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Use of PCR-DGGE to investigate the trophic ecology of marine suspension feeding bivalves

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ABSTRACT: Polymerase chain reaction denaturing gradient gel electrophoresis (PCR-DGGE) approaches have previously been used to characterize marine plankton communities, but have rarely been used to investigate the trophic ecology of marine organisms. Here we use PCR-DGGE to obtain complex dietary profiles (often >20 bands) of eukaryotic organisms ingested by various species of bivalves. Sequence-based identification of individual phylotypes revealed ingestion of diatoms, dinoflagellates and other groups of organisms consistent with their known feeding ecology. Simultaneously profiling the seawater (plankton) allowed direct comparison to the dietary profiles. In *Mytilus edulis*, 50% of the detected plankton community was observed in the dietary profiles. Conversely, 34% of the phylotypes detected in the dietary profiles were not observed in seawater samples. Similarity-based cluster analysis of the dietary profiles from 6 sympatric species (4 epifaunal, 2 infaunal) of bivalves revealed a distinct, species-specific clustering pattern in 5 species, indicating a partial division of food based resources. Interestingly, both infaunal species investigated had dietary profiles that clustered not only at the species-specific level, but also as a distinct infaunal group. Trophic overlap was also present as evidenced by multiple shared phylotypes across all species. *Mimachlamys varia* did not group in a species-specific manner, suggesting a more generalist feeding strategy. Together, these results demonstrate the utility of a PCR-DGGE approach to study the feeding ecology of marine bivalves. This method offers a fast and accurate way to investigate the trophic interactions of marine bivalves (and presumably other invertebrates) across both large spatial and temporal scales.

KEY WORDS: DGGE · Bivalve · Trophic interaction · Suspension feeding · Dietary biomarker

INTRODUCTION

Understanding the degree to which sympatric suspension feeders interact is critical to the conceptual models through which we describe coastal ecosystems and develop sustainable shellfish aquaculture practices. Suspension feeding bivalves are important members of the macrobenthic community and can play a major role in benthic–pelagic coupling and nutrient recycling processes in nearshore marine ecosystems (Dame 1996, Newell 2004). Much of their contribution to ecosystem functioning is a result of suspension feeding activities. Despite their importance, food web dynamics such as predator–prey and trophic interactions at the species and cross-species levels are poorly defined. This paucity in data is partially due to complex temporal and spatial elements as well as the diverse nature of the seston from which the bivalves feed. The extent to which bivalves can select particles of higher nutritional value from the seston has long been debated (Loosanoff 1949, Jørgensen 1996), but it is generally accepted that many bivalves have the ability to feed selectively (Ward & Shumway 2004). Selectivity has a profound impact at both the individual and

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ecosystem levels and could serve as an important mechanism limiting resource competition between species through trophic plasticity.

Specific predator–prey interactions have been established through morphological examination of gut contents, and this approach has contributed significantly to our understanding of the position and importance of marine bivalves in nearshore food webs. Morphologically based gut content analyses have demonstrated the importance of phytoplankton in natural diets, notably diatoms, both pelagic and benthic, and seasonally abundant dinoflagellates (Shumway et al. 1987, Kamermans 1994, Rouillon et al. 2005). More recently, such investigations have revealed significant predation on zooplankton and invertebrate larvae, highlighting the impact that large assemblages of suspension feeders can have at higher trophic levels (Davenport et al. 2000, Lehane & Davenport 2004, Alfaro 2006). Despite these valuable insights, morphological analysis is limited to recently ingested organisms that retain identifiable characteristics, potentially overlooking the importance of soft-bodied prey or smaller, less conspicuous organisms.

Stable isotope analysis offers an alternative approach to investigate trophic structure and can delineate longer-term patterns of dietary assimilation. Due to the complexity of potential food sources, stable isotope investigations of marine suspension feeders tend to consolidate organic matter sources into broad categories, typically including particulate organic matter (phytoplankton) from freshwater and marine sources, marine and terrestrial detritus, microphytobenthic material and macroalgae. Stable isotope studies, like morphological-based gut content analyses, have demonstrated the primary importance of phytoplankton (Yokoyama et al. 2005, Marín Leal et al. 2008) and microphytobenthic material (Sauriau & Kang 2000, Kang et al. 2006) in the diets of suspension feeding bivalves, and have also shown that spatial, temporal and hydrodynamic features can influence the relative contributions of various organic matter sources (Decottignies et al. 2007a). Moreover, interspecific comparisons of isotopic studies have shown variable degrees of competition and resource partitioning within populations of suspension feeders (Decottignies et al. 2007b, Dubois et al. 2007). While these studies are suggestive that qualitative selection can reduce feeding competition, stable isotope data provides only categorical information with respect to assimilated organisms, with limited taxonomic insight.

A DNA-based approach has the potential to provide both taxonomically and ecologically informative data. Central to this approach is PCR. The nature of PCR allows flexibility in the experimental approach through the design of primers that would preferentially amplify dietary organisms at the species level or higher taxonomic groupings. Further, universal primers can be used to gain insights into the feeding ecology of organisms without any prior knowledge of dietary composition. In fact, universal PCR and cloning approaches have been successfully used to characterize both prokaryotic (Giovannoni et al. 1990) and eukaryotic (López-García et al. 2001, Moon-van der Staay et al. 2001) community structure of marine plankton. Subsequent investigations and those using slight methodological variations have developed a large number and various types of primer sets while greatly adding to the pool of sequence data available in public databases. More recently, DGGE (Fischer & Lerman 1983) has been used to investigate the community structure of aquatic microeukaryotes based on universal amplification of 18S rRNA gene fragments (van Hannon et al. 1998, Díez et al. 2001, Gast et al. 2004). This combined approach (PCR-DGGE) offers a flexible method for the simultaneous detection of a diverse assemblage of organisms based on the presence of unique DNA signatures.

