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Feeding preference and deterrence in rabbitfish *Siganus fuscescens* for the cyanobacterium *Lyngbya majuscula* in Moreton Bay, south-east Queensland, Australia

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Rabbitfish *Siganus fuscescens* preferences for *Lyngbya majuscula* collected from three bloom locations in Moreton Bay, Queensland, Australia, were tested along with a range of local plant species in the laboratory. Consumption of *L. majuscula* by fish did not differ between wild and captive-bred fish ($P = 0{\cdot}152$) but did differ between bloom location ($P = 0{\cdot}039$). No relationship was found between consumption rates and lyngbyatoxin-a concentration ($r^2 = 0{\cdot}035$, $P = 0{\cdot}814$). No correlation existed between C : N and proportion of food consumed when all food types were analysed statistically, whereas a clear correlation was observed when *L. majuscula* was removed from the calculations. In simulated bloom conditions, fish avoided ingestion of *L. majuscula* by feeding through gaps in the *L. majuscula* coverage. Both wild and captive-bred *S. fuscescens* showed a distinct feeding pattern in 10 day no-choice feeding assays, with less *L. majuscula* being consumed than the preferred red alga *Acanthophora spicifera*. *Lyngbya majuscula* however, was consumed in equal quantities to *A. spicifera* by wild *S. fuscescens* when lyngbyatoxin-a was not detectable. Wild fish probably do not preferentially feed on *L. majuscula* when secondary metabolites are present and are not severely impacted by large *L. majuscula* blooms in Moreton Bay. Furthermore, poor feeding performance in both captive-bred and wild *S. fuscescens* suggests that they would exert little pressure as a top-down control agent of toxic *L. majuscula* blooms within Moreton Bay. © 2006 The Fisheries Society of the British Isles

Key words: chemical deterrence; debromaplysiatoxin; lyngbyatoxin-a; Siganidae; top-down control.

INTRODUCTION

The naturally occurring cyanobacterium *Lyngbya majuscula* has been blooming with increased frequency and longevity at a number of coastal sites in subtropical south-east Queensland, Australia, particularly in Moreton Bay (Dennison *et al.*, 1999; Albert *et al.*, 2005; Watkinson *et al.*, 2005). Over 100

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biologically active compounds have been isolated from *L. majuscula* worldwide (Faulkner, 1984, 1997; Nagle & Paul, 1999; Burja *et al.*, 2001; Osborne *et al.*, 2001), with two major secondary metabolites identified in blooms of *L. majuscula* in Moreton Bay: debromoaplysiatoxin and lyngbyatoxin-a (SEQRWQMS, 2001; Capper *et al.*, 2005, N.T. Osborne, pers. comm.). Production of these secondary metabolites can be highly variable on small spatial and temporal scales, with highest levels observed during favourable growth phases (N.T. Osborne, pers. comm.). Regional variation of *L. majuscula* compounds is not uncommon (Hashimoto *et al.*, 1976; Cardellina *et al.*, 1979; Hay & Fenical, 1988; Paul & Pennings, 1991; Nagle & Paul, 1999) and may be produced as a defence mechanism (Paul, 1992; Paerl & Millie, 1996), rendering blooms unpalatable to a wide array of herbivores, including fishes (Paul *et al.*, 1990; Nagle *et al.*, 1996; Pennings *et al.*, 1996; Thacker *et al.*, 1997; Nagle & Paul, 1998), sea urchins (Nagle *et al.*, 1996; Nagle & Paul, 1998), crabs (Pennings *et al.*, 1996) and amphipods (E. Cruz-Rivera, pers. comm.). In spite of this defensive capability, *L. majuscula* mats can support high epifaunal diversity, providing both refuge and food for small grazers (Cruz-Rivera & Paul, 2002). Some macrograzers have also capitalized on *L. majuscula* as a source of food, balancing a trade-off between a nitrogen-rich diet and an ability to tolerate unpalatable and possibly toxic secondary metabolites (O'Neil, 1999). *Lyngbya majuscula* forms a major constituent in the diet of Australian gregory *Stegastes apicalis* (Devis) (Klumpp & Polunin, 1989) and adult siganids (von Westerhagen, 1973; Bryan, 1975; Lundberg & Lipkin, 1979). Whilst *Siganus fuscescens* (Houttuyn) has been observed feeding upon *L. majuscula* with no apparent harm (Hashimoto *et al.*, 1976), it is not known whether siganids have the ability to store or detoxify secondary metabolites. Food poisoning incidents involving *S. fuscescens* were reported amongst the indigenous population of Okinawa in Japan where fish were reputed to be toxic after settling upon reefs covered in seasonal blooms of *L. majuscula* (Hashimoto *et al.*, 1976).

In laboratory bioassays in Guam, juvenile siganid species were deterred by compounds present within *L. majuscula* (Nagle *et al.*, 1996; Thacker *et al.*, 1997; Nagle & Paul, 1998), and siganids may only consume *L. majuscula* during periods of hunger-stress or once all preferred food sources (*i.e.* those not chemically defended) have been depleted (Thacker *et al.*, 1997). *Siganus fuscescens* have often been observed feeding in sites with dense *L. majuscula* coverage in Moreton Bay, Queensland, Australia (G. Savige, pers. obs.). Preliminary field trials in Australia suggested siganids may exert a top-down control on this cyanobacterium (W. Knibb, unpubl. data), fuelling speculation that overfishing of this commercially important species (with an export market to South East Asia) may have exacerbated bloom proliferation in this region. Aboriginal communities on North Stradbroke Island have also mentioned the possibility of such a link (E. Webb, pers. comm.). Further, suggestions that captive-bred siganids should be released to augment wild populations, and thus mitigate nuisance blooms, led to a series of trials to assess feeding preference and deterrence in both wild and captive-bred siganids and to assess the role these macro-grazers may play if any, in top-down control of blooms.

In this study the following questions were raised: (1) Is there a correlation between plant nutritional value and proportion of food consumed by

S. fuscescens? (2) Does hunger-stress (*i.e.* higher levels of hunger following starvation) alter feeding preference and consumption levels in wild *S. fuscescens*? (3) Do wild and captive-bred *S. fuscescens* show the same preferences in multiple choice feeding assays and do consumption rates of *L. majuscula* vary with bloom location and secondary metabolite concentration? (4) Does the presence of *L. majuscula* entwined around 'favoured' food (*i.e.* simulated bloom conditions) deter feeding? (5) Does chronic exposure to *L. majuscula* lead to feeding deterrence? (6) Are captive-bred fish more likely to consume *L. majuscula* (as they have not encountered it previously)? On the basis of these investigations, the advisability of the proposed use of captive-bred *S. fuscescens* as a biocontrol agent of *L. majuscula* was determined.

