expression is responsible for the *cre1 ahk3* phenotype, because loss of AHP6 function in *ahp6-1 cre1 ahk3* was able to suppress the ectopic protoxylem (91%; *n* = 45) (Fig. 1B). In both *wol* and *cre1 ahk2 ahk3*, the *AHP6* expression pattern expands throughout the vascular bundle (Fig. 4A) (*12*). The expanded expression pattern in *wol* is already evident by the early torpedo stage of embryogenesis when it occupies one broad domain within the embryonic root as opposed to two narrow strands in wild-type (Fig. 3F and fig. S8C). This indicates that cytokinin signaling specifies the spatial domain of *AHP6* expression upstream of protoxylem differentiation, which occurs after embryogenesis. Next, we examined the effect of exogenous cytokinins on *AHP6* expression. We observed down-regulation of the *AHP6* transcript after a 6-hour treatment with cytokinins (Fig. 4B). Likewise, the level of fluorescence in the *AHP6prom::GFP* line was reduced by cytokinins, and the reduction occurred at lower levels of cytokinin in *ahp6-1* than in wild-type roots. (Fig. 4C and fig. S9). In the absence of applied cytokinin, the levels of *AHP6* transcript in *ahp6-1* were slightly lower than in the wild type (*12*).

We report a regulatory circuit between cytokinin signaling and its newly identified inhibitor, AHP6, which specifies the meristematic versus differentiated nature of procambial cell files (Fig. 4D). In this sense, our results are consistent with requirement of cytokinins for transdifferentiation of xylem observed in *Zinnia* mesophyll cell culture (*5*, *19*). AHP6 can be considered the founding member of a new "pseudo" subclass of HPt proteins within the wider group present in prokaryotes and eukaryotes.

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Fishing, Trophic Cascades, and the Process of Grazing on Coral Reefs

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Since the mass mortality of the urchin *Diadema antillarum* in 1983, parrotfishes have become the dominant grazer on Caribbean reefs. The grazing capacity of these fishes could be impaired if marine reserves achieve their long-term goal of restoring large consumers, several of which prey on parrotfishes. Here we compare the negative impacts of enhanced predation with the positive impacts of reduced fishing mortality on parrotfishes inside reserves. Because large-bodied parrotfishes escape the risk of predation from a large piscivore (the Nassau grouper), the predation effect reduced grazing by only 4 to 8%. This impact was overwhelmed by the increase in density of large parrotfishes, resulting in a net doubling of grazing. Increased grazing caused a fourfold reduction in the cover of macroalgae, which, because they are the principal competitors of corals, highlights the potential importance of reserves for coral reef resilience.

Caribbean reefs were acutely disturbed in 1983 when the herbivorous urchin *Diadema antillarum* experienced mass disease-induced mortality (*1*). In the absence of *Diadema*, parrotfishes (Scaridae) have become the dominant grazer on most Caribbean reefs (*2*). Grazing performs several critical functions in this ecosystem, including the conversion of primary production to fish-based trophic pathways (*3*), the provision of suitable settlement substrata for new corals (*4*), and the mediation of competition between corals and macroalgae (*5*). Parrotfishes are exploited in many parts of the region, and several studies

have reported increases in their density when fishing intensity was reduced inside reserves (*6*, *7*). However, although reserves may benefit parrotfish populations in the short term, these impacts may be reversed on longer time scales. Most reserves aim to restore the biomass of large piscivores (*8*) such as the Nassau grouper (*Epinephelus striatus*), but this might eventually cause cascading top-down limitation on the biomass of their prey (*9*, *10*), which includes parrotfishes (*11*, *12*). Therefore, with the continued scarcity of *Diadema* (*13*), the realization of a successful reserve may impair levels of grazing and its associated ecosystem functions.

Few reserves are either large, old, or effective enough to have had a significant impact on large predators (*6*, *14*). An exception is the Exuma Cays Land and Sea Park (ECLSP), which lies near the center of the Bahamas archipelago. The ECLSP is large (456 km²) and was established in 1959. A ban on fishing has been enforced there since 1986, and the current biomass of *E. striatus* is seven times greater in the ECLSP than that observed in three other regions of the archipelago (*15*). The ECLSP is, therefore, one of the few places in the Caribbean where the long-term impacts of reserves can be investigated (*16*). We sampled four island systems at hierarchical scales and contrasted the results in and around the reserve with those found at equivalent spatial scales in systems that lacked reserves. Specifically, fish communities of *Montastraea* reefs were sampled at scales of sites (hundreds of

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meters, $n = 3$ to 4), reefs (or reserve) (tens of kilometers, $n = 3$), and island systems (hundreds of kilometers, $n = 4$). The reserve is located between two nonreserve reefs, to the north and to the south of the reserve, in the Exuma Cays island system. To examine the influence of reserves on grazing, we created and tested a model of parrotfish grazing intensity that was sensitive to species, size, and life phase (*16*).