DNA-based studies have been used to study the feeding ecology of marine invertebrates in a comparatively limited capacity, but the availability of published data is steadily expanding. Initial investigations, focused on group-specific PCR-based identification of ingested prey, have proved successful in delineating a trophic pathway in copepod feeding (Nejstgaard et al. 2003) and characterizing organisms ingested by Antarctic krill (Passmore et al. 2006). Universal primers and cloning approaches have been used to describe the dietary composition of deep-sea amphipods and bivalves (Duplessis et al. 2004, Blankenship & Yayanos 2006) and lobster phyllosoma larvae (Suzuki et al. 2006). Results of these universal approaches are based on clone library data, an intensive method that severely limits sample sizes. Alternatively, separation of universally amplified fragments with DGGE offers a more efficient means of evaluating sample diversity and is amenable to larger sample sizes. To our knowledge, only one PCR-DGGE study of marine invertebrate feeding has been reported. Martin et al. (2006) demonstrated a diverse mixotrophic diet of Antarctic krill through sequencing of unique phylotypes present in DGGE dietary profiles. Dietary profiles were also compared to environmental profiles (water column and sea ice) to assess their similarity, from which the authors suggested that the krill were selectively feeding.

In the case of marine suspension feeders, the ability to simultaneously profile the dietary composition and the seawater with PCR-DGGE offers a unique and powerful approach for trophic investigations. Here we demonstrate the ability of PCR-DGGE to obtain both

seawater (plankton) and dietary (gut contents) profiles from suspension feeding bivalves. We show that these profiles are representative of a wide diversity of eukaryotic organisms and are consistent with known feeding ecology. Comparing seawater and dietary profiles provides an assessment of selectivity. In addition, we show for the first time the utility of a PCR-DGGE approach to evaluate interspecific trophic interactions.

MATERIALS AND METHODS

Collection and fixation of adult animals. Adult bivalves were collected from local intertidal flats and transferred to a suspended lantern net in Mulroy Bay, County Donegal, Ireland on 8 March 2008 (used for phylotype identification and interspecific variation experiment). The mean shell heights (SH) for each species were: *Mytilus edulis*, 55.0 ± 5.9 mm; *Mimachlamys varia*, 50.5 ± 2.4 mm; *Cerastoderma edule*, 25.6 ± 1.1 mm; *Venerupis senegalensis*, 26.4 ± 2.1 mm; *Crassostrea gigas*, 88.0 ± 16.2 mm; *Ostrea edulis*, 72.9 ± 5.7 mm (n = 5 for each species). After 5 d in the lantern net, the adductor muscle(s) of each bivalve was severed and the 2 valves eased apart. Using a syringe and sterile needle, 1 to 2 ml of 95% EtOH was immediately injected into the digestive gland prior to fixation in 95% EtOH. All animals were processed within 1 h of removal from the water. Fixed animals were stored at 4°C and transferred to fresh 70% EtOH after 24 h. Additionally, adult *M. edulis* (57.2 ± 5.7 mm SH, n = 7) were collected from a pier piling prior to their emergence on a falling tide in Lough Swilly, County Donegal, Ireland on 15 May 2008 (used in selectivity experiment) and processed as above. Water samples of 200 ml (Mulroy Bay) and 1 l (Lough Swilly) were obtained from approximately 20 cm below the surface at the same time as animal collection. Though 200 ml samples had been taken during the initial 5 d trial it was considered that this volume was underestimating the total diversity of the water column so the volume was increased to 1 l during the sampling at Lough Swilly. The 200 ml water sample was stored overnight at 4°C before processing while the 1 l sample was held on ice and processed within 1 h of collection.

DNA isolation. ***Gut samples:*** The posterior mantle margins of each bivalve were detached from the valve surface and the residual ethanol allowed to drain for 15 to 20 min. Each bivalve was sectioned longitudinally exposing the stomach and digestive diverticulum. Under a low-powered (10 to 20×) dissecting microscope the stomach cavity was identified and a microspoon was used to collect the contents from each side. When possible, large portions of the crystalline style and gastric shield were removed prior to material being transferred to sterile 1.5 ml microfuge tubes. All bivalves were dissected using aseptic techniques; dissection tools were cleaned and flame sterilized prior to processing each bivalve. Recovered stomach contents were then pelleted (2 min at 10 000 × *g*) and processed with a DNeasy® Blood and Tissue Kit (Qiagen) following the gram positive bacteria protocol. Each pellet was mechanically disrupted with a sterile pellet pestle after the addition of enzymatic lysis buffer. Both the increased lysis stringency of the gram-positive bacteria protocol and use of mechanical disruption aided cell lysis. DNA was eluted in a final volume of 150 µl and stored at –20°C.

Adductor muscle: A positive DNA control was isolated from the adductor muscle of each species of bivalve used in the present study. Tissue was isolated with clean, sterile dissection tools and processed with a DNeasy® Blood and Tissue Kit following the manufacturer's animal tissue protocol.

Water samples: The organisms contained in the 200 ml water samples were pelleted at 6000 rpm for 15 min and the supernatants removed. The pellet was resuspended in 250 µl filter-sterilized seawater, transferred to a 1.5 ml microcentrifuge tube and pelleted at 10 000 × *g* for 3 min. The supernatant was removed by pipetting and DNA from the remaining pellet (representing the contents of 200 ml of raw seawater) extracted with a DNeasy® Blood and Tissue Kit following the gram-positive bacteria protocol. DNA was eluted in a final volume of 150 µl and stored at –20°C. In water samples used to evaluate feeding selectivity, 1 l of raw seawater was filtered (0.45 µm). To accommodate the increased quantities of DNA anticipated in 1 l samples, DNA was purified using the larger capacity UltraClean™ Water DNA Isolation Kit (MoBio Laboratories) according to the manufacturer's instructions.