MATERIALS AND METHODS

STUDY SITES

Lyngbya majuscula was collected at four study sites in Moreton Bay, Australia, during 2001 and 2002 (Fig. 1): Eastern Banks (EB) situated in Eastern Moreton Bay (27° 26′ S; 153° 22′ E) in clear oceanic waters; Deception Bay (DB) situated near the western shore of Moreton Bay (27° 05′ S; 153° 09′ E) where it often receives pulse runoff events from terrestrial and riverine sources (Albert *et al.*, 2005); Adams Beach (AB) situated in a sheltered bay (27° 51′ S, 153° 41′ E), subjected to localized runoff during storm events from North Stradbroke Island and turbid runoff from Southern Moreton Bay during periods of elevated rainfall. Blooms at these sites produce either lyngbyatoxin-a, debromoaplysiatoxin, or both. A fourth bloom site was observed at Wellington Point (WP) in 2003. This site is situated at the western shores of Moreton Bay (27° 47′ S; 153° 24′ E) and receives continual runoff from Hilliards' Creek and a storm water outflow. All sites have extensive seagrass and macroalgal coverage (Abal *et al.*, 2001).

STUDY ORGANISMS

All animal collection and experimentation met with guidelines provided by The Australian Code of Practice for the Care and Use of Animals for Scientific Purposes. Wild *S. fuscescens* were captured from Moreton Bay using both seines and trawl nets. Wild fish were maintained in a 1000 l re-circulatory seawater tank system at Moreton Bay Research Station (MBRS), North Stradbroke Island. Captive-bred fish were obtained from Bribie Island Aquaculture Research Centre (BIARC) and maintained in a 5000 l re-circulatory seawater tank system at MBRS. Prior to testing, captive-bred siganids were maintained in an indoor enclosed system and had no known experience with any native macroalgae or seagrass species resident in the Bay. Both wild and captive-bred fish were fed a diet of Ridleys Aqua-feed Native Starter (Brisbane, Australia) in the form of 2 mm food pellets prior to testing. Fish were transferred to 50 l aquaria with a re-circulatory flow-through 96 h prior to testing to allow acclimation. The sides of aquaria were masked to eliminate between-tank interactions. Fish appeared less stressed if two or more fish were included in each replicate aquaria, which may a result of the schooling behaviour of this species (Bryan, 1975). Because dominance and agonistic behaviour was observed prior to testing in fish of the same size as well as those of differing sizes, fish were allocated to three size categories: small (15–25 g), medium (26–40 g) and large (41–100 g) for each test replicate (where numbers permitted) to obviate any feeding differences associated with size dominance. Salinities were kept at 34–36 and temperature consistent at ambient (*c.* 24° C) with 12L : 12D photoperiod.

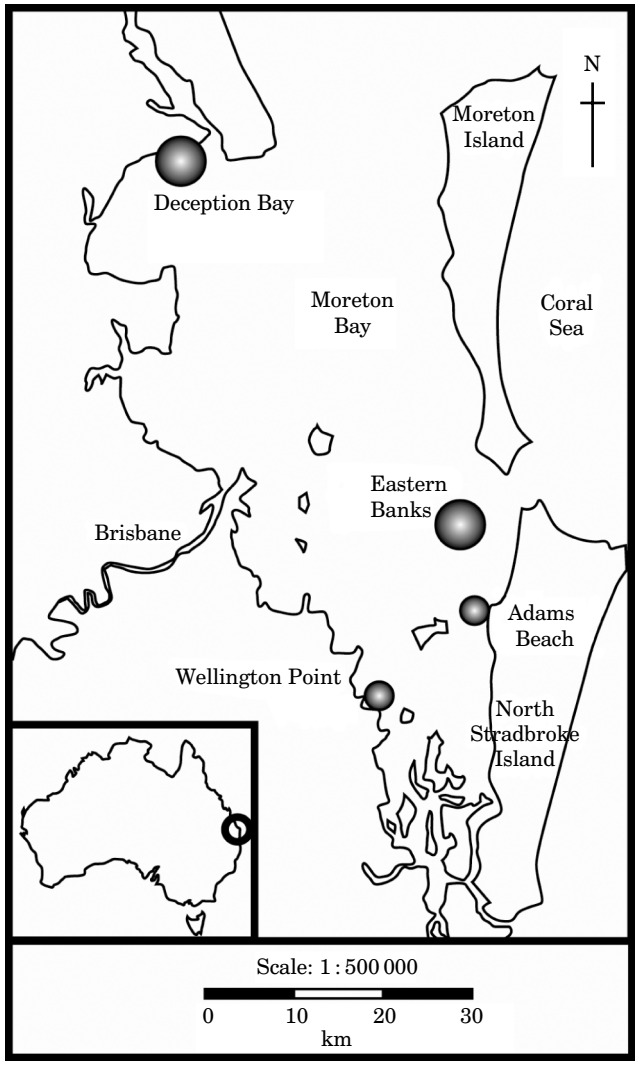

FIG. 1. Satellite map of *Lyngbya majuscula* bloom sites in Moreton Bay between 2001 and 2003.

Lyngbya majuscula was collected when available at four different locations and maintained in 50 l aquaria at MBRS. Macroalgae and seagrasses were collected from the shore at Dunwich, North Stradbroke Island, close to the Adams Beach site. A representative range of locally abundant rhodophytes, phaeophytes and chlorophytes were collected on a daily basis to ensure only healthy robust samples were used in feeding trials. Algal availability differed seasonally leading to different choices being presented in some experiments. Plant species tested included seagrasses *Zostera capricorni*, *Halophila spinulosa* and *Halophila ovalis*, red alga *Acanthophora spicifera*, green alga *Caulerpa taxifolia*, and brown algae *Dictyota dichotoma*, *Sargassum flavicans* and *Lobophora variegata*. These macrophytes provided a representative cross-section of siganid diet in subtropical regions (Tsuda & Bryan, 1973; von Westerhagen, 1973; Bryan, 1975; Lundberg & Lipkin, 1979; Hay *et al.*, 1988; Pillans *et al.*, 2004).

CHEMICAL EXTRACTION

To determine concentrations of *L. majuscula* secondary metabolites, voucher samples from all assays were frozen at −20° C and then freeze-dried. Plant material was then extracted three times in acetone, sonicated and filtered under vacuum. Three replicates were pooled and dried by rotary evaporation. Concentrations of secondary metabolites (mg kg^{-1}) were determined by HPLC-MS/MS using a PE/Sciex API 300 mass spectrometer equipped with a high flow electrospray interface (TurboIonspray) coupled to a Perkin Elmer series 200 HPLC system. Separation was achieved using a 150 × 4·6 mm Altima C_{18} column (Alltech) run at 35° C, with a mobile phase consisting of 80 : 20 acetonitrile : hi-pure water containing 0·1% formic acid and 2 mM ammonium formate at a flow rate of 0·8 ml min^{-1}. The flow was split-post column such that the flow to the mass spectrometer interface was 250 μl min^{-1}. Under these conditions, the retention times were 11·72 and 9·3 min for lyngbyatoxin-a and debromoaplysiatoxin, respectively. The mass spectrometer was operated in the positive ion, multiple ion monitoring mode. Ions monitored with dwell times of 300 ms were 410·3 and 438·3 $(M + H)^+$ for lyngbyatoxin-a and 543·3 for debromoaplysiatoxin. Quantification was achieved by comparison to standards of debromoaplysiatoxin and lyngbyatoxin-a (Calbiochem) run under the same conditions. Using a 20 μl injection the detection limit for both toxins is typically 0·01 mg l^{-1} in the extracted solution.

