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Feeding responses of the bivalves *Crassostrea gigas* and *Mytilus trossulus* to chemical composition of fresh and aged kelp detritus

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Abstract The chemical composition of kelps (e.g. polyphenolics) deters grazing by herbivores, but kelp detritus is potentially a source of nutrition for suspension feeders. The effects of kelp detritus derived from two species [*Agarum fimbriatum* Harvey and *Costaria costata* (Turner) Saunders] on feeding of oysters, *Crassostrea gigas* Thunberg, and mussels, *Mytilus trossulus* Gould, were examined in feeding experiments. Fresh and aged kelp particles were sequentially presented in combination with the microalga *Rhodomonas lens* at an initial total concentration of 5×10^{-4} ml^{-1}. Aging of kelp particles for 4 days in seawater significantly reduced the concentration of polyphenolics without changing the total carbon or nitrogen content. Clearance rates of both mussels and oysters were significantly lower in the presence of fresh versus aged kelp particles, and clearance rates declined overall with declining polyphenolic concentrations. Video endoscopy was used to examine feeding selectivity at the level of the gill in oysters in the same food treatments used in the clearance rate experiments. Comparison of particle composition in the water versus the pseudofeces in both oysters and mussels was also used as a measure of feeding selectivity. When presented with *R. lens* in combination with fresh and aged kelp particles selectivity for *R. lens* tended to be greater against fresh than aged particles, and there was some indication that this was stronger for *A. fimbriatum* than for *C. costata* particles. The ability to select was lower at very high polyphenolic concentrations, which may reflect poisoning of sensory binding sites. These data suggest that bivalves distinguish among particles of varying chemical composition and respond by changing their clearance rates and their selectivity.

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Introduction

Although seaweeds may be consumed directly by herbivores, a great deal of macroalgal production enters marine ecosystems as particulate organic matter (Mann 1988). Some of this material is captured by herbivores as large pieces of drift material, especially in kelp beds (Mattison et al. 1977; Bernstein et al. 1981), but much is broken down into smaller detrital particles that might be deposited on the bottom, close to the living seaweed source (Levinton and McCartney 1991) and available to deposit feeders (Levinton 1985; Lopez and Levinton 1987). Suspension feeders are often exposed to a mixture of particles, ranging from highly digestible phytoplankton to more toxic species, less digestible dead particulate organic matter, and resuspended inorganic matter (Rhoads and Young 1970; Findlay and Tenore 1982; Mann 1988; Duggins et al. 1989; Bustamante and Branch 1996; Ward et al. 1998a,b).

A number of studies suggest that dead particulate organic matter (POM) derived from plants may be ingested and assimilated to some degree by suspension feeders (Newell and Langdon 1990; Newell et al. 1995; Duggins and Eckman 1997; Kreeger and Newell 2001). POM from angiosperms is rich in indigestible materials such as cellulose and lignins, which are resistant to mechanical and microbial attack (Fenchel 1970; Harrison and Mann 1975; Tenore and Hanson 1980; Tenore 1983; Mann 1988). As a result, a relatively small fraction of particulate carbon from such sources is assimilated by suspension feeders (Crosby et al. 1989; Newell and Langdon 1990). In past work, we have demonstrated that bivalves can select nutritionally useful particles from POM of low value (Ward et al. 1998a,b).

Seaweeds contain smaller proportions of refractory materials (Mann 1988), and are potentially nutritionally

rich, as has been demonstrated by experimental additions of seaweed detritus, which stimulate growth of benthic invertebrates (Tenore 1977; Tenore and Hanson 1980; Levinton 1985). While particulates from kelp forests have been shown to contribute to growth in mussels (Duggins et al. 1989), the chemical content (e.g. polyphenolics) of many kelps and other seaweeds deters grazing by herbivores (Buchsbaum et al. 1984; Van Alstyne and Paul 1990; Paul 1992; Tugwell and Branch 1992; Hay 1996; Targett and Arnold 1998) and may exert negative effects on herbivore assimilation (Boettcher and Targett 1993). Duggins and Eckman (1994) could detect no effect of the presence of kelp on suspension feeder growth in the field, but ascribed their results partially to the regional abundance of the kelp *Agarum fimbriatum*, whose high polyphenolic content is believed to deter herbivore grazing (Steinberg 1985). In a later study, Duggins and Eckman (1997) were able to enhance somatic growth of mussels by addition of kelp particles, but mussel growth declined with increasing polyphenolic content.

In the field, bivalves exposed to complex mixtures of particles with widely varying nutritive content are capable of rejecting particles of poor nutritive content before ingestion (Newell and Jordan 1983; Hawkins et al. 1998; Ward et al. 1998a,b) and may respond by lowering clearance rates (Loosanoff and Tommers 1948; Ward et al. 1998a,b). Studies combining flow cytometry and video endoscopy demonstrate that preingestive selection may occur on the gills (oysters and zebra mussels: Baker et al. 1998, 2000; Ward et al. 1998a,b) or is confined to selection by the palps (*Mytilus* spp.: Ward et al. 1998a).

The present study investigated the mechanisms by which bivalves might respond to particles with varying polyphenolic content derived from kelps. Two major null hypotheses were addressed:

1. Because polyphenolic concentration declines with age of particles, the null hypothesis states that particle clearance rate for fresh kelp particles should not differ from clearance rate for aged kelp particles.
2. When given a choice, bivalves capable of selection should show no difference in selection between fresh kelp particles and aged particles.

These null hypotheses were tested in laboratory experiments using kelp detrital particles with different polyphenolic concentrations, using a laboratory-based aging process to stimulate breakdown of polyphenolics in the particles. We used standard measurements of particle clearance rate, combined with video-endoscopy-enabled sampling to study selection for or against particles of differing polyphenolic concentrations, derived from two different kelp species.