DGGE analysis. ***PCR and DGGE preparation:*** A portion of the 18S rRNA gene was amplified from each DNA sample using the eukaryotic-specific GC-clamped primer 960FbGC and universal primer 1200R (Gast et al. 2004). Each 50 µl PCR reaction contained PCR buffer at 1×, 2.25 mM $MgCl_2$, 0.8 µM of each primer, 200 µM of each deoxynucleoside triphosphate, 0.1 mg ml^{-1} BSA, 2.5 U *Taq* polymerase (Invitrogen) and 1.5 µl of template DNA. Reactions were run under the following thermocycler conditions: 3 min at 94°C followed by 35 cycles of 45 s denaturing (94°C), 45 s annealing (63°C), 45 s elongation (72°C) and an additional 7 min elongation at 72°C. Amplification quality was assessed on 1.5% agarose gels prior to DGGE analysis. The remaining 45 µl of PCR products were run on an 8% acrylamide (37.5:1, acrylamide/bis-acrylamide) gel containing a 35 to 55% denaturing gradient of formamide and

urea, where 100% denaturing equals 40% formamide and 7 M urea. Samples were run in a Bio-Rad DCode™ system at 60 V for 16 h at a constant temperature of 60°C. Gels were stained with ethidium bromide and visualized under UV excitation.

Phylotype identification: Bands were excised from DGGE-based dietary profiles of *Mytilus edulis* and *Mimachlamys varia* and seawater profiles using clean, flame-sterilized forceps and razor blades. Each band was placed in 150 µl of nuclease-free H_2O and held overnight at 4°C. A 0.25 µl aliquot was used as template for reamplification with primers 960F and 1200R (Gast et al. 2004). Each 50 µl PCR reaction contained PCR buffer at 1×, 1.5 mM $MgCl_2$, 0.8 µM of each amplification primer, 200 µM of each deoxynucleoside triphosphate, 2.5 U *Taq* polymerase and 0.25 µl of template. Thermocycler conditions were as stated previously. PCR amplicons were purified with a Qiagen mini elute gel extraction kit following the manufacturer's protocol and commercially sequenced (Cogenics). Sequence reads were assembled and manually edited using Chromas Pro software (version 1.41, Technelysium). Contiguous sequences were compared to the nt database provided by the National Center for Biotechnology Information (NCBI) using BLAST (Altschul et al. 1997). For each sequenced phylotype, BLAST results generated an assignment to a putative taxonomic affiliation and placement into a higher level taxon.

DGGE image analysis: Gel images were captured using an Alpha DigiDoc RT system (AlphaEase® 4.0.1, Alpha Innotech) equipped with a 5.1 megapixel Olympus CAMEDIA C-5060 digital camera. Raw TIFF files were imported and analyzed with TotalLab™ TL120 DM 1-D gel analysis software (Nonlinear Dynamics). Lanes were detected automatically and slight manual adjustments were made to achieve a better fit. The rolling ball method (radius 150) was used for background subtraction and bands were detected under the following settings: minimum slope = 150; noise reduction = 10; and 5% maximum peak (per gel). Manual adjustments were made to remove obvious misidentifications. In addition, the band within each dietary profile that comigrated with the positive tissue control for that species was removed from subsequent analyses. For feeding selectivity measurements (an intragel comparison), 4 retardation factor (R_f) reference points were inserted manually to account for any warping of the gel lanes. Anchor locations were chosen only where bands in the same position were present across all lanes of the gel. Band matching was carried out using an R_f vector of 0.005 (i.e. only bands with a ≤0.5% variation in gel position were considered a match). In this case, the profile obtained from the water column was used as a standard to which all dietary profiles were matched. To assess the dietary profiles between the 6 species (an intergel comparison), gels were normalized using 3 standard lanes per gel, each containing 7 bands that served as the R_f reference points. The similarities (Dice coefficients) of all normalized lanes (n = 35, 5 individuals per species and 5 replicate seawater samples) were compared to each other in a pairwise fashion and graphically assessed with an UPGMA cluster analysis.

RESULTS

DGGE profiles

After evaluating a range of concentrations (20 to 80%), a denaturing gradient of 35 to 55% was empirically determined to provide the best resolution while maximizing the observable phylotypic diversity (data not shown). Phylotypic diversity was high (often over 20 bands) and complex profiles were observed from both the water column and bivalve gut samples (Fig. 1). Individual sample profiles were highly reproducible between PCR reactions and DGGE runs. Inclusion of an adductor muscle control for each species clearly demonstrated the migration point and band position corresponding to the self phylotype within dietary profiles (Figs. 1 & 2). This nondietary component was excluded from all subsequent matching analyses.

Phylotype identification

To verify that amplification products observed in DGGE profiles were originating from expected sources, 2 species were chosen for a detailed evaluation. BLAST-based identification of organisms ingested by *Mytilus edulis* and *Mimachlamys varia* were consistent with the known feeding behavior of adult bivalves (Table 1). Sequence identities ranged from 96 to 100% similarity over a length of 217 to 234 bp. Due to multiple tied and/or low percent identity scores, results were interpreted only in a conceptual sense and to assign higher level taxonomic designations from which specific conclusions were drawn. The most frequently observed phylotypes were those belonging to Bacillariophyta. When present, comigrating Bacillariophyta phylotypes were generally observed in all bivalves (and the water column) suggesting the dietary importance of this group (e.g. Fig. 1a, bands 6 & 11 and Fig. 1b, bands 13, 14, 16 & 18). Other less common dietary phylotypes belonged to Dinophyceae, Cnidaria, Gastropoda and Crustacea. Cryptophytes were identified from the water column and

