



## FAU Institutional Repository

<http://purl.fcla.edu/fau/fauir>

This paper was submitted by the faculty of FAU's Harbor Branch Oceanographic Institute

Notice: ©2004 Elsevier B.V. This is the author's version of a work accepted for publication by Elsevier. Changes resulting from the publishing process, including peer review, editing, corrections, structural formatting and other quality control mechanisms, may not be reflected in this document. Changes may have been made to this work since it was submitted for publication. The definitive version has been published at <http://www.elsevier.com/locate/jembe> locate and may be cited as Barile, Peter J., Brian E. Lapointe, Thomas R. Capo (2004) Dietary nitrogen availability in macroalgae enhances growth of the sea hare *Aplysia californica* (Opisthobranchia: Anaspidea), *Journal of Experimental Marine Biology and Ecology* 303(1):65–78 doi:10.1016/j.jembe.2003.11.004

# Dietary nitrogen availability in macroalgae enhances growth of the sea hare *Aplysia californica* (Opisthobranchia: Anaspidea)

Peter J. Barile<sup>a,\*</sup>, Brian E. Lapointe<sup>a</sup>, Thomas R. Capo<sup>b</sup>

<sup>a</sup>*Division of Marine Science, Harbor Branch Oceanographic Institution, 5600 US 1, Ft. Pierce, FL 34946, USA*

<sup>b</sup>*Division of Marine Biology and Fisheries, Rosenstiel School of Marine and Atmospheric Sciences, University of Miami 4600 Rickenbacker Causeway, Miami, FL 33149, USA*

---

## Abstract

Evidence suggests that marine herbivores select for prey items with elevated nitrogen content. We tested this hypothesis with experimental growth studies of the herbivorous gastropod *Aplysia californica* offered diets of the rhodophyte *Gracilaria ferox* with varying nitrogen content. *A. californica* had a sevenfold feeding preference by weight for *G. ferox* with higher tissue % N and % protein, and lower % C, % carbohydrate and carbon-to-nitrogen ratio (C/N) as compared to treatments with lower nitrogen and protein content. *A. californica* provided treatment diets of varying nitrogen availability (high, medium and low) also had significantly different growth rates within 30 days. The high N diet supported a two-fold higher growth rate than that of the medium N diet and four-fold higher than the low N treatment, with mean weights over the experiment reaching 20.4, 9.8, and 5.6 g, respectively. The evolutionary value of this dietary preference may facilitate faster growth to reproductive size, outgrowth of predators and sequestration of organic compounds from nitrogen-enriched macroalgae to deter predators. Despite recent attention, the importance of diet-derived compounds as anti-predatory chemical defenses in Aplysiids remains equivocal. Nitrogen-enriched macroalgal diets, however, may provide “primary metabolites” that serve as alternative defenses for Aplysiids.

*Keywords:* Nitrogen; Diet composition; *Aplysia*; *Gracilaria*

---

---

\* Corresponding author: Tel.: +1-772-465-2400x325; fax: +1-772-468-0757.

E-mail address: pbarile@hboi.edu (P.J. Barile).

## 1. Introduction

The importance of food quality of prey items in herbivore-plant interactions has been recognized in marine food webs for several decades (Paine and Vadas, 1969; Carefoot, 1973; Himmelman and Carefoot, 1975). However, little attention has been given to understanding the importance of specific biochemical constituents of prey and their relation to secondary production in marine ecosystems, particularly for the Aplysiid gastropods (Carefoot, 1979). In contrast, diet formulation is an important facet of most marine aquaculture activities and considerable attention has been focused on the development of diets that result in maximum growth rate for food production. In particular, diets of varying protein to carbohydrate ratios are formulated as a function of life history, in order to maximize growth in culture conditions (Guillaume et al., 2001).

In the marine environment, nitrogen is generally recognized as a primary limiting nutrient not only for plant production, but also for herbivores consuming such prey (Mattson, 1980; Sterner and Hessen, 1994). In the marine ecology literature, Tenore (1977) first recognized that irrespective of total caloric content, the most important determinant for secondary production is the amount of nitrogen content in prey items. In feeding assays, biomass of the polychaete *Capitella capitata* increased to maximum levels as a result of a diet composed of the high nitrogen content red alga *Gracilaria* as compared to lower nitrogen diets of either saltmarsh grass, seagrass, or the brown alga *Fucus*. More recently, several workers demonstrated in the field that herbivores selectively feed upon macrophytes with enriched N biochemistry (Vince et al., 1981; Yates and Peckol, 1993; Hauxwell et al., 1998). However, prey preference of marine herbivores under field conditions may become uncoupled with the specific biochemical value of prey as a result of the effects of morphology (Littler and Littler, 1980; Nicotri, 1980; Steneck and Watling, 1982), chemical defenses (Hay and Fenical, 1988) and habitat refugia (Duffy and Hay, 1991; Wakefield and Murray, 1998).

While the availability of nitrogen in the marine environment is known to affect growth rates and biochemistry of macroalgae, much less is known about how the storage of protein versus carbohydrate ratios within the plant tissues translates to secondary production. Analyses of tissue biochemistry in the rhodophyte *Gracilaria foliifera* by Lapointe and Ryther (1979), Lapointe (1981) and Lapointe and Duke (1984) all indicated that when cultured under saturating N and P conditions, carbon-to-nitrogen ratio (C/N) ratios decreased below 10, while protein content and concentration of the pigments phycoerythrin and chlorophyll *a* increased. In contrast, when grown under N-limited conditions, C/N ratios and carbohydrate content increased with corresponding decreases in N-based photosynthetic pigments. Based on the dynamics of protein and carbohydrate synthesis reported in the literature for *Gracilaria* sp., nitrogen enrichment of prey macroalgae should support increased herbivore growth. Here, we tested the hypothesis that *Gracilaria ferox* cultured under conditions of nitrogen enrichment (C/N < 10) will cause measurable growth increase in the herbivorous opisthobranch gastropod *Aplysia californica* (Aplysiidae) relative to growth with nitrogen-limited (C/N > 20) macroalgal diets.

