Coral Reefs (2004) 23: 352–356
DOI 10.1007/s00338-004-0394-x

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"Sublethal effects of coral bleaching on an obligate coral feeding butterflyfish"

Received: 16 December 2002 / Accepted: 25 June 2003 / Published online: 20 August 2004

Keywords *Chaetodon lunulatus* · Disturbance · Hepatocyte vacuolation · Resource depletion · Physiological condition

Introduction

Coral bleaching is a significant and increasingly prevalent source of coral mortality, representing one of the most severe and widespread disturbances affecting coral reef ecosystems (Hoegh-Guldberg 1999; Pockley 2000). In the last few years (mostly since 1998), major episodes of coral bleaching have occurred on many coral reefs throughout the world, killing 20–80% of zooxanthellate corals (including both scleractinians and alcyonaceans) across expansive reef areas (e.g., Great Barrier Reef, Baird and Marshall 1998; Japan, Shibuno et al. 1999; eastern Pacific, Glynn et al. 2001; Caribbean, Ostrander et al. 2000). In addition to killing zooxanthellate corals, severe large-scale bleaching events may cause significant declines in the abundance of coral reef fishes, particularly among reef fish species that depend on live coral for food or shelter (Shibuno et al. 1999; Kokita and Nakazono 2001; Adjeroud et al. 2002).

Among those fishes with the greatest reliance on live corals are butterflyfish from the genus *Chaetodon* (family Chaetodontidae), most of which feed primarily (if not exclusively) on living tissue from scleractinian and alcyonacean corals (e.g., Birkeland and Neudecker 1981; Harmelin-Vivien and Bouchan-Navaro 1983; Anderson et al. 1981). Further, spatial and temporal variation in the abundance of *Chaetodon* butterflyfish is often associated with variation in the abundance of live corals (Findley and Findley 1985). Most notably, significant declines in the abundance of *Chaetodon* butterflyfish have been observed following coral depletion by crown-of-thorns starfish (Bouchan-Navaro et al. 1985; Williams 1986; Sano et al. 1987). Therefore, similar declines would be expected where bleaching causes extensive coral mortality. Even if there is no immediate decline in the abundance of butterflyfish, the reduced availability of prey corals is likely to affect the physiological condition and subsequent fitness of coral-feeding butterflyfish (Jones and McCormick 2002).

In this study, we explored the effects of coral bleaching on the redfin butterflyfish, *Chaetodon lunulatus* Quoy and Gaimard 1824. This species is distributed throughout the Indo-Pacific Archipelago and eastern Pacific Ocean (Allen et al. 1998), and is among the most abundant butterflyfish species on reefs within this region (e.g., Anderson et al. 1981; Bouchan-Navaro et al. 1985). Previous studies have shown that *Chaetodon lunulatus* feeds exclusively on living tissues from scleractinian corals (Harmelin-Vivien and Bouchan-Navaro 1983), and is likely therefore, to be very susceptible to changes in the availability of scleractinian corals (Kokita and Nakazono 2001). The purpose of this study was to investigate changes in the (1) abundance, (2) dietary habits (specifically, the proportional consumption of different prey corals), and (3) physiological condition of *C. lunulatus*, during a major episode of coral bleaching. The specific effects of coral bleaching on *C. lunulatus* were investigated over a 2-year period (May 2000 to March 2002), on the Great Barrier Reef (GBR), Australia. During this period, the GBR was subject to the most severe episode of coral bleaching ever recorded (Dennis 2002).

