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Lack of dietary specialization in adult *Aplysia californica*: evidence from stable carbon isotope composition

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Natural abundance, $^{13}C/^{12}C$ ratios ($\delta^{13}C$) of Californian sea hares, *Aplysia californica*, (Gastropoda: Opisthobranchia) were measured for comparison with the animals preferred red algal diets, *Plocamium cartilagineum* and *Laurencia pacifica*. Isotopic compositions of animals collected from around Santa Catalina Island, did not reflect those of the favoured red algal diets, nor the majority of algal species found in the field. However, animals held in seawater tanks and fed diets with a constant carbon isotopic composition exhibited $\delta^{13}C$ values similar to their food. Fast turnover tissue, such as the muscle tissue of actively growing juveniles or egg masses laid by adults, showed $\delta^{13}C$ values within ±2‰ of the algal diet. Slow turnover tissue, such as the muscle tissue of large adults, reflected the diet over several months. Stable carbon isotope ratios thus proved a useful tool to clarify the extent to which specialized feeding in *A. californica* can occur upon algae which are only moderately to rarely common.

INTRODUCTION

The Californian sea hare, *Aplysia californica* Cooper, is a large, marine, herbivorous opisthobranch mollusc which is common along the west coast of the United States. Halogenated secondary metabolites and pigments are obtained from its red algal diet and are incorporated into body tissue and ink, potentially serving as protective mechanisms against predators (Nolen et al., 1995; Pennings, 1990a). Thus, chemical defence may be an important factor contributing to the dietary choice of sea hares. Laboratory experiments have demonstrated that juvenile *A. californica* are specialist feeders, consuming only the red algae *Plocamium cartilagineum* (L.) P.S. Dixon and *Laurencia pacifica* Kylin (Pennings, 1990a,b). Adults have been shown to feed and grow on more species than juveniles (Pennings, 1990a,b, 1991), including the green algae *Ulva* sp. and *Codium fragile* (Sur.) Hariot. However, actual patterns of resource use in the field are not well known.

At Santa Catalina Island, California, adult *Aplysia californica* show a patchy distribution but large aggregates of individuals are commonly observed (personal observation). Subtidal reefs in this area are dominated by a variety of brown algae including *Sargassum palmerii* Grunow, *Dictyopteris undulata* Holmes, *Dictyota flabellata* (Coll.) Setchell & Gardner, *Zonaria farlowii* Setchell & Gardner and *Cytoseira osmundacea* (Turn.) C. Agardh (Pennings, 1990a,b). In contrast, the favoured diets of *P. cartilagineum* and *L. pacifica* almost never comprise more than 10% and 1% respectively of the total macroalgal bottom cover on rocky reefs (Pennings, 1990a,b, 1991). *Ulva* sp. and *Codium fragile* are also rare (personal observation). Therefore, it appears that the majority of algal species in the underwater environment of Santa Catalina would not support growth of *A. californica*.

A number of terrestrial studies have shown that the distinct stable carbon isotope signatures of C_3 and C_4 plants ($\delta^{13}C$ values of −21 to −35‰ and −10 to −14‰ respectively, Smith & Epstein, 1971) are reflected in the carbon isotopic composition of animals which derive their carbon source primarily from one, or other, of the photosynthetic types (Minson et al., 1975; Haines, 1976; DeNiro & Epstein, 1978). In the marine environment, macroalgae, which have C_3 type photosynthesis, show a wide range of $\delta^{13}C$ values from −2.5 to −35‰ for different species (Maberly et al., 1992; Raven et al., 1995; Raven, 1997). A number of studies in the marine environment (reviewed in Fry & Sherr, 1984) have also tested the isotopic similarity between consumers and their foods and have found that animals usually have $\delta^{13}C$ values within ±2‰ of their food source.

Of the preferred red algal diets of *A. californica*, *P. cartilagineum* is isotopically depleted in ^{13}C resulting in a very distinct $\delta^{13}C$ value of approximately −32‰. In comparison to *Plocamium*, *L. pacifica* is enriched in ^{13}C with values typically around −14‰. Brown algae comprise the majority of algal species around Santa Catalina Island, and the $\delta^{13}C$ values for Phaeophyceae collected from the wild are in the range of −10 to −28‰ (Maberly et al., 1992; Raven et al., 1995; Raven, 1997). Therefore, carbon isotope analysis may clarify the extent to which specialized feeding in *A. californica* can occur upon rare species.

