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Algal nitrogen and phosphorus content drive inter- and intraspecific differences in herbivore grazing on a Caribbean reef

Andrew A. Shantz[a,b,c,*], Mark C. Ladd[b], Deron E. Burkepile[b,c]

[a] Department of Biology, Florida International University, 3000 NE 151st St, North Miami, FL 33181, United States
[b] Department of Ecology, Evolution, and Marine Biology, University of California Santa Barbara, Santa Barbara, CA 93106, United States
[c] Marine Science Institute, University of California Santa Barbara, Santa Barbara, CA 93106, United States

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ABSTRACT

Identifying how herbivorous reef fish select resources is an important aspect of understanding how coral reef communities are structured. Ecological stoichiometry, built around the mass balance of elements, necessitates that to build tissue, maintain growth, and reproduce, animals must maintain a homeostatic balance of elements within their body. Nitrogen (N) and phosphorus (P) are abundant in animal tissue relative to most plants and algae. Accordingly, N and P are often limiting for herbivores, which should therefore focus their foraging efforts on resources rich in these elements. Here, we explore how variation in N and P concentrations in algae impact herbivore resource selectivity on a coral reef. We conducted feeding assays using a brown alga from the genus *Sargassum* grown under elevated nitrogen (N), phosphorus (P) or both (N + P). Overall bite rates were higher on N enriched, but not P enriched, assays but individual foraging efforts varied among species and fish size. Individual *Sparisoma aurofrenatum* increased the number of bites taken per feeding foray on N enriched algae while *Acanthurus coeruleus* and *Sparisoma rubripinne* fed more on P enriched assays. *Sparisoma chrysopterum* increased their foraging effort on both N and P enriched assays. However, the influence of algal nutrient content on foraging effort declined with increasing fish size, such that full grown fishes did not respond to assay nutrient content. Overall, preference for enriched algae resulted in ~20% more consumption of N and N + P enriched algae relative to unenriched controls. Our study is the first to link the elemental nutrient content of algae to species-specific feeding decisions of reef herbivores and demonstrates the utility of ecological stoichiometry to explore herbivory on coral reefs.

1. Introduction

On coral reefs, herbivores consume algae that would otherwise compete with corals. Because algae can poison corals (Rasher and Hay, 2010), reduce coral growth rates (Vega Thurber et al., 2012), alter coral microbiomes (Zaneveld et al., 2016), facilitate diseases (Nugues et al., 2004), and impede the recruitment of coral larvae (Dixson et al., 2014), herbivory is a vital ecosystem process on coral reefs (Adam et al., 2015a). Unfortunately, overfishing and the size selective harvest of fishes has drastically altered herbivore communities on many reefs (Sandin et al., 2008; Jackson et al., 2014), compromising the ability of herbivores to control algae on these reefs. While herbivore biomass is frequently considered the primary driver of algal abundance on reefs, there are often different functional roles among herbivore species, and changes in the functional diversity of herbivores can be just as important as changes in their overall biomass (Burkepile and Hay, 2008). For example, while reefs with a high degree of functional redundancy may be resilient to the loss of an individual species of herbivore, functional complementarity among herbivore species is essential to maintain control over more diverse primary producer communities (Rasher et al., 2013; Adam et al., 2015b). Consequently, a greater understanding of how different species and sizes of herbivorous fishes select and partition the algae they consume is needed to understand how herbivores shape coral reef communities.

Herbivores are often limited by the low nutritional quality of their food and frequently target nutrient-rich primary producers when possible (Mattson, 1980). Nitrogen (N) is a major constituent of amino acids and proteins, while phosphorus (P) is an essential component in RNA, bone, and scales (Sterner and Elser, 2002). For herbivores, the acquisition of these nutrients creates a unique challenge because N and P are far more abundant in animal tissue than in plant tissue. Accordingly, the N or P content in food often limits herbivore growth (Sterner and Elser, 2002; Anderson et al., 2005). However, overconsumption of these elements can carry an energetic cost, both in terms of wasted and

* Corresponding author at: Department of Biology, Pennsylvania State University, 311 Mueller Laboratory, University Park, PA 16802, United States.
E-mail address: shantz@psu.edu (A.A. Shantz).