PLANT NUTRITIONAL VALUE

To determine plant nutritional values, three replicate specimens of each plant used in feeding assays were rinsed, dried at 60° C for 48 h in a mechanical convection oven, ground to a powder using a mortar and pestle and placed in an airtight container. Samples were analysed by the School of Land and Food Sciences, University of Queensland, to determine mass-based carbon and nitrogen content.

HUNGER-STRESS ASSAY

A multiple-choice feeding assay was used to determine the effect of hunger-stress on dietary selectivity in wild *S. fuscescens*, using a range of plant species and *L. majuscula* collected from Eastern Banks (Table I). Fish were weighed (g wet mass) and allocated to 50 l aquaria, using one fish from each size range per replicate (Table I), and acclimated for 48 h. These aquaria formed two groups: (1) fish fed *ad libitum* for 48 h prior to testing and (2) fish starved for 48 h prior to testing. Each treatment group consisted of three replicate aquaria, which were masked to eliminate behavioural interactions. Macroalgae, seagrass and *L. majuscula* samples were rinsed with clean sea water (all visible epiphytes and particulate matter were removed by hand) and blotted dry. The number of blots was standardized for each algal species depending upon its water retention capacity. The plant material was then weighed (±0·5 g) and tied into a bundle using monofilament fishing line (0·05 mm). Six or seven simultaneous choices of plant material (depending upon seasonal availability at time of testing) were randomly assigned to predetermined positions, secured to a weight and placed on the tank base. Control tanks (those without siganids) with plant material were randomly interspersed between treatment tanks (those with siganids) to determine autogenic changes in the food mass (Peterson & Renaud, 1989). Preliminary observations showed that wild fish expressed a feeding preference within 1 h (*i.e.* >50% of one food item had been removed in this time period), resulting in both a fixed-consumption and a fixed-time experimental design (Lockwood, 1998). A 1 h trial period was thus allocated to wild fish in all multiple choice feeding assays, after which the test was terminated and food items were removed, re-blotted and re-weighed.

TABLE I. Experimental design for wild and captive-bred *Siganus fuscescens* feeding assays

Fish status	*Lyngbya majuscula* location	Plant species	Duration of test (h)	Number of treatment replicates	Number of control replicates	Mean ± S.E. animal mass[a] (g wet mass)
Hunger stress assay						
Wild – starved	EB (LTA)	*Lm, Ho, Zc, As, Lv, Hs, Sf*	1	3	3	1 × small 14·90 ± 1·42 1 × medium 34·16 ± 4·36 1 × large 59·15 ± 1·89
Wild – satiated	EB (LTA)	*Lm, Ho, Zc, As, Lv, Hs, Sf*	1	3	3	1 × small 16·83 ± 1·22 1 × medium 31·73 ± 4·20 1 × large 52·70 ± 5·30
Multiple choice feeding assays (intraspecific dietary selectivity)						
Wild	EB (LTA)	*Lm, Zc, As, Lv, Hs, Sf, Ho*	1	3	3	1 × small 14·90 ± 1·42 1 × medium 34·16 ± 4·36 1 × large 59·15 ± 1·89
Wild	AB (LTA)	*Lm, Dd, Zc, As, Ct, Sf, Ho*	1	5	5	2 × small 14·50 ± 0·72 2 × medium 31·52 ± 1·65
Captive-bred	DB (LTA)	*Lm, Dd, Zc, As, Ct, Lv, Ho*	24	5	5	2 × small 21·88 ± 2·13 2 × medium 34·09 ± 1·57
Captive-bred	AB (LTA)	*Lm, Dd, Zc, As, Ct, Lv, Ho*	24	5	5	2 × small 24·69 ± 1·29 2 × medium 38·23 ± 0·80
Simulated bloom assay						
Wild	EB (LTA)	*As*, *Zc*, *Ho*, *Hs* all entwined with *Lm*	1	3	3	1 × small 15·01 ± 1·39 1 × medium 36·23 ± 2·25 1 × large 58·16 ± 0·69
Wild	–	*As*, *Zc*, *Ho*, *Hs* no Lm present	1	3	3	1 × small 16·37 ± 1·76 1 × medium 34·36 ± 4·25 1 × large 56·40 ± 4·84
Acute no-choice feeding assay						
Wild	–	*As*	4	5	5	5 × large 104·53 ± 15·59

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Wild	WP (n/d)	*Lm*	4	5	5	5 × large 107·04 ± 6·59
Wild	WP (n/d)	*As, Lm*	4	5	5	5 × large 111·91 ± 9·48
Chronic no-choice feeding assay						
Wild	AB (LTA)	*As*	10	4	4	2 × small 13·73 ± 0·98 2 × medium 34·96 ± 283
Wild	AB (LTA)	*Lm*	10	4	4	2 × small 14·76 ± 1·58 2 × medium 37·58 ± 2·83
Captive-bred	AB (LTA)	*As*	10	5	5	5 × medium 35·70 ± 1·28
Captive-bred	AB (LTA)	*Lm*	10	5	5	5 × medium 37·28 ± 0·95

EB, Eastern Banks; AB, Adams Beach; DB, Deception Bay; WP, Wellington Point. Letters in parentheses refer to type of secondary metabolite detected in *L. majuscula* sample post-exposure: LTA, lyngbyatoxin-a; n/d, not detected using HPLC-MS/MS.

Plant species are those used in experimental feeding trials according to seasonal availability. *Lm*, *Lyngbya majuscula*; *Dd*, *Dictyota dichotoma*; *Zc, Zostera capricorni*, *As*, *Acanthophora spicifera*; *Lv*, *Lobophora variegata*; *Sf*, *Sargassum flavicans*, *Ho*, *Halophila ovalis*; *Ct*, *Caulerpa taxifolia.*

[a] Number of animals per size group used in each treatment replicate is indicated as *n* × category of fish, *i.e.* small (15–25 g); medium (26–40 g) and large (>41 g).

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MULTIPLE CHOICE FEEDING ASSAYS

Dietary preference of *S. fuscescens* was determined by conducting multiple choice feeding assays using *L. majuscula* from three different locations in Moreton Bay *i.e.* Adams Beach, Eastern Banks and Deception Bay (Table I). Previous analysis of *L. majuscula* from these sites showed differences in both secondary metabolite concentrations and types (Capper *et al.*, 2005; N.T. Obsorne, pers. comm.). Three or five replicate 50 l aquaria (depending upon availability of fish at time of testing) containing four fish each were used as treatment tanks as outlined above. Preliminary observations showed that captive-bred fish were much slower in exhibiting a feeding preference (*i.e.* where >50% of one food was consumed); therefore, a fixed-time design (Lockwood, 1998) of 24 h was allocated to captive-bred fish and 1 h to wild fish. After the test was terminated, food items were removed, re-blotted and re-weighed.

