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Charismatic microfauna alter cyanobacterial production through a trophic cascade

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Abstract The trophic ecology of cyanobacterial blooms is poorly understood on coral reefs. Blooms of toxic cyanobacteria, *Lyngbya majuscula*, can quickly form large mats. The herbivorous sea hare, *Stylocheilus striatus*, and the predatory nudibranch, *Gymnodoris ceylonica*, often associate with these blooms, forming a linear food chain: nudibranch—sea hare—cyanobacteria. Using laboratory studies, this study quantified (1) the functional response of nudibranchs, (2) the effect of sea hare size on predation rates, and (3) the strength of the indirect effect of sea hare predation on cyanobacteria (i.e., a trophic cascade). Nudibranchs consumed on average 2.4 sea hares d^{-1} , with the consumption of small sea hares 22 times greater than the consumption of large sea hares. Predation of sea hares reduced herbivory. Cyanobacterial biomass was 1.5 times greater when nudibranchs were present relative to when nudibranchs were absent. Although sea hare grazing can substantially reduce cyanobacterial biomass, predation of sea hares may mitigate grazing pressure, and therefore increase the abundance of cyanobacteria.

Keywords Cyanobacteria · *Gymnodoris ceylonica* · *Lyngbya majuscula* · *Stylocheilus striatus* · Trophic cascade

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Introduction

Cyanobacteria are an important, but understudied member of coral reef ecosystems capable of affecting reef ecosystem structure, function, and services. In recent years, blooms of cyanobacteria (*Lyngbya majuscula*), driven by a combination of increasing water temperatures and eutrophication, have increased in size and frequency (Elmetri and Bell 2004; Ahern et al. 2008; Paul 2008). Cyanobacterial blooms form large, dense mats in coral reef and associated habitat (Elmetri and Bell 2004; Paul et al. 2005; Watkinson et al. 2005; Ahern et al. 2008). For example, Stielow and Ballantine (2003) recorded a cyanobacterial density of $600 \text{ g}_{\text{dw}} \text{ m}^{-2}$ on shallow seagrass beds of the Caribbean Sea in Puerto Rico during 1998–2000. Cyanobacterial mats can have a range of deleterious effects on reefs and local human populations, including smothering corals and seagrass, inhibition of coral recruitment, killing fish, and skin, eye and respiratory irritation (Osborne et al. 2001; Elmetri and Bell 2004; Kuffner and Paul 2004; Watkinson et al. 2005). The longevity of cyanobacterial blooms is thought to be enhanced by the production of active secondary metabolites that chemically deter generalist herbivores including crabs, sea urchins, and fish (Thacker et al. 1997; Nagle and Paul 1998, 1999; Osborne et al. 2001); however, some herbivorous sea hares can tolerate these toxins and are therefore capable of grazing on cyanobacteria.

The herbivorous sea hare (*Stylocheilus striatus*) exhibits strong settlement and dietary preferences for cyanobacteria (Switzer-Dunlap and Hadfield 1977; Capper et al. 2006). Dense swarming aggregations of sea hares often form in cyanobacterial blooms (Paul and Pennings 1991; Pennings and Paul 1993a; Capper and Paul 2008). For example, in 1999 Cruz-Rivera and Paul (2002) found the average

density of *S. striatus* was 0.5 individuals g^{-1} of cyanobacteria in Guam. Such large aggregations of *S. striatus* are capable of consuming hectare-sized fields of cyanobacteria in days (Pennings and Paul 1993b). The rapid consumption of cyanobacteria may, in turn, reduce its negative effects on the reef; however, for this to be realized, sea hares must avoid predators. Although fish predators may be deterred by chemical defences sea hares sequester from cyanobacteria (Pennings et al. 2001), nudibranchs (*Gymnodoris ceylonica*) can withstand these defences. Nudibranchs are voracious predators of sea hares that increase in abundance within cyanobacterial blooms shortly after sea hares become abundant, and are capable of rapidly reducing the density of sea hares (Gosliner et al. 1996). Thus, these three species comprise a simple food chain (predator: nudibranchs, herbivore: sea hares, and primary producer: cyanobacteria; Fig. 1) that can potentially influence cyanobacterial dynamics; however, there are currently no empirical studies examining these relationships.

Here, a series of laboratory experiments explore the effects of predation of sea hares by nudibranchs and the potential for cascading effects on cyanobacteria through release from herbivory. First, the predatory behaviour of nudibranchs relative to sea hare density is described. Second, the effect of sea hare size on predation risk is examined. Finally, the indirect effect of nudibranchs on cyanobacteria is tested.

