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Changing feeding preferences of butterflyfishes following coral bleaching

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Abstract. Climate-induced coral bleaching poses a significant threat to reef fishes, especially for those fishes that rely on corals for food. Aside from the direct effects of coral loss, whereby many fishes decline in abundance in accordance with loss of live coral, coral-feeding fishes often exhibit a reduction in feeding intensity upon bleached colonies. These observations suggest that the nutritional quality of bleached corals may be compromised, but this is yet to be examined. In this study, we compared feeding preferences of coral-feeding butterflyfishes on bleached versus unbleached corals, and quantified total lipid content of the corals to assess whether changes in prey preferences reflected changes in food quality. The study was conducted in experimental aquaria with two species of corallivorous butterflyfish *Chaetodon plebeius,* and *Chaetodon lunulatu*s. Only *C. plebeius* reduced feeding on bleached coral colonies compared with healthy (unbleached) colonies, while *C. lunulatus* showed no preference in feeding between bleached and unbleached corals. However, no decline in total lipid content of corals was observed following bleaching, suggesting that it is not changes in prey quality (measured based on total lipid content) that led to observed changes in patterns of prey use.

Key words: Bleaching, Prey Quality**,** Feeding Preferences**,** Chaetodontidae.

Introduction

Coral reef ecosystems are experiencing extensive and increasing loss of scleractinian corals (Wilkinson et al. 2004), partly due to climate-induced coral bleaching. Extensive coral loss and associated declines in habitat complexity often lead to declines in the abundance of coral reef fishes (Jones et al. 2004; Wilson et al. 2006). The reef fishes that are most susceptible to declining coral cover are highly specialized coral-dependent species (Pratchett et al. 2008), such as the obligate coral-feeding butterflyfishes (Wilson et al. 2006; Pratchett et al. 2008). Several studies have documented significant declines in the abundance of coral-feeding fishes following severe coral bleaching and coral loss (Kokita and Nakazono 2001; Sano 2004; Pratchett et al. 2006; Graham 2007; Graham et al. 2009). However, coral loss may also lead to changes in the distribution or behavior of coral-feeding fishes (Pratchett et al. 2004; Berumen et al. 2005).

Pratchett et al. (2004) documented prey switching in *Chaetodon lunulatus* associated with declines in the abundance of preferred prey, while Cole et al. (2009) showed a decline in feeding intensity on bleached colonies, suggesting that prey quality declines during a bleaching event long before the coral actually dies. The most likely explanation to account for limited consumption of bleached corals, is that bleaching leads to an immediate decline in the nutritional quality of the coral which would directly influence prey quality. Indeed, numerous studies have reported declines in total lipid content following bleaching (Glynn et al. 1985; Grottoli et al. 2004; Yamashiro et al. 2005; Rodrigues and Grottoli 2006; Rodrigues and Grottoli 2007; Rodrigues et al. 2008; Rodríguez-Troncoso et al. 2010a, b). For bleached corals, the loss of endosymbiotic zooxanthellae and subsequent decline in the photosynthetically fixed carbon may lead most coral species to utilize their energy rich lipid reserves to meet their daily metabolic energy needs (Porter et al. 1989; Grottoli et al. 2004, 2006; Yamashiro et al. 2005; Rodrigues and Grottoli 2007). However, a direct link between declining lipid content in bleached colonies and declining feeding effort in corallivorous fishes has not been demonstrated.

The purpose of this study was to quantify feeding preferences of two obligate coral feeding butterflyfish (*Chaetodon lunulatus* and *Chaetodon plebeius*) for bleached versus unbleached corals, and test whether changes in feeding preferences correspond with changes in total lipid content of the different prey corals. This study also tested whether chronic predation by butterflyfishes exacerbated effects of bleaching on individual corals. At the colony level the chronic removal of live tissue is likely to represent a significant energetic cost for prey corals that are frequently consumed (Cole et al. 2011). The compounding effects of chronic predation may reduce fitness, survivorship and recovery of colonies that are already stressed from bleaching (Bellwood et al. 2006; Cole et al. 2009; Rotjan and Lewis 2009).