SIMULATED BLOOM ASSAY

Four plant species consumed by wild *S. fuscescens* (*A. spicifera*, *Z. capricorni*, *H. ovalis* and *H. spinulosa*) were chosen from the Eastern Banks *L. majuscula* multiple choice feeding assay. Food items were subjected to the same weighing protocol outlined above and assigned into groups: (1) food entwined with a known quantity of *L. majuscula* from Eastern Banks and (2) and food without *L. majuscula*. Two groups of fish were separated into three replicate aquaria, each containing three fish: (1) those offered four plant species entwined with *L. majuscula* and (2) those offered four plant species without *L. majuscula* (Table I). Incidental consumption of *L. majuscula* was calculated post-trial by weighing remaining *L. majuscula*.

ACUTE AND CHRONIC NO-CHOICE FEEDING ASSAYS

Lyngbya majuscula was collected from Wellington Point and used in an acute (<24 h) no-choice feeding assay with wild *S. fuscescens*. Five fish were allocated to each of the five replicate 50 l aquaria and offered: (1) *L. majuscula* alone, (2) *A. spicifera* alone or (3) a 50 : 50 mixture of *L. majuscula* and *A. spicifera* over a 4 h period (Table I).

Lyngbya majuscula collected from Adams Beach was used in chronic (10 day) no-choice feeding assays. *Acanthophora spicifera* was chosen as a control food type in this assay as it contains no secondary metabolites (Ginsburg & Paul, 2001). Four fish were allocated to either four (wild) or five (captive-bred) replicate 50 l aquaria and offered *A. spicifera* only. A second group of fish were offered *L. majuscula* only (Table I). Food was removed and replaced with freshly weighed food every 24 h for 10 days. A depuration period of 10 days followed where *A. spicifera* was offered as a food source for both treatment and control tanks to see whether fish previously fed *L. majuscula* would resume normal feeding patterns.

STATISTICAL ANALYSIS

The effect of feeding regimes (*i.e.* hunger-stress: starved *v.* satiated) data were analysed using paired-sample *t*-tests on individual plants. The normality of hunger-stress and subsequent data sets were subjected to Levene's homogeneity of variance tests. Data that deviated from normal distribution in this assay were transformed using the square root or logarithm of proportion. Results of multiple choice feeding assays were analysed using paired-sample *t*-tests on individual plants. Data that deviated from normal distribution were transformed using square root, arcsine or logarithmic transformations. Factorial ANOVA was used to assess the effect of fish environmental origin (wild *v.* captive-bred) upon *L. majuscula* consumption rates and the effect of bloom location (AB *v.* EB *v.* DB). Simulated bloom and no-choice feeding assays data were analysed using single factor ANOVA. Data from no-choice feeding assay using captive-bred fish were subjected to

sine transformation to meet the parametric criterion. Fisher's LSD pair-wise comparison tests were used to compare means. Statistica® software was used to analyse all data.

RESULTS

PLANT NUTRITIONAL VALUE

The highest levels of nitrogen (%N) were observed in *L. majuscula* (1·97% Deception Bay and 1·52% Eastern Banks) and *A. spicifera* (1·5%) resulting in a relatively low C : N ratio, indicative of a higher food quality than other plants tested (Table II). A significant positive correlation was observed for wild *S. fuscescens* between proportion of food consumed and C : N when *L. majuscula* was removed from statistical analysis (Fig. 2). No relationship was apparent for captive-bred *S. fuscescens* (Adams Beach all plants $r^2 = 0{\cdot}003$, $P = 0{\cdot}771$, minus *L. majuscula* $r^2 = 0{\cdot}007$, $P = 0{\cdot}666$; Deception Bay all plants $r^2 = 4{\cdot}53 \times 10^{-5}$, $P = 0{\cdot}969$, minus *L. majuscula* $r^2 = 0{\cdot}024$, $P = 0{\cdot}417$).

HUNGER-STRESS ASSAY

Hunger-stress did not significantly alter the feeding preference of wild *S. fuscescens* for any plant species tested [ANOVA, d.f. = 1,6, $P = 0{\cdot}296$, Fig. 3(a)]. Fish were subsequently starved for 24 h prior to testing in all other assays.

MULTIPLE CHOICE FEEDING ASSAYS

Distinct feeding preferences were observed in both groups of wild *S. fuscescens* [Fig. 3(a), (b)]. In comparison, captive-bred *S. fuscescens* consumed very little of any food type presented, with a maximum proportion of 0·12 [Fig. 3(c)] to 0·16 [Fig. 3(d)], thus exhibiting feeding patterns different from that of wild *S. fuscescens*. The quantity of *L. majuscula* consumed did not differ between fish type, *i.e.* wild *v.* captive (*t*-test, d.f. = 8, $P = 0{\cdot}064$), but did differ between bloom location, *i.e.* Adams Beach *v.* Deception Bay *v.* Eastern Banks (*t*-test, d.f. = 2, $P = 0{\cdot}016$). No relationship however, was apparent between consumption rates and lyngbyatoxin-a concentration ($r^2 = 0{\cdot}035$, $P = 0{\cdot}814$). Debromoaplysiatoxin was detected only in *L. majuscula* used in the Deception Bay assay.

SIMULATED BLOOM ASSAY

The presence of *L. majuscula* did not appear to affect feeding preference with distinct feeding preferences still demonstrated amongst food types presented (*t*-test, d.f. = 4 $P > 0{\cdot}05$ for all plants, Fig. 4). Some incidental consumption of *L. majuscula* occurred when feeding on *A. spicifera* and *Z. capricorni* (0·10 and 0·17 g wet mass of proportions, respectively); however, in general, fish oriented themselves to avoid ingestion of *L. majuscula* by feeding through gaps in the *L. majuscula* coverage. Therefore, the presence of *L. majuscula* did not deter feeding by wild *S. fuscescens* in the laboratory.

TABLE II. Mean ± S.E. percentage carbon and nitrogen values for algae, seagrass and *Lyngbya majuscula* from Moreton Bay used in feeding assays

Food type	Date of analysis	Carbon (%)	Nitrogen (%)	C : N
Lyngbya majuscula[a] (Deception Bay)	1998	17·0 ± 1·5	1·97 ± 0·2	8·66 ± 0·40
Lyngbya majuscula[a] (Eastern Banks)	2000	13·8 ± 4·4	1·52 ± 0·5	8·96 ± 0·24
Acanthophora spicifera[b]	1999	20·9 ± 1·3	1·5 ± 0·1	13·93 ± 0·10
Halophila ovalis	2004	24·3 ± 1·0	1·6 ± 0·1	15·11 ± 0·18
Caulerpa taxifolia	2004	26·4 ± 0·5	1·6 ± 0·0	15·73 ± 0·27
Zostera capricorni	2004	26·1 ± 0·7	1·2 ± 0·1	21·45 ± 0·36
Lobophora variegata	2004	16·0 ± 0·2	0·7 ± 0·0	23·56 ± 0·47
Sargassum flavicans	2004	22·4 ± 0·2	0·9 ± 0·0	25·88 ± 0·91
Dictyota dichotoma	2004	21·4 ± 0·7	0·8 ± 0·0	26·71 ± 0·46
Halophila spinulosa	2004	19·4 ± 0·2	0·7 ± 0·0	26·75 ± 0·57

[a] J. O'Neil, S. Albert & K. Ahern, unpubl. data.
[b] Pillans *et al.*, 2004.