This paper examines whether natural abundance ratios of the herbivore *A. californica* reflect that of its diet using a combination of field and laboratory animals of different age-classes. The results suggest that neither *P. cartilagineum* nor *L. pacifica* alone can account for the isotopic composition of adult sea hares in the field. The ecological implications of these findings are discussed in terms of food availability.

Table 1. *$\delta^{13}C$ values of* Aplysia californica *and field collected algae. Values are given as the mean ±1 SD.*

	N	δ^{13}C (‰)
Sea hares		
Adults—field diet	8	−19.96 ±0.34
Adults—*L. pacifica* diet	6	−18.43 ±0.72
Juveniles—*L. pacifica* diet	3	−15.88 ±1.17
Adults—*P. cartilagineum* diet	9	−25.31 ±1.09
Eggs—*P. cartilagineum* diet	9	−29.84 ±0.77
Algae		
Codium fragile	*	−12.59
Colpomenia peregrina	3	−12.91 ±0.43
Laurencia pacifica	6	−14.36 ±1.14
Plocamium cartilagineum	6	−31.94 ±0.43
Rhodymenia californa	3	−29.80 ±0.51
Sargassum palmerii	3	−13.25 ±0.67
Zonaria farlowii	3	−13.66 ±0.47

*, value taken from Maberly et al., 1992.

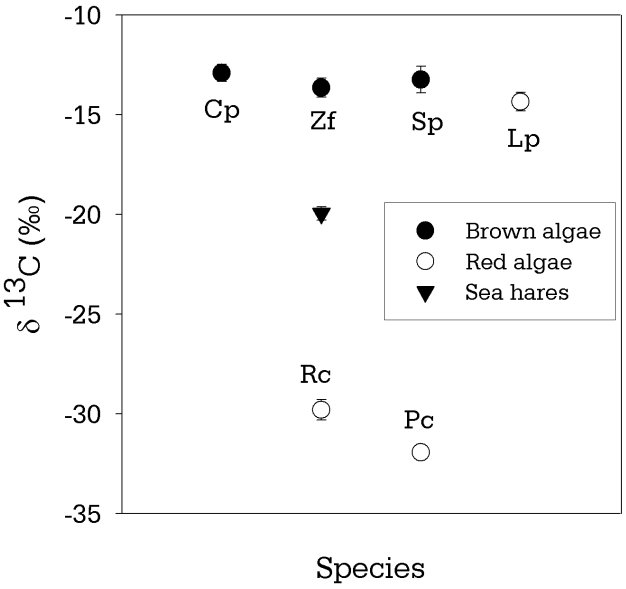

Figure 1. Mean δ^{13}C values (±1 SE) of adult *Aplysia californica* (N=8) and macroalgae (N=3) collected from Santa Catalina Island. Closed circles represent brown algae, open circles red algae, and triangles represent animal tissue. Algae tested for isotopic composition were *Colpomenia peregrina* (Cp), *Zonaria farlowii* (Zf), *Sargassum palmerii* (Sp), *Laurencia pacifica* (Lp), *Rhodymenia californa* (Rc), and *Plocamium cartilagineum* (Pc).

MATERIALS AND METHODS

Adult *Aplysia californica*, approximately 20–30 cm in length, were collected in May 2000 using SCUBA from a shallow reef (~8 m depth) near the Wrigley Institute for Environmental Studies, Santa Catalina Island, California (33°27′N 118°29′W). The reason for using this particular site was that large numbers of animals could be easily found. Animals were placed in cool boxes at ambient water temperature (~19°C) and immediately transported to the laboratory. Upon arrival at the laboratory, sea hares were transferred to holding tanks with running seawater. Eight individuals were randomly chosen from the tanks and a small piece of tissue removed from the muscular foot for stable carbon isotope analysis. These animals were then returned to the field. The remaining animals were used for feeding experiments.

Macroalgal samples were also taken from the collection site for stable carbon isotope analysis. Common algae on the reef included the browns, *Colpomenia peregrina* (Sauv.) Hamel, *Sargassum palmerii*, and *Zonaria farlowii*. Neither *Plocamium cartilagineum* nor *Laurencia pacifica* were found at the collection site; the only red alga found on the reef was the small *Rhodymenia californa* Kylin covering less than 0.1% of the substratum.

