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ORIGINAL ARTICLE

$\delta^{13}C$ and $\delta^{15}N$ changes after dietary shift in veliger larvae of the slipper limpet *Crepidula fornicata*: an experimental evidence

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Abstract $\delta^{13}C$ and $\delta^{15}N$ measurements are still poorly conducted in benthic invertebrate larvae. To assess the $\delta^{13}C$ and $\delta^{15}N$ changes occurring after a dietary shift, experiments were conducted on veliger larvae of *Crepidula fornicata* fed with two cultured microalgae (*Isochrysis galbana* and *Pavlova lutheri*) of known isotopic composition, ^{13}C-enriched and ^{15}N-depleted compared to the initial values of the larvae. Rapid changes in larval $\delta^{13}C$ and $\delta^{15}N$ were observed after the dietary shift, with an increase in $\delta^{13}C$ and a decrease in $\delta^{15}N$. After 19 days of feeding, isotopic equilibrium was still not reached, a period which is close to the duration of the pelagic life of the larvae. This implies that the isotopic composition measured in field-collected larvae might only partly reflect actual larval feeding but also the parental isotopic signature, especially during the early developmental stages. Isotopic measurements in marine invertebrate larvae should thus be interpreted cautiously. In planktonic food web investigations, the study of field-collected larvae of different size/developmental stage may reduce potential misinterpretations.

Keywords Stable isotopes · *Crepidula fornicata* · Larvae · $\delta^{13}C$ · $\delta^{15}N$

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Introduction

The use of natural stable isotope ratios of both carbon ($\delta^{13}C$) and nitrogen ($\delta^{15}N$) has improved our understanding of food web structures and functioning in marine coastal ecosystems (Peterson 1999). Most isotopic studies deal with field-collected samples of consumers and potential food sources. They thus complement observations of feeding behaviour, gut content analyses, fecal pellets observations and growth studies. However, growing evidence indicates that trophic step enrichment which occurs at each trophic level may vary depending on (1) the efficiency of assimilation of different dietary components, (2) the food quality (e.g. C:N ratio), and (3) the differential allocation of nutrients to specific tissues (Gannes et al. 1997; Adams and Sterner 2000; Post 2002; McCutchan et al. 2003; Yokoyama et al. 2005). Laboratory experiments are thus still needed to assess the validity of the assumptions used to infer food-web relationships (Gannes et al. 1997). In particular, such investigations are needed to determine the time necessary to reach the new isotopic equilibrium between a source and its consumer after a dietary shift, which depends on the metabolic rate of the consumer (Olive et al. 2003).

Experimental data are even more required when dealing with early developmental stages as, for a given species, isotopic fractionation may vary during ontogenesis due to changes in metabolic rate (Hentschel 1998; Rossi et al. 2004). However, isotopic variations in early developmental stages are still poorly documented, despite their role in ecosystem functioning. This is especially true in marine benthic species with a bentho-pelagic life cycle, for which only a few recent

studies have dealt with larvae (Schwamborn et al. 1999, 2002; Rossi et al. 2004; Sommer and Sommer 2004). Such lack of data is mostly related to practical limitations as the need to get a sufficient amount of material for isotopic analyses. To our knowledge, only one study has reported results of feeding experiments, and that with carnivorous decapod larvae fed *Artemia* (Schwamborn et al. 2002), but data for molluscan veliger larvae, which are a major component of the meroplankton, are still lacking.

In the present study, we report the results of feeding experiments on veliger larvae of the slipper limpet *Crepidula fornicata* fed phytoplankton, by using $\delta^{13}C$ and $\delta^{15}N$. Larvae of this species are easy to obtain as females brood their embryos within ovigerous capsules until the veliger stage, and are easy to culture in the laboratory (Pechenik 1980; Pechenik and Lima 1984; Marty et al. 2003). They are large at hatching (ca. 450 μm) and reach ca. 950 μm at settlement. In addition, *C. fornicata* is a key species which has become invasive in numerous areas of Europe causing detrimental effects to these ecosystems (Blanchard 1997; Thieltges et al. 2003). Its reproductive system and dispersal abilities (both natural by larval dispersal and human-mediated) are factors that might explain its invasion success (Dupont et al. 2003; Viard et al. 2006). In this context, the study of its larvae is of major interest (Pechenik 1999).