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potentially dangerous time spent foraging (Catano et al., 2016) and the physiological costs of eliminating excess nutrients to maintain homeostatic balance (Anderson et al., 2005). As a result, herbivores should selectively feed on plants and algae that most closely match their nutritional needs (e.g. Sterner and Elser, 2002; Simpson and Raubenheimer, 2012).

However, the elemental recipe for every animal is slightly different and body stoichiometry can vary widely among species (Vanni et al., 2002; Allgeier et al., 2014), suggesting potentially strong interspecific differences in foraging based on stoichiometry. Furthermore, as individuals age they undergo changes in size, physiology, and morphology that can lead to substantial variation in their elemental composition throughout their life (Pilati and Vanni, 2007; Persson et al., 2010). For instance, rapidly growing animals, such as those in their early life-stages, often have a high proportion of P, due to the abundance of ribosomal RNA needed to maintain rapid growth rates, but the proportion of P declines as individuals age and growth slows (Elser et al., 2003). Accordingly, ontogenetic changes in body stoichiometry could lead to changes in resource selection to meet specific nutritional or elemental targets as consumers age.

On coral reefs, numerous studies have explored how consumer morphology, digestive physiology, and algal chemical and physical defenses influence herbivore feeding (e.g. Hay et al., 1994; Choat et al., 2004; Bellwood et al., 2006). However, while the selective removal of macroalgae enriched with N and P is common (e.g. Diaz-Pulido and McCook, 2003; Fong et al., 2006; Shantz et al., 2015), comparatively few studies address how different species of reef herbivores respond to enrichment or the relative influence of N versus P in dictating foraging decisions. For instance, several studies report that nutrient enriched macroalgae are removed from reefs faster than un-enriched conspecifics but do not identify which herbivore species responded to enrichment or whether algal N or P content drove this pattern (Boyer et al., 2004; Fong et al., 2006; Chan et al., 2012). Similarly, studies that identify species-specific preferences towards enriched resources typically focus on a single nutrient, primarily N, and individual herbivore species, while failing to capture the effect of enrichment on other members of the herbivore community (e.g. Goecker et al., 2005; Holzer et al., 2013). In fact, while both N and P are critical for fish nutrition (Watanabe, 2002; Benstead et al., 2014), there has been little emphasis on disentangling the relative importance of N and P in the feeding decisions of herbivorous coral reef fishes.

Here, we explore the impact of algal N and P content on herbivore feeding and the consumption of a palatable species of *Sargassum*. We grew *Sargassum* in aquaria under ambient nutrient conditions or enriched with N, P, or both N + P. Each week, we transplanted nutrient-enriched algae to the reef and filmed the feeding activity. We predicted that enriched *Sargassum* would be consumed faster than algae grown under ambient conditions and that stoichiometric mismatch between fishes and algae would lead all fish species to increase their consumption of N-enriched algae. Additionally, P is important for bone mineralization, scale formation, and as a component of RNA needed to support rapid growth. Thus, we predicted that juvenile fishes, which are fast growing and have a greater surface area:volume ratio (and hence a higher proportion of scales:soft tissue), would increase consumption of P-enriched algae, whereas slower-growing adults would not.