Feeding experiments—adults

For experimental treatments, ten *Aplysia californica* were placed individually in shaded outdoor tanks, dimensions of 59×85×28 cm (0.14 m^{-3}), with a running seawater system. Adults were fed a packed volume of approximately 1 l of *P. cartilagineum* per day, collected from reefs in the vicinity of the Wrigley laboratory. Samples of this alga were taken for stable carbon isotope analysis. The daily food allowance was sufficient to allow the animals to increase or maintain weight. Blotted wet weight was measured once a week. Tanks were cleaned thoroughly every day to remove faecal matter, detritus, eggs and diatoms. Animals were held under these conditions for 60 days at which time a tissue sample for stable carbon isotope analysis was taken from the muscular foot. Samples for carbon isotope analysis were also taken from eggs laid 46 days from the start of the experiment, as well as from the macroalgal diet.

In an experiment examining the possibility of maintaining adult sea hares on a *Laurencia* diet, a further ten adult *A. californica* were maintained in running seawater tanks, with a diameter of 155 cm and a depth of 82 cm (1.5 m^{-3}), at a stocking density of five animals per tank. The limited number of aquaria available prevented proper replication of this experiment. To minimize tank effects, animals were rotated between tanks on a daily basis. A packed volume of approximately 1 l of *L. pacifica* per animal was placed into each tank daily. While the animals readily consumed this alga, it is not common around Santa Catalina and this experiment had to be terminated after 14 days. At this time, a small piece of tissue was removed from the muscular foot area and prepared for stable carbon isotope analysis. Samples for carbon isotope analysis were also taken from the macroalgal diet.

Feeding experiments—juveniles

Plocamium cartilagineum was collected every 2–3 days from reefs in the vicinity of the Wrigley laboratory and maintained in tanks with running seawater. Periodically juveniles, 1–3 cm in length, would be found in these tanks. These animals would be removed and placed into individual circular tanks (12×30 cm) with running seawater. Thereafter, juveniles were fed a diet of *L. pacifica*. Blotted wet weight was measured once a week. Tanks were cleaned daily and fresh algae replaced after cleaning. After two weeks, animals were starved for 24 hours and then sacrificed by freezing at −80°C. Whole animals

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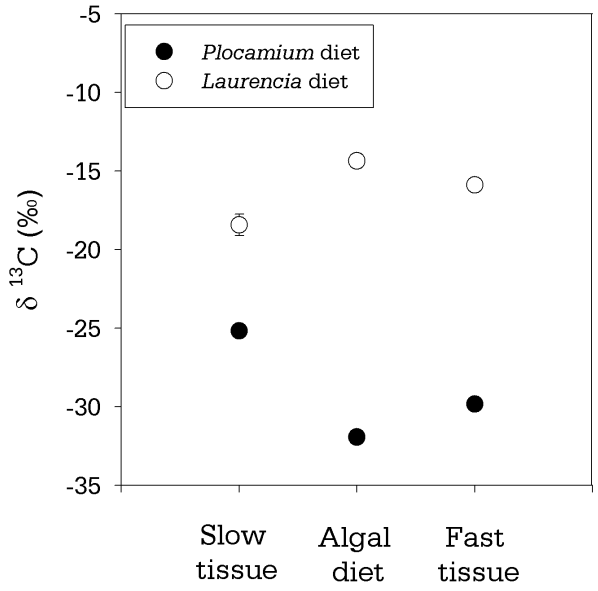

Figure 2. Mean δ^{13}C values (±1 SE) of *Aplysia californica* fed on diets of either *Laurencia pacifica* or *Plocamium cartilagineum*. The term 'fast' tissue indicates samples taken from egg masses (*Plocamium* diet, N=9) or juveniles (*Laurencia* diet, N=3), 'slow' tissue indicates muscle tissue from adults (N=6).

Table 2. *Growth rates of adult (mean values ±1 SD, N=9) and juvenile (individual values)* Aplysia californica *fed on red algal diets of either* Plocamium cartilagineum *or* Laurencia pacifica. *Values represent the growth rate of the individual over the whole time it was held in captivity. Growth rates were not measured for adults fed on* Laurencia.

Life stage	Diet	Growth rate (% d^{-1})
Adult	*Plocamium*	0.78 ±0.24
Juvenile 1	*Laurencia*	5.643
Juvenile 2	*Laurencia*	14.591
Juvenile 4	*Laurencia*	12.07

were prepared for stable carbon isotope analysis, as well as samples of their diet.