## 2. Methods

### 2.1. Macroalgae culture and tissue analysis

The rhodophyte *G. ferox* J. Agardh was cultured in outdoor 180-l aquaria according to the methods of Lapointe and Ryther (1979). Thalli from stock cultures maintained at the National Institutes of Health (NIH) National Resource for Aplysia at the Rosenstiel School of Marine and Atmospheric Sciences, University of Miami, were used to initiate the cultures (see Capo et al., 1999 for description of culture conditions). Thalli were cleaned of visible epiphytic growth, stocked in experimental aquaria at a density of 2 kg wet wt. m<sup>-2</sup>, and seawater flow rates adjusted to six turnovers per day. Three nutrient enrichment treatments were established using these cultures. First, ambient seawater alone was used for culture media for the low N treatment. These cultures were maintained under high light (full natural irradiance) to induce nitrogen deficiency (C/N>20) by growth stimulation. A second treatment (medium N) with pulsed nutrient concentrations to simulate eutrophic conditions in the field (3 μM DIN, 0.3 μM SRP) was established with 50% light attenuation by neutral density screening. A third treatment (high N) consisted of growth-saturating nutrient concentrations (50 μM DIN and 10 μM SRP) with 50% light attenuation with neutral density screening. The two nutrient-enriched treatments (medium N and high N) were pulsed once per week to yield ample resource allocation for maximum growth of this macroalga and to avoid a constant nutrient residual that leads to epiphytic overgrowth (Lapointe, 1985). Because *Gracilaria* spp. can store major macronutrients for up to 2 weeks (Lapointe and Ryther, 1979), the cultures were grown for 14 days in advance of feeding trials with the *Aplysia* to subvert antecedent nutritive conditions.

Cultures composed of the three nutrient enrichment treatments described above were maintained from 15 June–5 August 2000 for the feeding trials. On a weekly basis during the *Aplysia* culture experiment, composite macroalgal tissue samples (>5 thalli) from each of the three treatments were cleaned of epiphytes, rinsed in deionized water and dried in an oven at 60° C for 48 h. Dried thalli were ground with a mortar and pestle and analyzed for C/N ratios on a Carlo–Erba elemental analyzer at the Chesapeake Biological Laboratory. Dried tissue was analyzed for soluble carbohydrate by the Dubois Phenol-Sulfuric Acid Method (Dubois et al., 1956) using a D-glucose standard and for soluble protein composition using the Bradford (1976) method with bovine serum albumin as a standard.

### 2.2. *Aplysia* culture and experimental design

Juvenile specimens of the opisthobranch gastropod *A. californica*, from the NIH National Resource for Aplysia, University of Miami, were utilized for feeding trials. At this facility, *A. californica* veliger larvae were homogeneously cultured in an incubator for 60 days until settlement and metamorphosis. Significant growth responses can be distinguished within the first 30 days of culture after settlement, before energy is shunted toward gametogenesis (Capo et al., 2002). Cultures were maintained in polycarbonate aquaria (~ 12 l) filled with filtered (50 μm) seawater from Bear Cut

Table 1  
Tissue biochemistry for *G. ferox* cultures sampled from 13 July–4 August 2000

Date	Treatment														
	High N					Medium N					Low N				
	% N	% C	% Protein	% Carb.	C/N	% N	% C	% Protein	% Carb.	C/N	% N	% C	% Protein	% Carb.	C/N
13 July	2.74	19.2	17.13	48.41	7.0	1.92	17.5	12.02	46.75	9.1	1.15	26.7	7.19	76.09	23.2
20 July	3.63	23.8	22.68	51.62	6.6	1.7	20.0	10.63	43.41	11.8	1.04	25.8	6.50	73.78	24.8
28 July	3.82	24.1	23.88	53.02	6.3	1.56	21.8	9.75	50.14	14.0	0.97	29.2	6.06	80.22	30.1
4 August	2.61	19.9	16.31	41.79	7.6	1.58	24.2	9.83	55.66	15.3	1.1	29.6	6.88	81.36	26.9
Mean ± S.E.	3.20 ± 0.31	21.75 ± 1.28	20.00 ± 1.92	48.71 ± 5.00	6.9 ± 0.4	1.69 ± 0.09	20.87 ± 1.42	10.56 ± 0.53	48.99 ± 2.61	12.6 ± 2.1	1.07 ± 0.04	27.83 ± 0.93	6.66 ± 0.24	77.86 ± 1.77	26.3 ± 2.3

Percent dry weight composition for nitrogen (N), carbon (C), protein, carbohydrate (carb.), and C/N are presented for cultures under high, medium and low nitrogen conditions. Means ± S.E. are presented for each parameter for treatments.