Communicated by: P.F. Sale

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Methods

This study was conducted at Trunk Reef (18°17′S, 146°53′E), in the central section of the Great Barrier Reef, Australia. All sampling was conducted on the shallow reef crest (2–3-m-water depth), along the exposed (southeast) margin of Trunk Reef. Densities of *C. lunulatus* were measured using five replicate 50-m×4 -m-belt transects at each of three randomly selected sites in May 2000 (before the bleaching episode) and at three randomly selected sites in March 2002 (after the bleaching). All sites (six in total) were non-overlapping and completely independent, but appeared very similar in their structure, aspect, and exposure to prevailing southeast trade winds. Variation in the abundance of *C. lunulatus* was compared between sampling occasions and among sites using a two-factor nested ANOVA (sites nested within sampling occasions). Changes in the abundance of *C. lunulatus* were then compared with changes in the availability of coral prey, where live cover and species composition of scleractinian corals were quantified using replicate 10-m long line-intercept transects. Ten replicate transects were sampled on the reef crest at each site in May 2000 and March 2002, giving a total of 60 transects. In order to assess changes in the dietary habits of *C. lunulatus*, we also recorded the number of bites taken from each coral species during 3-min long field-observations of replicate individuals. Feeding observations were conducted for a total of 24 individuals in May 2000 (before the bleaching), and for 20 individuals in March 2002 (after the coral bleaching). All feeding observations were conducted between 11:00 a.m. and 2:00 p.m. to minimize variation that might be attributable to diurnal feeding patterns.

Physiological condition of *C. lunulatus* was quantified using hepatocyte vacuolation (the proportion of hepatic tissues occupied by intracellular vacuoles), following Pratchett et al. (2001). Hepatocyte vacuolation was measured for 20 replicate fish collected from the exposed reef crest at Trunk Reef in both May 2000 and March 2002. All fish were collected by spearing, and placed on ice for 2–4 h prior to processing. The entire liver from each fish was then removed and placed into 10% calcium-buffered formalin (FAACC) for 4 days. After fixing, hepatic tissues were dehydrated in a graded ethanol series and embedded in paraffin wax. Wax blocks of hepatic tissues were sectioned at 5 μm, and sections stained using Mayer's hematoxylin and eosin to emphasize hepatocyte vacuoles. The proportion of vacuoles in hepatic tissues was then quantified using a Weibel eyepiece, recording the proportion of points (out of 121) that intersected hepatocyte vacuoles viewed at ×40. Three estimates of hepatocyte vacuolation (the proportion of 121 points intersecting hepatocyte vacuoles) were recorded for each of three different sections through the liver of each fish, giving a total of nine estimates for each fish. The mean proportion of vacuoles in hepatic tissues of each fish was then calculated, and a one-way ANOVA was used to compare the mean hepatocyte vacuolation of fish collected in May 2000 ($n = 20$), versus fish collected in March 2002 ($n = 20$).

Although hepatocyte vacuolation is increasingly used as an indicator of physiological condition in coral reef fishes (e.g., Green and McCormick 1999; Pratchett et al. 2001), it is currently not known if, or how, hepatocyte vacuolation relates to absolute liver lipid content. Therefore, we used a random subsample of the *C. lunulatus* ($n = 9$) collected from Trunk Reef to compare estimates of hepatocyte vacuolation with absolute liver lipid content. The entire liver was removed from each fish and coarsely sectioned into six parts. Three of these sections were fixed in 10% buffered formalin for histological processing (as described previously), while the remaining sections from each fish were frozen at −40 °C to later measure absolute liver lipid content. Absolute liver lipid content was measured using the Bligh and Dyer (1959) gravimetric technique, whereby frozen liver sections from each individual fish were ground in a glass mortar and pestle then transferred to a Teflon vial. Ground liver samples were weighed and extracted using the single-phase chloroform, methanol, and water technique (Bligh and Dyer 1959). After addition of solvents and water, each sample was vortexed for 1 min and then left for 12 h. Chloroform and water were then added to separate samples into two phases. Samples were then vortexed for 1 min and centrifuged at 3,000 rpm for 5 min. The upper aqueous phase was removed with a glass pipette and discarded. The lower chloroform layer was poured through a glass funnel lined with a combusted GF/C filter paper to remove particulate matter. The filtrate was concentrated by rotary evaporation and transferred to a combusted 2-ml-glass vial. A 10-μl aliquot of concentrated lipid extract was placed on an aluminium pan and the solvent evaporated at approximately 30 °C. The remaining lipid was weighed using a Perkin Elmer microbalance. This procedure was repeated 3–4 times for each sample and then the total lipid content was calculated from the mean lipid content in replicate 10-μl aliquots. Absolute liver lipid content was then compared with estimates of hepatocyte vacuolation for each individual fish, using simple linear regression.