Materials and methods

Adults of the prosobranch gastropod *Crepidula fornicata* (L.) were collected in the bay of Morlaix (48°40′N, 3°53′E) on 21 June 2002, by dredging on board of N/O Mysis. Larvae released in the laboratory the day after collection were placed in 0.45 μm-filtered seawater. Larvae from several females were pooled but all had been released on the same day (i.e. same developmental stage). Larvae were transferred to three 1.5-l plastic tanks at an initial concentration of 10 larvae ml^{-1} in 0.45 μm-filtered seawater. Oxygenation was achieved with atmospheric air, filtered on 0.2-μm Millex-FG_{50} filters. Water was changed and larvae were fed every 2 or 3 days with either *Isochrysis galbana* T-ISO, *Pavlova lutheri*, or both at a final concentration of 10^5 cells ml^{-1}. Larvae were first fed the day after release. Feeding experiments were stopped after 19 days because (1) larval numbers had decreased due to sampling and mortality, and (2) metamorphosis had begun in a few larvae. The feeding experiment with the mixed diet was stopped already after 11 days due to high mortality.

The two algal strains used as food for *C. fornicata* larvae, *Isochrysis galbana* strain T-ISO (later T-ISO) (Prymnesiophyceae) and *Pavlova lutheri* strain PLY75 (later *Pavlova*) (Pavlophyceae), were obtained from the Roscoff Culture Collection (RCC; http://www.sb-roscoff.fr/Phyto/RCC/) with reference numbers RCC179 and RCC180, respectively. Both strains were cultured (non axenic) in K medium (Keller et al. 1987) in 2-l flasks at room temperature and light. Cultures were aerated by bubbling with atmospheric air, filtered on 0.2-μm Millex-FG_{50} filters. Before collection for larval feeding, algal concentrations were measured using a Malassez cell in order to feed the larvae with the adequate concentration. Carbon and nitrogen isotope ratios of the algal cultures were measured at the beginning of the experiment.

About 950 larvae were collected for stable isotope analyses immediately after their release by the females. A total of 170–300 larvae were further subsampled from each culture tank 2 to 19 days after their release. On day 2, two replicates were subsampled. Larvae were collected by filtration on precombusted Whatman GF/F glass fiber filters. The filters were then briefly acidified with 0.1 N HCl in order to remove carbonates, rinsed with Milli-Q water, freeze-dried and kept at –20°C until analysis.

Carbon and nitrogen isotope ratios were determined using a CHN analyser (ThermoFinnigan 1112 Series) interfaced with a mass spectrometer (ThermoFinnigan MAT Deltaplus) via a Conflow III open split interface. Data are expressed in standard δ-unit notation $\delta X = [(R_{sample}/R_{reference})–1] \times 10^3$, where X = C (carbon) or N (nitrogen) and $R = {}^{13}C/{}^{12}C$ for carbon and ${}^{15}N/{}^{14}N$ for nitrogen. These values are reported relative to the Vienna Pee Dee Belemnite standard (PDB) for carbon and to air N_2 for nitrogen. A laboratory working standard (Peptone) was run for every ten samples. Average reproducibilities based on replicate measurements, using the Peptone standard, for $\delta^{13}C$ and $\delta^{15}N$ were less than ± 0.10‰.

Results and discussion

About 170–300 veliger larvae (>400 μm), corresponding to about 200 μg dry weight of organic material, were needed for an accurate measure of $\delta^{13}C$ and $\delta^{15}N$. These values are close to those of other studies: ca. 200 polychaete and bivalve larvae (Sommer and Sommer 2004), 100–150 late-stage larvae (300–500 μm) of the hydrothermal vent bivalve *Bathymodiolus azoricus* (Trask and Van Dover 1999), 20–85 spionid polychaete

larvae (Hentschel 1998), 20–30 zoeae and 5–10 megalopae of decapods (Schwamborn et al. 2002).