Table 2

Results of one-way ANOVA for tissue % nitrogen (N), % carbon (C), % protein, % carbohydrate, and C/N of *G. ferox* when compared as high, medium and low nitrogen culture treatments

Source	df	MS	F	p
% Nitrogen	2	4.82	35.9	<0.0001*
Residual	9	0.14		
% Carbon	2	57.32	9.53	0.006 <sup>#</sup>
Residual	9	6.01		
% Protein	2	188.26	35.20	<0.0001*
Residual	9	5.35		
% Carbohydrate	2	1122.34	51.91	<0.0001 <sup>#</sup>
Residual	9	21.62		
C/N	2	396.86	71.83	<0.0001*
Residual	9	5.53		

Fisher's test for multiple treatment group comparisons when ANOVA main treatment effects are significant ( $p < 0.05$ ). Asterisk denotes significance difference ( $p < 0.05$ ) for all multiple comparisons between high N, medium N and low N treatments. <sup>#</sup>Denotes significance difference ( $p < 0.05$ ) of multiple comparisons between treatments except high and medium N treatments.

at Virginia Key. The culture media in this flow-through seawater system was chilled and maintained at 13–15 °C among all treatment containers within the confines of the culture facility.

### 2.2.1. Growth (no choice) assay

This experimental design consisted of four replicate culture containers for each of the three macroalgal treatment diets described above. Five juvenile *A. californica* were

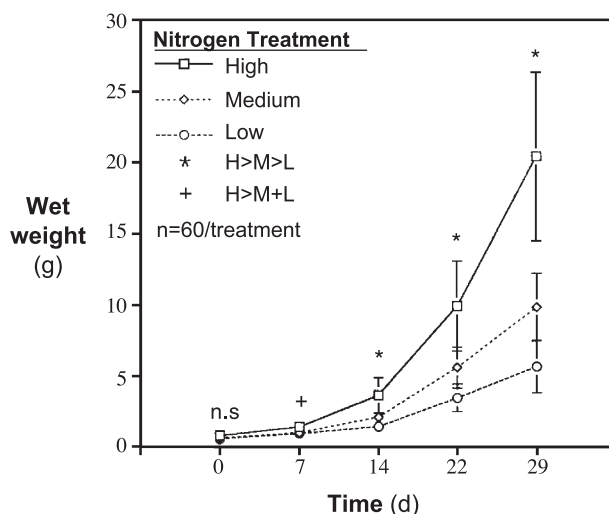


Fig. 1. Growth (wet weight in grams) of *A. californica* as a function of nitrogen content of *G. ferox* diet. Asterisk denotes significant ( $p < 0.05$ ) differences between treatment groups, and (+) denotes significant ( $p < 0.05$ ) higher treatment mean of high nitrogen treatment over medium and low treatments.

cultured in each container with the appropriate macroalgal diet supplied *ad libitum* throughout the 30-day experiment. Culture containers were cleaned daily to remove waste material and unconsumed macroalgae. All specimens were blotted dry and weighed every 5 days during the experiment. A nested two-way ANOVA was used to test for treatment and time effects after an *F*-test for homogeneity of replicate means within groups was utilized. Log transformations were utilized as necessary to achieve homogeneity of variances of wet weights within treatment groups.

### 2.2.2. Feeding preference (choice) assay

An additional experiment was conducted to determine feeding preference among the high, medium and low nitrogen macroalgae. This was accomplished by placing 10 *A. californica* (<5 g wet wt. each) within a culture chamber while offering partitioned choices of the three macroalgal treatments at 40 g wet weight each. Macroalgae of different nitrogen content were weighed before and after 48 h of experimental treatment in these assays. Autogenic changes (Peterson and Renaud, 1989) in wet weight of the *G. ferox* treatments were not tested as production of this tropical species is suppressed at <20 °C (see Lapointe et al., 1984; Capo et al., 1999) particularly at the low temperatures (13–15 °C) required for culture of *A. californica*. Consumption of the total wet weight biomass from treatment groups were compared with one-way ANOVA.

Table 3

Results of nested ANOVA for *A. californica* growth under high, medium and low nitrogen *G. ferox* cultures

Source	<i>df</i>	MS	<i>F</i>	<i>p</i>
Treatment	2	0.324	8.899	0.0005
Replicate	3	0.034	0.946	0.4258
Treatment × Replicate	6	0.036	0.984	0.4467
Residual	48	0.036		
<i>July 13</i>				
High N–medium N				<0.0001
High N–low N				<0.0001
<i>July 20</i>				
High N–medium N				<0.0001
High N–low N				<0.0001
Medium N–low N				0.0017
<i>July 28</i>				
High N–medium N				<0.0001
High N–low N				<0.0001
Medium N–low N				<0.0001
<i>August 4</i>				
High N–medium N				<0.0001
High N–low N				<0.0001
Medium N–low N				0.0004

Fisher's multiple comparisons test for significant ( $p < 0.05$ ) main effects of ANOVA.

### 3. Results

#### 3.1. Biochemistry of cultured macroalgae

Tissue biochemistry for all *G. ferox* cultures utilized during the *Aplysia* feeding trials are summarized in Table 1. Significant gradients in tissue nutrient composition were induced by the three nutrient treatments in cultures of *G. ferox*. Tissue % N and % protein were highest in the high N treatments and decreased stepwise in the medium N and low N treatments (Table 1). In contrast, % C, % carbohydrate and C/N were significantly higher in the treatments with the lowest nitrogen enrichment (Table 2). C/N ratios averaged  $6.9 \pm 0.4$  in high N treatments, indicating nitrogen saturation for *G. ferox*, but were near the known N limitation threshold of 10 (Lapointe, 1981) for the medium N treatment ( $12.6 \pm 2.1$ ) and over twice as high for the low N treatment ( $26.3 \pm 2.3$ ). The mean C/N values for the three nitrogen treatments (high, medium, low) were all significantly different ( $p < 0.0001$ , see Table 2).