Methods

This research was conducted in 2008 at the Gump Biological Research Station, Moorea, French Polynesia (17°30' S,

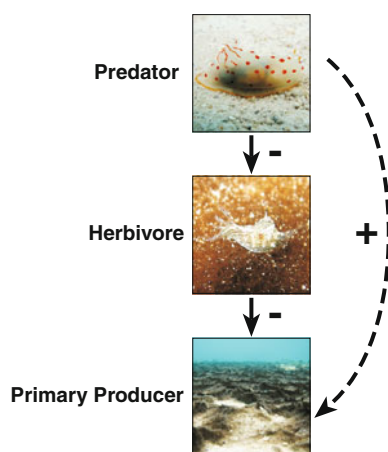


Fig. 1 The three trophic levels examined. Primary producer: cyanobacteria (*Lyngbya* spp.) mat covering a sand bar within the northern lagoon of Moorea, French Polynesia. Herbivore: sea hare (*Stylocheilus striatus*) grazing on the cyanobacteria mat. Predator: nudibranch (*Gymnodoris ceylonica*) hunting for sea hares on the sand flat

149°50' W). All experiments were conducted in plastic containers (190 mm × 190 mm × 90 mm; W × L × H). To allow the exchange of fresh seawater, ~80% of the surface area of the lids, and two opposing sides of containers were cut away and covered with 0.5 mm mesh screening. Containers were placed within large (2,670 l) outdoor flow-through seawater tanks (10 containers per tank). In each experiment, sea hares, nudibranchs, and cyanobacteria were collected from the field. Prior to experiments, nudibranchs were starved for 3 days, and cyanobacteria were rinsed with filtered seawater, which removed associated flora or fauna.

Functional response

Nine density treatments (1, 2, 3, 4, 5, 6, 7, 8 or 9 sea hares: mean length 20.0 mm ± 3.8 SD) were randomly assigned to 18 containers ($n = 2$ per treatment), each containing one adult nudibranch (mean length 36.2 mm ± 5.6 SD). The number of sea hares eaten was recorded after 1 day (24 h). Attack rates and handling time were estimated using a Holling Type II functional response. Because prey organisms were not replaced as they were consumed, attack rate and handling time were estimated using Roger's 'random predator equation' (Rogers 1972; Juliano 2001):

$$N_e = N_0 \left[1 - e^{-a(N_e h - P t)} \right] \quad (1)$$

where N_e is the number of sea hares eaten, N_0 is the initial sea hare density (per container), a , h , and P are, respectively, the per predator instantaneous attack rate, handling time, and predator density ($P = 1$ in this study). The duration of the experiment (t) is 1 day in this study. Following Bolker (2008) and McCoy and Bolker (2008), the random predator equation was fit using the Lambert W function (Corless et al. 1996):

$$N_e = N_0 \frac{W(a h N_0 e^{-a(P t - h N_0)})}{a h} \quad (2)$$

A general-purpose maximum likelihood function was used to estimate the attack rate and handling time parameters from Eq. 2. Nudibranchs died in three containers (sea hare densities: 6, 8, and 9), and these trials were therefore excluded from the analysis.

The effects of sea hare size on predation risk

Using a fully factorial design, the presence of nudibranchs (one individual present [mean length 40.5 mm ± 9.4 SD] vs. absent) and the size of sea hares (small [mean length 20.3 mm ± 1.98 SD]; medium [mean length 32.9 mm ± 2.5 SD]; or large [mean length 61.7 mm ± 5.4 SD]) were manipulated. The six treatments were randomly assigned to

30 containers, each containing a ~ 75 g tuft of cyanobacteria and 10 sea hares. Survival of sea hares was recorded after 15 h. Sea hares in treatments without nudibranchs exhibited 100% survival; therefore, analysis focused on effects of sea hare size in affecting survival in the presence of nudibranchs. Proportion survival of the three size classes was modelled using a generalized linear model with a quasibinomial error distribution (residual deviance = 36.73, residual $df = 12$) and a *logit* link function. An analysis of deviance was used to test for a main effect of size, and Tukey's post hoc comparisons were used to compare proportional survival among the three different size classes.