The overall biomass of all parrotfish predators within the reserve was approximately double that found in nonreserve areas within the same reef tract and was more than five times greater than that in other regions of the archipelago (Fig. 1). *E. striatus* accounted for 45% of this predator biomass, with the remainder being composed of other large groupers, including *Mycteroperca tigris* (27%) and a combination of Sphyraenidae, Muraenidae, and large Lutjanidae (28%).

At the scale of reefs within an island system (tens of kilometers), the community structure of parrotfishes showed significant variation only between the reserve and its immediate nonreserve reefs (analysis of similarities, $R = 0.29$, $P < 0.05$). Several parrotfish species exhibited significant differences in size across reserve boundaries (Fig. 2). Individuals of the smaller bodied scarid species, whose maximum length rarely exceeds 23 cm (*Scarus iserti* and *Sparisoma aurofrenatum*), were smaller inside the reserve (Fig. 2), but their densities were indistinguishable from those elsewhere [analysis of variance (ANOVA), $P > 0.05$]. In contrast, scarids that reached consistently large adult sizes, such as the terminal-phase (TP) males of *Sc. vetula* and *Sp. viride*, exhibited no difference in their size across the reserve boundary (Fig. 2), but their mean density was nearly doubled inside the reserve. Parrotfishes that occupied a wide range of size categories (6 to 32 cm) were either larger in the reserve [*Sp. viride* intermediate phase (IP)] or larger outside the reserve (*Sc. vetula* IP). Mean parrotfish sizes outside the reserve did not differ from those found elsewhere in the archipelago ($P > 0.05$).

Large-bodied parrotfishes appear to escape predation by the dominant piscivore, *E. striatus* (Fig. 3). The distribution of mouth sizes in the grouper population is such that few groupers (typically <0.5 individuals per 1000 m²) are large enough to swallow TP parrotfish of the larger bodied species *Sc. vetula* and *Sp. viride* (Fig. 3), and such large groupers were observed only in the reserve. In contrast, between 60 and 90% of the *E. striatus* in the reserve are able to consume adults of the smallest bodied parrotfish, *Sc. iserti*, and 30 to 60% of groupers are able to swallow the medium-sized scarid, *Sp. aurofrenatum*. Adults of both parrotfish species were significantly smaller inside the reserve (Fig. 2). An increase in natural predator-caused mortality within the reserve would be expected to reduce their average longevity and therefore length (*17*) and grazing capacity (*18*).
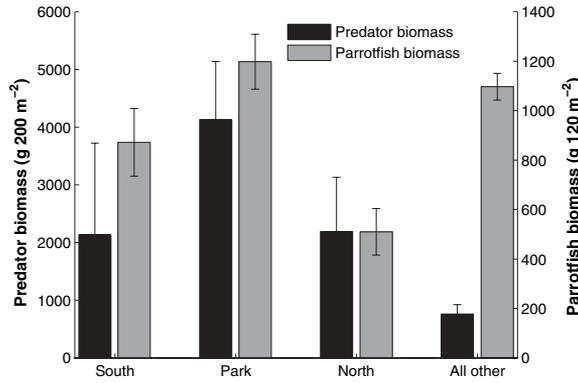

