial was conducted for 17 wk, from August to December 2012. Urchins were fed ad libitum with 160 g of *Nereocystis* (fresh or degraded) or 100 g of *Agarum* (fresh or degraded) per urchin per week based on previous observations of kelp consumption rates (Vadas 1977, McBride et al. 1997). During feedings, cages were cleaned of fouling organisms to prevent urchins from eating food sources other than the assigned diet. Uneaten kelp was collected from the cages at the end of each week and weighed before being discarded. We then determined the mass of the consumed kelp to calculate feeding rate for the urchins in each treatment group.

At the end of the 17 wk feeding trial, all urchins were dissected and measured for total wet mass and wet gonad mass (blotted-damp weight). GI (n = 12 per treatment) was calculated as the ratio of gonad mass to total mass and reported here as a percentage

([wet gonad mass/wet total mass] × 100; Vadas 1977, Rogers-Bennett et al. 1995, McBride et al. 1997, Minor & Scheibling 1997). Data were averaged for each of the 4 diet treatments and 2 wild reference groups. Approximately 5 g (wet weight) of gonad material from a random subset of urchins from each treatment (n = 5, one per replicate cage plus one chosen at random from replicates for each treatment) was taken and frozen (–20°C) for SI and FA analysis. In addition, samples (~5 g wet weight, n = 5 per treatment) for SI and FA were taken from the 4 kelp diets throughout the feeding trial at 1 to 3 wk intervals and frozen for analysis to assess potential changes in diet FA and SI over the 17 wk.

Stable isotopes and fatty acids

Each gonad and kelp tissue sample was analysed for both SI and FA content. All samples were lyophilized for 24 h and then ground using a stainless steel mortar and pestle. Ground gonad and kelp tissue was weighed using a microbalance, placed in a tin capsule and sent to Washington State University's Stable Isotope Core lab for analysis of $\delta^{13}C$ and $\delta^{15}N$ (for detailed methods see e.g. Dethier et al. 2013, Galloway et al. 2013). Isotope ratios are reported in per mille as the ratio of heavy to light isotope relative to Vienna Peedee Belemnite standard (δ‰). We did not extract lipids from our samples before SI analysis, because the paired FA profiles and SI values of gonad lipids were of primary interest for assessing the effects of diet treatments on gonad biomarkers and urchin GI. Fatty acid methyl esters (FAME) were extracted at FHL from 10 mg of tissue using a modified Folch method (see e.g. Taipale et al. 2011, Galloway et al. 2013). To analyze FAME, we used gas chromatography-flame ionisation detection (GC-FID; HP 6958, Agilent DB-23 column), with an 85 min 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. To quantify summary FA category sample weights (mg FAME [g dry tissue]$^{-1}$), we ran dilutions of known concentrations of FAME from the Nu-chek standard (2, 1, 0.5, 0.25, 0.1, 0.05, and 0.0025 mg ml^{-1}) through the GC-FID and correlated the known concentrations with areas from the chromatogram for all FA in the standard. After accounting for hexane volume and dry weight of the sample, an estimate of the proportion of FAME per unit mass of dry tissue was calculated (Taipale et al. 2011).

Statistical analyses

The effect of kelp diet species and degradation state on urchin GI was evaluated using 2-way ANOVA on untransformed data ($\alpha = 0.05$). Since the wild reference groups' diet was unknown, we performed an all pairwise comparisons procedure (*t*-test with a Bonferroni correction) to compare the GI of the 4 diet treatments plus the 2 wild reference groups to each other. The effects of kelp diet species and degradation state on $\delta^{13}C$ and $\delta^{15}N$ in kelp diets and in urchin gonads were evaluated using 2-way ANOVA on untransformed data ($\alpha = 0.05$). All statistical analyses related to GI and SI were performed in the R statistical environment (R Development Core Team 2013).

Proportional FA (percentage of total FAME) data were arcsine square-root transformed (e.g. Galloway et al. 2012, Dethier et al. 2013), and treatment effects of kelp species and kelp degradation state were analyzed with 2-way permutational multivariate analysis of variance (PERMANOVA; Anderson et al. 2008) using Euclidean distance on kelp diets and urchin gonads. All PERMANOVAs used 9999 permutations with Type III sums of squares and fixed factors. Post hoc pairwise comparisons with Monte Carlo correction (Anderson et al. 2008) were also performed to evaluate the differences between individual treatments for both kelp diets and urchin gonads. We performed similarity percentage analyses (SIMPER; Clarke & Gorley 2006) to identify those FA most important for differentiating urchin gonad FA among treatments. Because the SIMPER analysis is used for interpreting differences in the mean FA values of each treatment, SIMPER tests were run on untransformed FA data (e.g. Galloway et al. 2013). We used non-metric multidimensional scaling (NMDS) for visualization of multivariate patterns in the urchin gonad FA profiles (Vegan package in R), using the 'ordiplot' and 'ordihul' functions, and we calculated FA loadings on each NMDS axis with the 'envfit' function. We show all FA that were significantly correlated with the 2 NMDS axes (Monte-Carlo permutation test, $p < 0.01$), and color-coded the vectors corresponding to any of the top 5 FA identified as important for driving group separation in the SIMPER. SIMPER and PERMANOVA were performed using PRIMER v.6.0 with PERMANOVA+ add on (Clarke & Gorley 2006, Anderson et al. 2008). The effect of kelp diet species and degradation state on both kelp diets and urchin gonads was also assessed using univariate ANOVA of arcsine square-root transformed ω-3 and ω-6 FA proportions (percentage of total FAME) and raw concentrations (sum mg FAME g^{-1}).

RESULTS

Feeding

Urchins fed Degr-*Nereocystis* had the highest estimated consumption rate, eating an average 22.0 g urchin^{-1} d^{-1} versus an average of 11.7 g urchin^{-1} d^{-1} of Fresh-*Nereocystis*. Urchins consumed an average of 2.4 and 6.6 g urchin^{-1} d^{-1} of Fresh- and Degr-*Agarum*, respectively. Analysis of gut-content weight per urchin upon dissection showed consistent amounts of ingested material among urchins within each treatment.

Gonad index

GI increased throughout the trial period in Wild and *Nereocystis*-fed urchins, but not in *Agarum*-fed urchins (Fig. 1). After 17 wk, there was a significant effect of kelp diet species on GI (2-way ANOVA, $F_{1,44}$ = 243.21, p < 0.001), where *Nereocystis*-fed urchins had much larger GI than *Agarum* treatments. Kelp diet degradation state did not affect GI ($F_{1,44}$ = 3.36, p = 0.074). However, there was a significant interaction between kelp species and kelp degradation state ($F_{1,44}$ = 4.59, p = 0.038), indicating that the effects of degradation differ between kelp diets. Separate pairwise comparisons among feeding trial urchins and wild reference urchins found that GI of Fresh- and Degr-*Nereocystis* treatments were higher than all other groups (p < 0.001). GI in Fresh- and Degr-*Agarum* treatments and Wild-Start urchins were not different (all p > 0.8250) but were significantly lower than in *Nereocystis* treatments and Wild-End urchins (all p < 0.001). GI of Wild-End urchins were significantly different from all other treatments (all p < 0.0160; Fig. 1).