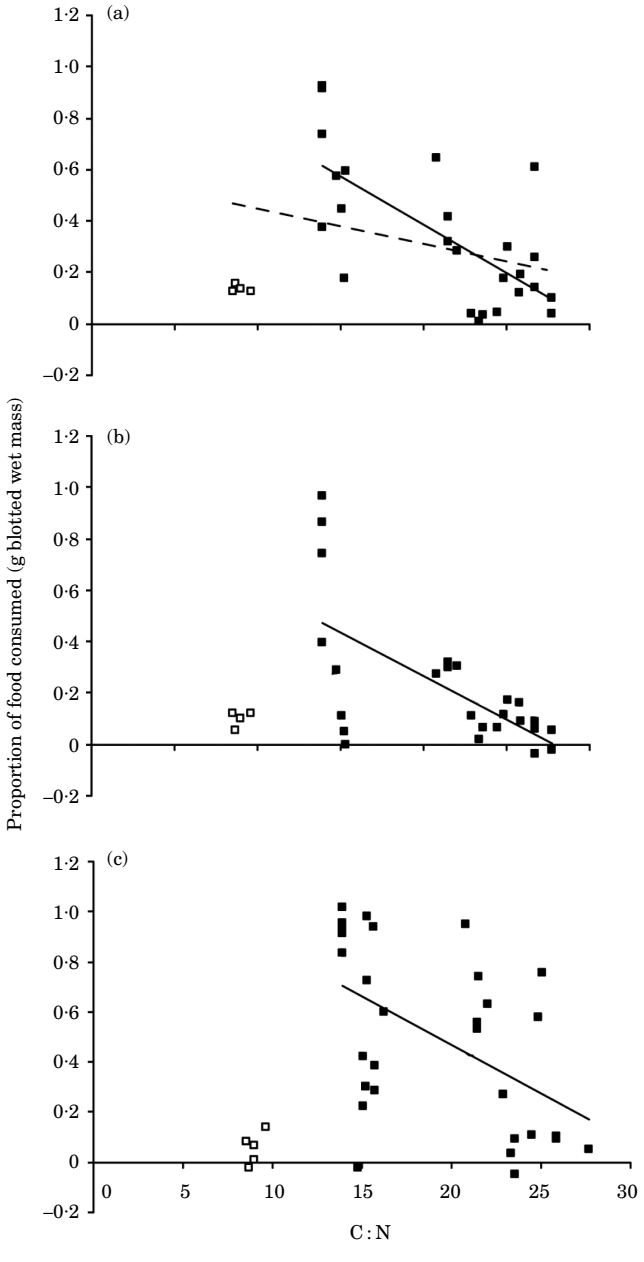

FIG. 2. Plots of carbon to nitrogen ratios (C : N) and proportion of food consumed for: (a) wild *Siganus fuscescens* (starved 48 h) using *Lyngbya majuscula* from Eastern Banks ($n = 3$), (b) wild *S. fuscescens* (satiated) using *L. majuscula* from Eastern Banks ($n = 3$) and (c) wild S. *fuscescens* (starved 24 h) using *L. majuscula* from Adams Beach ($n = 5$). Data are proportion of *L. majuscula* (□) and all other plants (■) consumed (minus *L. majuscula*) that have been control adjusted and plotted against C : N. The curves were fitted by: (a) all foods (--) $y = -0{\cdot}0127x + 0{\cdot}56$ ($r^2 = 0{\cdot}097$, $P < 0{\cdot}05$), minus *L. majuscula* (—) $y = -0{\cdot}0377x + 1{\cdot}1398$ ($r^2 = 0{\cdot}503$, $P < 0{\cdot}001$), (b) minus *L. majuscula* (—) $y = -0{\cdot}0337x + 0{\cdot}9404$ ($r^2 = 0{\cdot}415$, $P < 0{\cdot}001$) and (c) minus *L. majuscula* (—) $y = -0{\cdot}0388x + 1{\cdot}2458$ ($r^2 = 0{\cdot}262$, $P < 0{\cdot}01$). The curves for all food in (b) and (c) were non-significant ($P > 0{\cdot}05$).