Newly-hatched unfed *Crepidula fornicata* larvae had isotopic values ($\delta^{13}C$ = –21.7‰; $\delta^{15}N$ = 8.2‰) in the range of those typically encountered in marine invertebrates feeding on phytoplankton (e.g. Riera et al. 1996). Although we did not measure the isotopic composition of the females which incubated the larvae, the value obtained for the veligers might reflect that of parental tissues. During the first few days after the dietary shift, substantial changes in larval $\delta^{13}C$ and $\delta^{15}N$ were observed in all the treatments (Fig. 1). This resulted in an increase in $\delta^{13}C$ and a decrease in $\delta^{15}N$ towards the isotopic composition of the newly-offered food (T-ISO, $\delta^{13}C$ = –16.8‰, $\delta^{15}N$ = –1.6‰; *Pavlova*, $\delta^{13}C$ = –19.2‰, $\delta^{15}N$ = 1‰). These changes were likely to result from the assimilation of the newly-provided food rather than starvation. Indeed, it has been reported that starvation often results in an increase in both $\delta^{13}C$ and $\delta^{15}N$ (Gannes et al. 1997; Adams and Sterner 2000; Olive et al. 2003), due to selective respiration of ^{12}C (DeNiro and Epstein 1978) or selective excretion of ^{14}N (Hobson et al. 1993). In contrast, no or little change in isotopic composition in starved animals was observed in larval krill (Frazer et al. 1997), mysids (Gorokhova and Hansson 1999) and bivalves (Yokoyama et al. 2005). However, in our case $\delta^{13}C$ and $\delta^{15}N$ showed opposite variation suggesting that isotopic changes related to starvation are very unlikely.

At the end of the two monoalgal experiments, carbon isotope ratios of the larvae were close to those of their diet, with differences of –1.5‰ between larvae and T-ISO and –0.4‰ between larvae and *Pavlova* (Fig. 1). Considering $\delta^{15}N$ variations, differences of 1.5 and 5.7‰ were observed between larvae and *Pavlova* and T-ISO, respectively (Fig. 1). These differences might reflect an isotopic fractionation and/or an incomplete isotopic equilibrium. Isotopic equilibrium with a new diet will be reached after the consumer has either replaced most of its tissue carbon and nitrogen or has increased greatly in mass. Four- to sixfold weight increases before reaching the isotopic equilibrium have been reported in a variety of animal species (Fry and Arnold 1982; Herzka and Holt 2000), including decapod larvae fed *Artemia* nauplii (sixfold increase after 12 days in *Petrolisthes armatus*; Schwamborn et al. 2002). Previous data on *Crepidula fornicata* larvae reared under similar conditions as those of the present study (temperature: 19–20°C; food: *Isochrysis galbana* at a concentration of 1.2×10^5 cells ml^{-1}) indicated that a sixfold increase in