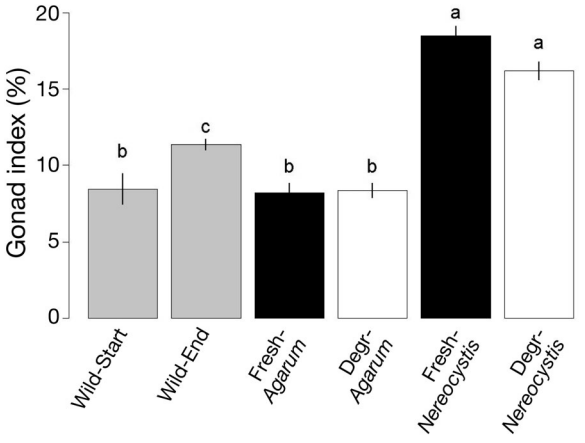

Fig. 1. *Mesocentrotus franciscanus*. Urchin gonad index expressed as a percentage for wild reference urchins and urchins fed either fresh or degraded kelp for 17 wk. Shared letters represent statistical similarity (p > 0.05) from all pairwise comparisons procedure. Error bars: ± SEM

Stable isotopes

Kelp $\delta^{13}C$ and $\delta^{15}N$ ratios were highly variable, with considerable change observed in *Nereocystis* over our sampling time (Fig. 2). Average kelp $\delta^{13}C$ values were not significantly different for either kelp species or degradation state, while kelp diet $\delta^{15}N$ values differed among kelp species but not degradation state (Table 1, Fig. 3a,c). There was a significant effect of kelp diet species on

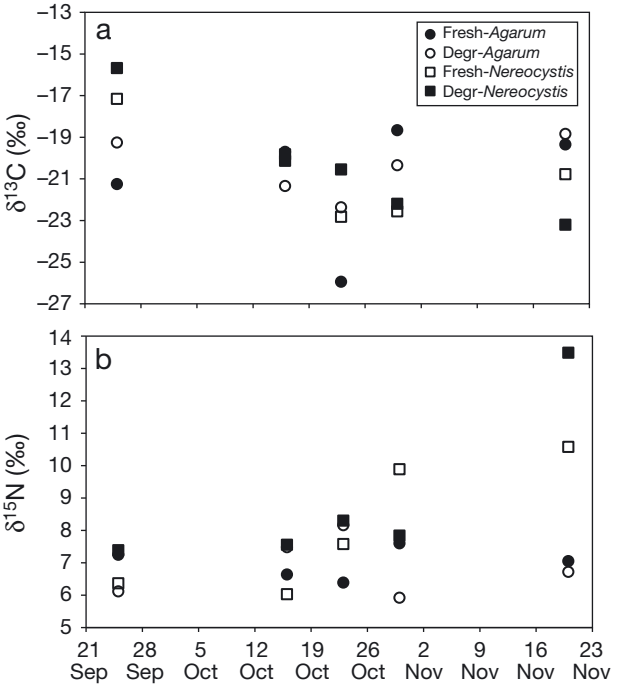

Fig. 2. *Agarum fimbriatum* and *Nereocystis luetkeana*. Stable isotope values of (a) $\delta^{13}C$ and (b) $\delta^{15}N$ in kelp diets through time. Each point represents a single tissue sample

Table 1. ANOVA results of the effect of kelp diet species and degradation state (Degr) on $\delta^{13}C$ and $\delta^{15}N$ in kelp diets and urchin gonads

Factor	df	$\delta^{13}C$				$\delta^{15}N$			
		Kelp diets		Urchin gonad		Kelp diets		Urchin gonad	
		F	p	*F*	p	*F*	p	*F*	p
Species	1,16	0.04	0.847	4.92	0.042	4.64	0.047	41.2567	<0.001
Degr	1,16	0.11	0.748	0.70	0.414	1.15	0.299	2.6074	0.126
Species × Degr	1,16	0.03	0.867	0.01	0.925	1.55	0.232	3.5181	0.079