#### 3.2. Growth (no choice) assay

The mean wet weight of *A. californica* increased significantly ( $p = 0.0005$ ) in the high N treatments compared to those from the medium and low N treatments after the first week of the assay and throughout the experiment (Fig. 1; Table 3). The mean medium N treatment group wet weight was higher than that of the low N treatment group by the second week and through the remainder of the assay. The final total biomass accumulation (wet weight<sub>f</sub> – wet weight<sub>i</sub>) measured after 4 weeks

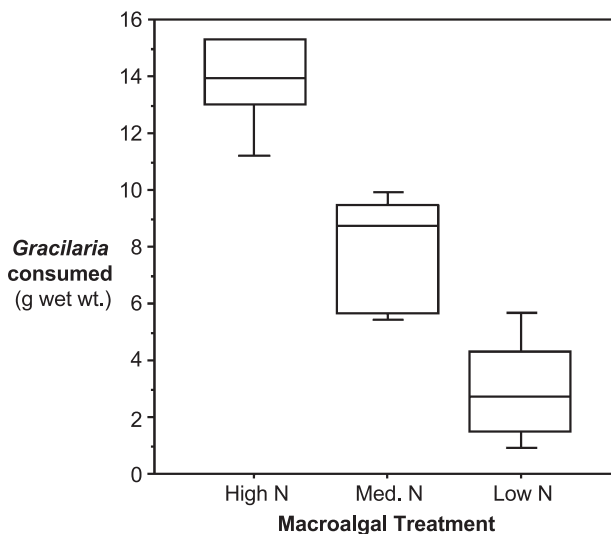


Fig. 2. Consumption of high, medium and low nitrogen (N) diets of *G. ferox* by *A. californica*. Box plots for treatments are means 95th confidence interval and S.E. Treatment means are significantly different at  $p < 0.05$ .



Table 4

Results of one-way ANOVA for feeding preference of high, medium and low nitrogen diets of *G. ferox* by *A. californica*

Source	df	MS	F	p
Treatment	2	143.42	42.10	<0.0001
Residual	12	3.41		

<i>Treatment</i>				
High N–medium N				0.0003
High N–low N				<0.0001
Medium N–low N				0.0015

Fisher's multiple comparisons test for treatment effects of diet where ANOVA main effects are significant ( $p < 0.05$ ).

of treatment was  $19.53 \pm 5.95$  (high N),  $9.12 \pm 2.38$  (medium N) and  $5.02 \pm 1.84$  g (low N).

### 3.3. Feeding preference (choice) assay

*A. californica* offered a choice of the three *G. ferox* treatment diets consumed significantly more ( $p < 0.0001$ ) of the high nitrogen thalli when offered a choice among all three diets (Fig. 2; Table 4). Of the 20-g wet weight offered of each diet, more biomass was consumed ( $13.62 \pm 0.73$  g) from the high N diet as compared to the medium N diet ( $7.68 \pm 0.92$  g,  $p = 0.0003$ ). Consumption of biomass from the medium N diet was also significantly higher ( $p = 0.0015$ ) than that consumed ( $2.93 \pm 0.81$  g) in the low N treatment.

## 4. Discussion

### 4.1. Nitrogen composition of diets and herbivore growth response

The “ecological stoichiometry” of plant–herbivore interactions may provide an opportunity to unify 25 years of research on the issue of consumption versus growth responses by marine herbivores. The emerging field of ecological stoichiometry considers how biogeochemistry of the physical environment places fundamental biochemical constraints upon biological communities. Early assumptions on the primary importance of “caloric value” (Paine and Vadas, 1969) of macroalgae for herbivore preference and fitness can be reconciled with the recognition that the specific biochemical composition of prey (with its respective caloric or energy value) may best explain food value (Tenore, 1983). Specifically, protein or organic nitrogen has been experimentally demonstrated to be the best measure of nutritive value for marine prey items (Tenore, 1977, 1981). When specific ingestion is considered, the quantity of protein consumed best explains growth of herbivores. This paradigm has been supported in studies of Capatellid polychaetes (Tenore, 1977) and echinoderms (Lily, 1975; Lowe and Lawrence, 1976; Fernandez and Boudouresque, 2000), as well as for their prodigy (de Jong-

Westman et al., 1995). Recent models relating food quality to optimal growth of herbivores consuming macrophytes also indicate protein as the most important factor for increased growth (Bowen et al., 1995). This study, which demonstrates nitrogen-enriched macroalgae supporting the fastest growth rate of *A. californica*, further supports this growing body of evidence.

The concept that invertebrate grazers prefer nitrogen-enriched macroalgae is supported by field work from ecosystem-scale nitrogen enrichment experiments. Hauxwell et al. (1998) reported highest grazing rates of macroalgae by amphipods from sub-estuaries of Waquoit Bay, MA, with the highest nitrogen loading, highest macroalgae % N composition and lowest C/N ratios. Amphipod feeding preference of nitrogen-enriched macroalgae in experimental conditions (Jimenez et al., 1996) corroborated Hauxwell et al.'s (1998) field results. Other field assays using invertebrate gastropod grazers indicated selective feeding upon macroalgae with higher nitrogen content (Yates and Peckol, 1993). Indeed, the selective consumption of nitrogen enriched macroalgae in the field may have important implications upon the larger scale transfer of carbon between trophic groups and may be important in the expression of macroalgal blooms in coastal ecosystems (Lapointe et al., 2004). In the same manner, *A. californica* clearly preferred the higher nitrogen content *G. ferox* when offered a choice. In field conditions, preference by Aplysiids for macroalgal species in the division Rhodophyta has been recognized for several decades (Carefoot, 1967a,b,c; Audesirk, 1979; Carefoot, 1987).

