Carbon isotope signatures reveal that diet is related to the relative sizes of the gills and palps in bivalves

Tanya J. Compton a,b,c,⁎, Rosemarie Kentie b, Andrew W. Storey d, Inka Veltheim d, Grant B. Pearson c, Theunis Piersma a,b

a Department of Marine Ecology and Evolution, Royal Netherlands Institute for Sea Research (NIOZ), P.O. Box 59, 1790 AB Den Burg, Texel, The Netherlands
b Centre for Ecological and Evolutionary Studies, University of Groningen, P.O. Box 14, 9750 AA Haren, The Netherlands
c Western Australian Department of Environment and Conservation (DEC), WA Wildlife Research Centre, P.O. Box 51, Wanneroo, WA 6065, Australia
d School of Animal Biology (M092), The University of Western Australia, Crawley, WA, 6009 Australia

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A B S T R A C T
In marine bivalves, the relative sizes of the gills and palps appear to be a useful functional trait that reflect feeding mode, i.e., suspension feeders have relatively larger gills than palps for pumping, whereas deposit feeders have relatively larger palps than gills for sorting. Also, within a species, the relative sizes of the gills and palps are related to changes in local food conditions. However, there is still no firm evidence showing that differences in the relative gill and palp sizes between species are related to diet selection. Based on the knowledge that carbon and nitrogen isotope signatures of an animal's tissues reflect past diet, we compared the relative gill and palp sizes of bivalves from Roebuck Bay, northwestern Australia with their carbon and nitrogen isotope signatures. The carbon isotope signatures distinguished clear differences in diet between bivalves along a gradient from suspension to deposit feeding, and strikingly this pattern was closely followed by the relative sizes of the gills and palps of the bivalves. This study confirms that relative gill and palp sizes in bivalves are a functional trait that can be used to compare resource use between species. Furthermore, these data may suggest that morphospace occupation, as determined by relative gill and palp sizes of bivalves, could reflect a gradient of resource use between species.

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1. Introduction

Functional traits have been proposed as necessary building blocks needed to provide insights into species adaptations and interactions including ecosystem properties (McGill et al., 2006). A trait is defined as any morphological, physiological or phenological feature measurable at the level of the individual, and a functional trait is a trait that impacts fitness indirectly via its effects on growth, reproduction or survival (Violle et al., 2007). It is expected that the most predictive functional traits will be those that relate directly to resource acquisition (McGill et al., 2006), e.g., beak size and shape in birds (Schoener et al., 1971) and feeding morphology in fishes (Bellwood et al., 2006). A revival of trait comparisons in community ecology should be useful (McGill et al., 2006); as previously, morphological traits have provided insights both as direct and indirect measures of an organism’s performance and/or resource use (Wainwright, 1994).

Contrary to the expectation that benthic bivalve species indiscriminately select their diet based solely on the size of food particles (see review by Ward and Shumway, 2004), there is evidence to show that bivalve species have the potential to select their diet based on particle quality via their feeding processes (Levinton et al., 1996; Ward and Shumway, 2004; Ward et al., 1997). Feeding processes, i.e., food uptake and selection, are optimized by the finely-tuned cooperation between the gills (ctenidia) and labial palps of the bivalves. The main role of the gills is to trap particles from a self-generated water current, and transfer these particles to the labial palps (Jones et al., 1992; Meyhöfer, 1985; Møhlenberg and Røisgard, 1978). The labial palps predominantly sort the organic from inorganic particles (Pohlo, 1967; Yonge, 1949). Organic particles are then accepted into the alimentary canal, whereas inorganic particles are rejected as pseudofaeces. Depending on the gill type in some suspension feeders sorting and rejection of particles can take place on the gill and the role of the labial palps can be reduced (Barillé et al., 2000; Dutertre et al., 2007; Shumway et al., 1985; Ward et al., 1998). Interestingly, the way in which bivalves deal with food quantity and quality appears to be species dependent (Ward et al., 2003).

The capability of the gills and palps to respond to food conditions and distinguish bivalve feeding modes, suggests that these organs are a unique functional trait. Within a species, the size of the gills and labial palps are
known to be flexible (Drent et al., 2004; Piersma and Drent, 2003) and related to changing food conditions over time (Honkoop et al., 2003) and space, e.g. turbidity and silt (Barille et al., 2000; Drent et al., 2004; Essink et al., 2000; Jones et al., 1992; Møhlenberg and Riisgård, 1998), whereas in deposit feeders the relatively larger labial palps than gills suggest sorting is important for purging inorganic material (Pohl, 1967; Reid and Reid, 1969; Young, 1949). Interestingly, the relative size of the gills versus the labial palps in multiple bivalve species form a gradient between suspension and deposit feeders within two tidal flat systems, suggesting that bivalves can filter a wide spectrum of feeding niches (Compton et al., 2007). Thus, relative gill and palp sizes of bivalves are known to relate to food acquisition. However, there is no direct evidence showing that the relative gill and palp sizes reflect diet selection.