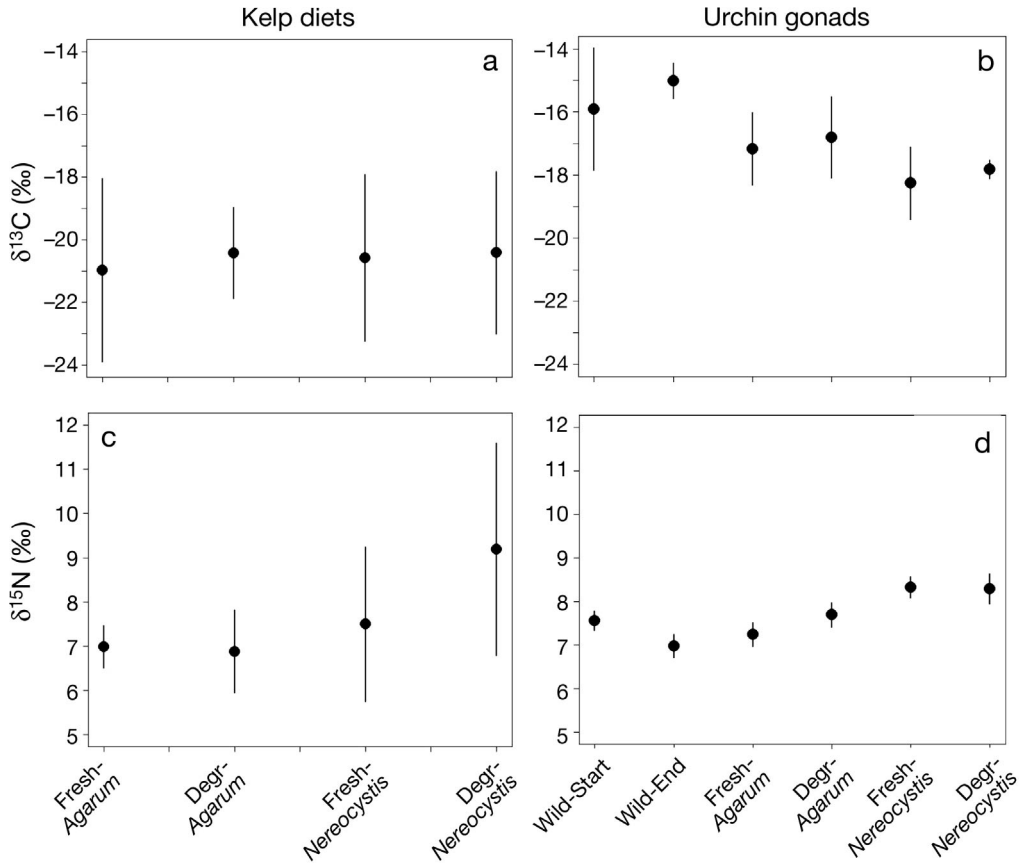

Fig. 3. *Agarum fimbriatum*, *Nereocystis luetkeana* and *Mesocentrotus franciscanus*. (a,b) $\delta^{13}C$ and (c,d) $\delta^{15}N$ stable isotope values for (a,c) 4 different kelp diets and (b,d) urchin gonads from the different experimental groups (wild reference urchins or urchins fed either fresh or degraded kelp for 17 wk). Data: means ± SD

urchin gonad $\delta^{13}C$ and $\delta^{15}N$, but no effect of kelp diet degradation state (Table 1, Fig. 3b,d).

Fatty acids

We identified 25 FA in the kelp diets and 40 FA in urchin gonads (Table 2). Multivariate FA signatures differed for kelp diet species (PERMANOVA, Pseudo-$F_{1,17}$ = 56.02, p < 0.001) and degradation state (Pseudo-$F_{1,17}$ = 12.09, p < 0.001), and there was a significant interaction of these 2 factors (Pseudo-$F_{14,17}$ = 12.51, p < 0.001). Similarly, multivariate FA signatures of urchin gonads also showed significant effects of kelp diet species (Pseudo-$F_{1,18}$ = 15.62, p < 0.001) and degradation state (Pseudo-$F_{1,18}$ = 11.61, p < 0.001), and there was an interaction between the 2 factors (Pseudo-$F_{15,18}$ = 12.54, p < 0.001). Post hoc multivariate pairwise comparisons showed that all urchin feeding trial treatments and wild reference groups were significantly different from one another in terms of multivariate FA signature (Degr-*Agarum* vs. Wild-End: p = 0.01; Wild-Start vs. Wild-End: p = 0.037, all other comparisons (n = 13): p < 0.01). The multivariate FA (n = 40 FA) ordination plot (Fig. 4a) shows all urchin gonad FA profiles (including the wild urchins for reference) and the FA vectors that are highly correlated with the 2 NMDS axes (p < 0.01, n = 21 FA; Fig. 4b).

The proportion of ω-3 FA in kelp diets differed between species, but not by kelp degradation state (Table 3). Fresh and degraded *Agarum* diets had a lower proportion of ω-3 FA, but similar concentrations (ω-3 mg [g dry tissue FAME]$^{-1}$; Tables 2 & 3, Fig. 5a,c) to those of *Nereocystis* diets. In contrast, the proportion of ω-6 FA in kelp diets did not differ between species, but did decrease significantly with degradation state (Table 3, Fig. 5a). Degraded kelp diets had significantly lower concentrations of ω-3 and ω-6 FA than their fresh counterparts (Table 3, Fig. 5c). Urchin gonads had similar absolute ω-3 concentrations regardless of diet (Tables 2 & 3). There were significant effects of species but not of degradation state on ω-3 FA in urchin gonads (Table 3). Urchins fed *Nereocystis* had significantly higher proportions of ω-6 FA than urchins fed *Agarum* (Table 3,

Table 2. Mean (±SD) fatty acid proportions in kelp diets as well as in the gonads of wild urchins and urchins fed fresh or degraded kelp diets for 17 wk. Σmg (g FAME)$^{-1}$ are mean mass ± SD