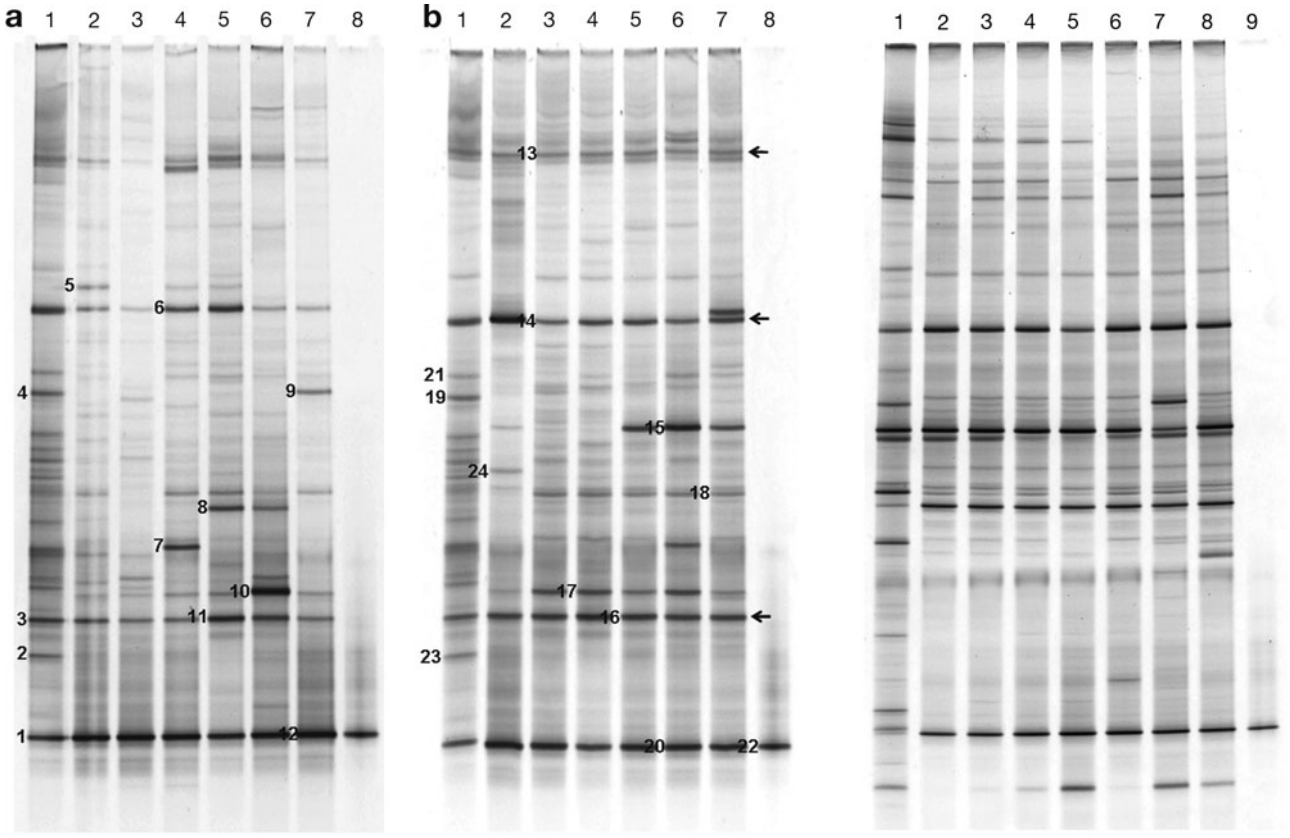

Fig. 1. DGGE profiles depicting sequenced phylotypes from *in situ* dietary profiles of (a) *Mytilus edulis* and (b) *Mimachlamys varia*. Lane 1: 200 ml raw seawater; Lanes 2 to 7: gut contents; Lane 8: adductor muscle control. Numbers indicate sequenced phylotypes detailed in Table 1. (←) phylotypes that correspond to those observed in all profiles from Fig. 4 (↑)

Fig. 2. DGGE profiles of *in situ* feeding selectivity of *Mytilus edulis*. Lane 1: 1 l raw seawater; Lanes 2 to 8: *M. edulis* gut contents; Lane 9: *M. edulis* adductor muscle control

comigrating phylotypes were observed in some of the dietary profiles. A Phaeophyte alga was also identified from the water column although comigrating phylotypes were not observed in dietary profiles.

Feeding selectivity

Initial attempts to assess feeding selectivity using 200 ml seawater samples underestimated the total diversity. To account for this, a larger 1 l sample was processed and feeding selectivity was assessed in *Mytilus edulis* by comparing the number of phylotypes in the water column that also had comigrating counterparts within the dietary profiles (Fig. 2, Table 2). A total of 33 phylotypes were detected in the 1 l water sample while dietary profiles (n = 7) averaged 21.1 ± 2.2 phylotypes. Of the 33 phylotypes present in the water column, an average of 49.6% were detected within the dietary profiles. By contrast, not all dietary phylotypes were detected in the sampled water. On average, 33.6% of the phylotypes were unique to the dietary profiles, while the remaining 66.4% could be matched to comigrating phylotypes in the water column.

Interspecific variation

The similarity of dietary profiles was compared between 6 co-occurring bivalve species and the water column. A 1-way ANOVA demonstrated a significant difference ($F = 3.45$, $p = 0.017$) in the mean number of dietary phylotypes detected (phylotypic richness) between species (Fig. 3). Subsequent pairwise comparisons (Tukey's test) of species-specific means indicated that only *Mytilus edulis* and *Ostrea edulis* differed in the detectable number of phylotypes. An UPGMA analysis using Dice similarity coefficients of dietary profiles revealed a distinct species-based clustering pattern. Three species

(*Venerupis senegalensis*, *Cerastoderma edule* and *Crassostrea gigas*) formed distinct clusters containing all 5 individuals sampled. Two additional species (*Mytilus edulis* and *O. edulis*) had distinct clusters containing 4 of the 5 individuals, while dietary profiles of *Mimachlamys varia* were dissimilar and did

Table 1. Taxonomic relatedness of excised DGGE phylotypes from water and *in situ* dietary profiles from *Mytilus edulis* and *Mimachlamys varia*. Relatedness based on BLAST search in NCBI's nt database. Band codes refer to phylotypes positions in Fig. 1. W: water; G: gut; A: adductor muscle. For samples with multiple tied percent identity matches, only one representative is listed. The 5 closest relatives were used to determine the higher level taxon designation