Previously, it was not easy to identify how feeding morphology was related to diet in bivalves because: (1) assimilated food sources were difficult to determine (Hummel, 1985; Kamermans, 1994), and (2) some bivalve species easily switch feeding mode, e.g. facultative deposit feeders (Brafield and Newell, 1961; Hughes, 1969; Ölafsson, 1986; Peterson and Skilleter, 1994; Thompson and Nichols, 1988). For example, stomach content analysis suggested that a suspension (Cerastoderma edule) and a deposit (Macoma balthica) feeder were eating similar proportions of the same food sources, i.e. benthic and pelagic algae (Kamermans, 1994). More recently, carbon and nitrogen stable isotopes have shown a clear–cut separation between bivalve species in their assimilated carbon sources (viz. dietary sources) (Decottignies et al., 2007b; Herman et al., 2000; Riera, 2007; Riera and Richard, 1996; Riera et al., 1999), such that the separation between bivalve species in their assimilated carbon sources has been used to test diet selection along estuarine gradients (Riera and Richard, 1996; Yokoyama and Ishii, 2003), and that bivalve diets reflect changing food conditions over time (Decottignies et al., 2007a; Decottignies et al., 2007b).

In this study, the gill and palp size of bivalves were related to the carbon and nitrogen isotope signatures of the bivalves from Roebuck Bay, northwestern Australia. We aimed to test whether the relative sizes of gills and palps of bivalves reflect diet, and thus can be used as a functional
trait for interspecies comparisons. Possible food sources were characterized using carbon and nitrogen isotopes and related to the isotopic signatures of the sampled bivalve species.

2. Materials and Methods

2.1. Collection methods

The field work for this study was carried out in the first two weeks of November 2003. Bivalves were collected from Dampier Flat, located on the tidal flat of Roebuck Bay, northwestern Australia (17°S and 122°E). The site is fringed by mangrove trees (Avicennia marina, Rhizophora sp.), and has tidal ranges that vary between 3.5 m at neap to 8.5 m at spring tide. The area has a wet–dry tropical climate, with a warm, dry season from May to November, followed by a hot, monsoonal–influenced wet season, with most rain arising from cyclonic activity (Rogers et al., 2003). In Roebuck Bay, chlorophyll–a values are generally low and average about 0.7 ± 0.4 mg/l measured over a three year period (Rose et al., 1990).

Aiming to sample a variety of feeding modes, we specifically chose adults of five suspension feeding bivalve species (Anadara granosa, Placamer berryi, Anomalocardia squamosa, Barbatia pistachia, Gafriarum tumidum), three deposit feeding bivalve species (Tellina capsoides, T. piratica and T. litoralis) and a lucinid bivalve (Diaricella irpex) for morphological and stable isotope analyses. One tellinid species (Tellina sp.) was sampled from another location on the tidal flat (One Tree). Species were sampled based on the knowledge of bivalve distributions (Pepping et al., 1999). Sampling was completed outside of the main reproductive periods of the bivalve species concerned (de Goeij et al., 2003, R. Kentie pers obs). For more detail on the sampling location, and sediment parameters at the point of bivalve species collection, please see Table 1 and the map of sampling points in Fig. 1.

Morphological and stable isotope analyses were completed on the same individual in all cases (n = 3 – 5 individuals per species), allowing direct comparison within individuals. The lucinid species was not analysed for its morphology, as it has modified gills and palps (Beelesly et al., 1998) that are likely to host endosymbiotic bacteria (Taylor and Glover, 2006). All species dwell within the sediment, except for B. pistachia which is a rocky intertidal species. Specimens were collected by either careful removal from rocks, passing sediment through a 1–mm sieve, or digging in the mud with hands. After collection all species were placed in ultra–violet irradiated filtered seawater for 48 hours to allow for depuration, prior to dissection.