Material and Methods

This study was conducted in September 2010 at Lizard Island (14°40'S, 145°27'E) on the northern Great Barrier Reef, Australia. Experiments were conducted in 12 large flow-through aquaria (~ 300 litres, 1.0 m diameter per 0.4 m deep) at the Lizard Island Research Station, with butterflyfishes and corals collected from back reef habitats immediately adjacent to the research station. A total of 12 individuals each of *C. lunulatus* and *C. plebeius*, as well as 24 colonies of similar size (20 cm maximum diameter) each of *Pocillopora damicornis* and *Acropora spathulata*, were collected from the field. To ensure a realistic representation of the natural environment, three conspecific butterflyfish of different sizes (between 20 and 122 mm total length) were introduced to each tank, such that there were 4 tanks containing *C. lunulatus* and 4 with *C. plebeius*. To test whether chronic predation exacerbates the effects of coral bleaching, we maintained 4 control tanks (no corallivores treatment). Coral colonies were randomly assigned to the 12 different tanks, with two colonies of each species (*P. damicornis* and *A. spathulata*) in each tank. Prior to placing the colonies in the aquaria, half of the colonies (one of each species) were bleached, by immersing them in low salinity water (10) for 20 minutes, following Cole et al. (2009).

To assess the relative consumption of bleached versus healthy corals by butterflyfishes, feeding observations were conducted every second day over a nine day period around 2 pm, recording the corals (species and treatment) from which each single fish took 50 consecutive bites.

To test for changes in condition and quality of different colonies, we monitored total lipid content and zooxanthellae density throughout the 9 days of experiment. A single branch was removed on each sampling day and then analyzed for total lipid content and zooxanthellae density. It was expected that lipid content would decline in accordance with declines in zooxanthellae densities, following bleaching (Rodríguez-Troncoso et al. 2010). To extract total lipids, coral branches were dried in the oven at 55°C for 24 h, weighed and placed in a solution of chloroform: methanol (2:1, v:v) to dissolve the lipids (Barnes and Blackstock 1973). The tissues were redried at 55°C overnight and reweighed. The difference in weight was due to lipids loss, with total lipid content then expressed as percentage of dry weight. Symbiont density (per unit surface area (cells/cm^2)) was quantified for each coral based on samples (5mm x 5mm) collected from branch tips and fixed in 10% buffered formalin every 1-2 day throughout the experiment. Each sample was homogenized and the ground solution was examined using slide glass under a microscope and counts were normalized to coral surface area, following McCowan et al. (2011). Symbiont densities were estimated by multiplying the number of zooxanthellae counted in each sample (N) by 10^4 (to account for 0.0001ml sampled in haemocytometer chamber) and the dilution factor (1ml of ethanol), and then divided by the surface area of the coral tissue sample (0.25 cm^2).

Data analysis

To test for changes in the relative number of bites on bleached versus unbleached colonies and among coral species, a MANOVA was conducted comparing the number of bites on different corals through time. Separate analyses were conducted for each species of butterflyfish (*C. lunulatus* and *C. plebeius*). Each tank was treated as a replicate, and the feeding response variable was generated from the average proportion of bites of each fish within a tank. The variation in feeding preferences for bleached and unbleached between individuals was low in both fish species, with the significance of the treatments being consistent whether mean tank or replicate fish were used. Feeding preferences were not analyzed using Repeated Measures ANOVA, as the assumption of sphericity was never met (Mauchley Sphericity Test). A Two-Way ANOVA was carried out to test for significant differences in symbiont density, while total lipid content was analyzed for each coral species with Repeated Measures ANOVA. Time was used as a dependent variable and treatment (bleached with and without corallivores and unbleached with and without corallivores) as a categorical variable.