Band code	Sample source	Sequence length (bp)	Sequence identity (%)	Closest relative	Higher taxon
Mytilus edulis					
1	W	231	100	*Mytilus edulis*	Bivalvia
2	W	233	98	*Ectocarpus* sp.	Phaeophyta
3	W	234	99	*Skeletonema* sp.	Bacillariophyta
4	W	228	100	*Teleaulax acuta*	Cryptophyta
5	G	229	98	*Ichthyophonida* sp.	Choanozoa
6	G	234	100	*Stephanodiscus minutulus*	Bacillariophyta
7	G	231	98	*Pisinna albizona*	Gastropoda
8	G	231	97	*Pisinna albizona*	Gastropoda
9	G	230	96	Uncultured eukaryote clone	Cnidaria
10	G	230	96	Uncultured copepod clone	Crustacea
11	G	230	96	*Skeletonema* sp.	Bacillariophyta
12	G	231	100	*Mytilus edulis*	Bivalvia
Mimachlamys varia					
13	G	234	99	*Cyclotella choctawhatcheeana*	Bacillariophyta
14	G	234	100	*Stephanodiscus minutulus*	Bacillariophyta
15	G	229	100	Uncultured eukaryote clone	Cnidaria
16	G	234	98	*Skeletonema* sp.	Bacillariophyta
17	G	233	97	*Heterocapsa pygmaea*	Dinophyceae
18	G	232	96	*Thalassiosira tenera*	Bacillariophyta
19	W	228	99	*Teleaulax acuta*	Cryptophyta
20	G	230	100	*Mimachlamys varia*	Bivalvia
21	W	217	100	Cryptophyta sp. CR-MAL01	Cryptophyta
22	A	230	100	*Mimachlamys varia*	Bivalvia
23	W	233	98	*Ectocarpus* sp.	Phaeophyta
24	G	232	99	Uncultured eukaryote clone	Dinophyceae

Table 2. Results of feeding selectivity based on DGGE-detected phylotypes in the water column and *Mytilus edulis* gut samples. Lane numbers correspond to the DGGE image depicted in Fig. 2. Band corresponding to self amplification within dietary profiles was excluded from analyses. NA: not applicable. The average number of phylotypes detected is calculated from Lanes 2 through 8

Lanes	Total no. phylotypes detected	Water column phylotypes detected within dietary profiles (%)	Dietary phylotypes detected within water column profiles (%)	Dietary phylotypes not detected within water column profiles (%)
1	33	NA	NA	NA
2	22	40.6	61.9	38.1
3	25	50.0	66.7	33.3
4	26	59.4	76.0	24.0
5	24	50.0	69.6	30.4
6	21	34.4	55.0	45.0
7	27	56.3	69.2	30.8
8	28	56.3	66.7	33.3
9	1	NA	NA	NA
Average	24.7 ± 2.5	49.6 ± 9.1	66.4 ± 6.6	33.6 ± 6.6

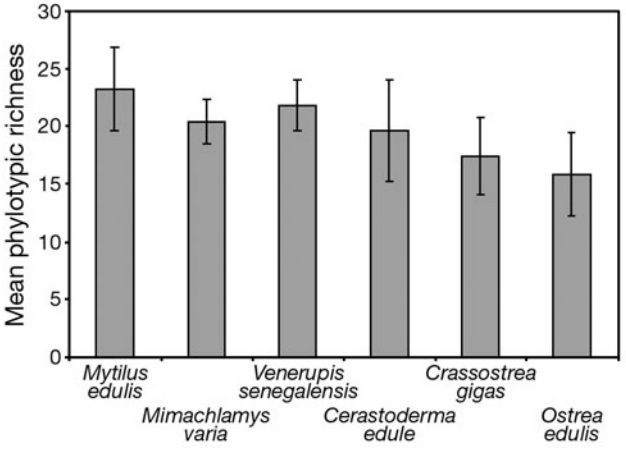

Fig. 3. Mean phylotypic richness detected in DGGE dietary profiles from the gut contents of 6 species of marine bivalve. n = 5 for each species. Error bars are ±1 SD

not cluster in a predictable manner. All water column profiles formed a distinct, distantly related cluster (Fig. 4).

DISCUSSION

PCR-DGGE approaches are being used to successfully characterize complex eukaryotic marine plankton communities and to correlate shifts in community structure with environmental variables (Díez et al. 2004, Gast et al. 2004, Marie et al. 2005). Here we adapted these techniques to explore the feeding ecology of adult suspension feeding bivalves. Using primers (960FbGC and 1200R, Gast et al. 2004) targeting a portion of the 18S rRNA gene, it was possible to obtain complex profiles representative of a wide diversity of ingested eukaryotic organisms. Sequencing of prominent phylotypes present in DGGE profiles (Fig. 1) and subsequent BLAST analysis (Altschul et al. 1997) confirmed the taxonomic affiliations. Due to the limitation of DGGE to relatively short fragments, generally <500 bp, multiple tied percent identity matches were common, some with relatively low (<98%) identities. For this reason, the closest relative was interpreted only in a conceptual sense and BLAST results were used to determine a higher level taxonomic identification (Table 1) from which insights into the feeding ecology were derived. The most frequently observed organisms in the dietary profiles of *Mytilus edulis* and *Mimachlamys varia* were diatoms (Bacillariophyta). When present, diatoms tended to have comigrating phylotypes present in all bivalves sampled. For example, comigrating diatom phylotypes (Fig. 1a, Bands 6 & 11) were present in all *Mytilus edulis* sampled as well