Stable isotope analysis

Algal specimens were freed of extraneous organisms. All samples for isotope analysis were rinsed in distilled water and dried at 80°C. Dried material was ground and approximately 0.35 mg samples were analysed. Mass spectrometry was performed at the University of Southern California, Los Angeles on a Micromass Isoprime isotope ratio mass spectrometer equipped with an elemental analyser. The $^{13}C/^{12}C$ of the samples are expressed as δ^{13}C values in parts per thousand (‰):

$$\delta^{13}\mathrm{C}(‰) = \frac{R_{sample}}{R_{standard}} - 1 \times 1000 \quad (1)$$

where R_{sample} is the $^{13}C/^{12}C$ ratio of the sample and $R_{standard}$ is the $^{13}C/^{12}C$ ratio of a secondary standard, related back to the standard reference material of Pee Dee Belemnite carbonate.

Statistical analysis

The δ^{13}C values of sea hares and potential algal diets from the field were compared using one-way analysis of variance. Significant differences were determined using Tukey tests. A Student's *t*-test was used to compare the δ^{13}C values of field and laboratory animals, as well as to compare laboratory animals and their algal food sources. For all tests, significance was tested at $P<0.05$.

RESULTS AND DISCUSSION

Animals generally bear a close isotopic resemblance to their diets as isotopic fractionations during assimilation and respiration are small (De Niro & Epstein, 1978). Marine animals resemble dietary values within ±0.7‰ (Fry & Sherr, 1984). This level of variability is small when compared to the differences in δ^{13}C values seen between food sources. The dominant brown, macroalgal species collected from the subtidal rocky reefs near the Wrigley laboratory showed little variation in carbon isotope ratios with values ranging less than 1‰, from −12.91 to −13.66‰ (Table 1). Mean δ^{13}C value of the red alga, *Laurencia pacifica*, was not significantly different ($P>0.05$, Tukey tests) to that of the browns at −14.36‰. In contrast, the δ^{13}C values of the reds *Plocamium cartilagineum* and *Rhodymenia california* were significantly ($P<0.05$, Tukey tests) more negative than any of the other algae analysed, at −31.94 and −29.80‰, respectively. The δ^{13}C values of adult *Aplysia californica* (−19.96‰, Table 1) collected from the field did not closely reflect the isotopic composition of the major carbon sources in their immediate natural environment, nor did they approximate the values of the supposed, preferred red algal diets. Instead, the isotopic composition of animals collected from the field fell between the algal extremes (Figure 1) and were significantly different ($P<0.05$, Tukey tests) to all possible food sources.

The isotopic composition of animals provides a good overall idea of the average diet over the previous several weeks or months (Gearing, 1990). Animals usually have δ^{13}C values within ±2‰ of their foods (Fry & Sherr, 1984). When multiple types of food are available, isotope ratios can indicate, but not prove, that a certain type of food was assimilated. While many laboratory studies have shown that *A. californica* preferentially feeds on *P. cartilagineum* (Pennings, 1990a,b, 1991) and to a lesser extent *L. pacifica*, actual feeding patterns in the field are not well known. The results of this study indicate that adult sea hares, at the particular reef sampled, were not feeding exclusively on either *Plocamium* or *Laurencia*. This result cannot simply be attributed to source variability. Although a few field populations of macroalgae have shown large differences in δ^{13}C values within species (Simsenstad et al., 1993), natural populations of *Plocamium* show little variation in δ^{13}C values regardless of its origin (Raven, 1997). Indeed, little variability was found in any of the field collected algae in this study (Table 1). To fully interpret the field

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data, it is vital to consider the biology of the animal and its environment.

In the laboratory, adult *A. californica* grew well on its preferred red algal diets as well as the green algae, *Codium fragile*, but not on other species of algae (mainly the Phaeophyceae) common around Santa Catalina Island (Pennings, 1990a). Of these favoured algae, *P. cartilagineum* is the only moderately common species found around the island. Simple mixing equations (Gearing, 1990) were used to estimate carbon contributions of the preferred algal diets to animal tissue with the assumption that only these diets were being consumed:

$$\mathrm{Fx} = \frac{\delta^{13}\mathrm{C\ animal} - \delta^{13}\mathrm{C\ algae\ y}}{\delta^{13}\mathrm{C\ algae\ x} - \delta^{13}\mathrm{C\ algae\ y}} \quad (2)$$

where algae y=*Plocamium cartilagineum* (−31.94‰) and algae x=*Codium fragile* (−12.59‰) or *Laurencia pacifica* (−14.36‰).