Results

Butterflyfishes (both *C. lunulatus* and *C. plebeius*) began feeding on experimental corals within 48 hours of being introduced to tanks. A different feeding behavior was observed in the two corallivorous fishes following bleaching. At the start of the experiment, *C. plebeius* fed mainly on bleached colonies, but over time feeding on bleached colonies declined (Fig. 1a, Table 1). While there was a strong difference in feeding preferences for unbleached versus bleached corals, there was no distinction between *P. damicornis* and *A. spathulata* (Fig. 1a, Table 1). Conversely, *C. lunulatus* did not show a clear feeding preference towards either unbleached or bleached colonies (Fig. 1b). Feeding intensity on bleached colonies was higher for the first two days of the experiments, but steadily declined, and there was approximately equal use of both bleached and unbleached corals by day 9 (Fig. 1b). Similarly, there were no significant differences in the proportional consumption of *P. damicornis* and *A. spathulata* (Table 1).

Table 1: Results of MANOVA to test for changes in number of bites in *C. plebeius* and *C. lunulatus* between days, between bleached and unbleached colonies and between coral species (*A. spathulata* and *P. damicornis*).

	Wilks	F	d.f.	P
Chaetodon plebius				
Day	0.5	2.43	3/10	0.12
Day*Treatment	0.3	5.41	3/10	0.01
Day*Coral Species	0.9	0.07	3/10	0.97
Day*Treatment* Coral Species	0.9	0.005	3/10	0.99
Chaetodon lunulatus				
Day	0.9	0.03	4/9	0.99
Day*Treatment	0.4	2.32	4/9	0.13
Day*Coral Species	0.6	1.03	4/9	0.43
Day*Treatment* Coral Species	0.4	2.48	4/9	0.11

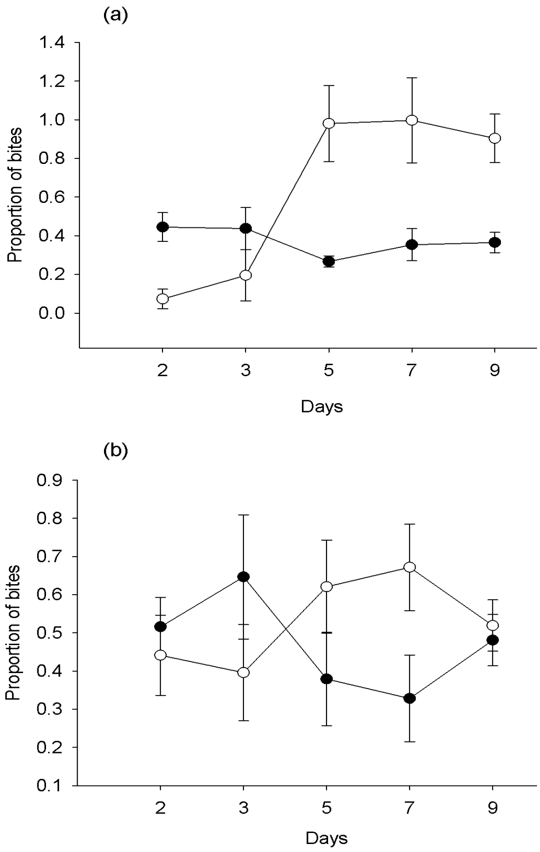

Figure 1: (a) Average proportion of bites of *C. plebeius* taken from bleached (black circles) and unbleached colonies (white circles). (b) Proportion of bites of *C. lunulatus* taken from bleached (black circles) and unbleached colonies (white circles).

Total lipid content did not decline in bleached colonies during the 9 days of the experiment in *A. spathulata* or in *P. damicornis*. At the end of the experiment lipid content did not differ significantly between bleached and healthy (i.e., unbleached) colonies for both coral species (Fig. 2, Table 2). While treatment did not have any effects on lipid content, there was significant temporal variation in lipid content in *A. spathulata* (Table 2). Change in the lipid content of bleached versus unbleached corals was consistent among tanks regardless of the presence of coral-feeding butterflyfishes (Fig. 2, Table 2).