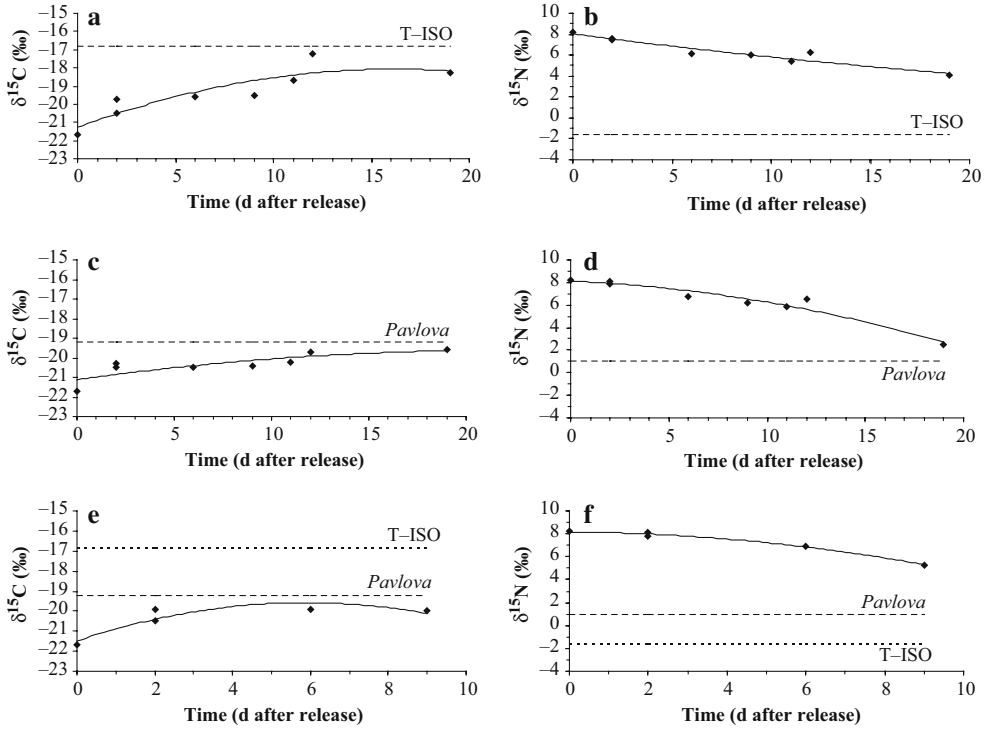

Fig. 1 $\delta^{13}C$ and $\delta^{15}N$ of *Crepidula fornicata* veliger larvae fed on *Isochrysis galbana* T-ISO (**a**, **b**), *Pavlova lutheri* (**c**, **d**), and both (**e**, **f**). *Dashed line* indicates C and N isotope ratios for algal cultures at the beginning of experiment. Note that the duration of the experiment with the algal mix (**e**, **f**) was only 10 days (see text for explanations)

weight was reached 10–14 days after hatching (Pechenik 1980). This suggested that isotopic equilibrium might have been reached at the end of the feeding experiment, although this was not observed from the present data. Whether the isotopic equilibrium was reached or not, did not affect our main result showing changes in both $\delta^{13}C$ and $\delta^{15}N$ in the course of larval development.

These results have strong implications for the interpretation of isotopic measurements in field-collected larvae, a challenging issue in pelagic food web studies. We showed that *C. fornicata* larvae needed at least 19 days before reaching isotopic equilibrium after a dietary shift, a period close to the duration of the free-swimming larval phase (2–3 weeks; Table 1). Larval development might thus be too short to reach isotopic equilibrium with larval food. Schwamborn et al. (2002) reported similar results for larvae of two decapod species, *Sesarma rectum* and *Petrolisthes armatus*. Their feeding experiments showed that isotopic equilibrium was reached after 6–9 days which is close to the duration of the pelagic larval stage of the two species (Table 1). Hence, our results suggest that the C and N isotopic composition measured in field-collected larvae might only partly reflect actual larval feeding, but also their initial (i.e. parental) isotopic signature, especially during the early developmental stages. In the light of these results, data from field-collected larvae should be interpreted with caution when investigating planktonic food webs and the trophic role of invertebrate larvae. In particular, the comparison of isotopic signatures between larvae of species with different larval life duration in order to determine their use of different available food sources may lead to misinterpretations if measurements are made while the isotopic composition is still changing. One way to overcome such problems would be to study larvae of different size/developmental stage within the same sample; this will allow for detecting potential changes in isotopic composition during larval growth in the field. Future investigations should also focus on the comparison between larval and parental isotopic composition because isotopic changes in larvae will depend on their value at the time of release. This is of particular interest in brooding species (like *C. fornicata*), in which larval isotopic composition could be different from that of the parents due to metabolic processes during brooding.

Table 1 Larval life duration of marine invertebrates for which feeding experiments with isotopic measurements at the larval stage are available (in Schwamborn et al. (2002) for *S. rectum* and *P. armatus*, in this study for *C. fornicata*)

	Duration (days)	Source
Decapod zoeae		
Sesarma rectum	9	Diesel et al. (2000)
Petrolisthes armatus	11–13	Gore (1970, 1972)
Molluscan veligers		
Crepidula fornicata	14–21	Coe (1949)[a]
	12–24 (18.5°C)	Ament (1979)
	11.3±2 (20°C)	Pechenik et al. (1996)[b]

[a]Original citation: 2–3 weeks

[b]Metamorphosis triggered by KCl supply

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