2. Methods

From June through August of 2013 we deployed feeding assays of *Sargassum* enriched with either N, P, N + P, or maintained as controls at 6–8 m on a forereef in the Florida Keys, USA (24.9614° N, 80.4539° W). We chose *Sargassum* for our experiment because it is a common macroalgae that is highly palatable, and stores excess nutrients within its tissue (Steinberg et al., 1991; Chan et al., 2012). To create assays of varying nutritional content, every week we collected ~150 g of a *Sargassum* sp. from an offshore patch reef. We collected thalli from the apical portions of morphologically identical *Sargassum* fronds that were minimally fouled by epiphytes. We transported the *Sargassum* sp. to shore in fresh seawater, where we removed any epiphytes present using a razor blade and tweezers and divided the cleaned algae into four, 50 l aquaria. Aquaria were maintained outdoors under shade cloth in a seawater bath chilled to 26 °C. Each tank was supplied with a continuous drip of seawater at ~500 ml h^{-1} from one of four independent reservoirs (one reservoir for each treatment). Every two days we refilled the reservoirs with fresh seawater collected from 2 km offshore. To achieve enrichments, every time that the reservoirs were filled we spiked the seawater in the N, P, and N + P reservoirs with commercial aquarium nitrogen and phosphorus supplements (Flourish™ Nitrogen, and Flourish TM Phosphorus, Seachem Laboratories, Madison, GA. USA) to increase nutrient concentrations by 10 μm N, 1 μm P, or 10 μm N + 1 μm P for the respective N, P, and N + P treatments. These values are higher than N and P levels typically found on healthy reefs but fall within the range of nutrient levels reported on reefs subjected to high levels of nutrient loading (Shantz and Burkepile, 2014).

We allowed the *Sargassum* to grow under the respective treatments for one week. After growing for one week, we collected a ~5 g portion of algae from each tank for tissue nutrient analysis. The remaining algae from each tank were spun in a salad spinner for 1 min, weighed out to ~40 g portions, and transported to the reef in water from the appropriate treatment tank for deployment. For each assay, we attached one pre-weighed section of *Sargassum* from a single treatment to an array of 5 clothes pegs wired together to form a ring and anchored to the benthos with a nail (Fig. S1). We used 5-peg arrays to ensure that the *Sargassum* was secured in multiple locations so that distal sections of the algae would not float away if bitten at a lower point on the thallus. Review of assay footage (see below) revealed that relatively little algae was dislodged during the deployments and the majority of algal biomass removed was consumed by herbivores.

Each week, we deployed four assays (1 Control, 1 N, 1 P, 1 N + P) separated by at least 10 m (n = 1 $week^{-1}$; n = 9 for each treatment over the study duration). However, during the deployments, one N-enriched replicate detached from the benthos and was lost, lowering our replication to n = 8 for the N enriched assays and creating an unbalanced design. Assays were deployed between the hours of 10:00 and 11:00 in the morning and filmed for 2 h with a GoPro camera secured to the substrate 1 m from the assay. After 2 h, we collected the assays and obtained a final weight as described above. We scored videos to record the species, size, and life phase (juvenile, initial, or terminal) of any fishes observed feeding during the deployment. Fish size was estimated visually from a marker of known length in each video. Additionally, we recorded the total number of bites taken by each herbivore and the number of bites $foray^{-1}$. We counted forays as feeding events with no discernable interval between successive bites other than the time it took to reapply the jaws to the food item (Bellwood and Choat, 1990). This metric eliminates subjective assessments as to whether a fish is actively foraging, thereby providing a conservative metric of foraging effort. To determine feeding rates, we divided the total number of bites observed by the full two-hour assay duration or the elapsed time until herbivores had consumed the *Sargassum* to the point it was no longer visible in the video.

2.1. Analysis

To document how our treatments increased algal nutrient content, we measured the N and P content of the *Sargassum* used in each assay. Samples collected before each assay deployment (n = 9 for each treatment) were dried at 60 °C to a constant weight, ground to a fine powder, and analyzed via a carbon-nitrogen elemental analyzer (Thermo Fisher Scientific, Waltham MA USA). Phosphorus content was measured using dry oxidation-acid hydrolysis extraction followed by colorimetric analysis (Fourqurean et al., 1992). Briefly, we weighed out duplicate subsamples from each sample. To each, we added 0.5 ml of