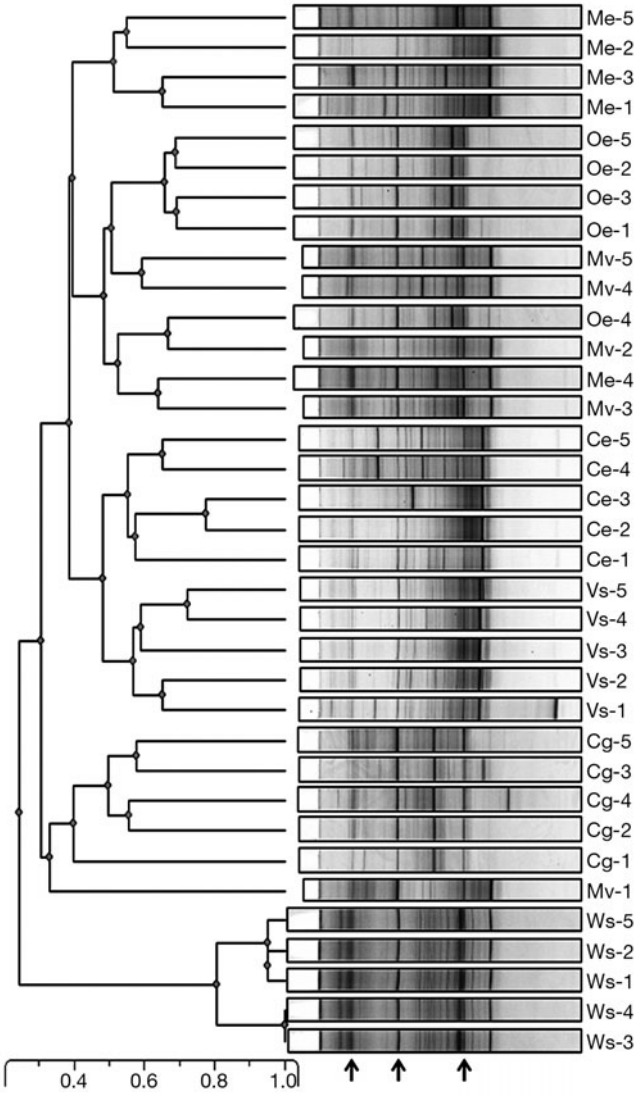

Fig. 4. UPGMA dendrogram based on DGGE dietary profiles from 6 species of intertidal marine bivalves and seawater samples. Me: *Mytilus edulis*; Mv: *Mimachlamys varia*; Ce: *Cerastoderma edule*; Vs: *Venerupis senegalensis*; Cg: *Crassostrea gigas*; Oe: *Ostrea edulis*; Ws: 200 ml water sample. n = 5 for each species, with 1 specimen represented per profile. The water sample profiles comprised 5 replicate reactions from a single DNA extract (↑) phylotypes common to all profiles. The abscissa shows Dice similarities

as the water column. In *Mimachlamys varia*, diatoms (Fig. 1b, Bands 13, 14 & 16) were again identified in all specimens and the water column, with Band 18 present in 5 of the 6 samples. Additionally, a dinoflagellate was identified (Fig. 1b, Band 17) with a corresponding phylotype in all *Mimachlamys varia* sampled. Together, diatoms and dinoflagellates were the most frequently observed phylotypes in the dietary profiles of *Mytilus edulis* and *Mimachlamys varia*, reflecting

their dietary importance. Phylotypes belonging to a cnidarian, a choanozoan, a gastropod and a crustacean were also identified from dietary profiles, although these organisms had a lower frequency of comigrating phylotypes in other samples. These results suggest that the dietary profiles obtained through PCR-DGGE are representative of the organisms ingested and accurately reflect the known feeding behavior of these bivalves.

It was not our intention to provide a full and complete characterization of dietary components by the sequencing of DGGE phylotypes, but to simply ground-truth the profiles themselves. Obtaining profiles representative of both the dietary elements and the environment allowed for a comparative analysis between profile types. On average, *Mytilus edulis* ingested 49.6% of the phylotypes detected within seawater profiles. Of the dietary profiles, 66.4% could be explained by the presence of matching phylotypes in the water column, leaving 33.6% unmatched. The presence of unmatched phylotypes in the dietary profiles could have several explanations. First, these phylotypes could represent organisms of low abundance in the water column that have been selectively fed upon, thus increasing their concentration in the gut. It has been estimated that a specific template DNA must represent approximately 1 to 1.6% of the total DNA extract to be detected by a PCR-DGGE approach (Muyzer et al. 1993, Murray et al. 1996). Therefore, it seems reasonable that selective feeding could lead to preferential detection of certain organisms in the dietary profiles. Second, detection of phylotypic signatures in the dietary profile could be biased towards organisms that have a longer retention time, if retention time exceeds the temporal variability of the plankton community. Finally, in this particular analysis, 1 l of raw seawater was processed, a volume that may not be sufficient to represent the total availability of prey organisms. Though these data suggest that *M. edulis* is selectively feeding, further experimentation with greater control over environmental variables is necessary.

The degree to which bivalves selectively feed may serve as an important mechanism limiting resource competition between species. If significant trophic plasticity does exist, it should be evident in the dietary profiles of various species. To evaluate this, we obtained dietary profiles from 6 species of sympatric intertidal suspension feeding bivalves. Animals were held in a suspended lantern net for 5 d prior to sampling to ensure uniform access to the plankton community and control for potential effects of spatial heterogeneity. Only the dietary profiles of *Mytilus edulis* and *Ostrea edulis* differed significantly in phylotypic richness (Fig. 3). Subsequent UPGMA cluster analysis was used to measure the similarities of dietary profiles between the 6 different species and the water column (Fig. 4). As expected, all water samples formed a distinct, distantly related cluster, while the dietary profiles clustered largely on a species-specific basis, indicating that profiles are more similar intraspecifically than they are interspecifically. Interestingly, infaunal dietary profiles were distinct from those of epifaunal species with both infaunal species, *Venerupis senegalensis* and *Cerastoderma edule*, forming distinct clusters nested within a larger one, though in this instance a behavioral artifact cannot be ignored as both species had been removed from their natural infaunal habitat. In contrast, dietary profiles of *Mimachlamys varia* were dissimilar and failed to group in a species-specific manner, suggesting a more generalist feeding strategy. The self phylotype was removed prior to matching analysis as it does not definitively represent ingested material. However, the PCR-DGGE technique does not allow discrimination between host tissue incidentally collected at sampling and cannibalistic larviphagy, reported to be common in some shellfish species (Lehane & Davenport 2004, Alfaro 2006).