Trophic cascade

The presence of a trophic cascade was examined using three treatments: (1) cyanobacteria alone ($n = 16$); (2) cyanobacteria and sea hares ($n = 8$); (3) cyanobacteria, sea hares, and nudibranchs ($n = 8$). Cyanobacteria (mean = 78.0 g, SD = 12.9) were added to each of the 32 containers. Ten sea hares (mean length 57.4 mm \pm 5.6 SD) were added to containers assigned to treatments 2 and 3, and one nudibranch (mean length 48.6 mm \pm 3.6 SD) was added to containers assigned to treatment 3. Each morning (~ 0700), the number of surviving sea hares in each container was recorded. Nudibranch densities were maintained at their original density by replacing any missing or dead nudibranchs throughout the duration of the study (two nudibranchs were replaced on day two, and no other replacements were necessary). After 4 days, cyanobacterial biomass (g) and the number of sea hares in each container were recorded.

A Wilcoxon rank sum test, with continuity correction, was used to test whether nudibranchs significantly reduced the number of sea hares. Finding nudibranchs significantly reduced sea hare abundance; the indirect effect of sea hare predation on cyanobacteria (i.e., a trophic cascade) was examined. Due to the short duration of the experiment, there was no discernable growth of cyanobacteria in each treatment. All treatments experienced significant loss in mass (though this change in mass was minimal in the control treatment), and the change in mass was not correlated with initial mass in the control treatment (Slope = 0.320 [± 0.345 SE], $t_{1,14} = 0.929$, $P = 0.368$). Therefore, the effect of treatments on cyanobacterial mass was examined using the change in wet mass of cyanobacteria between the initial and final day of the experiment ($\delta M = M_i - M_f$). δM was approximately normally distributed (visual examination of quantile–quantile plots; Shapiro–Wilk normality test, $W = 0.95$, $P = 0.128$) with equal variances (Leven's test for homogeneity of variances, $F_{2,29} = 0.37$, $P = 0.700$); therefore, a one-factor ANOVA

was used to test whether δM significantly differed among treatments. Two orthogonal contrasts were conducted to examine (1) the effect of sea hares (control vs. sea hare only and sea hare + nudibranch); and (2) the effect of nudibranchs (sea hare only vs. sea hare + nudibranch). Least-squares regression was used to determine the degree to which the number of sea hares present at the end of the study predicted the change in cyanobacterial biomass (δM). All statistical analyses were conducted in R 2.8.0 (R Development Core Team 2008).

Results and discussion

Nudibranchs are suction feeders, consuming sea hares one individual at a time. During an attack, a sea hare would attempt to evade the nudibranch by excreting purple “ink” and undulating its body, occasionally freeing itself from the nudibranch's grip. Predation by nudibranchs approximated a Holling Type II functional response (Holling 1961). The mean attack rate (α ; lower 95% CI, upper 95% CI) was 12.21 (3.25, ∞) per day, and the mean handling time (h) was 0.285 (0.190, 0.400; Fig. 2a) days. Because the Rogers ‘random predator equation’ integrates the Holling Type II functional response through time, the infinite upper bound on the attack rate describes a circumstance where the slope of the relationship between the N_0 and time is equal to $1/h$. The survival of sea hares increased with increases in sea hare size ($F_{2,12} = 15.29$, $P = 0.005$; Fig. 2b). Relative to the treatment with small sea hares, survival was 12.5 times ($Z = 2.47$, $P = 0.035$) and 22 times ($Z = 3.69$, $P < 0.001$) greater in treatments with medium and large sea hares, respectively (Fig. 2b). Therefore, effects of nudibranchs will likely depend on the both density and size structure of sea hare populations.

In the trophic cascade experiment, the presence of nudibranchs reduced sea hare survival by 68% ($W = 64$, $P < 0.001$), and there was a significant treatment effect on cyanobacterial biomass (δM ; $F_{1,14} = 83.69$, $P < 0.001$). Cyanobacterial biomass (δM) was 3.5 times greater when sea hares were absent relative to when they were present ($t_{1,29} = -11.96$, $P < 0.001$). In the presence of sea hares, cyanobacterial biomass (δM) was 1.5 times greater when nudibranchs were also present ($t_{1,29} = 4.94$, $P < 0.001$; Fig. 3a). Furthermore, δM was positively correlated with the final number of sea hares ($F_{1,29} = 167$, $P < 0.001$; Fig. 3b). These results suggest that nudibranchs can substantially reduce sea hare abundance, which in turn promotes the growth of cyanobacteria via a reduction in grazing pressure (i.e., a trophic cascade).