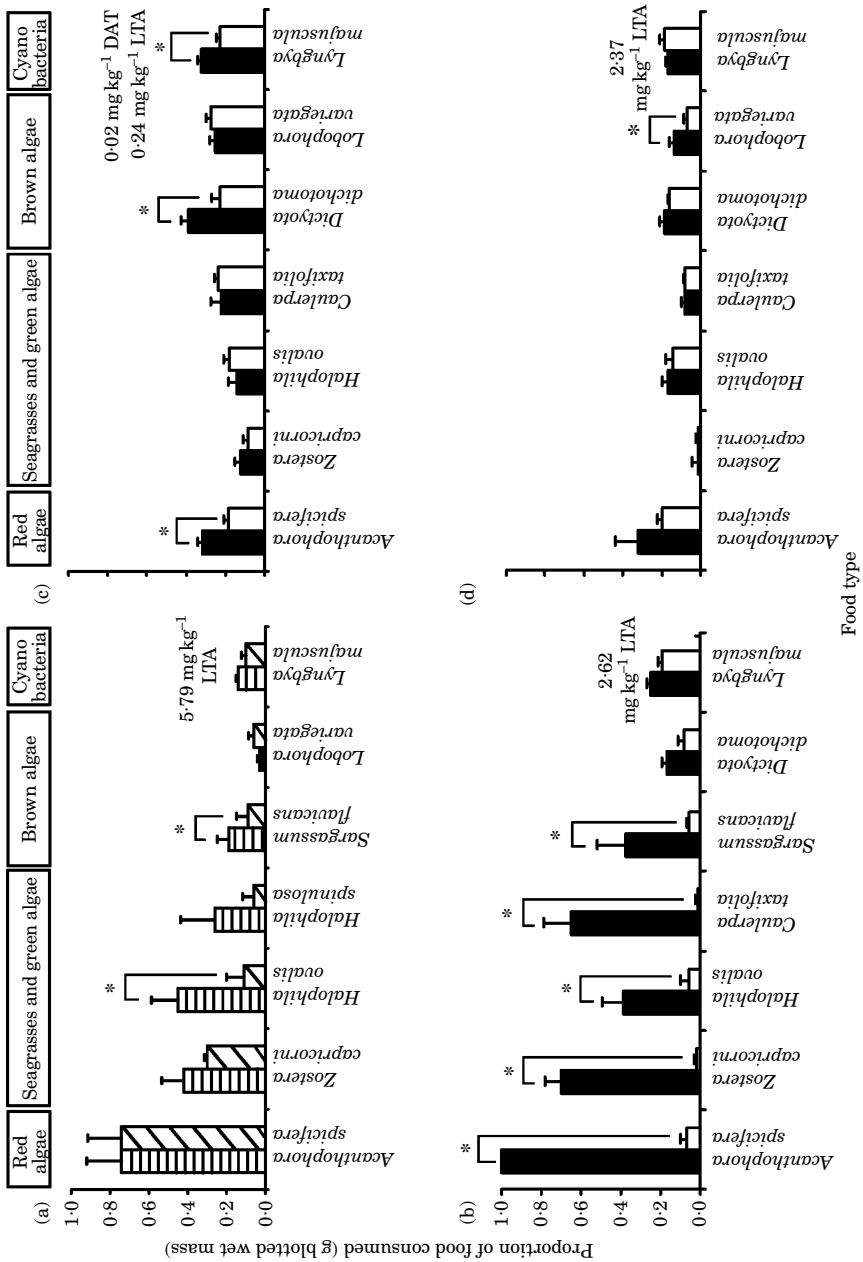

FIG. 3. Wild and captive-bred *Siganus fuscescens* multiple choice feeding assays. Proportion of plant material consumed by: (a) starved (▤) *v.* satiated (▨) wild *S. fuscescens*, (b) wild *S. fuscescens* using Adams Beach *L. majuscula* (■) *v.* control (□), (c) captive-bred *S. fuscescens* using Deception Bay *L. majuscula* (■) *v.* control (□) and (d) captive-bred *S. fuscescens* using Adams Beach *L. majuscula* (■) *v.* control (□). (a) Data are mean + S.E. proportion of plants consumed control adjusted. One way ANOVA was used to analyse data. *, significant difference ($P < 0{\cdot}05$, $n = 3$). (b)–(d) Paired sample *t*-tests were used to analyse data. * significant difference ($P < 0{\cdot}05$; $n = 5$). Homogeneity of variance was verified for all data sets using Levene's test. Lyngbyatoxin-a (LTA) and debromoaplysiatoxin (DAT) concentration in *L. majuscula* is shown above bars. *n*, number of replicates.

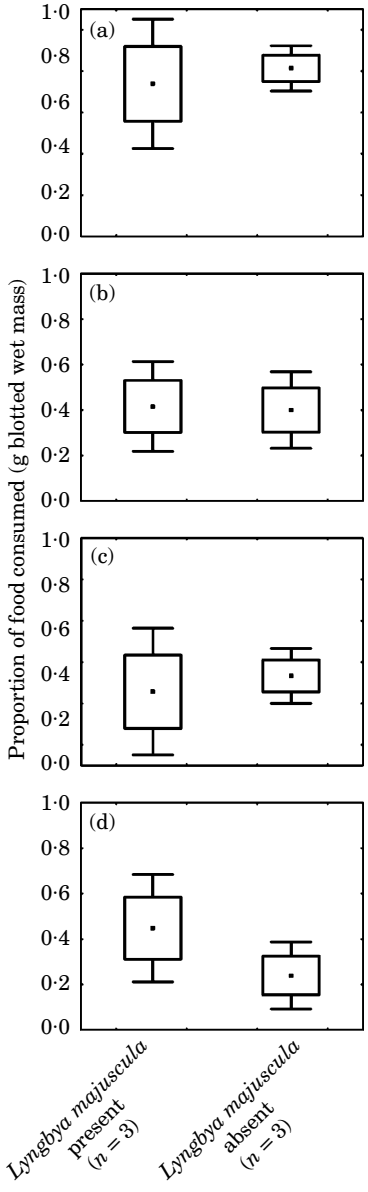

FIG. 4. Wild *Siganus fuscescens* simulated *Lyngbya majuscula* bloom assay. Mean (□, S.D.; I, S.E.) proportion (■) of seagrass and alga consumed when entwined with or without *L. majuscula*: (a) *Acanthophora spicifera*, (b) *Zostera capricorni*, (c) *Halophila spinulosa* and (d) *Halophila ovalis*. Paired sample *t*-tests were used to analyse data (all $P > 0{\cdot}05$). Homogeneity of variance was verified using Levene's test. n = number of replicates.

ACUTE AND CHRONIC NO-CHOICE FEEDING ASSAY

No feeding deterrence was observed in wild *S. fuscescens* fed either Wellington Point *L. majuscula* alone or mixed with the favoured food *A. spicifera* (*t*-test, $P < 0{\cdot}001$ for all plant types, Fig. 5), and all were consumed in equal quantities in acute no-choice feeding assays. Neither lyngbyatoxin-a nor debromoaplysiatoxin was detected post-trial in *L. majuscula* used in this assay.

Both wild and captive-bred *S. fuscescens* showed a distinct feeding pattern in chronic no-choice feeding assays (ANOVA, d.f. = 1, 1, $P < 0{\cdot}001$ and d.f. = 1, 1, $P < 0{\cdot}001$ respectively), with small quantities of *L. majuscula* being consumed [2–10% for wild fish and 1–12% for captive fish, Fig. 6(a)] compared to *A. spicifera* [87–99% for wild fish and 63–81% for captive fish, Fig. 6(b)]. Levels of *L. majuscula* consumption were not significantly different between wild and captive-bred fish (ANOVA, d.f. = 1, 1, $P = 0{\cdot}635$). When *A. spicifera* was offered *ad libitum* to both groups, those previously fed *L. majuscula* resumed normal feeding patterns within 2 days post-exposure. Therefore, *L. majuscula* was consumed in very low levels compared to the preferred alga *A. spicifera*, and environmental origin (*i.e.* wild *v.* captive-bred) may have influenced

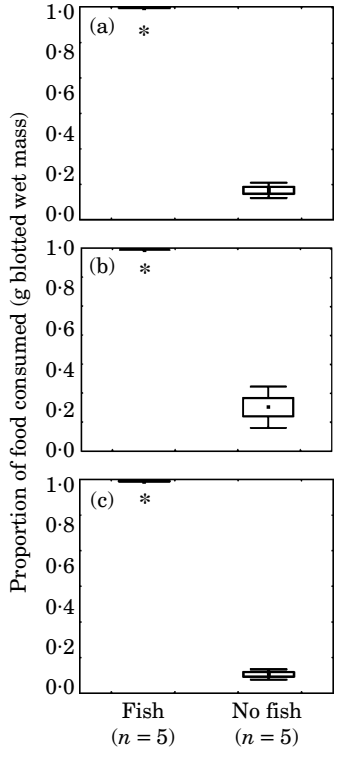

FIG. 5. Wild *Siganus fuscescens* acute no-choice feeding assay. Data are mean (□, S.D.; I, S.E.) proportion (■) of plants consumed by wild *S. fuscescens* (a) *Lyngbya majuscula*, (b) *Acanthophora spicifera* and (c) *L. majuscula* and *A. spicifera*. Paired sample *t*-tests were used to analyse data (all $P < 0{\cdot}001$). Homogeneity of variance was verified using Levene's test. *, a proportional value of one. n = number of replicates.