Materials and methods

Choice of kelp species and particle preparation

We chose the sieve kelp *Agarum fimbriatum* Harvey and the seersucker kelp *Costaria costata* (Turner) Saunders to prepare particles to feed to bivalves. Both species grow luxuriantly around Friday Harbor, Washington, USA, producing fronds up to 2 m in length with similarities in mechanical toughness. *A. fimbriatum* is known to deter feeding by grazing sea urchins, owing to its high concentrations of polyphenolics (Steinberg 1985). In the course of the study we found that *C. costata* can have lower concentrations. *A. fimbriatum* and *C. costata* were collected from the floating dock or subtidally near Friday Harbor Laboratories at depths < 10 m. After maintenance for < 2 days in a shaded running seawater aquarium, fronds were cut into 4- to 5-cm squares and placed in a bucket of flowing seawater in the dark for about 20 h, to allow leaching of mucus-like material. After conditioning, algal squares were placed in a blender with filtered (0.45 μm) seawater and ground for 3–4 min. The resulting slurry was passed through a 250 μm sieve and then a 20 μm sieve to obtain a final particle distribution (3–20 μm) overlapping that of algal cultures (described below). The particles that passed through both sieves were then collected, pelleted out of suspension, and resuspended in 0.45 μm filtered seawater, in order to remove the high concentration of solubilized metabolites that drastically reduced the feeding in bivalves in preliminary experiments with *A. fimbriatum*. The collected particles were split into a "fresh" particle fraction and another that was refrigerated for 4 days and then fed to the bivalves as "aged" particles. In previous experiments this time was sufficient for substantial degradation of the polyphenolics (up to ca. 78%, Duggins and Eckman 1997). During aging, flasks were gently aerated and on day 3, the particles were removed by centrifugation and resuspended in fresh filtered seawater. Prior to feeding assays, aged particles were pelleted out of suspension and resuspended in 0.45 μm filtered seawater. Particles were observed to be fully suspended. Of necessity, responses of bivalves were examined first to fresh particles, then to 4-day-aged particles.

Determination of polyphenolic and carbon–nitrogen content of kelp

Samples of kelp taken immediately after fronds were cut into squares, prior to grinding, from the ground residue on the 250 μm sieve, and from the material passing through the 20 μm sieve and were pelleted by centrifugation. All samples were stored at –70°C until assayed for phenolic concentration. Total phenolic concentration of kelp particles was determined with a modified micro-Folin–Denis assay, which can be carried out on as little as 1 mg of tissue (Arnold et al. 1995; Hatch and Tanner, unpublished data). All glassware was acid-washed in 50% nitric acid prior to use. Kelp squares were ground with a mortar and pestle in liquid nitrogen prior to use. A subsample of all kelp samples (100–200 mg wet mass) was transferred to dried and preweighed 2 ml microcentrifuge tubes, macerated with a Teflon rod in liquid nitrogen, macerated in trichloroacetic acid to precipitate proteins, and then extracted in 50% acetone. Folin–Denis reagent was used to develop the samples, and the absorbance of the supernatant was read on a Beckman DU-650 spectrophotometer at 725 nm. Extracted samples (each in their own microcentrifuge tube) were then dried to constant weight at 60°C. Phenolic concentrations are given as phloroglucinol equivalents times dry tissue per mass by comparing samples to a standard curve of desiccated phloroglucinol (Hatch and Tanner, unpublished data).

Carbon and nitrogen analyses were performed on particles from the fresh and 4-day-aged *A. fimbriatum* and *C. costata* samples from 1998; these samples were frozen and stored at –75°C and run in July 2001. They were defrosted and dried at 60°C for over 2 weeks and analyzed (three replicates each) using a CE Instruments Flash EA 1112 combustion elemental analyzer.

Experimental bivalves and protocol

The oyster *Crassostrea gigas* Thunberg and the mussel *Mytilus trossulus* Gould were obtained in July 1998 and July 2000 from Westcott Bay Oyster Farm, Friday Harbor, Washington. They

were transported to the Friday Harbor Laboratories and maintained in ambient, running seawater (ca. 12°C) until they were prepared for observation. Investigations of particle selection and preference were conducted by two methods: (1) traditional particle clearance experiments and (2) feeding assays using endoscope-directed, in vivo sampling. In all experiments, two different particle types were presented to the bivalves. The first was the cryptophyte *Rhodomonas lens* Pascher and Ruttner (6–13 μm in greatest diameter), obtained from the Provasoli-Guillard Center for Culture of Marine Phytoplankton (clone CCMP – 739), and grown in *f*/2 at 18°C under an 18 h light:6 h dark regime (Guillard 1975). The second particle type was ground kelp, as described above. Particle suspensions were prepared by diluting stock solutions of microalgal cultures and ground kelp particles with filtered seawater (0.45 μm) to form suspensions with a total concentration of $4–5\times10^4$ particles ml^{-1} of kelp and *R. lens*. Working suspensions were then combined in equal volumes and delivered to bivalves in clearance and endoscopy assays.

We conducted a series of particle clearance experiments using traditional clearance methods (e.g. Shumway et al. 1985). Bivalves (3–6 cm in shell length) were cleaned of fouling material and placed in a 700 ml suspension in individual 900 ml plastic containers, which were filled with the mixed *R. lens*/kelp particle suspensions at concentrations of $4–5\times10^4$ particles ml^{-1}. The suspension in each container was gently aerated and maintained at ambient temperature (ca. 13–14°C). Control vessels were maintained without bivalves to correct for microalgal cell division and settling during experiments. Changes in counts in the controls were used to correct numbers of algae in the experimental treatments. Experiments lasted for 0.75 h for mussels and 1.5 h for oysters. During the experiments, water samples were taken periodically and analyzed using flow cytometry to determine feeding activity. At the end of the experiment, pseudofeces were collected and analyzed using flow cytometry (see below). Experimental bivalves were then opened, their soft tissue was separated from the shell, blotted with a paper towel and then dried to constant weight at 65°C. Clearance rates of bivalves ($l\ h^{-1}$) were calculated using the methods of Coughlan (1969) and standardized to 500 mg dry soft tissue mass (Bayne 1976).