Fatty acid	Kelp diets				Wild urchins		Feeding trial urchins			
	Fresh-*Agarum*	Degr-*Agarum*	Fresh-*Nereocystis*	Degr-*Nereocystis*	Wild-Start	Wild-End	Fresh-*Agarum*	Degr-*Agarum*	Fresh-*Nereocystis*	Degr-*Nereocystis*
N	4	5	5	5	5	5	5	5	4	5
c14:0	4.3 ± 0.9	5.3 ± 1.3	9.2 ± 1.0	10.0 ± 1.6	16.5 ± 1.8	18.4 ± 1.4	15.7 ± 2.9	14.8 ± 1.6	14.6 ± 1.0	14.9 ± 2.1
14:1	0.1 ± 0.1	0.3 ± 0.2	0.1 ± 0.1	0.2 ± 0.3	1.8 ± 1.1	2.5 ± 0.5	1.5 ± 0.6	1.4 ± 0.8	1.1 ± 0.2	1.0 ± 0.4
c15:0	0.2 ± 0.1	0.4 ± 0.2	0.2 ± 0.1	0.1 ± 0.1	0.4 ± 0.0	0.3 ± 0.1	0.4 ± 0.1	0.4 ± 0.1	0.2 ± 0.1	0.2 ± 0.0
i-16:0	–	–	–	–	0.5 ± 0.1	0.5 ± 0.1	0.5 ± 0.1	0.5 ± 0.0	0.5 ± 0.1	0.6 ± 0.0
c16:0	21.1 ± 4.1	20.6 ± 4.8	19.2 ± 1.4	25.5 ± 2.7	17.3 ± 0.7	15.9 ± 1.9	17.4 ± 2.1	17.0 ± 1.8	17.5 ± 0.9	18.0 ± 1.0
16:1ω7	11.0 ± 4.3	13.1 ± 2.3	0.8 ± 0.2	1.5 ± 1.0	5.0 ± 1.7	5.0 ± 0.3	4.8 ± 0.9	4.2 ± 0.8	4.0 ± 0.5	3.9 ± 1.0
16:1ω5	0.3 ± 0.3	0.6 ± 0.2	1.1 ± 0.2	1.3 ± 0.2	6.5 ± 2.2	7.4 ± 1.2	6.1 ± 0.8	5.4 ± 1.6	4.7 ± 0.7	4.9 ± 1.2
16:2ω4	6.4 ± 3.6	5.6 ± 1.5	0.3 ± 0.3	–	0.4 ± 0.1	0.2 ± 0.2	0.8 ± 0.2	0.6 ± 0.2	0.2 ± 0.1	0.1 ± 0.1
16:2ω7	0.2 ± 0.1	–	–	–	0.1 ± 0.0	0.2 ± 0.0	0.1 ± 0.1	0.2 ± 0.1	0.1 ± 0.1	–
c17:0	–	–	–	–	0.1 ± 0.0	0.1 ± 0.0	0.1 ± 0.1	0.2 ± 0.1	0.1 ± 0.0	0.1 ± 0.0
16:3ω4/5	3.0 ± 1.0	5.1 ± 1.5	–	–	0.5 ± 0.3	0.4 ± 0.2	0.8 ± 0.3	0.6 ± 0.2	0.1 ± 0.1	0.2 ± 0.1
16:4ω3	–	–	–	–	0.5 ± 0.3	0.5 ± 0.3	0.2 ± 0.2	0.6 ± 0.4	0.1 ± 0.2	0.2 ± 0.2
16:4ω1	0.4 ± 0.4	1.2 ± 0.8	–	–	0.3 ± 0.1	0.3 ± 0.1	0.3 ± 0.1	0.3 ± 0.1	0.1 ± 0.1	0.0 ± 0.0
c18:0	2.4 ± 1.3	2.1 ± 0.5	1.5 ± 0.4	2 .0 ± 0.6	1.8 ± 0.3	1.6 ± 0.1	1.8 ± 0.2	2.2 ± 0.6	1.9 ± 0.3	1.8 ± 0.3
18:1ω9	7.4 ± 5.5	3.9 ± 2.5	16.6 ± 1.9	18.8 ± 0.9	3.3 ± 0.4	2.9 ± 0.2	2.9 ± 0.5	2.5 ± 0.3	4.5 ± 1.0	3.5 ± 0.3
18:1ω7	0.6 ± 0.1	1.4 ± 0.5	–	–	4.0 ± 0.6	3.8 ± 0.5	4.4 ± 0.4	4.0 ± 0.4	3.4 ± 0.4	3.4 ± 0.6
18:1ω5	–	–	–	–	0.1 ± 0.3	0.6 ± 0.1	0.6 ± 0.1	0.5 ± 0.1	0.3 ± 0.2	0.5 ± 0.1
18:2ω6 (LIN)	10.8 ± 2.9	8.3 ± 1.8	7.1 ± 0.2	6.2 ± 0.3	0.8 ± 0.2	0.7 ± 0.1	0.8 ± 0.2	0.7 ± 0.1	1.2 ± 0.7	1.1 ± 0.1
18:3ω6	1.2 ± 0.4	1.7 ± 0.4	1.0 ± 0.3	0.2 ± 0.2	0.2 ± 0.0	0.3 ± 0.2	0.2 ± 0.1	0.3 ± 0.1	0.5 ± 0.5	0.2 ± 0.1
18:3ω4	0.7 ± 0.4	1.2 ± 0.2	–	–	–	0.1 ± 0.2	0.6 ± 0.5	–	–	0.1 ± 0.1
18:3ω3 (ALA)	1.3 ± 0.9	–	5.8 ± 1.3	4.9 ± 0.6	1.8 ± 0.4	1.6 ± 0.2	1.0 ± 0.3	1.5 ± 0.4	1.6 ± 0.2	1.7 ± 0.3
18:4ω3 (SDA)	0.8 ± 0.4	0.9 ± 0.4	10.3 ± 3.3	6.0 ± 1.4	2.5 ± 1.5	3.2 ± 0.5	2.1 ± 0.4	2.7 ± 1.0	4.1 ± 0.5	3.9 ± 0.8
18:4ω1	–	–	–	–	0.1 ± 0.1	0.1 ± 0.1	0.2 ± 0.0	0.2 ± 0.0	0.0 ± 0.1	0.1 ± 0.1
20:1ω11	0.7 ± 0.5	0.7 ± 0.4	–	–	4.8 ± 0.6	4.2 ± 0.5	4.9 ± 0.9	5.2 ± 0.7	4.1 ± 0.5	4.7 ± 0.7
20:1ω9	–	–	–	–	1.2 ± 0.1	1.0 ± 0.1	1.3 ± 0.3	1.3 ± 0.1	1.0 ± 0.1	1.2 ± 0.1
20:1ω7	1.5 ± 0.9	1.7 ± 0.4	–	–	4.7 ± 0.9	4.2 ± 0.5	5.6 ± 2.0	5.1 ± 1.5	5.2 ± 0.7	5.1 ± 0.6