Although this study focuses solely on top–down effects, studies of trophic dynamics in coral reefs have shown substantial support for both bottom-up and top–down

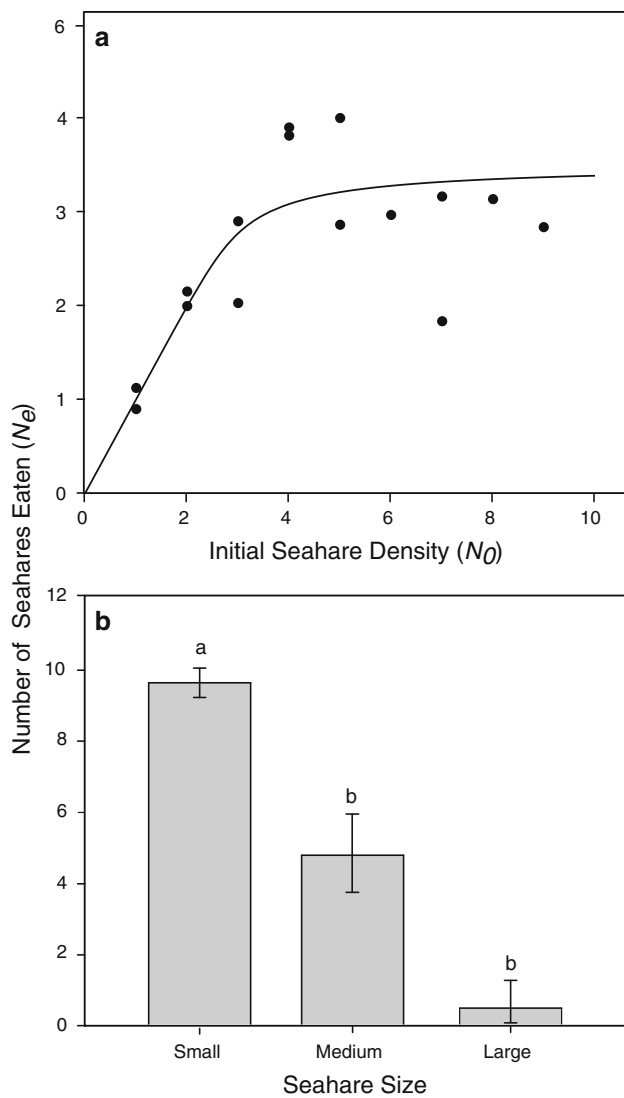


Fig. 2 Number of sea hares (*Stylocheilus striatus*) eaten (N_e) by nudibranchs (*Gymnodoris ceylonica*) in a 24-h feeding trial as a function of sea hare density. The curve represents predicted means from a fitted Holling Type II functional response. Points are jittered for clarity (Panel a). Mean number of sea hares eaten (± 1 SE) in three sea hare size classes. Letters indicate statistically different treatments as determined by Tukey's post hoc comparisons (panel b)

structuring of communities. For example, Elmetri and Bell (2004) identified daily growth of cyanobacteria (*L. majuscula*) to be 4.7 times greater when phosphate concentrations were $0.18 \mu\text{M}$ (common in sites affected by anthropogenic inputs) relative to when they were $0.07 \mu\text{M}$ (background phosphate concentrations). Increased cyanobacterial production resulting from phosphorus addition may increase the carrying capacity of cyanobacteria. When sea hares are absent, substantial increases in the standing biomass of cyanobacteria may occur. When sea hares are present, sea hare populations may respond to increases in the carrying capacity of cyanobacteria by shifting from a