Results and discussion

Mass bleaching of zooxanthellate corals occurred throughout much of the Great Barrier Reef from November 2001 to February 2002, causing very extensive and widespread coral mortality (Dennis 2002). At Trunk Reef, in March 2002, there were conspicuous signs of recent coral mortality, with large areas of dead but intact coral skeletons (mostly corymbose and digitate *Acropora* spp.) covered with fine filamentous algae. Furthermore, many (30–40%) of the remaining coral colonies were very pale in colour, showing mild or early signs of coral bleaching (*sensu* Marshall and Baird

2000). Live coral cover declined by 55% during this study; from a mean of 33.4% live coral cover (±1.3 SE) in May 2000, down to just 15.0% (±1.3 SE) in March 2002 (Fig. 1). However, declines in live coral cover were not equal across different coral taxa. *Acropora* corals were the most severely affected coral taxa, and declined in abundance by 72% during the study (Fig. 2). There were also significant declines in live cover of Poritidae and *Montipora* spp. However, there was comparatively little change in the abundance of *Pocillopora*, *Goniastrea*, *Favia*, *Stylophora*, *Seriatopora*, or *Isopora* (Fig. 2). Different coral taxa are widely known to differ in their susceptibilities to bleaching (see review by Hoegh-Guldberg 1999), and findings from this study concur with previous studies showing *Acropora* to be among the most susceptible of scleractinian corals (e.g., Glynn 1996, Marshall and Baird 2000).

Despite significant declines in live coral cover at Trunk Reef (ANOVA, $F = 52.98$, $df = 1$, $p < 0.01$), there was no corresponding decline in the abundance of *C. lunulatus* (Fig. 1). Mean densities of *C. lunulatus* on the exposed reef crest at Trunk Reef were 7.63 (±0.5 SE) fish per 200 m^2 (per transect), and did not differ significantly between sampling occasions (ANOVA, $F = 0.10$, $df = 1$, $p = 0.76$), or among sites within each sampling occasion (ANOVA, $F = 2.12$, $df = 4$, $p = 0.11$). These findings concur with Shibuno et al. (1999) who found little change in the abundance of *C. lunulatus* during significant declines in live coral cover (93% coral mortality) at Ishigaki Island, Japan (but see Kokita and Nakazono 2001).

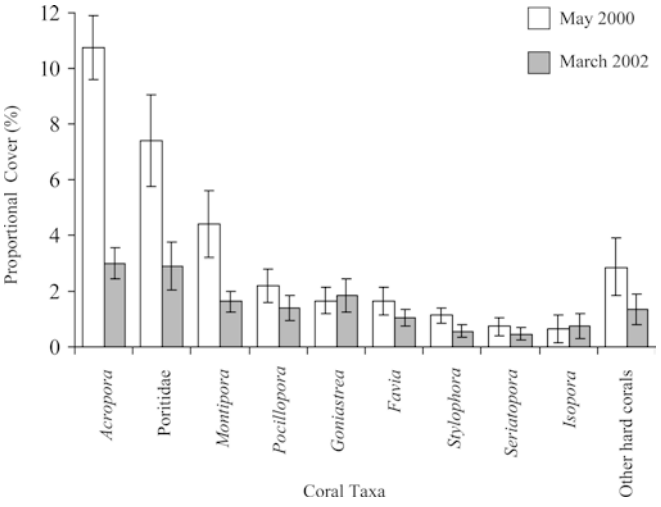

Fig. 2 Temporal variation in live cover of different coral taxa. Data show the mean cover (±SE) of each coral taxon sampled using ten replicate line-intercept transects at each of three sites in May 2000, and March 2002 ($n = 10$ transects per site)