Using a PCR-DGGE approach it was possible to obtain dietary profiles that contained sufficient information to allow comparative analysis. Based on the largely species-specific clustering patterns, some degree of trophic partitioning was evident. Trophic competition was also present, as evidenced by multiple shared phylotypes across all species sampled (Fig. 4, arrows). Although obtained from different PCR reactions and gel runs, these 3 common phylotypes correspond to phylotypes identified as diatoms (Fig. 1b, arrows). The temporal stability of this trophic pattern is unknown. Decottignies et al. (2007b) observed a partial and seasonally variable level of isotopic overlap between *Crepidula fornicata* and *Crassostrea gigas*. Competition was most pronounced during periods of food limitation and partitioning was evident during bloom conditions. This shift was attributed to qualitative selection by *Crassostrea gigas* during periods of higher food availability (Decottignies et al. 2007b), while *Crepidula fornicata* showed no selective feeding capacity (Beninger et al. 2007). It remains unclear as to the mechanism driving the trophic patterns observed in the present study. Qualitative selection on a facultative basis may serve to reduce competition during periods of seasonal abundance, but further studies are necessary to understand the patterns and processes shaping the trophic structure of suspension feeding bivalves.

Several areas of potential bias and limitation are apparent with the application of a PCR-DGGE approach to understanding the feeding ecology of bivalves. DNA-based detection of organisms present

in gut contents eliminates the need for morphologically identifiable characteristics, but is still limited to recently ingested prey. Organisms detected in the gut contents may not necessarily represent important components of the diet as they may pass through the digestive system unassimilated. In addition, ingested organisms do not all have the same gut retention time (Bricelj et al. 1984), hence detection could be biased towards those retained for longer periods. Resource competition assessed through DGGE profiles of ingested organisms does not account for organisms rejected as pseudofeces, although it is possible that pseudofeces (or undigested feces) could be made available through subsequent hydrodynamic resuspension. The intensity of a DGGE band (PCR bias aside) should be proportional to the starting number of 18S rDNA fragments available in the initial PCR reaction. However, this starting number can vary for several reasons: (1) ingestion of a larger quantity of one species relative to another, (2) variation in gene copy number between species and (3) ingestion of multicellular vs. single-celled organisms. These factors limit a universal type approach to a qualitative assessment, although quantification may be possible in a limited capacity by targeting specific groups of organisms with known rRNA gene copy numbers. Such attempts using quantitative PCR have been successful in quantifying a species-specific level of feeding in copepods (Durbin et al. 2008, Nejstgaard et al. 2008).

The PCR-DGGE method described here provides a fast and accurate means to investigate the feeding ecology of a diverse range of suspension feeding bivalves. Little *a priori* knowledge of dietary composition is necessary and detailed characterization of ingested organisms is possible through DNA sequencing of detected phylotypes. This approach allows for the simultaneous detection of a wide range of ingested organisms and, in the case of suspension feeders, potential prey in the environment, providing comparable and informative DNA-based profiles. The *in situ* ingestion profiles from 6 species of marine bivalves were successfully obtained and it is expected that with slight modifications this technique could be adapted to a wide range of marine organisms. Coupling PCR-DGGE feeding studies with multiple environmental variables and application across large spatial and temporal scales is expected to provide further insights into the complex structure of nearshore marine food webs.

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LITERATURE CITED

Alfaro CA (2006) Evidence of cannibalism and benthic-pelagic coupling within the life cycle of the mussel, *Perna canaliculus*. J Exp Mar Biol Ecol 329:206–217

Altschul SF, Madden TL, Schaffer AA, Zhang J, Zhang Z, Miller W (1997) Gapped BLAST and PSI-BLAST: a new generation of protein database search programs. Nucleic Acids Res 25:3389–3402

Beninger PG, Decottignies P, Guiheneuf F, Barillé L, Rincé Y (2007) Comparison of particle processing by two introduced suspension feeders: selection in *Crepidula fornicata* and *Crassostrea gigas*. Mar Ecol Prog Ser 334: 165–177

Blankenship LE, Yayanos AA (2006) Universal primers and PCR of gut contents to study marine invertebrate diets. Mol Ecol 14:897–899

Bricelj VM, Bass AE, Lopez GR (1984) Absorption and gut passage time of microalgae in a suspension feeder: an evaluation of the ^{51}Cr:^{14}C twin tracer technique. Mar Ecol Prog Ser 17:57–63

Dame RF (1996) Ecology of marine bivalves: an ecosystem approach. CRC Press, Boca Raton, FL

Davenport J, Smith RJJ, Packer M (2000) Mussels *Mytilus edulis*: significant consumers and destroyers of mesozooplankton. Mar Ecol Prog Ser 198:131–137

Decottignies P, Beninger PG, Rincé Y, Robins RJ, Riera P (2007a) Exploitation of natural food sources by two sympatric, invasive suspension-feeders: *Crassostrea gigas* and *Crepidula fornicata*. Mar Ecol Prog Ser 334:179–192

Decottignies P, Beninger PG, Rincé Y, Riera P (2007b) Trophic interaction between two introduced suspension-feeders, *Crepidula fornicata* and *Crassostrea gigas*, are influenced by seasonal and qualitative selection capacity. J Exp Mar Biol Ecol 342:231–241

Díez B, Pedrós-Alió C, March TL, Massana R (2001) Application of denaturing gradient gel electrophoresis (DGGE) to study the diversity of marine picoeukaryotic assemblages and comparison of DGGE with other molecular techniques. Appl Environ Microbiol 67:2942–2951

Díez B, Massana R, Estrada M, Pedrós-Alió C (2004) Distribution of eukaryotic picoplankton assemblages across hydrographic fronts in the Southern Ocean, studied by denaturing gradient gel electrophoresis. Limnol Oceanogr 49:1022–1034

Dubois A, Orvain F, Marin-Léal JC, Ropert M, Lefebvre S (2007) Small-scale spatial variability of food partitioning between cultivated oysters and associated suspension-feeding species, as revealed by stable isotopes. Mar Ecol Prog Ser 336:151–160