20:2ω6	0.4 ± 0.2	–	–	–	1.2 ± 0.3	1.0 ± 0.2	1.4 ± 0.8	1.9 ± 0.4	1.3 ± 0.3	1.9 ± 0.3
20:3ω6	1.0 ± 0.4	0.3 ± 0.3	0.7 ± 0.4	0.2 ± 0.3	0.8 ± 0.1	1.0 ± 0.0	0.3 ± 0.0	0.9 ± 0.2	0.9 ± 0.1	0.3 ± 0.3
20:4ω6 (ARA)	14.3 ± 4.1	14.1 ± 3.8	18.3 ± 0.8	15.7 ± 1.9	5.7 ± 1.0	5.3 ± 0.5	5.6 ± 1.3	5.2 ± 0.6	9.1 ± 1.6	8.5 ± 1.6
20:3ω3	–	–	–	–	1.6 ± 0.5	1.5 ± 0.3	1.3 ± 0.2	1.5 ± 0.5	1.5 ± 0.2	1.1 ± 0.5
20:4ω3	0.1 ± 0.1	0.0 ± 0.0	0.4 ± 0.1	0.6 ± 0.4	2.0 ± 0.6	2.1 ± 0.3	1.5 ± 0.3	1.9 ± 0.5	2.2 ± 0.2	2.5 ± 0.4
20:5ω3 (EPA)	8.9 ± 1.3	10.8 ± 2.0	7.5 ± 1.1	6.7 ± 1.7	8.6 ± 2.2	9.3 ± 0.6	8.4 ± 1.2	10.1 ± 2.5	9.8 ± 0.9	9.6 ± 1.5
c22:0	–	–	–	–	2.2 ± 0.4	1.7 ± 0.2	2.1 ± 0.3	1.6 ± 0.9	2.1 ± 0.5	2.3 ± 0.6
22:1ω11	–	–	–	–	0.2 ± 0.0	0.2 ± 0.0	–	0.3 ± 0.1	0.2 ± 0.0	0.1 ± 0.0
22:1ω9	–	–	–	–	0.9 ± 0.1	0.9 ± 0.1	–	0.9 ± 0.1	0.6 ± 0.2	0.6 ± 0.2
22:3ω6	–	–	–	–	0.2 ± 0.1	0.2 ± 0.0	–	0.2 ± 0.1	0.3 ± 0.0	–
22:5ω3	–	–	–	–	–	0.1 ± 0.1	0.1 ± 0.1	0.1 ± 0.1	–	0.1 ± 0.1
22:6ω3 (DHA)	–	–	–	–	0.2 ± 0.1	0.3 ± 0.1	0.4 ± 0.1	0.4 ± 0.1	0.3 ± 0.1	0.4 ± 0.1
c24:0	–	–	–	–	0.3 ± 0.1	0.3 ± 0.1	0.4 ± 0.2	0.5 ± 0.1	0.3 ± 0.0	0.2 ± 0.1
24:1ω9	–	–	–	–	0.9 ± 0.4	0.3 ± 0.1	1.8 ± 1.7	1.9 ± 1.2	0.5 ± 0.4	0.5 ± 0.3
Σ proportions										
SAFA	29.0 ± 5.1	29.0 ± 5.0	30.1 ± 2.7	37.7 ± 3.8	39.1 ± 1.5	38.8 ± 1.8	38.5 ± 3.7	37.2 ± 1.5	37.2 ± 1.7	38.2 ± 2.3
MUFA	21.5 ± 2.0	21.4 ± 1.8	18.5 ± 2.0	21.6 ± 1.1	31.4 ± 4.1	30.1 ± 1.0	32.5 ± 2.1	31.1 ± 3.6	28.3 ± 1.0	28.4 ± 2.8
PUFA	49.2 ± 5.1	49.3 ± 6.5	51.3 ± 4.6	40.4 ± 2.8	27.1 ± 6.6	27.8 ± 1.4	27.1 ± 4.3	29.5 ± 4.9	32.9 ± 2.8	32.2 ± 5.3
ω-3	11.1 ± 1.8	11.7 ± 2.2	23.9 ± 5.7	18.1 ± 2.7	17.2 ± 4.8	18.6 ± 1.7	15.2 ± 1.8	19.1 ± 5.0	19.6 ± 1.4	19.7 ± 3.8
ω-6	27.5 ± 1.7	24.4 ± 5.6	27.2 ± 1.3	22.3 ± 1.7	8.7 ± 1.6	8.3 ± 0.6	9.4 ± 2.1	8.9 ± 1.2	12.9 ± 1.7	12.0 ± 1.8
Σ n-3 PUFA	11.1 ± 3.5	11.7 ± 4.4	23.8 ± 4.5	18.1 ± 3.2	15.2 ± 3.2	16.6 ± 3.4	13.6 ± 3.1	16.8 ± 3.7	18.0 ± 3.6	18.2 ± 3.5
EPA/DHA	–	–	–	–	45.35	29.01	27.79	25.84	34.37	26.50
EPA/ARA	0.63	0.76	0.41	0.42	1.51	1.75	1.50	1.94	1.08	1.13
Σmg (g FAME)$^{-1}$										
Total FAME	25.4 ± 14.4	10.2 ± 2.9	15.0 ± 2.9	8.7 ± 3.5	180.0 ± 35.9	196.4 ± 38.8	182.3 ± 39.2	224.2 ± 94.7	178.5 ± 33.5	177.3 ± 40.5
SAFA	7.9 ± 5.1	2.9 ± 0.2	4.6 ± 0.8	3.3 ± 1.1	73.8 ± 4.3	80.9 ± 13.8	73.3 ± 8.9	88.0 ± 39.6	69.4 ± 12.5	70.3 ± 14.1
MUFA	5.3 ± 2.5	2.1 ± 0.3	2.5 ± 0.5	1.7 ± 0.7	55.9 ± 7.4	58.9 ± 7.6	58.6 ± 7.9	68.8 ± 29.4	49.4 ± 8.2	49.3 ± 11.1
PUFA	12.2 ± 4.4	5.2 ± 1.5	7.8 ± 1.0	3.7 ± 1.5	50.3 ± 13.2	56.6 ± 9.5	50.4 ± 7.4	67.3 ± 27.9	59.7 ± 5.6	57.7 ± 9.9
ω-3	2.8 ± 1.4	1.2 ± 0.3	3.6 ± 0.8	1.7 ± 0.8	31.6 ± 9.4	37.3 ± 6.4	28.0 ± 4.0	42.1 ± 15.8	35.0 ± 4.1	34.9 ± 7.0
ω-6	7.0 ± 2.9	2.6 ± 0.9	4.2 ± 0.6	2.0 ± 0.7	16.5 ± 3.2	17.2 ± 3.2	17.7 ± 3.6	21.3 ± 10.8	23.8 ± 1.5	22.0 ± 2.9