Because multiple factors are generally involved in the expression of ecological phenomenon (Quinn and Dunham, 1983), the relation between nitrogen content and specific growth rate, with respect to other evolutionary forces affecting diet selection, is an important consideration in discriminating ultimate prey choice. From an evolutionary perspective, the feeding preference of nitrogen-enriched *G. ferox* may have multiple roles in the ecology of this species. Because *A. californica* is an annual species, consumption of a specialized diet may affect survival by (1) increasing growth rate to more quickly reach reproductive maturity, (2) increasing growth rate as a means to escape predators and (3) acquiring chemical defenses that may be sequestered to deter predators (Pennings, 1990a). Enhanced growth rate, as a consequence of specialized diet selection, may result in faster removal from predation pressure and may be an important evolutionary selective pressure for *Aplysia* species in the field. For *A. californica* in southern California waters, the predatory gastropod *Navanax* sp. has difficulty ingesting opisthobranchs larger than 10 cm (Pennings, 1990b). Likewise, adult *Aplysia* spp. are generally free from predation by anemones, sea stars and crustaceans above a specific size (Carefoot, 1987; Johnson and Willows, 1999).

#### 4.2. Relating primary tissue biochemistry to primary and secondary chemical defenses of macroalgae and herbivores

Increasing evidence suggests that prey preference by Aplysiids is related to feeding specialization on algae from which they acquire secondary metabolites to protect them from predators. Several reports (Carefoot, 1987; Pawlik, 1989; Pennings, 1990a; Ginsburg and Paul, 2001) indicated the dietary preference of chemically rich and high

nitrogen-demanding rhodophytes (e.g., *Laurencia*, *Plocamium*, *Gracilaria*) which, in many cases, contain photosynthetic pigments known to cue gastropod larvae settlement and are often the primary habitat choice and diet for adults. Protein-rich pigments, such as phycoerytherin, are known to be important settlement cues for gastropod larvae, specifically for *Strombus gigas* and *Aplysia brasiliana* (Mianmanus, 1988). The storage of protein-rich photosynthetic pigments by macroalgae of the division Rhodophyta in the phycobilisomes (e.g., phycoerytherin) indicates the utilization of specialized metabolic pathways (Lapointe, 1981). Under conditions of N-limitation, carbohydrate synthesis predominates and accounts for increased storage of agarose polysaccharides (Dawes et al., 1974). Alternatively, during N-enriched exponential growth, protein synthesis predominates as evidenced by high protein pigment concentrations (phycoerythrin) and chlorophyll in *Gracilaria* (Lapointe, 1981; Lapointe and Ryther, 1979). Terpene chemical precursors (e.g., meralonate) are required for the production of chlorophyll and carotenoid photopigments.

Because isoprenoids are also precursors for halogenated terpene-based secondary metabolites of the Rhodophyta (Fenical, 1975), changes in nitrogen availability (resulting in changes in carbohydrate synthesis) should also be reflected in the production of this class of metabolites. To date, little information is available on how nitrogen availability affects production of secondary metabolites in the red algae. Puglisi and Paul (1997) reported that production of the monoterpene Ochtodene is not influenced by N or P enrichment. However, this investigation did not demonstrate nutrient-limited control conditions through analysis of primary tissue biochemistry, either in the field or in experimental conditions that might otherwise suggest a mechanism for alteration in the production of these C-based secondary metabolites. Several reports indicate widespread nitrate enrichment via ground waters in surface waters surrounding Guam (Marsh, 1977; Matson, 1991, 1993; Gamble et al., 2003), where blooms of fleshy macroalgae and filamentous cyanobacteria are common. *In situ* growth studies utilizing artificial nutrient enrichment (see Thacker et al., 2001) on these blooms indicate, specifically, that ambient water column nutrient concentrations are commonly above saturating concentrations (see Matson, 1991; Thacker and Paul, 2001) for maximum productivity ( $P_{\max}$  or  $\mu_{\max}$ ) known for coral reef macroalgae (Lapointe, 1997). These complications bring to light the importance of utilizing the best available control conditions (i.e., nutrient-limited growth conditions, analysis or primary tissue biochemistry) for testing the importance of nutrient additions to plant growth in field-based experiments or manipulation of macroalgal biochemistry in culture conditions (see Lapointe et al., 2004), particularly when used as prey items in feeding or feeding deterrent experiments. Other issues that confound experimental feeding studies utilizing field captured herbivores or their macroalgal prey include: (1) antecedent adaptation of the herbivore to a particular prey item and (2) heterogeneous field conditions that may confound primary or secondary biochemical content of field-collected macroalgae and hence, disposition as a prey item.

Macroalgae high in nitrogen-based photosynthetic pigments may simultaneously provide secondary metabolites critical for predator defense. Diet mixing has been reported by Rogers et al. (1995) where adult *Aplysia juliana* specialize on the chlorophyte *Ulva lactuca* (with lower % N, and lower food conversion efficiency) but

also consume highly chemically defended (and higher % N) rhodophytes *Laurencia obtusa* and *Delisea pulchra*. Pennings (1990a) compared the growth of this sea hare when offered 12 different species of macroalgae and has also reported ontogenic shifts in macroalgal preference when offered a complementary suite of macroalgal prey items (Pennings, 1990b).