Preliminary analyses demonstrated strong differences in particle clearance rates among oysters. To compensate for this, we used an experimental design to account for differences among individuals. Clearance rate was measured in the same bivalve for fresh and aged detritus, presented in that order, prepared from a given kelp species. This design allowed the use of a repeated measures analysis of variance design, where within-bivalve variation could be factored into estimates of the effects of detrital aging, kelp species, and year of analysis. We used the design described by Kirby (1993) using SYSTAT software (SPSS, Chicago, Illinois, USA).

Adult *C. gigas* (8–12 cm shell length) were prepared for endoscopy using the methods of Ward et al. (1993, 1994). Valves were scrubbed to remove debris and encrusting organisms. A small section of shell was trimmed from the inhalant margin of the right and left valves, taking care not to damage the underlying soft tissues. Trimming produced a permanent opening, which facilitated insertion and movement of the endoscope optical insertion tube (OIT) and prevented damage to the OIT when the oyster closed its valves. After preparation the oysters were placed in ambient flowing seawater for at least 24 h before feeding experiments were initiated. Bivalves showed no sign of damage and usually began to resecrete shell material within a day or two of the trimming process.

Endoscopy was performed according to methods described previously (Ward et al. 1991, 1998a,b; Beninger et al. 1992). During endoscopic examination, oysters were placed in an aerated container (ca. 1.0 l) filled with seawater at ambient temperature. Individual oysters were exposed to mixtures of equal concentrations of kelp particles and *R. lens* adjusted to a total concentration of $4–5\times10^4$ particles ml^{-1} and were allowed to feed for 15–20 min prior to sampling. Particle concentration in the assay chamber was monitored during the exposure period using an electronic particle counter, and the chamber was flushed with additional volumes of the appropriate particle suspension when needed. We used different oysters for each replicate and therefore did not need to use a repeated measures design as was done in the clearance rate experiments. Mussels were not sampled with the aid of endoscopy.

Samples from the dorsal and ventral tracts of the gill were collected using a micropipet connected to a low-flow peristaltic pump (ca. 50 $\mu l\ min^{-1}$). The micropipet was mounted on a micromanipulator, and its position in the pallial cavity was observed by means of endoscopy.

Analyses of samples and data

Flow-cytometry was used to enumerate particle abundance in all samples (water, pseudofeces, ventral grooves, dorsal tracts, etc.). Particles were differentiated based on their optical properties by means of a FACScan bench top flow cytometer (Becton Dickinson, San Jose, Calif.) equipped with a 15 mW, 488 nm, air-cooled argon laser. Detection of microalgal cells was derived from chlorophyll fluorescence (> 650 nm) and phycoerythrin fluorescence (560–590 nm) emissions. All other particles (i.e. kelp particles) were detected by the simultaneous measurements of their forward scatter and 90° light scatter optical properties. All particles between 3 and 35 μm can be enumerated based on the forward scatter (a sizing parameter) signal from polystyrene microspheres (3.15 μm). Prior to analysis, each sample was vigorously agitated on a vortex mixer to disrupt particle aggregates. The volume of sample analyzed was calculated gravimetrically by weighing the sample immediately before and after analysis. Samples were run at low (ca. 30 $\mu l\ min^{-1}$) or high (ca. 50 $\mu l\ min^{-1}$) flow rates, depending on initial particle concentration. The photomultiplier detectors were in logarithmic mode, providing four decades of signal detection, and signal peak integrals were measured.

To determine particle selection for or against the kelp detrital particles relative to *R. lens*, we calculated a modified electivity index (EI) (Jacobs 1974; Bayne et al. 1977). This index was defined as:

$$EI = (r - p)/[(r + p) - 2rp]$$

where r is the proportion of *R. lens* cells in the postcapture samples (ventral groove, dorsal tracts, or pseudofeces where appropriate), and p is the proportion of cells in the water (i.e. precapture samples). A positive EI signifies enrichment of *R. lens* in the sample relative to the water, which, in turn, means selection against the kelp particles. This approach is analogous to another study of feeding of oysters and mussels on algae as compared to *Spartina alterniflora* detritus (Ward et al. 1998a,b).

Our methods allowed us several options to evaluate electivity. We evaluated electivity in both bivalve species by comparing pseudofeces to particle proportions in the water to assess total selection during preingestive processes. We used endoscope-aided sampling of oysters to estimate electivity for *R. lens* relative to kelp particles by analyzing data for particles collected from the dorsal tract, which contains particles more likely to be ingested (Ward et al. 1998a). Endoscope-aided sampling also allowed comparisons of electivity for *R. lens* relative to kelp particles in the dorsal tract to the same value in the ventral tract, which our previous studies show reflects ingested versus rejected particles, respectively (Ward et al. 1998a). This estimate was calculated as:

$$\Delta EI = EI_{dorsal} - EI_{ventral}$$

which is the difference in EI values for *R. lens*, calculated from the dorsal (EI_{dorsal}) and ventral tracts ($EI_{ventral}$). Increasing values of ΔEI indicate an increasing degree of enrichment of *R. lens* in the dorsal, particle-accepting, tract.

Results

Polyphenolic concentrations

Phenolic concentrations of the kelp particles varied with species and year (Table 1). In 1998, *Agarum fimbriatum*

Table 1. *Agarum fimbriatum*, *Costaria costata*. Mean phenolic concentrations (±SE, μg mg^{-1}) in fresh and aged (4 days) kelp detrital particles using modified Folin–Denis assay

Year	Kelp species	Age	Concentration (μg mg^{-1})	SE	*n*
1998	*A. fimbriatum*	Fresh	54.6	4.8	4
		Aged	30.9	1.8	4
	C. costata	Fresh	31.6	5.6	11
		Aged	9.2	1.4	4
2000	*A. fimbriatum*	Fresh	32.4	2.5	18
		Aged	17.1	1.9	7
	C. costata	Fresh	33.5	2.5	17
		Aged	14.6	1.2	8

generally had higher concentrations than *Costaria costata*, but the concentration in *A. fimbriatum* was dramatically lower in 2000 and was approximately equal to that of *C. costata*, which did not change. The effect of particle aging was also apparent. Four-day-aged particles had far lower phenolic concentrations than the fresh particles (Table 1).