Table 2 Results of repeated measures ANOVA to test for changes in total lipid content in *A. spathulata* and *P. damicornis*, between days, and between bleached colonies with and without corallivores and unbleached colonies with and without corallivores (Treatment).

	d.f.	SS	F	P
A. spathulata				
Day	5/100	561.27	2.93	0.01
Treatment	30/20	30.22	0.16	0.91
Day*Treatment	15/100	610.54	1.06	0.39
P. damicornis				
Day	5/100	336.35	2.04	0.07
Treatment	30/20	194.71	1.01	0.40
Day*Treatment	15/100	491.46	0.99	0.46

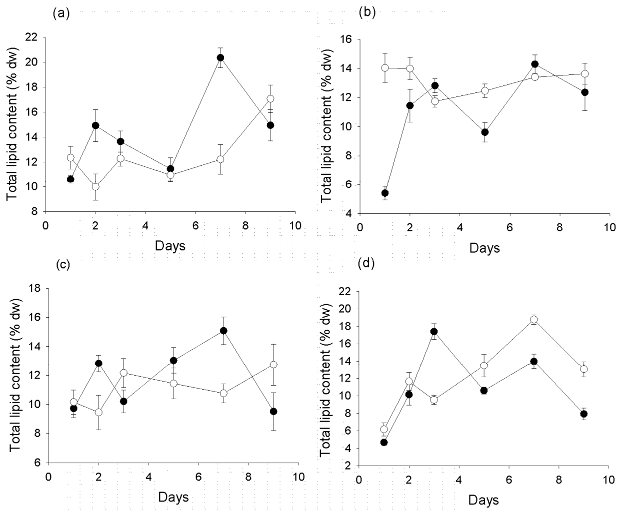

Figure 2: Averages across all tanks of total lipid content (% loss of dry weight) in unbleached and bleached colonies of *P. damicornis* with corallivorous fishes (a), and without corallivorous fishes (b), and in unbleached and bleached colonies of *A. spathulata* with corallivorous fishes (c), and without corallivorous fishes (d). White circles represent unbleached colonies, while black circles represent bleached colonies.

Symbiont density declined approximately 48% from day 1 to day 9 in both bleached *P. damicornis* (day 9: $0.26 \times 10^6 \pm 0.01$ cells cm^2) and *A. spathulata* (day 9: $0.41 \times 10^6 \pm 0.02$ cells cm^2) (Fig. 3). Time and treatments were found to have a significant effect on symbiont density in both *P. damicornis* and *A.*

spathulata (Table 3).

Table 3: Results of two-way ANOVA to test for changes in symbiont density in *A. spathulata* and *P. damicornis*, between days, and between bleached and unbleached colonies (Treatment). * denotes significant effect (P<0.05).

	d.f.	SS	F	P
A. spathulata				
Day	5/110	1.03	18.02	*
Treatment	1/22	1.39	24.2	*
Day*Treatment	5/110	5.34	9.3	*
P. damicornis				
Day	5/110	3.89	7.19	*
Treatment	1/22	1.26	46.0	*
Day*Treatment	5/110	4.08	7.53	*