A δ^{13}C value of −19.96‰ for adult sea hares would require approximately 62–68% of body carbon to be derived from *C. fragile* or *L. pacifica* and only 32–38% from *P. cartilagineum* (a δ^{13}C value of −12.59‰ for *C. fragile*, taken from Maberly et al., 1992, was used in the mixing equation). Clearly this is not likely, given the availability of these diets and the fact that adults eat prodigious quantities of algae, being capable of completely consuming small patches of *Plocamium* in the field (Pennings, 1990b). It is possible that the reef sampled in this study had been cleared of *Plocamium* in such a manner. The addition of ^{13}C-rich algae to the diet would increase the proportion of carbon which would have to have been contributed by *Plocamium*. For example, consumption of an alga such as *Codium hubsii* (found in the lower intertidal at Santa Catalina Island) with a δ^{13}C value of −8‰ (J. Raven, personal communication) would then require 50% of carbon assimilation to be derived from *Plocamium*. While there are no data confirming whether or not *C. hubsii* may be eaten by sea hares, even if it were to be consumed, it is unlikely to be a major carbon source to adult *Aplysia* due to its small size and relative scarcity. Similarly, microalgal turfs may be eaten by *Aplysia* but could not solely support growth of such a large animal. While the use of isotope ratios in this study could not determine the exact diet of *A. californica* in the field, it did indicate that the animals did not feed exclusively on *Plocamium*. Field data was supported by studies on captive animals. Adult sea hares fed on a diet of *P. cartilagineum* or *L. pacifica*, had δ^{13}C values significantly different (P values <0.05, t-test) to adults collected from the field (Table 1).

Captive animals showed isotopic compositions similar to algal diets (Figure 2). However, δ^{13}C values of muscle tissue from adults remained approximately 4 and 7‰ different from the algal diets of *L. pacifica* and *P. cartilagineum*, respectively (Table 1). Egg masses laid by adults proved to be a better indicator of a *Plocamium* diet; δ^{13}C values were only 2‰ different from the algae (Table 1).

Aplysia species are some of the biggest opisthobranch molluscs in the marine environment. These large animals are annuals which die in late autumn (Audesirk, 1975) and reproduce mainly in the summer and autumn. Adults collected for this study were approximately 20–30 cm in length, weighing approximately 0.75–1 kg wet mass. Growth rates remained low throughout the study period at approximately 0.8% d^{-1} (Table 2). In addition, the feeding experiment was carried out during June and July and egg masses were frequently found in tanks. It is probable then, that the majority of newly assimilated carbon was being invested into reproductive, rather than cellular, constituents.

While muscle tissue constitutes the majority of the body mass of sea hares, in adults it may be defined as a slow turnover tissue indicating the average isotopic composition of diet over the long term (Tieszen et al., 1983). It is likely that if the feeding experiments had been extended, tissue samples would increasingly reflect that of assimilated carbon sources. In contrast, egg masses may be defined as a fast turnover tissue providing a useful indicator of diet on a short timescale. Unlike adults, muscle tissue samples from rapidly growing juveniles may also reflect newly assimilated food sources.

Juveniles are highly susceptible to predation, especially by navanax (*Aglaja inermis*), a specialist feeder on opisthobranchs, and their rapid growth may be viewed as a mechanism to escape a vulnerable life stage (Nolen, 1995). Juvenile sea hares fed on *L. pacifica* doubled their weight within the first week of feeding on a controlled diet, with growth rates ranging between 6 and 14% d^{-1} during the experimental period (Table 2) and isotopic composition of the muscle tissue was not significantly different (t-test, $P>0.05$) to that of their algal diet (Figure 2, Table 1). Clearly, the majority of assimilated carbon was being allocated to body growth.

This study demonstrates that natural abundance, stable carbon isotopes can be a useful, non-destructive method for examining dietary preferences of specialist feeders such as *Aplysia californica*. Although the precise diet of adult sea hares in the field could not be determined, isotope ratios provided evidence that these animals had not been feeding on either *P. cartilagineum* or *L. pacifica* alone. Indeed the reef from where they were collected was devoid of any preferred algal diet and it is possible that the animals had completely consumed any *Plocamium* available and then subsisted on other seaweeds later on. Alternatively, the animals may have been eating seaweeds which were not tested for isotopic composition. It would be interesting to examine the isotope ratios of animals from reefs with more *Plocamium* coverage as during the time of the present study, reefs with a high coverage of *Plocamium* or *Laurencia* were devoid of *Aplysia*. In addition, while muscle tissue reflects the average diet over many months, examination of egg masses found in the field may be a useful indicator of animal diet in the short term as well as switching to an isotopically novel diet.

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