A three-way analysis of variance procedure, testing for effects of kelp species, aging, and year of experiment, demonstrated a strongly significant kelp species effect due to the higher polyphenolic concentrations of *A. fimbriatum* samples in 1998 (Table 2). Phenolic concentrations did not differ significantly, however, in 2000, resulting in the strong kelp species×year interaction. The effect of particle aging was also significant ($P \approx 0.00002$), as expected. Concentrations appeared to have dropped proportionally more with aging for *C. costata* than for *A. fimbriatum* although this effect was not significant (kelp×aging interaction: $P \approx 0.07$).

Carbon–nitrogen analyses

Figure 1 shows the results for carbon and nitrogen contents for the 1998 samples of fresh and 4-day-aged particles of *A. fimbriatum* and *C. costata*. Percent carbon, percent nitrogen, and the C/N ratio did not change substantially over the 4 days.

Table 2. Analysis of variance for Folin–Denis estimates of phenolic concentration in detrital particles, as a function of year of analysis (1998, 2000) kelp species (*Agarum fimbriatum*, *Costaria costata*), and degree of aging (fresh, 4 days)

Source	SS	*df*	MS	*F*-ratio	*P*
Year	2288.4	1	2288.4	14.9	0.00024
Kelp species	4204.6	1	4204.6	27.4	< 0.00001
Aging	3295.1	1	3295.1	21.5	0.00002
Kelp species×Year	3834.7	1	3834.7	25.0	< 0.00001
Kelp species×Aging	493.9	1	493.9	3.2	0.07686
Year×Aging	81.07	1	81.1	0.5	0.46941
Kelp species×Year×Aging	240.4	1	240.4	1.6	0.21445
Error		71	153.2		

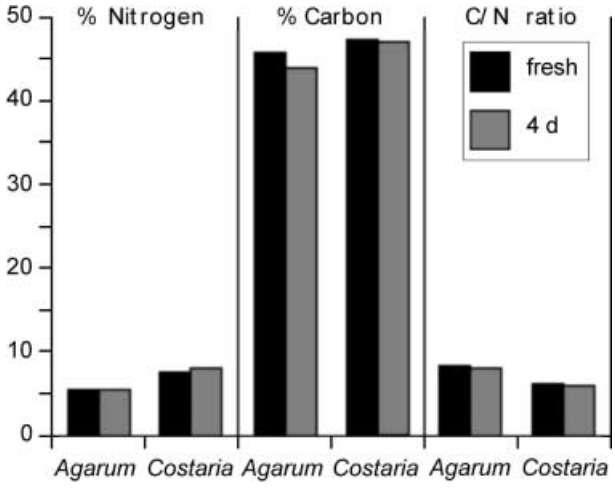

Fig. 1. *Agarum fimbriatum*, *Costaria costata*. Nitrogen, carbon, and C/N contents of fresh (day 1) and aged (day 4) kelp particles. Each measure is the mean of three subsamples of a pooled sample

Clearance rate

A repeated measures analysis of variance (Table 3) demonstrated that the effect of particle aging on oyster clearance rate was highly significant ($P < 0.000001$). Clearance rate increased significantly when oysters (*Crassostrea gigas*) were presented with aged particles (Fig. 2). The year of experiment strongly influenced the results, which was expected, owing to the strong differences in polyphenolic concentrations observed in *A. fimbriatum* between years. This difference rendered the kelp species effect statistically insignificant.

Particle aging also strongly affected clearance rates of mussels (*Mytilus trossulus*) (Table 4; Fig. 3). Clearance rate increased when mussels were presented with aged particles containing lower polyphenolic concentrations (Fig. 3). We only have data for the year 2000, and in this case clearance rate was not significantly different for either kelp species ($P \approx 0.1$), as was the case for the oyster clearance data for the year 2000.

There was no marked difference in clearance rate between mussels and oysters.

Analysis of clearance rate, using particles from both kelp species for combined mussel and oyster data as a function of phenolic concentration, indicated that clearance rate declined significantly with increasing phenolic concentrations (Pearson's correlation coefficient of means: $r = -0.70$, $P = 0.008$; Fig. 4).

Particle selection

Electivity data, calculated for samples from the dorsal margin of oysters, were analyzed using a three-way ANOVA, which considered the fixed effects of year of experiment, kelp species, and degree of aging (Table 5). In this case, year had no significant effect, but selectivity was notably affected by kelp species and age of the

Table 3. *Crassostrea gigas*. Repeated measures analysis of variance of depletion rates. Three main fixed factors are year of experiment (1998, 2000), kelp species (*Agarum fimbriatum*, *Costaria costata*), and age of particles (fresh, 4 days). Each oyster was exposed to fresh and aged detritus prepared from a given kelp species

Source	SS	*df*	MS	*F*-ratio	*P*
Between oysters					
Year	112.722	1	112.722	7.520	0.009
Kelp species	11.233	1	11.233	0.749	0.392
Kelp species×Year	70.413	1	70.413	4.697	0.036
Error	584.593	39	14.990		
Within oysters					
Aging	44.069	1	44.069	28.583	< 0.000001
Aging×Kelp species	3.021	1	3.021	1.959	0.170
Aging×Year	0.659	1	0.659	0.427	0.517
Aging×Kelp species×Year	8.135	1	8.135	5.276	0.027
Error	60.129	39	1.542		

detritus ($P \approx 0.06$ in both cases). Electivity index values (Fig. 5) were greatest for the cryptophyte *Rhodomonas lens* when oysters were fed fresh *A. fimbriatum* particles, which had higher phenolic loads, especially in 1998. In the overall ANOVA, there also were strong interaction effects between year of experiment, kelp species, and age of particles, which complicated estimates of the effect of particle aging alone.