More often than not, however, *Aplysia* spp. specialize upon a single particular species that supports maximal nutritional value. This dietary choice may also provide some means of anti-predatory defense. Pennings (1990a) reported that *A. californica* grew fastest when fed the red macroalgae *Plocamium cartilagineum* compared to other macroalgal diets. *A. californica* specializes upon this alga because it may likewise acquire secondary metabolites from the alga for use against predators. Considerable evidence, however, suggests that sequestration of algal secondary metabolites may not be a primary anti-predator defense mechanism for *Aplysia* (Pennings, 1994). A multitude of secondary metabolites from algal prey items are known to be sequestered in the digestive gland of *Aplysia* spp. (Stallard and Faulkner, 1974a; Pennings and Paul, 1993). *Aplysia* may modify specific algal metabolites by acid catabolism, specifically noted for the conversion of laurinterol to aplysin (Stallard and Faulkner, 1974b). Several reports indicate *Aplysia* spp. that consume chemically defended macroalgae are themselves generally resistant to predators (Pennings, 1994; Rogers et al., 1995; Ginsburg and Paul, 2001). However, anti-predator chemicals stored in the gut may not be an appropriate defense, as they are not detected by predators until ingestion, after the *Aplysia* gut content is exposed and the animal is killed (Pennings, 1994). Secondary metabolites from *Portieria hornemannii* appear to be stored primarily in the digestive gland at concentrations of an order magnitude higher than in mantle tissue (Ginsburg and Paul, 2001).

Alternatively, nudibranchs sequester secondary metabolites in mucus located in surficial tissues and are widely reported to utilize this chemistry as an anti-predator defense (e.g., Paul and Alstyne, 1988; Avila and Paul, 1997). Despite the apparent under-utilization of algal secondary metabolites for defense, *Aplysia* tissue remains, in general, distasteful and unpalatable to most predators (Pennings, 1990b, 1994). Evidence suggests that some factor other than secondary metabolites, which are low or often below detection limits in surface “skin” or mantle tissue, may serve to deter predators (Pennings, 1994; Paul and Pennings, 1991). *Aplysia* egg masses appear to lack secondary metabolites (Faulkner, 1992), but are generally not ingested by predators. Alternatively, photosynthetic pigments derived from parental macroalgal prey appear to be important to the conspicuous nature of egg masses and may provide a chemical deterrent. Likewise, a diet composed primarily of rhodophytes with high photosynthetic pigment and terpene chemistry may provide adult *Aplysia* with an alternative chemical deterrent. Chapman and Fox (1969) found that the chromoprotein phycocyanin was transported to the skin and may play a role in unpalatability of somatic tissue.

Our results, utilizing the macroherbivore *A. californica*, support the conclusion of Mattson (1980) that nitrogen content of prey items plays a significant role in feeding preference of marine herbivores. However, the importance of diet as a mechanism supporting predator defense in *Aplysia* species, despite recent attention, remains unclear,

without any general rules that categorically apply to generalist predators (Pennings and Paul, 1993; Ginsburg and Paul, 2001).

## Acknowledgements

The University of Miami, NIH National Resource for *Aplysia* (RRI0294) and their staff are thanked for supporting laboratory space for this research. Additional assistance provided by Nicholas, Ken, and Barbie Abruzzini and Tony and Dan Barile. Support for P.J.B. provided by a graduate scholarship from the NOAA Florida Sea Grant Program. This is contribution number 1533 from Harbor Branch Oceanographic Institution. [SS]

## References

- Audesirk, T.E., 1979. A field study of growth and reproduction in *Aplysia californica*. Biol. Bull. 157, 407–421.
- Avila, C., Paul, V.J., 1997. Chemical ecology of the nudibranch *Glossodoris pallida*: is the location of diet-derived metabolites important for defense? Mar. Ecol. Progr. Ser. 150, 171–180.
- Bowen, S.H., Lutz, E.V., Ahlgren, M.O., 1995. Dietary protein and energy as determinants of food quality: trophic strategies compared. Ecology 76 (3), 899–907.
- Bradford, M.M., 1976. A rapid and sensitive method for the quantification of microgram quantities of protein utilizing the principle of protein-dye binding. Anal. Biochem. 72, 248–254.
- Capo, T., Jaramillo, J., Boyd, A., Lapointe, B., Serafy, J., 1999. Sustained high yields of *Gracilaria* (Rhodophyta) grown in intensive large-scale culture. J. Appl. Phycol. 11, 143–147.
- Capo, T.R., Fieber, L.A., Stommes, D.L., Walsh, P.J., 2002. The effect of stocking density on growth rate and maturation time in laboratory-reared California sea hares (*Aplysia californica*; Mollusca: Gastropoda). Contemp. Topics Lab. An. Sci. 41, 18–23.
- Carefoot, T.H., 1967a. Studies on a sublittoral population of *Aplysia punctata*. J. Mar. Biol. Assoc. U.K. 47, 335–350.
- Carefoot, T.H., 1967b. Growth and nutrition of *Aplysia punctata* feeding on a variety of marine algae. J. Mar. Biol. Assoc. U.K. 47, 565–589.
- Carefoot, T.H., 1967c. Growth and nutrition of three species of opisthobranch molluscs. Comp. Biochem. Physiol. 21, 627–652.
- Carefoot, T.H., 1973. Feeding, food preference, and the uptake of food energy by the supralittoral isopod *Ligia pallasii*. Mar. Biol. 18, 228–236.
- Carefoot, T.H., 1979. Artificial diets for sea hares. Can. J. Zool. 57, 2271–2273.
- Carefoot, T.H., 1987. *Aplysia*: it's biology and ecology. Oceanogr. Mar. Biol. Ann. Rev. 25, 167–284.
- Chapman, D.J., Fox, D.L., 1969. Bile pigment metabolism in the sea hare *Aplysia*. J. Exp. Mar. Biol. Ecol. 4, 71–78.
- Dawes, C.J., Lawrence, J.M., Cheney, D.P., Mathieson, A.C., 1974. Ecological studies of Floridian *Eucheuma* (Rhodophyta, Gigartinales): III. Seasonal variation of carrageenan, total carbohydrate, protein and lipid. Bull. Mar. Sci. 24, 286–299.
- de Jong-Westman, M., Qian, P.-Y., March, B.E., Carefoot, T., 1995. Artificial diets in sea urchin culture: effects of dietary protein level and other additives on egg quality, larval morphometrics, and larval survival in the green sea urchin, *Strongylocentrotus droebachiensis*. Can. J. Zool. 73, 2080–2090.
- Dubois, M.K., Giles, A., Hamilton, J.R., Rebers, P.A., Smith, R., 1956. Calorimetric method for determination of sugars and related substances. Anal. Chem. 28, 350–356.
- Duffy, J.E., Hay, M.E., 1991. Food and shelter as determinants of food choice by an herbivorous marine amphipod. Ecology 72 (4), 1286–1298.
- Faulkner, D.J., 1992. Chemical defenses of marine molluscs. In: Paul, V.J. (Ed.), Ecological Roles of Marine Secondary Metabolites. Cornell Univ. Press, Ithaca, NY, pp. 119–163.