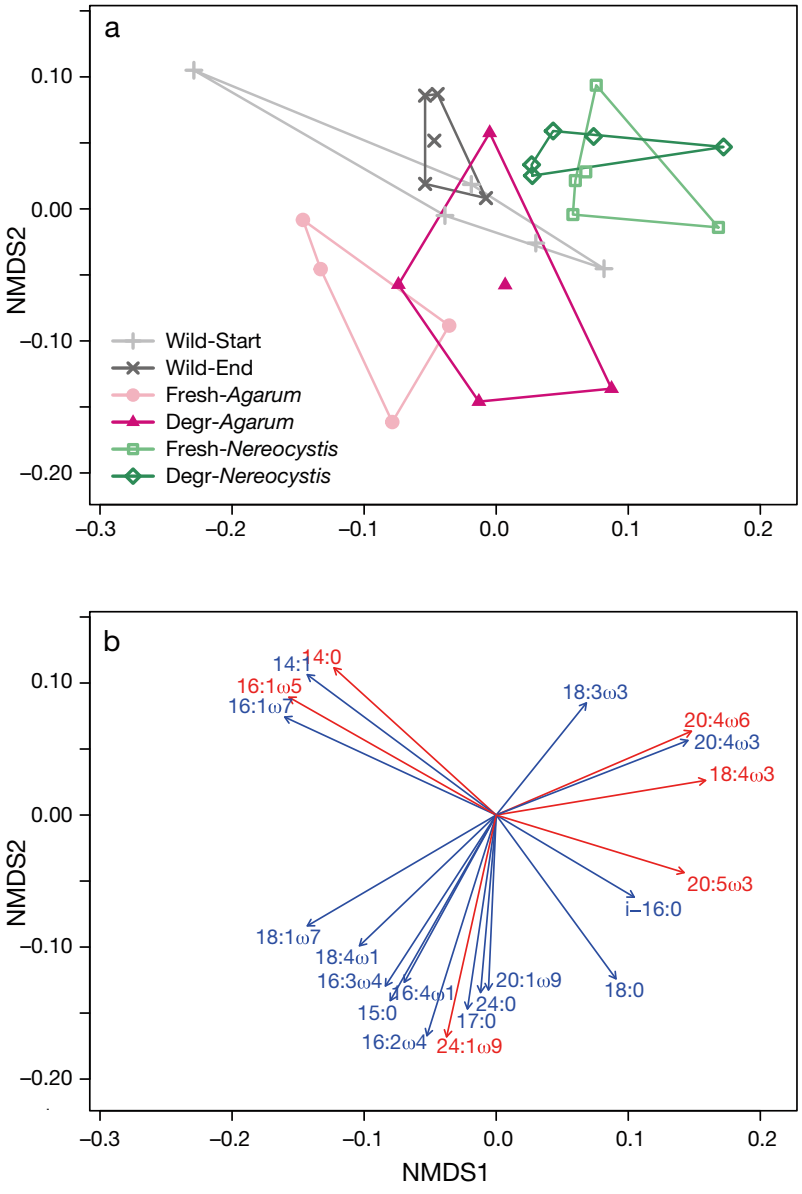

Fig. 4. *Mesocentrotus franciscanus*. (a) Nonmetric multidimensional scaling (NMDS) plot (stress = 16.6) of urchin gonad FA from the 4 feeding treatments and 2 wild reference groups. (b) FA that are significantly correlated with the NMDS axes (Monte-Carlo permutation test, $p < 0.01$; see 'Materials and methods: Statistical analyses'). Red vectors: 6 FA that were significantly correlated to NMDS axes and also identified as being in the top 5 FA for differentiating all groups in the SIMPER analysis (see Table 4)

Fig. 5b). Neither kelp diet species nor degradation state affected total ω-3 or ω-6 FA concentration in urchin gonads (Table 3, Fig. 5d).

Nereocystis diets were enriched in the relative proportions of the essential PUFA 20:4ω6 (ARA) compared to *Agarum* diets (Table 2). In addition, ARA and the PUFA 18:4ω3 (SDA) both declined with degradation state in *Nereocystis* diets but did not change in *Agarum* diets (Table 2). Degraded *Nereocystis* kelp diets had lower proportions of ARA and 20:5ω3 (EPA) than fresh ones (Table 2); the SIMPER analysis indicated that these 2 FA accounted for ~28% of the difference between the urchin gonads in the fresh and degraded *Nereocystis* diet treatments (Table 4). SIMPER results showed that ARA was the most important FA driving differences in FA content of gonads of urchins fed *Nereocystis* or *Agarum*, accounting for ~25% of the differences for both the fresh and

Table 3. ANOVA results on the effect of kelp diet species (*Agarum fimbriatum* vs. *Nereocystis luetkeana*) and degradation state (Degr; fresh vs. degraded) on the proportion and concentration of ω-3 and ω-6 fatty acids in both kelp diets and urchin gonads

Factor	df	Kelp diets				Urchin gonads				
		ω-3		ω-6			ω-3		ω-6	
		F	p	*F*	p	df	*F*	p	*F*	p
FA proportion										
Species	1,14	39.68	<0.001	0.581	0.459	1,15	4.64	0.047	41.26	<0.001
Degr	1,14	2.32	0.150	6.295	0.025	1,15	1.15	0.299	2.61	0.126
Species × Degr	1,14	3.81	0.071	0.232	0.638	1,15	1.55	0.232	3.52	0.079
FA concentration										
Species	1,14	2.04	0.175	4.76	0.047	1,15	0.04	0.838	1.27	0.278
Degr	1,14	17.48	0.001	20.85	<0.001	1,15	2.34	0.147	0.06	0.803
Species × Degr	1,14	0.12	0.729	2.33	0.149	1,15	2.74	0.118	0.95	0.346

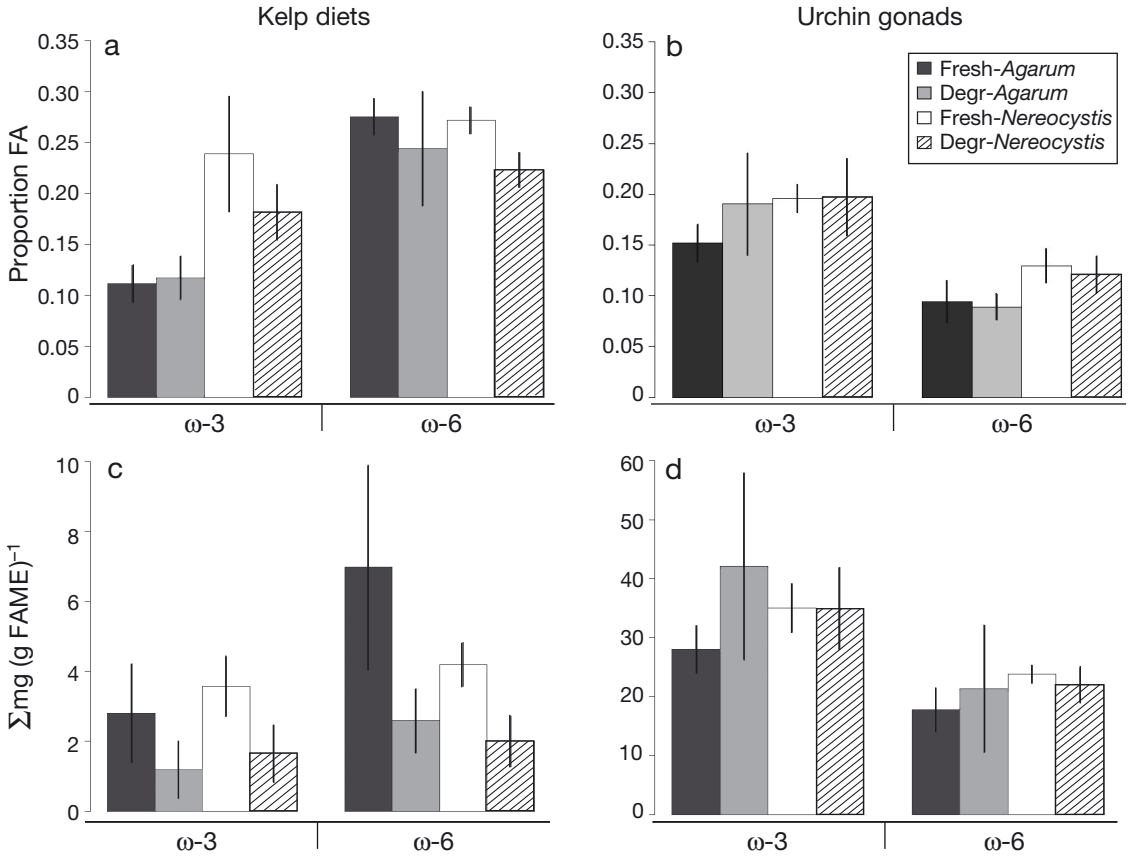

Fig. 5. Proportions of total ω-3 and ω-6 fatty acids in (a) kelp diets and (b) urchin gonads. Concentrations of total ω-3 and ω-6 fatty acids in (c) kelp diets and (d) urchin gonads. Note the difference in *y*-axis scale between (c) and (d). Error bars: ± SD

degraded treatments (Table 4). Wild urchin gonads (both Start and End) had similar ARA content (~5.5%) to urchins fed *Agarum* (Table 2). There was an increase in proportion of EPA in urchin gonads from Wild-Start to Wild-End (Table 2).