Sampling with the aid of endoscopy allowed us to examine selectivity on the gills of the oyster for *R. lens* relative to kelp particles as a function of phenolic concentration (Fig. 6). Most notable is that all values were significantly positive, suggesting that kelp particles were being discriminated against and more *R. lens* were being directed to the dorsal tract. Our previous work on nutritionally poor *Spartina alterniflora* particles (Ward et al. 1998a) found electivity values approximately in the middle of the range of values for kelp particles (ca. 0.45). There appeared to be a trend towards increased selectivity against kelp particles with increasing phenolic content, but the trend was not statistically significant ($r = 0.217$, $P \approx 0.147$, $n = 46$).

Total selection by the gill of the oyster is best examined by comparing the electivity on the dorsal versus the ventral margins, which is plotted as a function of phenolic concentration (Fig. 7). Values were always positive and significantly > 0, indicating that *R. lens* was being directed preferentially to the dorsal tract and kelp detritus to the ventral groove for eventual rejection. ΔEI appeared to increase at intermediate concentrations of phenolics, then decreased. A one-way ANOVA demonstrates that the highest and lowest concentrations have significantly lower ΔEI than those for the intermediate concentrations ($F = 13.46$, $P = 0.00066$). Previous work on aged *S. alterniflora* detritus (Ward et al. 1998a) suggests that there is more dorsal enrichment of *R. lens* relative to *S. alterniflora* particles than we have found in the present study for kelp particles.

Electivity data, calculated for samples of pseudofeces of oysters, were analyzed using a three-way ANOVA, taking into account year of experiment, kelp species, and aging of particles (Table 6). Electivity showed a consistent preference for *R. lens*, relative to kelp particles. Electivity was strongly affected by kelp species. A Tukey post hoc analysis showed electivity to differ significantly between the two kelp species (difference = 0.2525, $P \approx 0.00001$). Electivity against *A. fimbriatum* was significantly less (EI = –0.169 ± 0.033, the negative value means selective ingestion of *R. lens* particles, relative to kelp particles) than for *C. costata* (EI = –0.466 ± 0.034). A strong between-year effect was also found, and this could be related to the strong difference in phenolic content of *A. fimbriatum* in the 2 years (Table 1), but the interaction effects were too complex for easy interpretation (Table 6). Degree of aging, however, had no significant effect on electivity as measured from pseudofeces, which is a measure of total net preingestive particle selection.

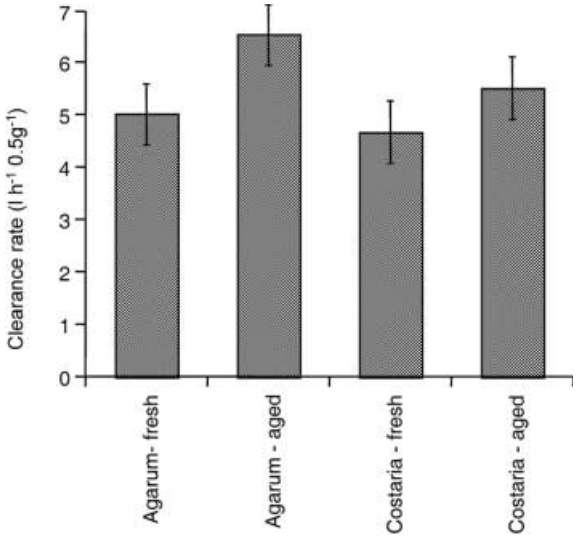

Fig. 2. *Crassostrea gigas*. Clearance rates (l h^{-1} 0.5 g^{-1}) of fresh and aged *Agarum fimbriatum* and *Costaria costata* particles

Table 4. *Mytilus trossulus*. Repeated measures analysis of variance of clearance rates. Two main fixed factors are kelp species (*Agarum fimbriatum*, *Costaria costata*) and age of particles (fresh, 4 days)

Source	SS	*df*	MS	*F*-ratio	*P*
Between mussels					
Kelp species	22.285	1	22.285	2.582	0.114
Error	483.255	56	8.630		
Within mussels					
Aging	51.485	1	51.485	20.049	0.00004
Aging×Kelp species	8.850	1	8.850	3.446	0.069
Error	143.805	56	2.568		

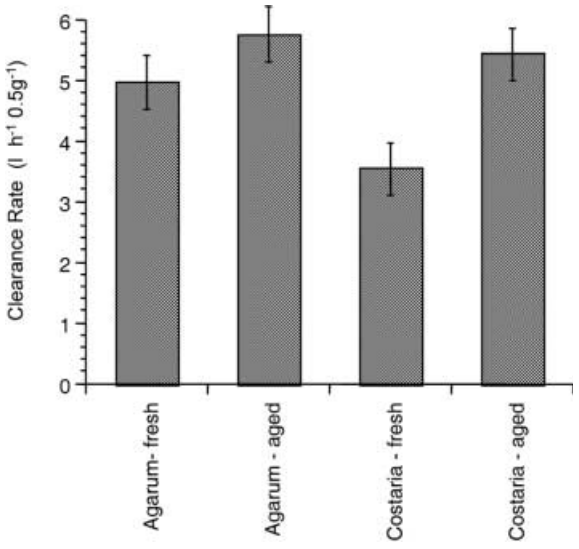

Fig. 3. *Mytilus trossulus*. Clearance rates (l h^{-1} 0.5 g^{-1}) of fresh and aged *Agarum fimbriatum* and *Costaria costata* particles

Electivity data, calculated for samples of pseudofeces of mussels, were analyzed using a two-way ANOVA because only year 2000 data were available (Table 7). Electivity differed significantly ($P \approx 0.002$) between kelp species: electivity against *C. costata* particles (mean = –0.188) was greater than against *A. fimbriatum* particles (mean = –0.075). This result was also found in the analyses of pseudofeces of oysters (see above). Electivity also significantly declined with age of particles ($P \approx 0.0001$). A significant kelp×aging interaction effect stemmed from the difference in response to particle aging between kelps: *M. trossulus* did not have a strong negative selection for *A. fimbriatum* particles, but electivity also did not change much with particle aging. By contrast (Fig. 8) electivity against *C. costata* particles strongly declined with aging, despite the fact that phenolic concentrations in fresh particles was about the same for both kelp species and the day 4 values were also not very different (Table 1). Qualitative observation suggested that some toxic element in the *A. fimbriatum* particles was affecting the mussels, which often did not feed on particles derived from this kelp species.