- Fenical, W., 1975. Halogenation in the Rhodophyta: a review. *J. Phycol.* 11, 245–259.
- Fernandez, C., Boudouresque, C.F., 2000. Nutrition of the sea urchin *Paracentrotus lividus* fed different artificial food. *Mar. Ecol. Prog. Ser.* 204, 131–141.
- Gamble, D.W., Taborosi, D., Mylroie, J.E., Jenson, J.W., Carew, J.L., Jocson, J.M.U., Mylroie, J., Vann, D.T., 2003. The use of water temperature to characterize groundwater discharge of a coastal fracture on Guam, USA. *J. Coast. Res.* 19 (2), 462–471.
- Ginsburg, D.W., Paul, V.J., 2001. Chemical defenses in the sea hare *Aplysia parvula*: importance of diet and sequestration of algal secondary metabolites. *Mar. Ecol. Prog. Ser.* 215, 261–274.
- Guillaume, J., Kaushik, S., Bergot, P., Metailler, R., 2001. Nutrition and feeding of fish and crustaceans. In: Laird, L., Stead, S. (Eds.), *Springer–Praxis Books in Aquaculture and Fisheries*. Springer-Verlag, New York. 407 pp.
- Hauxwell, J., McClelland, J., Behr, P.J., Valiela, I., 1998. Relative importance of grazing and nutrient controls of macroalgal biomass in three temperate shallow estuaries. *Estuaries* 21 (2), 347–360.
- Hay, M.E., Fenical, W., 1988. Marine plant–herbivore interactions: the ecology of chemical defense. *Ann. Rev. Ecol. Syst.* 19, 111–145.
- Himmelman, J.H., Carefoot, T.H., 1975. Seasonal changes in calorific value of three Pacific coast seaweeds, and their significance to some marine invertebrate herbivores. *J. Exp. Mar. Biol. Ecol.* 18, 139–151.
- Jimenez, E., Hauxwell, J., Heckscher, E., Rietsma, C., Valiela, I., 1996. Selection of nitrogen-enriched macroalgae (*Cladophora vagabunda* and *Gracilaria tikvahiae*) by the herbivorous amphipod (*Microdeutopus gryllotalpa*). *Biol. Bull.* 191, 323–324.
- Johnson, P.M., Willows, A.O.D., 1999. Defense in sea hares: multiple layers of protection from egg to adult. *Mar. Fresh. Behav. Physiol.* 32, 147–180.
- Lapointe, B.E., 1981. The effects of light and nitrogen on growth, pigment content and biochemical composition of *Gracilaria foliifera*. *J. Phycol.* 17, 90–95.
- Lapointe, B.E., 1985. Strategies for pulsed nutrient supply to *Gracilaria* cultures in the Florida Keys: interactions between concentration and frequency of nutrient pulses. *J. Exp. Mar. Biol. Ecol.* 93, 211–222.
- Lapointe, B.E., 1997. Nutrient thresholds for bottom-up control of macroalgal blooms on coral reefs in Jamaica and southeast Florida. *Limnol. Oceanogr.* 42 (5-2), 1119–1131.
- Lapointe, B.E., Duke, C.S., 1984. Biochemical strategies for growth of *Gracilaria tikvahiae* (Rhodophyta) in relation to light intensity and nitrogen availability. *J. Phycol.* 20, 488–495.
- Lapointe, B.E., Ryther, J.R., 1979. The effects of nitrogen and seawater flow rate on the growth and biochemical composition of *Gracilaria foliifera* var. *angustissima* in mass outdoor cultures. *Bot. Mar.* 23, 529–537.
- Lapointe, B.E., Dawes, C.J., Tenore, K.R., 1984. Interactions between light and temperature on the physiological ecology of “*Gracilaria tikvahiae*” (Gigartinales: Rhodophyta) II: nitrate uptake and levels of pigments and chemical constituents. *Mar. Biol.* 80, 171–178.
- Lapointe, B.E., Barile, P.J., Yentsch, C.S., Littler, M.M., Littler, D.S., Kakuk, B., 2004. The relative importance of nutrient enrichment and herbivory on macroalgal communities near Norman’s Pond Cay, Exumas Cays, Bahamas: a “natural” experiment. *J. Exp. Mar. Biol. Ecol.* (in press).
- Lily, G.R., 1975. The influence of diet on the growth and bioenergetics of the tropical sea urchin *Tripneustes ventricosus*. PhD thesis. Univ. British Columbia, Vancouver.
- Littler, M.M., Littler, D.S., 1980. The evolution of thallus form and survival strategies in benthic marine macroalgae: field and laboratory tests of a functional form model. *Am. Nat.* 116, 25–44.
- Lowe, E.F., Lawrence, J.M., 1976. Absorption efficiencies of *Lytechinus variegatus* for selected marine plants. *J. Exp. Mar. Biol. Ecol.* 21, 223–234.
- Marsh, J.A., 1977. Terrestrial inputs of nitrogen and phosphorus on fringing reefs of Guam. *Proc. 3rd Int. Coral Reef Symp.* University of Miami, FL, pp. 331–336.
- Matson, E.A., 1991. Nutrient chemistry of the coastal waters of Guam. *Micronesica* 24 (1), 109–135.
- Matson, E.A., 1993. Nutrient flux through soils and aquifers to the coastal zone of Guam, Mariana Islands. *Limnol. Oceanogr.* 38 (2), 361–371.
- Mattson, W.J., 1980. Herbivory in relation to plant nitrogen content. *Ann. Rev. Ecol. Syst.* 11, 119–161.
- Mianmanus, R., 1988. Induction of settlement and metamorphosis in larvae of *Aplysia brasiliana* and *Strombus gigas*. PhD dissertation. University of Miami. 186 pp.