DISCUSSION

GI of urchins differed greatly among kelp diet treatments, with the greatest differences observed between the 2 kelp species making up the diet. This finding is consistent with previous feeding trials, which found that urchins had higher GI when fed *Nereocystis* than those fed *Agarum* (Vadas 1977, Vadas et al. 2000, McBride et al. 2004). GI of urchins fed *Agarum* remained unchanged during the feeding trial, while GI of wild and *Nereocystis*-fed urchins increased. The absence of gonad development in *Agarum*-fed urchins could be due to the high phlorotannin content in this alga acting as an anti-herbivory compound (Steinberg 1985, Duggins & Eckman 1997, Van Alstyne et al. 1999a,b), causing either reduced consumption or ineffective digestion of kelp tissue (Tugwell & Branch 1992, Boettcher & Targett 1993). We also observed much lower consumption rates in the *Agarum*-fed urchins than in the *Nereocystis*-fed urchins.

The degradation-related changes of *Agarum* resulted in increased consumption; however, this did not translate into greater gonad development over the course of the 17 wk feeding trial. While all urchins were fed ad libitum, the differences in consumption rate among kelp diets resulted in sizable differences in the amount of material available for allocation to gonad development over the course of the feeding trial. These differences likely played a strong role in gonad development in our urchins (compare e.g. Rogers-Bennett et al. 1994, Minor & Scheibling 1997, Garrido & Barber 2001, Britton-Simmons et al. 2009, Dodge & Edwards 2012). While a 2-fold difference in consumption rate was observed between urchins in the Fresh- and Degr-*Nereocystis* treatment groups, the difference in GI between these treatments was small compared to the difference in GI between urchins fed either *Nereocystis* or *Agarum*. This sug-

Table 4. SIMPER results showing the top 5 FA that are primarily responsible for driving differences in urchin gonad FA signatures among treatments. The urchin gonad treatment type is listed, followed by the comparison of interest, and the total number of FA that account for at least 90% of the total variation in this comparison ($n_{FA} \geq 90\%$). For the top 5 FA, proportion relative to total FA content (Mean FA) and % contribution to differences between treatments are shown

Treatment	Comparison (a, b)	n_{FA} ≥ 90%	Top 5 FA	Mean FA (%) a	Mean FA (%) b	Contribution (%)
Wild	Start, end	11	c14:0	16.5	18.4	21.3
			16:1ω5	6.5	7.4	15.5
			c16:0	17.3	15.9	14.3
			20:5ω3 (EPA)	8.6	9.3	12.6
			18:4ω3 (SDA)	2.5	3.2	6.8
Agarum	Fresh, degraded	14	c14:0	15.7	14.8	18.9
			20:5ω3 (EPA)	8.5	10.1	18.1
			c16:0	17.4	17.0	12.4
			20:1ω7	5.6	5.1	10.0
			24:1ω9	1.8	1.9	7.1
Nereocystis	Fresh, degraded	15	c14:0	14.6	14.9	18.9
			20:4ω6 (ARA)	9.1	8.5	17.7
			20:5ω3 (EPA)	9.8	9.6	10.8
			18:1ω9	4.5	3.5	7.5
			c16:0	17.5	18.0	7.3
Fresh	*Agarum*, *Nereocystis*	13	20:4ω6 (ARA)	5.6	9.1	25.2
			c14:0	15.7	14.6	14.2
			c16:0	17.4	17.5	6.8
			24:1ω9	1.8	0.5	6.8
			18:4ω3 (SDA)	2.2	4.1	6.7
Degraded	*Agarum*, *Nereocystis*	13	20:4ω6 (ARA)	5.2	8.5	24.7
			20:5ω3 (EPA)	10.1	9.6	13.6
			c14:0	14.8	14.9	10.9
			c16:0	17.0	18.0	8.3
			24:1ω9	1.9	0.5	6.6

gests a greater importance of the type of diet available (i.e. species) than of the diet's degradation state (Fig. 1).

The $\delta^{13}C$ and $\delta^{15}N$ signatures of urchin gonads were influenced by the species of kelp consumed during the feeding trial. The variability in diet SI signature over our sampling time frame confounds analysis of the effect of diet species and degradation state in this study (Fig. 2). This observation highlights the need to account for variability in diet SI signature when investigating SI assimilation from diets in organisms collected from the field over long time periods. We expected to find enrichment of $\delta^{13}C$ and $\delta^{15}N$ values in urchins eating degraded kelps, similar to organisms living in deep habitats (~100 m) in the San Juan Islands (Galloway et al. 2013). Instead, we found a consistent pattern of $\delta^{13}C$ depletion with increased consumption rate and increased GI relative to the Wild-Start (Figs. 1 & 3).

The $\delta^{13}C$ values of gonads from urchins in the *Nereocystis* treatment groups (with high consumption rates and gonad growth) were lowest compared to the Wild-Start urchins and most similar to those of the kelp diets. Urchins in the *Agarum* treatment groups (with lower consumption rates and little increase in gonad size) had only slightly lower $\delta^{13}C$ values than the Wild-Start urchins. Increased lipid content is expected to result in a depleted $\delta^{13}C$ signature (Logan et al. 2008); however, this pattern is inconsistent with the relationship between $\delta^{13}C$ (Fig. 3) and lipid concentration of the gonads in this study (Table 2). This evidence suggests that the magnitude of $\delta^{13}C$ change was driven by assimilation of the diets into the gonads. However, gonad tissue may not entirely reflect the signature of the diet even when fed a single diet for 17 wk. The lack of any clear patterns in $\delta^{15}N$ and trophic enrichment from kelp diets to urchins (Fig. 3c,d) was unexpected and is currently unexplained. The $\delta^{15}N$ variation among our sampling times (Fig. 2) and the relatively fast growth rate of gonad tissue may have contributed to the lack of $\delta^{15}N$ fractionation.