Fig. 1. Patterns of parrotfish biomass and their predators (±SE) within the Exuma Cays and for all other surveyed areas combined. "Park" denotes the ECLSP.
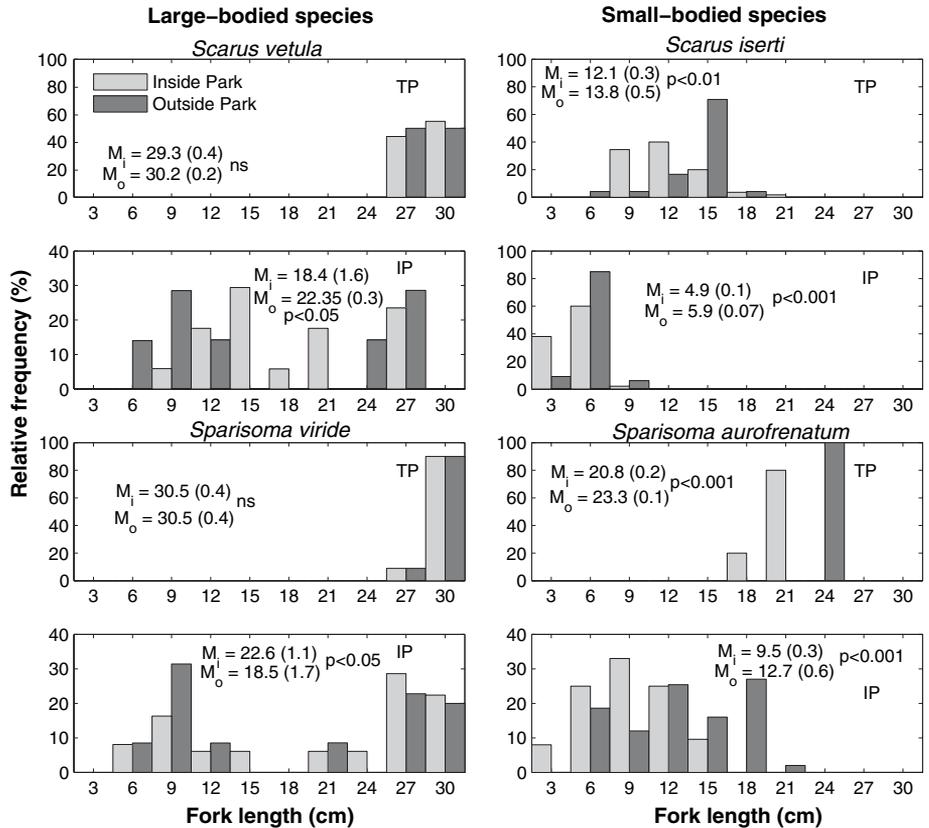
Fig. 2. Relative size frequency distributions of four parrotfish species in two life phases (TP and IP). Data were pooled for the *Montastraea* reef habitat inside and outside the ECLSP. Also shown are the mean (±SE) length of species inside (M_i) and outside (M_o) the reserve and the probability of a *t* test finding no significant difference between sites. "Fork length" is measured from the snout to the fork of the tail.

The optimal size of prey for many piscivorous fishes is 0.6 times that of the predator's jaw width (*19*); chasing larger prey may be uneconomic because of the greater evasive potential of larger individuals (*20*). Therefore, predator capacity was also plotted for the assumption that prey choice was restricted to the most preferred individuals [those with body height not exceeding 60% of grouper jaw width (Fig. 3)]. Under this scenario, a grouper's capacity to consume the smaller bodied species remains greater in the reserve, whereas the likelihood of consuming TP *Sc. vetula* or *Sp. viride* is even less, emphasizing

the potential for a size escape from predation. However, foraging by *E. striatus* is primarily nocturnal and crepuscular (*12*, *21*) and is therefore coincident with the nocturnal resting of parrotfishes (*22*), often in vulnerable locations on the reef. Prey may be much less likely to escape under these circumstances, thereby increasing the probability that groupers will prey on scarids with dimensions approaching the upper limits of mouth size (though it appears that *Sc. vetula* and *Sp. viride* are able to avoid this increased vulnerability).

Our data suggest that the fishing mortality of large-bodied parrotfish is considerable in

Fig. 3. Consumptive capacity of Nassau grouper populations inside (bold lines) and outside (light lines) the ECLSP. Solid lines assume that prey consumption is constrained by the full width of grouper mouthparts, whereas dotted lines represent an optimal prey size equivalent to 60% of the full gape. Dorsoventral heights of parrotfish (prey) species were plotted for the observed range of scarid size in the Exuma Cays. Dark and light shading denote the size ranges of small- and large-bodied parrotfishes, respectively.
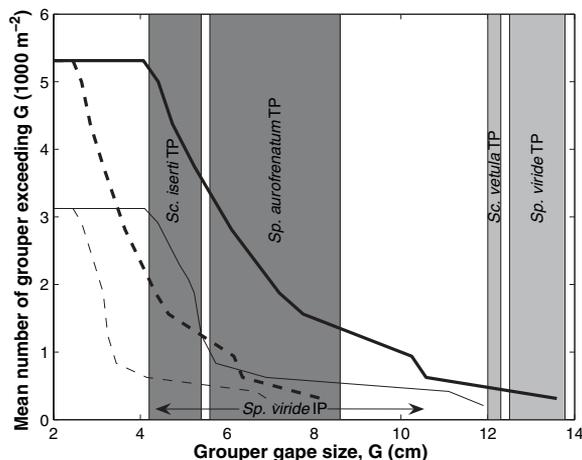
Fig. 4. Mean grazing intensity of parrotfishes (black bars) and macroalgal cover (gray bars) (±SE) inside and outside the ECLSP. Reserve impacts are significant ($P < 0.01$) for each variable.
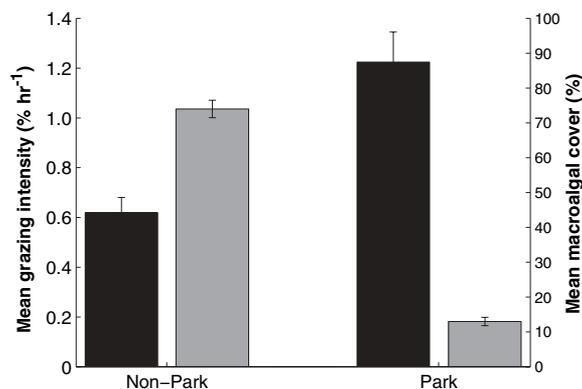
fished areas of the Exuma Cays. Of the large commercial fishing vessels registered as using fish traps in the Bahamas, 40% (14) have sufficient size (>10 m) and proximity (Nassau to Exuma Cays) to fish around the reserve (23). An additional 30 traps are deployed locally to the south of the reserve. Large-bodied parrotfish are highly susceptible to fish traps (6), which may be left unattended during periods of high winds and cause considerable, though not quantified, mortality of parrotfishes. Studies in Barbados found traps to be disproportionately selective for *Scarus vetula* (6), and the biomass of this species was almost seven times greater inside the reserve. Overall, the total biomass of parrotfishes was significantly greater inside the reserve (Fig. 1, $P < 0.001$). We conclude, therefore, that larger parrotfishes have benefited numerically from a reduction in fishing pressure within the ECLSP.