- Nicotri, M.E., 1980. Factors involved in herbivore food preference. J. Exp. Mar. Biol. Ecol. 42, 13–26.
- Paine, R.T., Vadas, R.L., 1969. Calorific values of benthic marine algae and their postulated relation to invertebrate food preference. Mar. Biol. 4 (2), 79–86.
- Paul, V.J., Alstyne, K.L., 1988. Use of ingested algal diterpenoids by *Elysia halimeda* as anti-predator defenses. J. Exp. Mar. Biol. Ecol. 119, 15–29.
- Paul, V.J., Pennings, S.C., 1991. Diet-derived chemical defenses in the sea hare *Stylocheilus longicauda*. J. Exp. Mar. Biol. Ecol. 151, 227–243.
- Pawlik, J.R., 1989. Larvae of the sea hare *Aplysia californica* settle and metamorphose on an assortment of macroalgal species. Mar. Ecol. Prog. Ser. 51, 195–199.
- Pennings, S.C., 1990a. Multiple factors prompting narrow host range in the sea hare, *Aplysia californica*. Oecologia 82, 192–200.
- Pennings, S.C., 1990b. Size related shifts in herbivory: specialization in the sea hare *Aplysia californica*. J. Exp. Mar. Biol. Ecol. 142, 43–61.
- Pennings, S.C., 1994. Interspecific variation in chemical defenses in the sea hares. J. Exp. Mar. Biol. Ecol. 180, 203–219.
- Pennings, S.C., Paul, V.J., 1993. Sequestration of dietary secondary metabolites by three species of sea hares: location, specificity and dynamics. Mar. Biol. 117, 535–546.
- Peterson, C.H., Renaud, P.E., 1989. Analysis of feeding preference experiments. Oecologia 80, 82–86.
- Puglisi, M.P., Paul, V.J., 1997. Intraspecific variation in the red alga *Portieria hornemannii*: monoterpene concentrations are not influenced by nitrogen or phosphorus enrichment. Mar. Biol. 128, 161–170.
- Quinn, J.F., Dunham, A.E., 1983. On hypothesis testing in ecology and evolution. Am. Nat. 122, 602–617.
- Rogers, C.N., Steinberg, P.D., de Nys, R., 1995. Factors associated with oligophagy in two species of sea hares. J. Exp. Mar. Biol. Ecol. 192, 47–73.
- Stallard, M.O., Faulkner, D.J., 1974a. Chemical constituents of the digestive gland of the sea hare *Aplysia californica*: I. Importance of diet. Comp. Biochem. Physiol. 49b, 25–35.
- Stallard, M.O., Faulkner, D.J., 1974b. Chemical constituents of the digestive gland of the sea hare *Aplysia californica*: II. Chemical transformations. Comp. Biochem. Physiol. 49b, 37–41.
- Steneck, R.S., Watling, L., 1982. Feeding capabilities and limitations of herbivorous molluscs: a functional group approach. Mar. Biol. 68, 299–319.
- Sterner, R.W., Hessen, D.O., 1994. Algal nutrient limitation and the nutrition of aquatic herbivores. Annu. Rev. Ecol. Syst. 25, 1–29.
- Tenore, K., 1977. Growth of *Capitella capitata* cultured on various levels of detritus derived from different sources. Limnol. Oceanogr. 22, 936–941.
- Tenore, K., 1981. Organic nitrogen and caloric content of detritus: I. Utilization by the deposit-feeding polychaete *Capitella capitata*. Est. Coast. Mar. Sci. 12, 39–47.
- Tenore, K., 1983. What controls the availability to animals of detritus derived from vascular plants. Organic nitrogen enrichment or caloric availability? Mar. Ecol. Prog. Ser. 10 (3), 307–309.
- Thacker, R.W., Paul, V.J., 2001. Are benthic cyanobacteria indicators of nutrient enrichment? Relationships between cyanobacterial abundance and environmental factors on the reef flats of Guam. Bull. Mar. Sci. 69 (2), 497–508.
- Thacker, R.W., Ginsburg, D.W., Paul, V.J., 2001. Effects of herbivore exclusion and nutrient enrichment on coral reef macroalgae and cyanobacteria. Coral Reefs 19, 318–329.
- Vince, S.W., Valiela, I., Teal, J.M., 1981. An experimental study of the structure of herbivorous insect communities in a salt marsh. Ecology 62, 1662–1678.
- Wakefield, R.L., Murray, S.N., 1998. Factors influencing food choice by the seaweed-eating marine snail *Norrisia norrisi* (Trochidae). Mar. Biol. 130, 631–642.
- Yates, J.L., Peckol, P., 1993. Effects of nutrient availability and herbivory on polyphenolics in the seaweed *Fucus vesiculosus*. Ecology 74, 1757–1766.