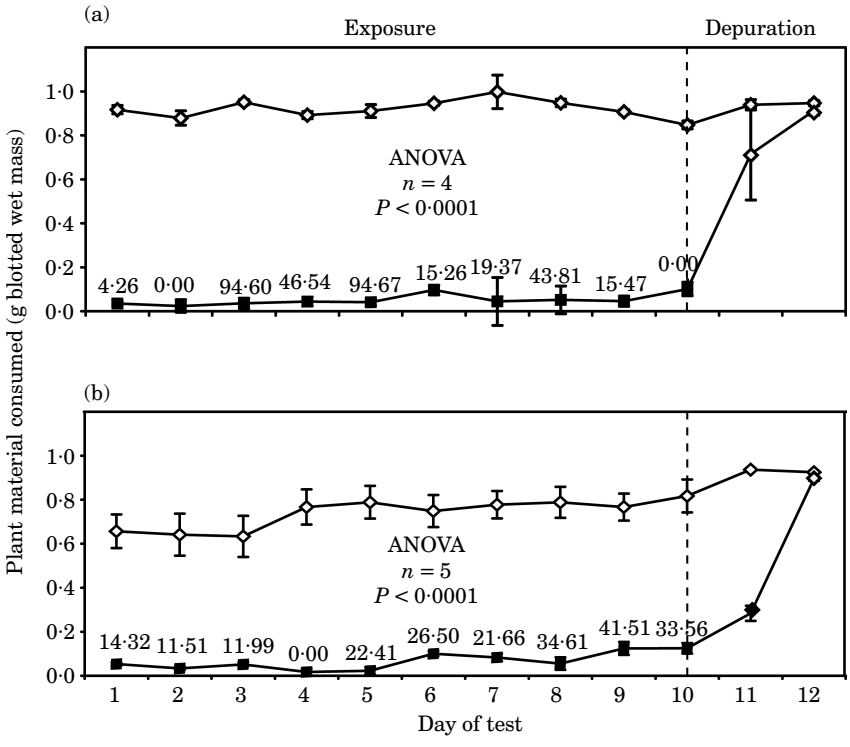

FIG. 6. Wild and captive-bred *Siganus fuscescens* chronic no-choice feeding assays. (a) Wild *S. fuscescens* and (b) captive-bred *S. fuscescens* offered a mono-specific diet of either *Lyngbya majuscula* (■) collected from Adam's Beach or *Acanthophora specifera* (◇). Data are mean ± S.E. proportion of plants consumed that have been control adjusted. Repeated measures ANOVA was used to analyse data. Numbers above ■ refer to lyngbyatoxin-a concentration (mg kg^{-1}). Filled symbols represent significant differences ($P < 0{\cdot}05$) in consumption of plant material (*i.e. L. majuscula v. A. spicifera*) on a daily basis using Fisher's LSD pair-wise comparisons. Homogeneity of variance was verified using Cochran and Levene's tests. n = number of replicates.

consumption patterns. Lyngbyatoxin-a concentrations observed in Adams Beach *L. majuscula* used in wild and captive-bred *S. fuscescens* no-choice assays ranged from 0 to 94·67 mg kg^{-1} and 0 to 41·51 mg kg^{-1}, respectively, over the 10 day trial period. No relationship was apparent between *L. majuscula* consumption and lyngbyatoxin-a concentration in wild *S. fuscescens* [$r^2 = 0{\cdot}09$, $P = 0{\cdot}39$, Fig. 7(a)], whereas a positive correlation was observed in captive-bred *S. fuscescens* [$r^2 = 0{\cdot}58$, $P = 0{\cdot}01$, Fig. 7(b)].

DISCUSSION

Wild *S. fuscescens* showed a distinct feeding preference for a range of algae and seagrass but only consumed small quantities of *L. majuscula* when lyngbyatoxin-a was present regardless of hunger-stress or prior learning experience. Captive-bred *S. fuscescens* showed no distinct preference for any plant type, including *L. majuscula*. *Lyngbya majuscula* was only consumed *ad libitum* when lyngbyatoxin-a was not detected. The proportion of food consumed by wild

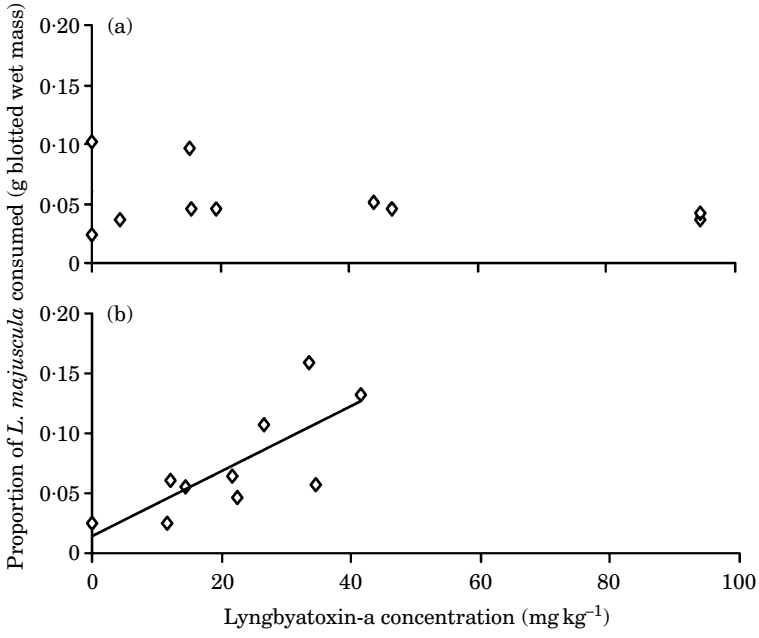

FIG. 7. Plot of lyngbyatoxin-a and consumption of *Lyngbya majuscula* in chronic no-choice feeding assays using (a) wild *Siganus fuscescens* and (b) captive-bred *S. fuscescens*. Data are mean proportion (◇) of plants consumed that have been control adjusted plotted against lyngbyatoxin-a concentration over a period of 10 days. (a) There was no significant relationship ($r^2 = 0{\cdot}2239$, $n = 4$, $P > 0{\cdot}05$). The curve in (b) was fitted by: $y = 0{\cdot}0023x + 0{\cdot}0159$($r^2 = 0{\cdot}563$, $n = 5$, $P < 0{\cdot}01$).