Discussion

Our results show strong effects of kelp detrital particle aging and total polyphenolic content on both clearance rates and selectivity for particles. In both *Crassostrea giga* and *Mytilus trossulus*, clearance rate increased when bivalves were presented with aged particles relative to fresh particles with higher polyphenolic concentrations. Clearance rate declined steadily with increasing polyphenolic concentration of kelp detrital particles, and the response of mussels showed no strong difference from oysters. This suggests that in a sea where detrital particles derived from seaweeds are laden with secondary compounds, bivalves might respond by feeding more slowly, which could reduce somatic growth rate. This was precisely the result obtained in the laboratory experiments by Duggins and Eckman (1997), who found reduced growth rates when mussels were fed with particles of higher polyphenolic content.

Much of our evidence for the role of polyphenolics depends upon the reduction of concentrations of polyphenolics in particles during the laboratory aging process. It is possible that other aspects of particle aging made the particles more attractive to the bivalves. Percent carbon, percent nitrogen, and the C/N ratio did not change substantially during aging, so it is unlikely that major trophic changes, such as colonization by abundant microorganisms, occurred during aging which are related to carbon and nitrogen content. Furthermore, the results presented on the overall trends in clearance rate (Fig. 6) and selectivity (Fig. 7) suggest that concentrations of polyphenolics per se have an overall effect on bivalve feeding responses.

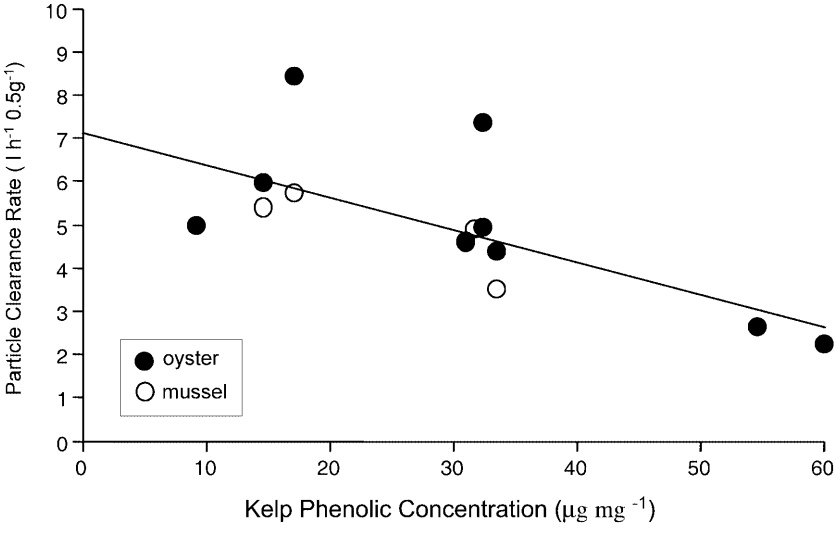

Fig. 4. *Crassostrea gigas*, *Mytilus trossulus*. Particle depletion rate as a function of polyphenolic concentration

Table 5. *Crassostrea gigas*. Analysis of variance of electivity for *Rhodomonas lens*, when oysters were fed approximately equal concentrations of *R. lens* and kelp-derived particles. Three main fixed factors are year of experiment (1998, 2000), kelp species (*Agarum fimbriatum*, *Costaria costata*), and age of particles (fresh, 4 days)

Source	SS	*df*	MS	*F*-ratio	*P*
Year	0.015	1	0.01	0.343	0.561
Kelp species	0.167	1	0.167	3.730	0.061
Aging	0.157	1	0.157	3.517	0.068
Year×Kelp species	0.227	1	0.227	5.067	0.030
Year×Aging	0.333	1	0.333	7.441	0.010
Kelp species×Aging	0.014	1	0.014	0.316	0.577
Year×Kelp species×Aging	0.156	1	0.156	3.487	0.070
Error	1.700	38	1.045		

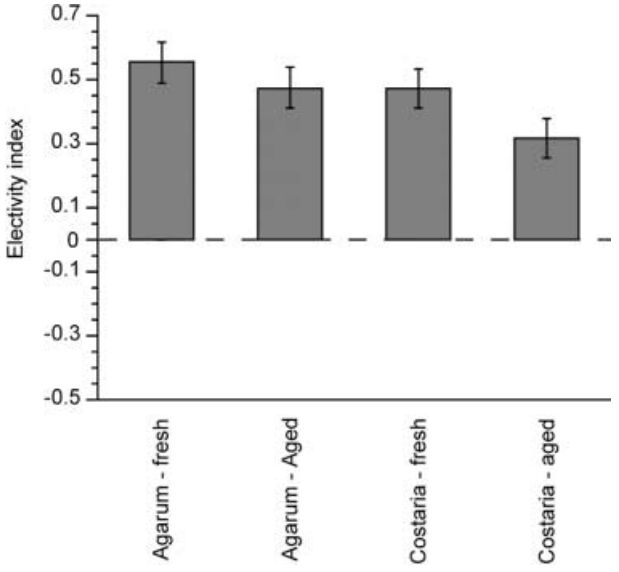

Fig. 5. *Crassostrea gigas*. Electivity for the flagellate *Rhodomonas lens*, relative to fresh and aged particles from *Agarum fimbriatum* and *Costaria costata*. Samples were taken by micropipet from the dorsal margin, where particles are known to be transported to the mouth (Ward et al. 1998a)

The use of video-endoscope-aided sampling allows for an understanding of the nature of preingestive selection in oysters. Our previous work (Ward et al. 1998a,b) demonstrated that particles moved to the dorsal margin were likely to be ingested. This study demonstrated that dorsal margin samples were more enriched with *Rhodomonas lens* when phenolics were higher in concentration; this was also true for the species of kelp used, since in 1998 *Agarum fimbriatum* particles had considerably higher polyphenolic concentrations than *Costaria costata* particles. Preference for *R. lens* declined when aged particles were presented, suggesting that the loss of phenolics made the kelp particles more palatable. These results suggest that oysters can use the gills to select for favored particles, which is consistent with results obtained previously for oysters when fed microalgae and aged *Spartina alterniflora* detritus (Ward et al. 1998a,b).