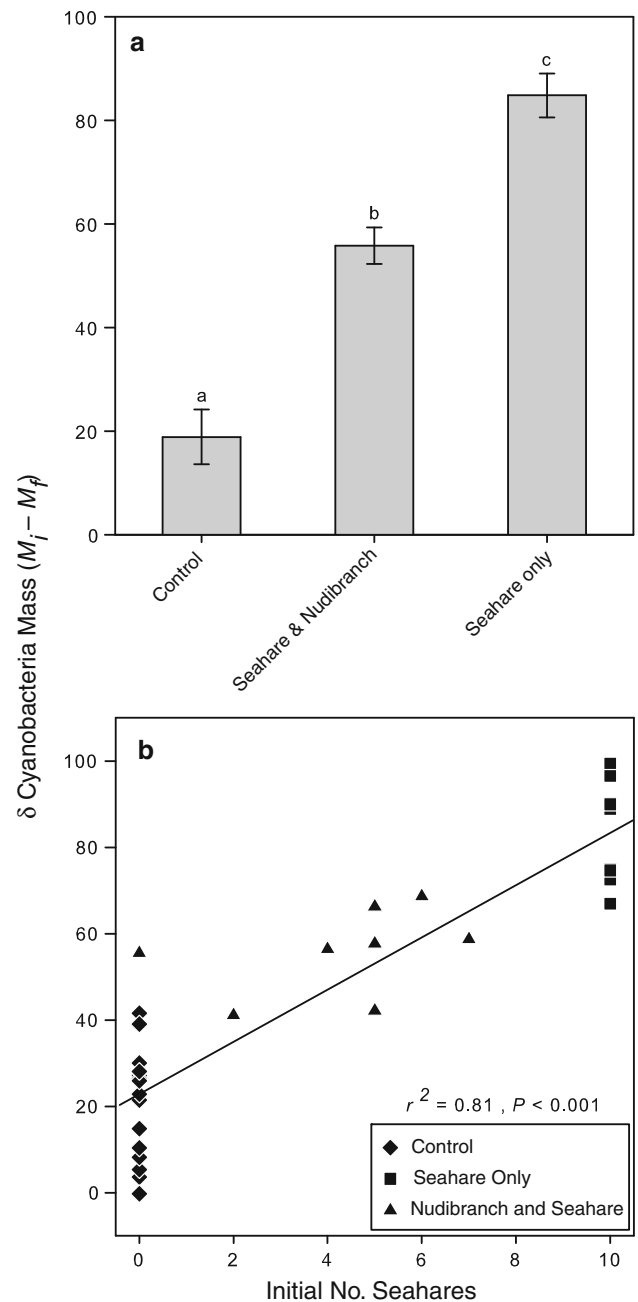


Fig. 3 A trophic cascade. The effect that predation of sea hares (*Stylocheilus striatus*) by nudibranchs (*Gymnodoris ceylonica*) has on change in cyanobacterial (*Lyngbya majuscula*) biomass (δM) as determined by a factorial experiment with three treatments: (1) control (cyanobacteria alone); (2) sea hares only (cyanobacteria and sea hares); and (3) sea hares and nudibranchs (cyanobacteria, sea hares and nudibranchs). Mean (± 1 SE) change in cyanobacterial biomass (δM), with letters indicating statistically different treatments as determined by Tukey's post hoc comparisons (panel a). Correlation between δM and the number of surviving sea hares after 4 d. The line represents predicted mean fit from least-squares regression (panel b)

stable equilibrium to unstable "outbreaks" (i.e., paradox of enrichment: Rosenzweig 1971). The potential for outbreaks of sea hares to regulate the biomass of cyanobacteria is

likely dependent upon the rate at which nudibranch populations respond to increases in sea hare abundance and the magnitude of the response.

Due to their toxic nature and capacity for rapid growth, cyanobacterial blooms have deleterious effects on coral reef ecosystem function and human health; therefore, understanding the factors that promote or limit cyanobacterial outbreaks has important implications. This study identifies sea hares as a natural agent that can reduce cyanobacterial blooms; however, predatory nudibranchs limit the numerical response of sea hares and thus promote the continuation of cyanobacterial blooms. In nature, this appears to be an inherently unstable system, with booms and busts occurring at all three trophic levels. Future research that quantifies population oscillations between cyanobacteria, sea hares, and nudibranchs, coupled with field experiments examining the concurrent influences of bottom-up nutrient additions and top-down manipulations of grazers and predators, are required to increase our understanding of the processes contributing to the population dynamics of cyanobacteria and its effects on reef ecosystem structure, function, and services.