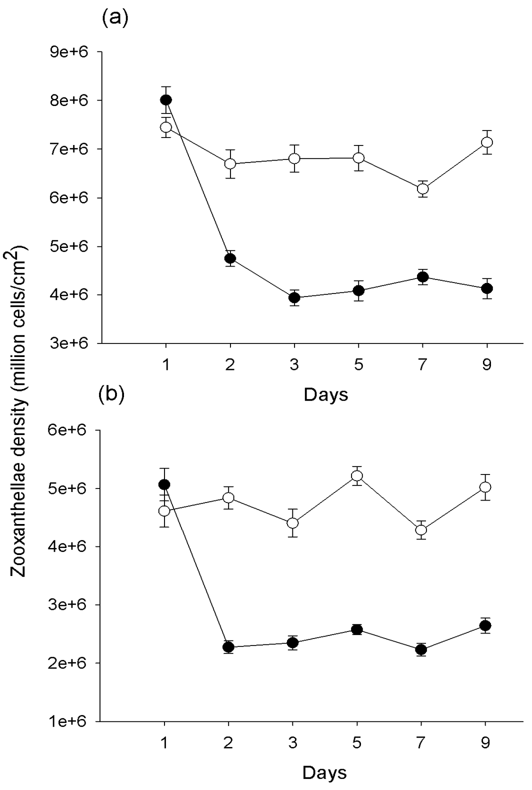

Figure 3: Densities of symbiotic zooxanthellae (in units of cells/cm^2) in (a) *A. spathulata,* and (b) *P. damicornis* during 9 days of experiment. Zooxanthellae density is expressed in millions (e^{+6} on the y axis). White circles represent unbleached colonies, while black circles represent bleached colonies.

Discussion

Bleaching appeared to induce feeding of *C. plebeius* initially, but over time the fish switched their preferences to unbleached corals. Similarly, Cole et al. (2009) documented that *Chaetodon baronessa* and the coral feeding wrasse, *Labrichthys unilineatus* initially increased their feeding intensity on bleached colonies before declining to zero feeding 5 to 7 days following experimental bleaching.

In this study, the change in feeding preference over time does not seem to reflect changes in prey quality, measured based on lipid content. In contrast to previous studies (e.g. Rodríguez-Troncoso et al. 2010a, b), which documented decline in lipid content from days to weeks, this study found no consistent decline in lipid content following bleaching. The variation in total lipid content through time observed in *A. spathulata* is likely driven by natural variability rather than by sublethal effects of bleaching. The disparity between these results may be due to the different bleaching triggers. The method used here to induce bleaching is an acute stress based on salinity stress rather than thermal bleaching, which is a chronic cumulative event. The different rate at which *Symbiodinium* density starts to decline in salinity shock compared to thermal bleaching, may result in subtle differences in the physiological response of corals (i.e. lipid conditions) (Douglas 2003). Is it also possible that a significant change in lipid content was not detected due to the short duration of the experiment.

Other changes in prey quality that are not apparent simply by measuring total lipid content may actually drive feeding preferences in butterflyfishes. The increased feeding effort towards bleached colonies may be due to an initial increase in the nutritional value of recently bleached colonies. Colonies release more mucous as a stress response to environmental changes (Riegl and Branch 1995) and this discharge may increase their desirability as food source (Cole et al. 2009). Butterflyfishes initially fed more heavily on bleached colonies because they may benefit from the highly nutritious mucus but then shifted to unbleached colonies due to a subsequent withdrawal of polyps in the corallum. Intense predation may cause polyp retraction into the corallum as a short-term response (Gochfeld 2004). Similarly, salinity bleaching as an acute stress, may cause colonies to fully retract their polyps in response to osmotic stress. Small-mouthed corallivores visually locate polyps and simply nip at their tentacles. Hence, these corallivores tend to prefer corals with expanded tentacles as fully expanded tentacles can provide higher energy content per bite (Gochfeld 1991, 2004). The increased predation combined with a delayed bleaching response may have caused bleached colonies to fully retract their polyps thereby explaining the reduction in feeding intensity upon bleached colonies over time.

Given fish avoidance of bleached corals over time, the energetic cost of predation on bleached corals was limited. No measurable effect of corallivores was observed on the total lipid content of colonies of either *P. damicornis* or *A. spathulata* during the 9 days of the experiment. Feeding pressure may not have been sufficient to reduce fitness of colonies already stressed from bleaching. However, this might differ if there are no unbleached corals available, such as during severe mass-bleaching. An important extension of this study will be to test whether butterflyfish constrained to feeding on bleached corals actually loose condition, which is fundamental in assessing the effects of coral bleaching on butterflyfishes.

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