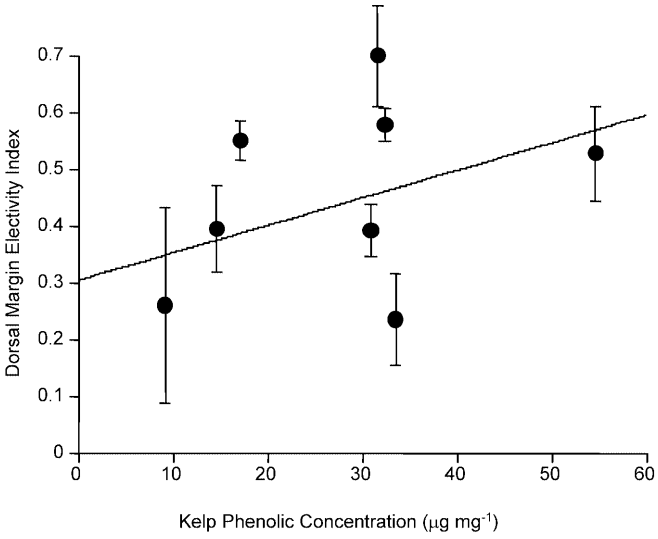

Fig. 6. *Crassostrea gigas*. Mean electivity (±SE) for *Rhodomonas lens* relative to kelp particles as a function of phenolic concentration, measured from particles collected in the dorsal margin of oyster gills

In contrast to dorsal margin sampling, proportions of particles in pseudofeces give us the total preingestive selectivity, including the activities of the palps. Here it was also found that the oysters were, in toto, selecting against polyphenolic-rich kelp particles, relative to microalgae. The *A. fimbriatum* particles, however, were being rejected less than *C. costata*, which differs from the dorsal margin results. We can only speculate on the reason for this difference, but it may relate to a poisoning reaction on palp binding sites, which resulted in an inability to reject particles in the palp area. We obtained similar results for electivity calculations based upon pseudofeces of *M. trossulus*: selection against *A. fimbriatum* was less than for *C. costata*. This result is not explainable by polyphenolic concentrations alone, because they did not differ between kelp species in the samples prepared in the year 2000. If this result is meaningful, it must relate to some other property of *A. fimbriatum* particles not determined by the Folin–Denis technique employed. There was, however, a major difference in electivity between fresh and aged *C. costata* particles. Aged particles were far more acceptable to the mussels.

Ward et al. (1998a,b) found that oysters transported more nutritious particles towards the dorsal margin and transported less nutritious particles towards the ventral margin, where they were often enmeshed in mucus, transported to the palps, and rejected as pseudofeces. Comparisons between particles in the dorsal and ventral margin might therefore be a sensitive indicator of selectivity. The results of the oyster experiments suggest a curvilinear response to polyphenolic content of kelp particles (Fig. 7). At first, increasing polyphenolic concentration results in a greater contrast in selection between dorsal and ventral tracts, but the difference declines at high polyphenolic concentrations. At high

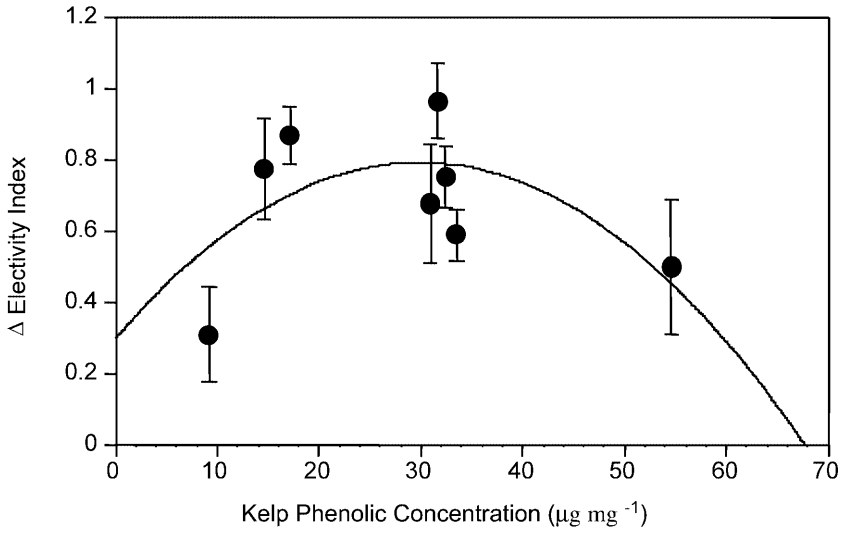

Fig. 7. *Crassostrea gigas*. Relationship between kelp phenolic concentration and the difference in electivity (mean ± SE, best-fit, second-degree polynomial, $r^2 = 0.41$) for *Rhodomonas lens* relative to kelp particles between the dorsal and ventral margins

Table 6. *Crassostrea gigas*. Analysis of variance of electivity for *Rhodomonas lens* versus kelp particles, when comparing particles in pseudofeces with those available in the water. Three main fixed factors are year of particle collection (1998, 2000), kelp species (*Agarum fimbriatum*, *Costaria costata*), and aging of particles (fresh, 4 days)