The results of nested analyses allow us to infer that the reserve has significant effects on predator biomass and on the community structure, size distribution, and grazing intensity of parrotfishes. Even if we assume that only 2 of the 12 inter-reef comparisons exhibit significant differences, the probability that both involve the reserve is ~1%. Fully functioning marine reserves in the Caribbean appear to have a negative impact on the size distribution of smaller bodied parrotfish through increased predation, but a positive impact on larger bodied species because of the release from fishing pressure. With the continued paucity of *Diadema* (13) and the nonlinear positive relationship between fish length and grazing intensity (18), any impact on larger bodied fishes will have a disproportionately large impact on grazing. In this system, the net impact of reserve implementation was a doubling of total scarid grazing (Fig. 4), and this reef-level effect was observed only across reserve boundaries (nested generalized linear model ANOVA, $P < 0.05$).

The cover of living coral on Bahamian reefs was severely reduced by bleaching during the exceptionally high sea temperatures of the 1998 El Niño–Southern Oscillation event (13). Given projected climate change, the resilience, or ability of reefs to recover from such disturbances, is a key societal concern (24). Recovery requires the successful recruitment and survival of new corals, but both processes are inhibited by macroalgae (5, 25). Our data reveal a strong negative relation between fish grazing intensity and macroalgal cover in the Exuma Cays; the cover of macroalgae was reduced fourfold inside the reserve (Fig. 4), whereas there were no reef-scale fluctuations in cover in systems with no reserve ($P > 0.40$). Although the biomass of herbivorous fishes has been negatively correlated with macroalgal

cover on a Caribbean-wide scale (26), the direct small-scale impacts of a reserve were previously undocumented. Although reductions in macroalgal cover inside reserves may enhance the recruitment and survival of corals, elevated parrotfish grazing may have complex impacts on reefs. Scarid grazing is an important source of bioerosion, and although only 4% of their bites are taken from adult corals (18), the role of parrotfishes as predators of juvenile corals is yet to be evaluated.

The "benefits" accrued from a reserve will largely depend on the intensity of fishing outside its boundaries. If Caribbean parrotfishes were not affected by exploitation at all, then a reserve would cause only negative impacts on parrotfishes because of enhanced predation. Rescaling the size of each parrotfish using the mean size discrepancies reported in Fig. 2, we calculate that the total grazing intensity would drop by 4 to 8% of its current value in several island systems (San Salvador, the Turks and Caicos Islands, and Andros) where parrotfish exploitation is relatively light (fish traps are used on <1% of fishing trips). A predation-based reduction in grazing of 4 to 8% would be fully compensated for if the reserve enabled parrotfish densities to increase by a mean level of 9% (approximately one individual of each life phase of each species in 1200 m²). To place this reserve impact in perspective, it represents just one-sixth that observed in the Exuma Cays, which by Caribbean standards is a relatively lightly fished system. For example, scarid grazing intensity in fished regions of the Exuma Cays was at least five times greater than in exploited areas of Jamaica (16). Because parrotfish form bycatch in fisheries and are easily targeted by commercial and recreational spear fishermen, reserves will almost always increase the level of fish grazing within their boundaries.

Many fisheries management agencies are adopting the principle of ecosystem-based management (EBM), in which the wider functioning and requirements of whole ecosystems are considered (27). Despite the appealing premise of EBM, its implementation is generally constrained by a lack of data or appropriate analyses. How do EBM tools, such as marine reserves, influence the key processes that drive an ecosystem? This question is particularly important when considering disturbed systems, because returning one process to its "natural" level may have unexpected and even deleterious consequences for the ecosystem overall. Our results indicate that the long-term impact of Caribbean no-take marine reserves is enhanced grazing, a process that is key to the ecosystem functioning of coral reefs.

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