2.2. Bivalve feeding morphology

It has been established that the mass of the gills and palps are an accurate measure of the size of these three–dimensional organs (Honkoop et al., 2003; Drent et al., 2004). Therefore gills and palps of bivalves were dissected, using surgical forcesps, under a binocular microscope within 48 h of collection. Dissected feeding organs were then placed in platinum crucibles and dried at 60 °C for three days. Following drying, the organs were cooled in a dessicator and weighed to the nearest 0.1 mg (Mettler Toledo AE 160 balance). After weighing, the organs were incinerated at 560 °C for five hours, and left to cool before weighing a second time. The difference between the dried and ashed mass being the ash–free dry mass (AFDM). To observe the variation in feeding morphology between species, we log transformed and ranked the gill–to–palp mass ratios.

2.3. Stable isotope analysis

In this study, stable isotopes of both carbon and nitrogen were measured, as when primary food sources overlap (Fry and Sherr, 1984;
deployed behind a slow moving boat adjacent to the fringes of the tidal
mangrove leaves (Avicennia marina, Rhizophora sp.), and mangrove
coarse particulate organic matter (CPOM) were sampled from the
fringes of the tidal flat. Mangrove-derived medium and fine
particulate organic matter (MPOM and FPOM) were obtained by
elutriating sediment samples in a bucket and passing the suspended
material through 180 μm and 1 mm mesh sieves. Macroalgae were
picked from the tidal flat surface and from objects projecting above
the flats (reef outcrops and stakes). Marine ‘plankton’ (a mixture of
phyto- and zooplankton with some seston/detritus) was collected from
the water column on an incoming tide using nested plankton nets
of three different mesh sizes: (1) 250 μm (x=250 μm), (2) 110 μm
(250 μm×x=110 μm) and (3) 60 μm (110 μm×x=60 μm) that were
deployed behind a slow moving boat adjacent to the flats. The
plankton was concentrated onto a Whatman glass–fiber filter (GF/C)
with a vacuum pump, and the filters frozen for storage. At low tide,
a brown–green sheen progressively developed across the exposed mud
flats, presumed to be diatoms moving to the surface as the tide receded. A two stage process was used to isolate the diatoms. Initially,
where the brown–green colour was most strongly developed, the top
few mm of material was carefully scraped and concentrated into a
container for return to the laboratory. In the laboratory, the sediment
was kept moist and covered by GF/C filters with glass cover slips
placed on top of the filter. A light gradient was then used to attract
the diatoms through the filter and onto the cover slips. The diatoms on
the cover-slip were then dried and scraped into a tin–foil crucible. This
process was repeated until sufficient mass was collected for isotopic
determination (n = 1).

Preparation and measurement of source material was done using
the same methods as the tissue stable isotope analysis, except that the
source material was weighed to ~5.5 mg to allow for lower carbon
content.

2.5. Analysis

Isotopic mass balance equations were used to estimate the range of
possible food sources contributing to the diet of each bivalve species
To estimate the predicted diet values of the bivalves, fractionation values
of 1% c13C and 3.4% c15N were subtracted from the isotopic signatures
of the bivalves prior to analysis. These fractionation values have been
determined previously in two bivalve species (Yokoyama et al., 2005)
and other organisms (Minagawa and Wada, 1984; Rau et al., 1983). In
the Isosource model, the diet of each species was calculated from the
average isotopic food source values: plankton (60–110 μm, 110–250
μm and >250 μm), diatoms and mangrove material (i.e. POM and
mangrove leaf material). Macroalgae were excluded from the model as
they are rare on this tidal flat. To test whether nitrogen isotope
signatures differed between feeding modes a one–way ANOVA was
used. Finally, a linear regression was used to examine whether there
was a significant correlation between the log gill–to–palp mass ratio and
the c13C ratios of the bivalves.

3. Results

At Roebuck Bay, the bivalve species were clearly differentiated into
three groups based on their carbon isotope signatures (Fig. 2, Table 2).
In contrast, the nitrogen isotope signatures (c15N) did not differ between
the suspension (from 6.33 to 7.50‰) and deposit feeding modes
(F1,34 =0.075, P =0.79, from 6.6 to 7.2‰, Table 2). At this site, the
suspension feeders had relatively depleted carbon isotope signatures
in the range of ~16.2 to ~17.0‰, and the deposit feeders had more enriched
carbon isotope signatures with values in the range of ~12.3 to ~13.7‰.