Source	SS	*df*	MS	*F*-ratio	*P*
Year	1.87762	1	1.87762	37.13053	< 0.00001
Kelp species	1.88928	1	1.88928	37.36118	< 0.00001
Aging	0.12800	1	0.12800	2.53134	0.11419
Year×Kelp species	0.03855	1	0.03855	0.76243	0.38429
Year×Aging	0.30658	1	0.30658	6.06275	0.01520
Kelp species×Aging	0.26457	1	0.26457	5.23196	0.02390
Year×Kelp species×Aging	0.56331	1	0.56331	11.13968	0.00112
Error	6.16930	122	0.05057		

Table 7. *Mytilus trossulus*. Analysis of variance of electivity for *Rhodomonas lens*, when mussels were fed approximately equal concentrations of *R. lens* and kelp-derived particles. Particles from pseudofeces are compared to those available in the water. Two main fixed factors are kelp species (*Agarum fimbriatum*, *Costaria costata*) and age of particles (fresh, 4 days)

Source	SS	*df*	MS	*F*-ratio	*P*
Kelp species	0.359	1	0.359	9.765	0.002
Aging	1.282	1	1.282	34.870	< 0.001
Kelp species×Aging	1.491	1	1.491	40.579	< 0.001
Error	4.006	109	0.037		

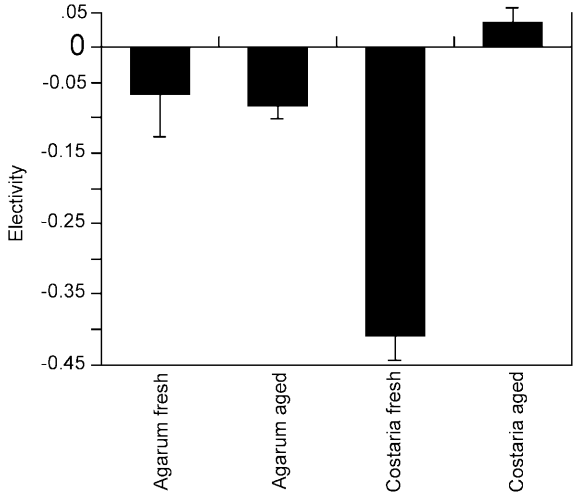

Fig. 8. *Mytilus trossulus*. Mean electivity (± SE) measured from pseudofeces for *Rhodomonas lens*, relative to fresh and aged particles of the two kelp species (*Agarum fimbriatum*, *Costaria costata*). More negative values indicate increased rejection of kelp particles. See Table 7 for statistical analysis

concentrations all binding sites may be saturated, blocking the gill from discriminating among particles. Curvilinear responses to selection for organic matter have been found in several bivalves (MacDonald and Ward 1994; Iglesias et al. 1996; Wong and Cheung 1999). Iglesias et al. (1992) argued that, when feeding on nutritively poor foods, increased selectivity is adaptive in gaining more organic matter for ingestion, but at high organic matter contents, bivalves might regulate clearance rates. Our data are obviously relevant to materials that are harmful to bivalves, and it is therefore not surprising that clearance rate might decline with increasing polyphenolic concentration. But one might expect increased selectivity to reduce ingestion of poisonous particles.

Bivalve populations are exposed to complex mixtures of particles, ranging from readily digestible algae to indigestible particles such as cellulose and silicates (Widdows et al. 1979; Berg and Newell 1986; Levinton et al. 1996, 2001; Ward et al. 1998a,b; Kreeger and Newell 2001). This is especially true in nearshore habitats and in marsh creeks where relatively indigestible detrital particles and even toxic cells are mixed with far more digestible microorganisms. Bivalves are well known to respond to varying conditions of turbidity and to variance of particle quality (Bayne et al. 1989; Iglesias et al. 1992; Navarro et al. 1998). Our results suggest that

oysters and mussels can, to varying degrees, respond to polyphenolic concentration in food particles by adjusting clearance rates and by selecting against unpalatable particles. It has not been established directly for bivalves that polyphenolics have a direct detrimental effect on processes such as assimilation efficiency, but these compounds appear generally toxic to many consumers (Paul and Hay 1986; Paul 1992) and *A. fimbriatum* is particularly inhibitory to consumer growth (Vadas 1977). It therefore appears reasonable that natural selection has selected for compensatory behavior in these bivalves. In areas where kelps with high polyphenolic loads are common, we would expect that bivalve feeding responses would result in rejection of such particles and also would result in lower clearance rates. This would create an indirect effect of increasing deposition of particles with high polyphenolic concentrations on the sediment surface via pseudofeces, and it might even result in a general reduction in feeding on the phytoplankton. Seaweeds might therefore indirectly affect the interactions between suspension feeders and shallow-water phytoplankton.

Previous studies using traditional techniques demonstrate that bivalves increase clearance rates with increasing food quality and preferentially reject poor-quality material in pseudofeces (Newell and Jordan 1983; Iglesias et al. 1992), sometimes reducing clearance rates when food is of poor quality (Widdows et al. 1979; Bricelj and Malouf 1984; Bayne et al. 1989). In the last two decades, the technique of flow cytometry has demonstrated strong selectivity for certain species of phytoplankton relative to others (Shumway et al. 1985). Our studies with mussels and oysters have used flow cytometry to demonstrate that bivalves can select against low-quality, cellulose-rich particles and preferentially ingest more nutritionally rich phytoplankton cells. Using direct endoscope-guided micropipet sampling, Ward et al. (1998a,b) demonstrated that this selectivity is mainly accomplished on the gills of oysters (*Crassostrea* spp.), but chiefly on the palps of *Mytilus* spp. Sorting by the gills for preferred particles has also been described from endoscopy-flow cytometry studies on the zebra mussel *Dreissena polymorpha* (Baker et al. 1998, 2000). Combined with the present results, these studies support growing evidence that bivalves can respond to variations in food quality and quantity by a variety of adaptive behaviors.

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