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References

- Ahern KS, Ahern CR, Udy JW (2008) In situ field experiment shows *Lyngbya majuscula* (cyanobacterium) growth stimulated by added iron, phosphorus and nitrogen. *Harmful Algae* 7:389–404
- Bolker BM (2008) Ecological models and data in R. Princeton University Press, New Jersey
- Capper A, Paul VJ (2008) Grazer interactions with four species of *Lyngbya* in southeast Florida. *Harmful Algae* 7:717–728
- Capper A, Tibbetts IR, O'Neil JM, Shaw GR (2006) Dietary selectivity for the toxic cyanobacterium *Lyngbya majuscula* and resultant growth rates in two species of opisthobranch mollusc. *J Exp Mar Biol Ecol* 331:133–144
- Corless RM, Gonnet GH, Hare DEG, Jeffrey DJ, Knuth DE (1996) On the Lambert W function. *Advances in Computational Mathematics* 5:329–359
- Cruz-Rivera E, Paul VJ (2002) Coral reef benthic cyanobacteria as food and refuge: Diversity, chemistry and complex interactions. *Proc 9th Int Coral Reef Symp* 1:515–520
- Elmetri I, Bell PRF (2004) Effects of phosphorus on the growth and nitrogen fixation rates of *Lyngbya majuscula*: implications for management in Moreton Bay, Queensland. *Mar Ecol Prog Ser* 281:27–35
- Gosliner TM, Behrens DW, Williams GC (1996) Coral reef animals of the Indo-Pacific. Sea Challengers, Monterey
- Holling CS (1961) Principles of insect predation. *Annu Rev Entomol* 6:163–182
- Juliano SA (ed) (2001) Non-linear curve fitting: predation and functional response curves. Oxford University Press, New York
- Kuffner IB, Paul VJ (2004) Effects of the benthic cyanobacterium *Lyngbya majuscula* on larval recruitment of the reef corals *Acropora surculosa* and *Pocillopora damicornis*. *Coral Reefs* 23:455–458
- McCoy MW, Bolker BM (2008) Trait-mediated interactions: influence of prey size, density and experience. *J Anim Ecol* 77:478–486
- Nagle DG, Paul VJ (1998) Chemical defense of a marine cyanobacterial bloom. *J Exp Mar Biol Ecol* 225:29–38
- Nagle DG, Paul VJ (1999) Production of secondary metabolites by filamentous tropical marine cyanobacteria: Ecological functions of the compounds. *J Phycol* 35:1412–1421
- Osborne NJ, Webb PM, Shaw GR (2001) The toxins of *Lyngbya majuscula* and their human and ecological health effects. *Environ Int* 27:381–392
- Paul VJ (2008) Global warming and cyanobacterial harmful algal blooms. *Adv Exp Med Biol* 619:239–257
- Paul VJ, Pennings SC (1991) Diet-derived chemical defenses in the sea hare *Stylocheilus longicauda* (Quoy et Gaimard 1824). *J Exp Mar Biol Ecol* 151:227–243
- Paul VJ, Thacker RW, Banks K, Golubic S (2005) Benthic cyanobacterial bloom impacts the reefs of South Florida (Broward County, USA). *Coral Reefs* 24:693–697
- Pennings SC, Paul VJ (1993a) Secondary chemistry does not limit dietary range of the specialist sea hare *Stylocheilus longicauda*. *J Exp Mar Biol Ecol* 174:97–113
- Pennings SC, Paul VJ (1993b) Sequestration of dietary secondary metabolites by three species of sea hares: location, specificity and dynamics. *Mar Biol* 117:535–546
- Pennings SC, Nastisch S, Paul VJ (2001) Vulnerability of sea hares to fish predators: importance of diet and fish species. *Coral Reefs* 20:320–324
- R Development Core Team (2008) R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna
- Rogers D (1972) Random search and insect population models. *J Anim Ecol* 41:369–383
- Rosenzweig ML (1971) Paradox of enrichment: Destabilization of exploitation ecosystems in ecological time. *Science* 171:385–387
- Stielow S, Ballantine DL (2003) Benthic cyanobacterial, *Microcoleus lyngbyaeus*, blooms in shallow, inshore Puerto Rican seagrass habitats, Caribbean Sea. *Harmful Algae* 2:127–130
- Switzer-Dunlap M, Hadfield MG (1977) Observations on development, larval growth and metamorphosis of four species of aplysiidae (Gastropoda: Opisthobranchia) in laboratory culture. *J Exp Mar Biol Ecol* 29:245–261
- Thacker RW, Nagle DG, Paul VJ (1997) Effects of repeated exposures to marine cyanobacterial secondary metabolites on feeding by juvenile rabbitfish and parrotfish. *Mar Ecol Prog Ser* 147:21–29
- Watkinson AJ, O'Neil JM, Dennison WC (2005) Ecophysiology of the marine cyanobacterium, *Lyngbya majuscula* (Oscillatoria-ceae) in Moreton Bay, Australia. *Harmful Algae* 4:697–715