S. fuscescens was positively correlated with decreasing C : N only when *L. majuscula* was omitted from analyses.

Nitrogen content is an important determinant of food quality in marine environments (Mattson, 1980; Montgomery & Gerking, 1980; Choat & Clements, 1998; O'Neil, 1999). Therefore, low C : N foods may be preferentially consumed. Selectivity for the red alga *A. spicifera* exhibited by wild *S. fuscescens* was probably related to its nutrient content. If this was the case, then *L. majuscula* should be preferred, however, it always ranked lowest in feeding preference assays for wild fish. No correlation existed between C : N and proportion of food consumed when all food types were analysed statistically, whereas a clear correlation was observed when *L. majuscula* was removed from the calculations. As a diazatroph (*i.e.* nitrogen-fixer), *L. majuscula* is a potentially valuable food resource in an often N-limited marine environment. It may be a 'high maintenance' food for grazers, however, because of: unpredictable secondary metabolite content, energy requirements for detoxification, hair-like morphology making it difficult to digest and assimilate, incomplete nutritional content (missing essential fatty acids) and presence of gas vesicles reducing overall nutrient content (O'Neil, 1999). These characteristics are probably responsible for the preferences observed in the feeding assays. Published research on wild siganids confirms the present results, *i.e.* red and green algae are preferred food sources followed by brown algae and cyanobacteria (Tsuda &

Bryan, 1973; von Westerhagen, 1973; Bryan, 1975; Lundberg & Lipkin, 1979). During natural foraging, fishes can maximize the nutritional value of foods consumed (Montgomery & Gerking, 1980) and reduce the likelihood of over-ingesting toxins from chemically defended plants (Thacker *et al*., 1997) by sampling small amounts of each food type.

Feeding trials in Guam showed that an increase in hunger can decrease feeding selectivity in siganids and diminish the deterrent effects of anti-feedant compounds (Thacker *et al*., 1997). Cronin & Hay (1996) also suggest that hunger-stress may alter learned aversions to chemically rich foods. Such aversions were not evident in the present assays as feeding preference was not altered by hunger-stress (*i.e*. 48 h starvation prior to testing), nor did starved *S. fuscesens* show an increase in consumption of *L. majuscula* that was found to contain the secondary metabolite lyngbyatoxin-a. Whilst siganids in Guam were more likely to consume food containing *L. majuscula* secondary metabolites when fed less frequently, this contrary result may be related to chemical properties of the secondary metabolites present and potential deleterious effects that ensue from their consumption. *Lyngbya majuscula* in Guam contains a suite of biologically active compounds known to act as feeding deterrents (Nagle *et al*., 1996; Thacker *et al*., 1997; Nagle & Paul, 1998), but these compounds may be less harmful to fishes than lyngbyatoxin-a and debromoaplysiatoxin found in Australian *L. majuscula*, as these are potent inflammatory agents with tumour-promoting capabilities in mice (Kato & Scheuer, 1975; Cardellina *et al*., 1979; Fujiki *et al*., 1985, 1990).

Feeding preferences differed between captive-bred *S. fuscescens* and wild *S. fuscescens* and also between captive-bred groups, with no distinct feeding pattern observed and only small quantities of food being consumed from any of the plant species offered. Consumption of *L. majuscula* did not appear to be affected by bloom location in either wild or captive-bred siganids. Lyngbyatoxin-a, however, was the only secondary metabolite detected in almost all voucher specimens used in these trials (debromoaplysiatoxin was detected at a low concentration in the Deception Bay *L. majuscula* used in the captive-bred *S. fuscescens* multiple choice assay); therefore it is difficult to make assumptions regarding differences between *L. majuscula* chemotypes. The reason for substantial shifts in lyngbyatoxin-a concentration recorded daily in *L. majuscula* retained in a 50 l holding tank is unclear but variability in toxin levels has been observed in the field (Banner, 1959; Carmichael, 1986; Izumi & Moore, 1987; Paul, 1992; Nagle & Paul, 1999; N.T. Osborne, pers. comm.) and may be attributable to natural variability influenced by a range of environmental and physical parameters (Shannon *et al*., 1992; Paerl & Millie, 1996; Sivonen 1996; Thacker & Paul 2001). Regardless of bloom location or the concentration of lyngbyatoxin-a and debromaplysiatoxin, *L. majuscula* was never a preferred food for wild or captive-bred siganids in the laboratory.

As *L. majuscula* is not the preferred food choice for wild siganids, what impact would extensive bloom coverage have upon their foraging behaviour? A mass mortality of juvenile siganids was attributed to starvation during widespread *L. majuscula* blooms in Guam (Nagle & Paul, 1998). The impact of *L. majuscula* blooms upon adult siganid foraging habitats is unknown. Extensive coverage of seagrass beds with mats of *L. majuscula* have affected large areas in Moreton

Bay in recent years (SEQRWQMS, 2002; pers. obs.). In simulated bloom trials in the laboratory, fish were observed feeding through gaps in the *L. majuscula* to gain access to seagrass and algae underneath, resulting in only minor incidental consumption of *L. majuscula*. Adult fish do not appear to be severely impacted by large *L. majuscula* blooms in Moreton Bay. These presumably mobile populations are likely to move to unaffected feeding grounds or feed around the *L. majuscula* when coverage is not relatively dense.

Thacker *et al.* (1997) suggested that fishes without prior learning experience may be more likely to consume chemically defended foods which, depending upon the post-ingestive consequences of this food, the fishes may then learn to avoid. A lack of experience with chemically defended foods did not appear to make captive-bred *S. fuscescens* more likely to consume *L. majuscula*. In fact, both wild and captive-bred *S. fuscescens* displayed the same feeding pattern when offered a mono-specific diet of *L. majuscula* containing lyngbyatoxin-a from the Adams Beach site, with only small quantities consumed over a 10 day trial period compared the group offered the control food, *A. spicifera*. While no relationship was observed between lyngbyatoxin-a concentration and consumption levels in wild *S. fuscescens*, a slight positive correlation in captive-bred *S. fuscescens* suggests that more *L. majuscula* was consumed when lyngbyatoxin-a concentrations were elevated. This result was surprising and may be related to lack of experience with chemically defended foods and the potential negative post-ingestive consequences that may be associated with lyngbyatoxin-a consumption.

Suggestions that captive-bred *S. fuscescens* be used to augment wild *S. fuscescens* populations in an attempt to mitigate severe *L. majuscula* blooms raises a number of points for consideration. Firstly, captive-bred *S. fuscescens* did not preferentially consume *L. majuscula* in the laboratory and only consumed small quantities during chronic exposure trials. Therefore, they would probably exert little pressure as a top-down control agent. Secondly, very little is known of the population size and dynamics of wild *S. fuscescens* currently within Moreton Bay. Wild siganids did not preferentially consume *L. majuscula* and were inconsistent in the quantities they did consume. Augmentation of wild populations by captive-bred siganids would therefore seem to offer an inadequate solution to mitigate nuisance blooms in Moreton Bay. Thirdly, very little is known of the consequences of *L. majuscula* consumption in siganids. Toxicity and deterrence of secondary metabolites will depend upon the physiological interactions between that metabolite and its consumer (Paul, 1992). Reports of siganids becoming 'toxic' after feeding on *L. majuscula* (Hashimoto *et al.*, 1976) may imply a similar fate for Moreton Bay siganids. The sale of the fish in export markets, and its potential toxicity require more detailed research. Whilst acute poisoning episodes have been documented that could be a potential threat to human health (Sims & Zandee van Rilland 1981; Nagai *et al.*, 1996; Yasumoto, 1998), little is known of the sublethal effects of tumour-promoting toxins such as lyngbyatoxin-a (Ito *et al.*, 2002).

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