The distinct differences in the multivariate FA signatures in kelps and urchin gonads (Table 2, Fig. 4) suggest that multivariate FA composition is sensitive to kelp species and degradation state. This contrasts with our GI and SI results, which were primarily affected by kelp species but not diet degradation state (Figs. 1 & 3). Degradation significantly reduced the proportion of ω-6 FA and the raw concentration of ω-3 and ω-6 FA in kelp diets; however, this diet degradation effect was not reflected in urchin gonad FA (Table 3, Fig. 5). Castell et al. (2004) found that varying amounts of certain ω-3 and ω-6 FA (e.g. 18:2ω-6 [LIN], 18:3ω-3 [ALA], EPA, and DHA) in diets did not correlate with growth in the green sea urchin *Strongylocentrotus droebachiensis*. This pattern indicates that urchins are likely able to elongate and desaturate low-carbon FA in sufficient quantities to meet physiological requirements regardless of diet quality (Takagi et al. 1980, Castell et al. 2004). For example, essential FA summary categories in urchin gonads remained similar even when the diets consumed were depleted in long chain essential ω-3 and ω-6 FA (Fig. 5). The very low proportion of ω-3 in

urchins fed fresh *Agarum* could be a result of urchins metabolizing FA stored in the gonads (Hughes et al. 2005, 2006). The trade off of maintaining consistent concentrations and proportions of ω-3 and ω-6 FA in gonads appears to be the reduced net growth of gonads on poorer quality diets, as evidenced by the similar ω-3 and ω-6 concentrations and proportions among urchins with very different rates of gonad growth.

Carboni et al. (2012) identified high ω-3 PUFA content, and high EPA:DHA and EPA:ARA ratios as FA indicators of increased larval growth and survival in the urchin *Paracentrotus lividus*. In our experiments, urchins that consumed fresh *Nereocystis* had the highest values of all 3 of these indicators, suggesting that larvae from these urchins would outperform larvae from other treatment groups (Table 2). The low EPA:DHA ratios of degraded *Nereocystis* and *Agarum* indicates reduced nutritional value of these diets to urchins, which could potentially lead to lower production of viable gametes (Minor & Scheibling 1997, Dodge & Edwards 2012). The EPA:DHA ratios of fresh and degraded *Agarum* and urchins fed degraded *Nereocystis* were consequently much lower than those of urchins fed fresh *Nereocystis* (Table 2). However, the large GI in urchins fed the degraded *Nereocystis* diet may show that urchins can compensate for the low EPA:DHA ratio in the gonads through increased reproductive output.

Enrichment of ARA in gonads of urchins fed *Nereocystis* diets (Table 2) may have direct implications for potential larval production of these urchins. ARA is the most common PUFA present in experimentally raised larvae of the urchin *P. lividus* and may be important for gonad development (Castell et al. 2004, Hughes et al. 2011). ARA was found to be the most important FA for discriminating between urchins fed *Nereocystis* and *Agarum* at both degradation state levels and was the second most important FA in separating urchins fed fresh and degraded *Nereocystis* (Table 4). GI differences among treatments were also consistent with these differences in ARA, suggesting that gonads, and potentially larvae, from urchins fed *Agarum* may be in a poorer condition owing to the deficiency of ARA.

Carboni et al. (2013) found that EPA content in urchin gonads increases as gonads develop, as seen in our wild urchins. Therefore we expected that all urchin treatments, especially urchins fed *Nereocystis*, should show an increase in EPA. However, we found that degradation state affected EPA differently depending on kelp diet species. The proportion of EPA was depleted in degraded versus fresh *Nereocystis* but enriched in degraded versus fresh *Agarum* for both kelp diets and urchin gonads (Table 2). Furthermore, SIMPER analysis identified EPA as an important discriminator between fresh and degraded *Nereocystis* (~10%) and between fresh and degraded *Agarum* (~18%; Table 4). This role of EPA may explain the significant interaction between species and degradation state seen in our PERMANOVA analyses. Moreover, the apparent importance of ARA and EPA identified here in discriminating between diet degradation states is consistent with results of SIMPER analyses comparing FA profiles of green urchins *Strongylocentrotus droebachiensis* living in shallow (15 m) and deep (~100 m) water at several sites in this same study system (Galloway et al. 2013).

The GI of wild urchins increased over the 17 wk feeding trial, as expected given the annual reproductive cycle of this species (Bernard 1977); however, the increase was significantly less than that observed in the *Nereocystis* treatments (Fig. 1). Red urchins can capture >20 g drift algae urchin^{-1} d^{-1} in the San Juan Archipelago (Lowe et al. 2014), an amount greater than they consumed in this study. The captured drift algae are composed of many species, among which *Agarum* is common, while *Nereocystis* is less frequently observed (Britton-Simmons et al. 2009). Observations from our feeding trial indicate that degradation state did not significantly affect the GI of *Nereocystis*-fed urchins. Degradation of the *Agarum* diet resulted in increased consumption rate and an increased proportion of gonadal EPA in urchins consuming this diet compared to those eating fresh *Agarum* (Table 2). Therefore, diet degradation may increase utilization of the abundant, yet less preferred, *Agarum* biomass.

The combination of somatic and biochemical metrics provided a unique perspective of the effects of diet species and degradation state on urchin nutritional condition. The degradation state (either fresh or degraded) of kelp diets did not cause changes in urchin GI, suggesting that the degradation state of kelp tissue does not significantly affect urchin gonad development. However, the species-specific fatty acid and stable isotope changes to kelps during degradation influenced aspects of gonad fatty acids. The observed depletion in gonadal $\delta^{13}C$ was related to the amount of food consumed rather than diet degradation state or kelp species. The patterns seen in $\delta^{15}N$ suggest that it may not be a useful tool in identifying producer–consumer connections over long time periods or in looking at the dietary pathways leading to gonad development. Responses of urchin gonad FA signatures to the different kelp

diets are consistent with results of previous work (e.g. Cook et al. 2000, Hughes et al. 2005, 2006, Kelly et al. 2008, Barberá et al. 2011), but diet degradation state affected certain individual FA and groups of FA differently depending on the species of kelp diet. The results gathered from GI, SI, and FA analyses in the present study suggest that the species-dependent degradation of kelp has important consequences for consumers relying on this carbon subsidy.

Acknowledgements. We thank D. O. Duggins and M. N. Dethier for support and guidance through all stages of this study. S. Weisner, L. Watson, R. McLaughlin, M. Eisenlord, A. Thomson, S. Yeung and K. Van Alstyne assisted with lab work, diving, boating and the experiment. We are also grateful to FHL staff for facilities and logistical support. This research was inspired by a National Science Foundation (NSF) Research Experience for Undergraduates awarded to W.W.R. and the undergraduate research of T. Stephens. Funding for the research came from the NSF (Biological Oceanography Division of Ocean Sciences grant 0925718 to all authors). Additional support to A.W.E.G. came from NSF (Division of Graduate Education Ocean and Coastal Interdisciplinary Science GK-12 fellowship grant 0742559). Comments from 2 anonymous reviewers significantly improved the manuscript.

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Editorial responsibility: James McClintock, Birmingham, Alabama, USA

Submitted: November 20, 2013; Accepted: May 27, 2014
Proofs received from author(s): August 18, 2014