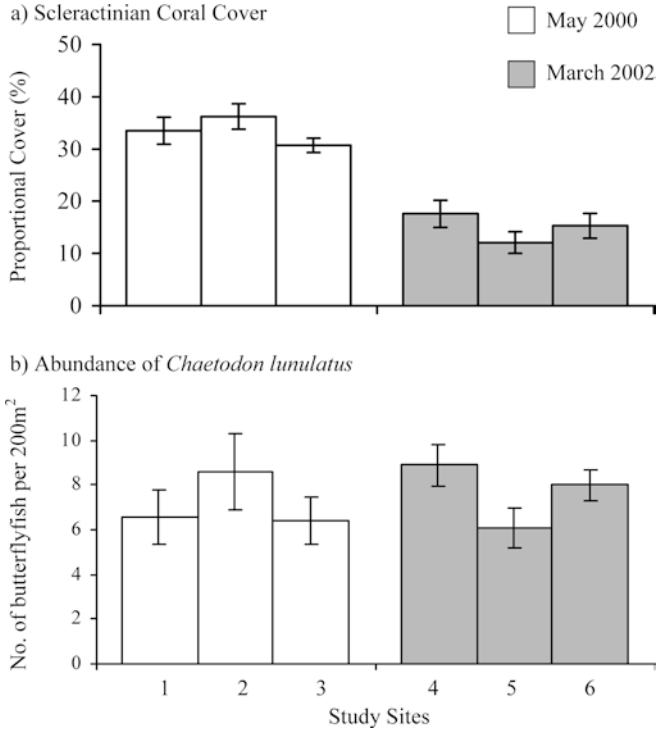

Fig. 1 Variation in: **a** the mean cover (±SE) of scleractinian corals ($n = 10$ transects) and **b** mean densities (±SE) of *Chaetodon lunulatus* ($n = 10$ transects) at each of six study sites, on the exposed reef crest at Trunk Reef. Sites 1–3 were sampled in May 2000 (before the bleaching), while sites 4–6 were sampled in March 2002 (after the bleaching)

At Trunk Reef, *Chaetodon lunulatus* responded to massive declines in the availability of coral prey by increasing their intake of corals that were relatively unaffected during recent bleaching events (Fig. 3). Consequently, there was a marked shift in the relative consumption of different coral species. Most notably, the proportion of bites taken from *Acropora* corals declined by 55%, from 37.0% (213–576 bites) in May 2000, down to just 16.7% (83–496) in March 2002. At the same time, there was an increase in the proportion of

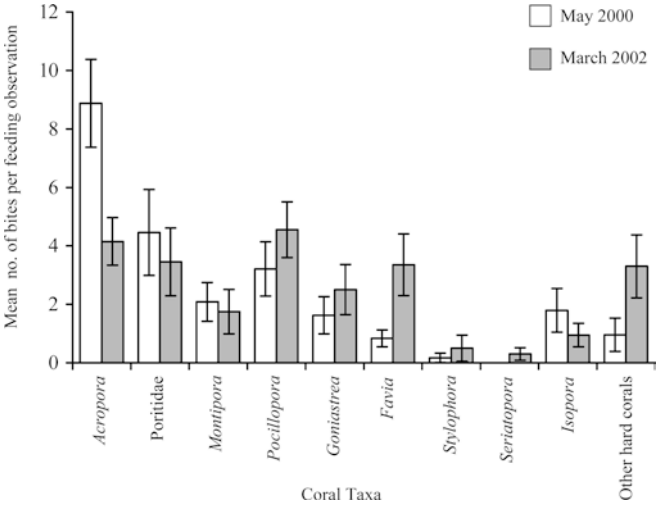

Fig. 3 Changes in the dietary composition of *Chaetodon lunulatus*. Data show the mean number of bites taken on each coral taxon in May 2000 ($n = 24$ feeding observations), and March 2002 ($n = 20$ feeding observations)