<table>
<thead>
<tr>
<th>Table 2</th>
<th>The mean carbon (c13C) and nitrogen (c15N) isotopic signatures (±SE) for the bivalve species sampled at Roebuck Bay, Dampier Flat in November 2003</th>
</tr>
</thead>
<tbody>
<tr>
<td>species</td>
<td>δ13C (‰)</td>
</tr>
<tr>
<td>Anadara granosa</td>
<td>6.65</td>
</tr>
<tr>
<td>Anomalocardia squamosa</td>
<td>6.77</td>
</tr>
<tr>
<td>Tellina piratica</td>
<td>7.50</td>
</tr>
<tr>
<td>Placamer bernyi</td>
<td>6.44</td>
</tr>
<tr>
<td>Tellina capsoides</td>
<td>6.58</td>
</tr>
<tr>
<td>Tellina sp.</td>
<td>7.20</td>
</tr>
<tr>
<td>Tellina piratica</td>
<td>6.59</td>
</tr>
<tr>
<td>Placamer bernyi</td>
<td>6.33</td>
</tr>
</tbody>
</table>

The number of bivalves per species sampled (n) and their average shell length (±SE) are indicated.

Table 3 | The mean nitrogen (c15N %) and carbon (c13C) isotopic signatures (±SE) of the possible primary food sources collected at Roebuck Bay, Dampier Flat |
<table>
<thead>
<tr>
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</thead>
<tbody>
<tr>
<td>source</td>
<td>δ13C</td>
</tr>
<tr>
<td>Rhizophora sp. (leaf)</td>
<td>−23.92</td>
</tr>
<tr>
<td>Avicennia marina (leaf)</td>
<td>−27.49</td>
</tr>
<tr>
<td>POM (Coarse)</td>
<td>−26.43</td>
</tr>
<tr>
<td>POM (Medium)</td>
<td>−24.91</td>
</tr>
<tr>
<td>POM (Fine)</td>
<td>−23.72</td>
</tr>
<tr>
<td>Macroalgae (green)</td>
<td>−11.04</td>
</tr>
<tr>
<td>Macroalgae (brown)</td>
<td>−13.81</td>
</tr>
<tr>
<td>Plankton (&lt;250 μm)</td>
<td>−19.35</td>
</tr>
<tr>
<td>Plankton (&gt;250 μm)</td>
<td>−13.00</td>
</tr>
<tr>
<td>Plankton (110 μm×x=60 μm)</td>
<td>−5.51</td>
</tr>
<tr>
<td>Diatoms</td>
<td>−6.74</td>
</tr>
</tbody>
</table>

In the table n is the number of replicates collected, and POM represent particulate organic matter.
Notably, each species had a distinct carbon isotope signature (Fig. 2). In a single case, the lucinid bivalve (D. irpex) showed a depleted carbon isotope value of $\delta^{13}C = -23.1\%_o$, as well as a depleted nitrogen isotope value of $\delta^{15}N = -0.9\%_o$ (Fig. 2, Table 2).

The Isosource model calculated the food source contributions to all bivalve species (Tables 3 and 4), except for the lucinid bivalve D. irpex. Notably, the model predicted that the suspension and deposit feeding bivalves were consuming very different proportions of available food sources in this system. Specifically, all suspension feeding species consumed a large proportion of mangrove material (average $\text{POM}=24.6\%$ and average leaf $=24\%$). Only in two suspension feeders (B. pistachia and A. squamosa) was oceanic plankton, in combination with mangrove material, an important dietary component. In the deposit feeding species, benthic diatoms were the most important diet component (average diatoms $=31\%$). The second most important dietary component for the deposit feeders was the smallest plankton fraction in two species (T. piratica and T. sp.) or mangrove particulate organic matter in a single species (T. capsoides).

In this system, the carbon isotope signatures of bivalve species differed both between the suspension and deposit feeders and within each feeding mode (Fig. 3). Notably, in parallel with the clear differentiation of carbon isotope signatures, the log gill–to–palp mass ratio and the carbon isotope signatures ($R^2=0.85, F_{1, 34}=203, P<0.01$, Fig. 3). This resulted in a significant correlation between the log gill–to–palp mass ratio and the carbon isotope signatures ($R^2=0.85, F_{1, 34}=203, P<0.01$, Fig. 3).

**Fig. 3.** The relationship between the log gill–to–palp mass ratios and the carbon isotope signatures ($\delta^{13}C$) is significant ($R^2=0.85, F_{1, 34}=203, P<0.01$), and shows a gradient of resource partitioning between the suspension and deposit feeding modes. The legend includes the species names.
4. Discussion

In this study, carbon isotope signatures clearly distinguished differences in diet both within and between the suspension and deposit feeding bivalves at Roebuck Bay, northwest Australia. Differences in carbon isotope signatures within a single feeding mode are noticeable in species that were sampled within a small distance of each other, and thus where similar food resources should be available (Table 1, Fig. 1). Similar to other dietary studies using bivalves (Riera, 1998, 2007; Rossi et al., 2004), carbon signatures showed greater differences in diet than nitrogen signatures. The small differences in nitrogen isotope signatures suggest that all bivalves belong to the same trophic level, with the exception of D. irpex.