Duplessis MR, Dufour SC, Blankenship LE, Felbeck H, Yayanos AA (2004) Anatomical and experimental evidence for particle feeding in *Lucinoma aequizonata* and *Parvilucina tenuisculpta* (Bivalvia: Lucinidae) from the Santa Barbara Basin. Mar Biol 145:551–561

Durbin EG, Casas MC, Rynearson TA, Smith DC (2008) Measurement of copepod predation on nauplii using qPCR of the cytochrome oxidase I gene. Mar Biol 153: 699–707

Fischer SG, Lerman LS (1983) DNA fragments differing by single base-pair substitutions are separated in denaturing gradient gels: correspondence with melting theory. Proc Natl Acad Sci USA 80:1579–1583

Gast RJ, Dennett MR, Caron DA (2004) Characterization of protistan assemblages in the Ross Sea, Antarctica, by denaturing gradient gel electrophoresis. Appl Environ Microbiol 70:2028–2037

Giovannoni SJ, Britschgi TB, Moyer CL, Field KG (1990) Genetic diversity in Sargasso Sea bacterioplankton. Nature 345:60–63

Jørgensen CB (1996) Bivalve filter feeding revisited. Mar Ecol Prog Ser 142:287–302

Kamermans P (1994) Similarity in food source and timing of feeding in deposit- and suspension-feeding bivalves. Mar Ecol Prog Ser 104:63–75

Kang CK, Lee YW, Choy EJ, Shin JK, Seo IS, Hong JS (2006) Microphytobenthos seasonality determines growth and reproduction in intertidal bivalves. Mar Ecol Prog Ser 315: 113–127

Lehane C, Davenport J (2004) Ingestion of bivalve larvae by *Mytilus edulis*: experimental and field demonstrations of larviphagy in farmed blue mussels. Mar Biol 145:101–107

Loosanoff VL (1949) On the food selectivity of oysters. Science 110:122

López-García P, Rodríguez-Valera F, Pedrós-Alió C, Moreira D (2001) Unexpected diversity of small eukaryotes in deep-sea Antarctic plankton. Nature 409:603–607

Marie D, Zhu F, Balagué V, Ras J, Vaulot D (2005) Eukaryotic picoplankton communities of the Mediterranean Sea in summer assessed by molecular approaches (DGGE, TTGE, QPCR). FEMS Microbiol Ecol 55:403–415

Marín Leal JC, Dubois S, Orvain F, Galois R and others (2008) Stable isotope ($\delta^{13}C$, $\delta^{15}N$) and modelling as tools to estimate the trophic ecology of cultivated oysters in two contrasting environments. Mar Biol 153:673–688

Martin DL, Ross RM, Quetin LB, Murray AE (2006) Molecular approach (PCR-DGGE) to diet analysis in young Antarctic krill *Euphausia superba*. Mar Ecol Prog Ser 319:155–165

Moon-van der Staay SY, De Wachter R, Vaulot D (2001) Oceanic 18S rDNA sequences from picoplankton reveal unsuspected diversity. Nature 409:607–610

Murray AE, Hollibaugh JT, Orrego C (1996) Phylogenetic composition of bacterioplankton from two California estuaries compared by denaturing gradient gel electrophoresis of 16S rDNA fragments. Appl Environ Microbiol 62:2676–2680

Muyzer G, de Waal EC, Uitterlinden AG (1993) Profiling of complex microbial populations by denaturing gradient gel electrophoresis analysis of polymerase chain reaction-amplified genes coding for 16S rRNA. Appl Environ Microbiol 59:695–700

Nejstgaard JC, Frischer ME, Raule CL, Gruebel R, Kohlberg KE, Verity PG (2003) Molecular detection of algal prey in copepod guts and fecal pellets. Limnol Oceanogr Methods 1:29–38

Nejstgaard JC, Frischer ME, Simonelli P, Troedsson C and others (2008) Quantitative PCR to estimate copepod feeding. Mar Biol 153:565–577

Newell RIE (2004) Ecosystem influences of natural and cultivated populations of suspension-feeding bivalve mollusks: a review. J Shellfish Res 23:51–61

Passmore AJ, Jarman SN, Swadling KM, Kawaguchi S, McMinn A, Nicol S (2006) DNA as a dietary biomarker in Antarctic krill, *Euphausia superba*. Mar Biotechnol 8: 686–696

Rouillon G, Guerra Rivas J, Ochoa N, Navarro E (2005) Phytoplankton composition of the stomach contents of the mussel *Mytilus edulis* L. from two populations: comparison with its food supply. J Shellfish Res 24:5–14

Sauriau PG, Kang CK (2000) Stable isotope evidence of benthic microalgae-based growth and secondary production in the suspension feeder *Cerastoderma edule* (Mollusca, Bivalvia) in the Marennes-Oléron Bay. Hydrobiologia 440: 317–329

Shumway S, Selvin S, Shick D (1987) Food resources related to habitat in scallop *Placopecten magellanicus* (Gmelin, 1791): a qualitative study. J Shellfish Res 6:89–95

Suzuki N, Murakami K, Takeyama H, Chow S (2006) Molecular attempt to identify prey organisms of lobster phyllosoma larvae. Fish Sci 72:342–349

van Hannen EJ, van Agterveld MP, Gons HJ, Laanbroek H (1998) Revealing genetic diversity of eukaryotic microorganisms in aquatic environments by denaturing gradient gel electrophoresis. J Phycol 34:206–213

Ward JE, Shumway SE (2004) Separating the grain from the chaff: particle selection in suspension- and deposit-feeding bivalves. J Exp Mar Biol Ecol 300:83–130

Yokoyama H, Tamaki A, Harada K, Koyama K, Ishihi Y, Shimoda K, Harada K (2005) Isotopic evidence for phytoplankton as a major food source for macrobenthos on an intertidal sandflat in Ariake Sound, Japan. Mar Ecol Prog Ser 304:101–116

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