bites taken from *Favia* corals and other less common scleractinian corals species (other hard corals), especially *Galaxea fascularis* (Fig. 3). Unlike some corallivorous butterflyfish (e.g., *Chaetodon trifascialis*, Irons 1989), *C. lunulatus* is a generalist feeder, able to feed on a wide range of different coral prey (including *Acropora*, *Pocillopora* , *Porites* , *Favia* , *Goniastrea* , *Montastrea* , *Symphyllia* , *Fungia,* and *Galaxea*). This ability to utilize a wide range of different coral prey appears fundamental to their ability to endure massive declines in the availability of coral prey. However, populations of *C. lunulatus* were not completely unaffected by declines in the abundance and compostion of coral prey.

Although there was no change in the abundance of *C. lunulatus*, hepatocyte vacuolation was much lower for individuals collected in March 2002, compared to May 2000 (Fig. 4). In May 2000, hepatocyte vacuoles occupied an average of 12.4% (± 1.0 SE) of the cross-sectional area of hepatic tissues of fish collected from Trunk Reef. By contrast, hepatocyte vacuoles occupied an average of just 5.2% (± 0.8 SE) of the liver area in butterflyfish collected in March 2002 (Fig. 4). Mean levels of hepatocyte vacuolation in *C. lunulatus* differed significantly between sampling occasions (ANCOVA, $F = 33.3$, $df = 1$, $p < 0.01$) and though there was considerable variation among individual fish, estimates of hepatocyte vacuolation were independent of differences in body size (ANCOVA, $F = 0.66$, $df = 1$, $p = 0.61$), or sex (ANCOVA, $F = 2.17$, $df = 1$, $p = 0.15$). These findings suggest that temporal changes in coral abundance and composition may have had an adverse effect on the physiological condition of *C. lunulatus*.

Variation in hepatocyte vacuolation among individual butterflyfish was strongly and positively correlated with differences in their absolute liver lipid content, showing that hepatocyte vacuolation provides a good proxy for levels of lipid stores in the liver of *C. lunulatus*. Absolute liver lipid content for *C. lunulatas* ranged from 5.8 to 13.0% ($n = 9$), whereas the proportion of hepatocyte vacuoles in the liver ranged from 0% to 8.47% for the same fish. In fish without hepatocyte vacuoles, liver lipid content was 5.9–6.3%, which may represent the proportion of structural lipids (phospholipids) within hepatic tissues of *C. lunulatus* (*sensu* Love 1980). At higher levels of liver lipid content (above 6.3%), hepatocyte vacuolation was directly proportional to liver lipid content ($y = 0.95x–6.04$), and the relationship was highly significant ($r^2 = 0.85$, $n = 9$, $p < 0.01$). This confirms that hepatocyte vacuolation is a useful measure of physiological condition, at least in the case of *C. lunulatus* (see also Theilacker 1978). Moreover, lipid (rather than carbohydrate) is the favored energy reserve of fish, and the liver is generally the first site for deposition of lipid stores (Cowey and Sargent 1977). Lipid stores within the liver are also rapidly mobilized during periods of high energy expenditure (Black and Love 1986), or reduced food intake (Green and McCormick 1999), such that liver lipid content (and thereby hepatocyte vacuolation) provides a very sensitive measure of the physiological condition and subsequent fitness for individual fish.