In this system, mangrove detritus and leaf material were the most important dietary components for all suspension feeders, and also an important component for the diet of the deposit feeders. The importance of mangrove detritus and leaf material to the diet of the bivalves is consistent with the close proximity of the bivalve species to the mangrove trees at Dampier flat and also One Tree (see Fig. 1). Furthermore, these results are supported by other studies that have shown plant organic matter can contribute to bivalve diets either directly (Peterson et al., 1985; Riera, 2007; Riera and Richard, 1997) or indirectly via trophic mediation by bacteria (Crosby et al., 1990; Langdon and Newell, 1990). In two suspension feeding bivalves (B. pistachia, A. squamosa) oceanic plankton was also an important dietary component. This is perhaps due to their physical location, which is further away from the mangroves than the other suspension feeders (Fig. 1). Consistent with the knowledge that deposit feeding bivalves gather their food from the surface of the sediment (Polo, 1967; Yonge, 1949), the deposit feeders in this study were mainly assimilating a diet of diatoms. In two of the three deposit feeders (T. pinitica and T. sp.) the smallest plankton fraction (110 µm–x–60 µm) was also an important dietary component. Whether this planktonic food source was obtained directly from the water column or taken from the sediment surface is difficult to ascertain.

A third feeding mode, chemosynthesis, was also identified at this system. In the lucinid species (D. irpex, Lucinidae) negatively depleted carbon and nitrogen isotope signatures identified the host as being a chemosymbiont, i.e. much of the host’s carbon is supplied by endosymbiotic bacteria that obtain their energy from carbon dioxide fixation by the oxidation of reduced sulphur compounds (Dando et al., 1985; Rau, 1981). Note that the depleted nitrogen isotope ratios are expected to reflect the use of pore–water ammonium (Conway et al., 1989).

In parallel with the different carbon isotope signatures of the bivalves, the relative gill and palp sizes of the bivalves in this study fell along a gradient from suspension to deposit feeding. The strong correlation between the relative gill and palp sizes and carbon isotope signatures at this system suggest that: (1) these species can select their diet, (2) they have different diets available to them, (3) a combination of (1) and (2), or (4) that there are differences in isotopic fractionation between species (Gannes et al., 1997; Tieszen et al., 1983). Because isotopic fractionation appears to be similar between the bivalve species studied so far (Dubois et al., 2007; Yokoyama et al., 2005), we do not consider fractionation accountable for the differences in isotopic signatures. Furthermore, as most species were sampled within a close proximity of each other (~250 m), and because the bay is well mixed as a result of large tidal ranges (~8 m), spatial variation in resources may only have a minimal impact on the carbon isotope signatures of the species. However, a spatial component might be present as two species living away from the mangroves, B. pistachia and A. squamosa, had a diet that contained a higher percentage of oceanic plankton. Nevertheless, the strong correlation between the relative size of the gills and palps and the carbon isotope signature of the bivalves strongly suggests that these feeding organs are involved in diet selection. Diet selection is consistent with the knowledge that bivalve species can sort qualitatively different food particles from a mixture using surface tracts on their gills and palps and by detection of the surface properties of the particles (Hemroth et al., 2000; Shumway and Cucci, 1987; Shumway et al., 1985; Shumway et al., 1997; Ward et al., 1997). In addition, preigestive particle processing has also been related to the relative size of the gills and the palps, both within and between species (Dutertre et al., 2007; Velasco and Navarro, 2002).

As bivalve species are known to select their diet via a fine–tuned cooperation between their gills and palps (for review see Ward and Shumway, 2004), it is notable that a strong correlation between the gills and palps of the bivalve species and their carbon isotope signature, which reflects diet, was observed. In light of these results, the continuous gradient of relative gill and palp sizes between suspension and deposit feeders in Roebuck Bay (Compton et al., 2007) could suggest that bivalves select different diets from a shared pool of resources and thus decrease competition for food. Furthermore, the observation that relative gill and palp sizes in bivalves appear to be matched to diet suggests that bivalve feeding morphology is a unique functional trait for examining differences between species (Compton et al., 2007) and organisinal performance (McGill et al., 2006).

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