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
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-Garcia 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 phytype 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 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 MgCl₂, 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 H2O 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 MgCl2, 0.8 µM of each amplification primer, 200 µM of each deoxynucleoside triphosphate, 2.5 U Tag 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 each species was removed from subsequent analyses. For feeding selectivity measurements (an intragel comparison), 4 retardation factor (Rf) 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 Rf 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 Rf 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
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 (phytotype 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>
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<th>Band code</th>
<th>Sample source</th>
<th>Sequence length (bp)</th>
<th>Sequence identity (%)</th>
<th>Closest relative</th>
<th>Higher taxon</th>
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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.

<table>
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<tr>
<th>Lanes</th>
<th>Total no. phylotypes detected</th>
<th>Water column phylotypes detected within dietary profiles (%)</th>
<th>Dietary phylotypes detected within water column profiles (%)</th>
<th>Dietary phylotypes not detected within water column profiles (%)</th>
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<td>49.6 ± 9.1</td>
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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 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.Phylootypes 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
phylootypes in other samples. These results suggest that
the dietary profiles obtained through PCR-DGGE are
representative of the organisms ingested and accur-
ately reflect the known feeding behavior of these
bivalves.

It was not our intention to provide a full and com-
plete characterization of dietary components by the
sequencing of DGGE phylootypes, but to simply
ground-truth the profiles themselves. Obtaining pro-
files 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 phylootypes detected within sea-
water profiles. Of the dietary profiles, 66.4 % could be
explained by the presence of matching phylootypes in
the water column, leaving 33.6% unmatched. The
presence of unmatched phylootypes in the dietary pro-
ciles could have several explanations. First, these phy-
lootypes 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 signa-
tures in the dietary profile could be biased towards
organisms that have a longer retention time, if reten-
tion time exceeds the temporal variability of the plank-
ton 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 neces-
sary.

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 sam-
ping to ensure uniform access to the plankton commu-
nity and control for potential effects of spatial hetero-
geneteity. Only the dietary profiles of Mytilus edulis and
Ostrea edulis differed significantly in phylotypic rich-
ness (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 dis-
tinct, distantly related cluster, while the dietary pro-
ciles clustered largely on a species-specific basis, indi-
cating 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 gener-
alist feeding strategy. The self phylootype 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 cannibal-
istic larviphagy, reported to be common in some shell-

Using a PCR-DGGE approach it was possible to
obtain dietary profiles that contained sufficient infor-
mation 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 phylootypes across all species sampled (Fig. 4,
arrows). Although obtained from different PCR reac-
tions and gel runs, these 3 common phylootypes corre-
spond to phylootypes identified as diatoms (Fig. 1b,
arrows). The temporal stability of this trophic pattern is
unknown. Decottignies et al. (2007b) observed a par-
tial 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 qualita-
tive 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 faculta-
tive basis may serve to reduce competition during peri-
ods of seasonal abundance, but further studies are nec-
essary 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 phyotypes. 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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