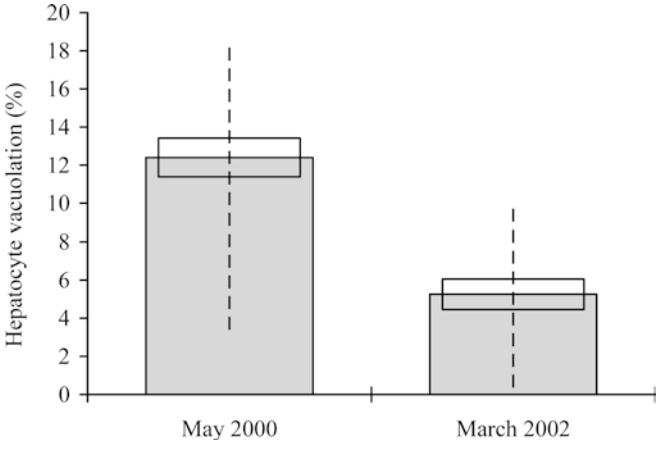

Fig. 4 Variation in the mean proportion of hepatocyte vacuoles in livers of *Chaetodon lunulatus* collected in May 2000 (before the bleaching) vs. March 2002 (after the bleaching). Estimates of hepatocyte vacuolation were averaged across replicate fish ($n = 20$), which were collected from the exposed reef crest at Trunk Reef. Box-and-whisker plots show the standard error and range around estimates of hepatocyte vacuolation

Declines in hepatocyte vacuolation observed in populations of *C. lunulatus* (from May 2000 to March 2002), are likely to have resulted from declines in the quantity and/or quality of coral prey. Feeding observations showed that bite rates of *C. lunulatus* varied very little from May 2000 ($\bar{x} = 24.0 \pm 1.3$ bites per 3-min observation, $n = 24$) to March 2002 ($x = 24.8 \pm 1.6$ bites per 3-min observation, $n = 20$). However, observed shifts in the dietary composition may have resulted in a lower energetic return, despite a similar rate of feeding. Other factors, such as increased incidence of competition, and/or variation in energy allocation, may have contributed to observed declines in the physiological condition of *C. lunulatus* (Jones and McCormick 2002). In particular, the timing of fish collections (relative to peak reproductive allocation in December/January) differed slightly between years, and fish collected in May 2000 would have had longer to amass liver lipid stores since last reproduction, compared to fish collected in March 2002. Even so, fish can restore liver lipid stores very quickly when prey is abundant (e.g., Pratchett et al. 2001), so it is likely that reduced prey availability (attributed to severe and wide-spread bleaching of scleractinian corals) played at least some part in observed declines in the condition of *C. lunulatus* (*sensu* Bruggemann et al. 1994; Green and McCormick 1999).

Most studies ascertain effects of disturbances on coral reef fishes by quantifying changes in their distribution and abundance (e.g., Harmelin-Vivien and Laboute 1986; Williams 1986; Kokita and Nakazono 2001), implicitly assuming that such disturbances will lead to widespread mortality and/or migration. In contrast, this study found that catastrophic coral bleaching had no immediate effect on the local abundance of *C. lunulatus*.

However, changes in the abundance and composition of coral prey did appear to have significant sublethal effects. Observed declines in the physiological condition of *C. lunulatus* are likely to have significant effects on their future growth and/ or reproductive output (e.g., Jones 1986; Kerrigan 1997). Ultimately, declines in physiological condition may also reduce survivorship of fishes, and may lead to eventual declines in population size (Jones and McCormick 2002). Continued monitoring of *C. lunulatus* populations at Trunk Reef will be carried out to assess whether observed declines in physiological condition are a precursor to population declines, or whether current populations can be sustained indefinitely despite significantly reduced prey availability.

Acknowledgements The authors are grateful to Oki Prajaksumi, who provided fish for histological examination in March 2002, and to Andrew Hoey and Josiah Pitt who assisted with all field collections. This study was funded by a Sturgis Fellowship awarded to MLB, as well as internal funding from James Cook University awarded to MSP. Andrew Baird, Eric Taylor, Geoffrey Jones, Joshua Madin, and two anonymous referees provided comments that greatly improved this manuscript. This is contribution number 73 from the Centre for Coral Reef Biodiversity, and contribution 198 of the Coral Ecology Group at James Cook University.

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