Vol. 307: 143–154, 2006 | MARINE ECOLOGY PROGRESS SERIES
Mar Ecol Prog Ser | Published January 24

Experimental manipulation of shade, silt, nutrients and salinity on the temperate reef sponge *Cymbastela concentrica*

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ABSTRACT: Discharge of sewage effluent into the ocean has been shown to cause changes in the structure and distribution of a range of biological assemblages, including those dominated by sponges. To date, the underlying mechanisms by which exposure to sewage alters such assemblages is unclear, although a number of potential models have been proposed. Here, a series of manipulative field experiments were done using the phototrophic sponge *Cymbastela concentrica*. Hypotheses from the general models that increased shade, silt, nutrients or salinity gradients were tested to find a cause for observed declines in populations exposed to sewage. Changes in the variables examined (i.e. growth and reproductive status of *C. concentrica* and concentrations of chl *a* associated with symbiotic micro-algae in *C. concentrica*) strongly supported the models showing that shading and siltation were a cause for decline. Nutrients did not affect any of the variables that were measured, whereas a decreasing salinity gradient caused a decline in growth, reproductive status and symbiotic algae (as measured by the concentration of chl *a*). This work makes a significant contribution to our understanding of the mechanisms that underpin the changes in patterns observed when sponges are exposed to physical factors associated with a sewage plume.

KEY WORDS: Sponges · Sewage · Symbiotic algae · Light · Growth · Reproduction

INTRODUCTION

The discharge of sewage effluent into the ocean can alter the chemical and physical nature of the receiving water (Baker et al. 1995) leading to changes in the structure of a wide range of biological assemblages (Smith et al. 1999, Hindell & Quinn 2000). Reported effects of sewage effluent on the structure and dynamics of sessile subtidal assemblages on temperate reefs have included changes to: (1) the fauna inhabiting the holdfasts of the kelp *Ecklonia radiata* (C. Agardh) in shallow water (Smith 1996); (2) sponge- and algae-dominated assemblages close to the shoreline at 15 to 20 m depth (Chapman et al. 1995, Roberts et al. 1998); and (3) sponge-dominated assemblages on deep-water reefs to 60 m depth (Roberts 1996).

The process by which the discharge of sewage alters subtidal assemblages is unclear, although there are a number of potential models. Sewage can increase the level of suspended solids and, therefore, turbidity in the water column at or near its point of discharge (Coade 1995). In turn, this has the potential to reduce the amount of light that reaches the substratum (Kirk 1983). Reductions in light availability can limit the growth and survival of subtidal primary producers such as seagrasses (Fitzpatrick & Kirkman 1995), benthic algae (Vadas & Steneck 1988), and symbiotic micro-algae (Maldonado & Young 1998). Indeed, the structure of shallow subtidal assemblages can be altered due to shading by kelp canopies (Kennelly 1989) and artificial structures (Glasby 1999), whilst depth- and light-limitation have been correlated with

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changes to the structure of sponge assemblages on coral reefs (Wilkinson & Vacelet 1979).

Suspended particles also increase sedimentation (Bickford 1996), which may, in turn, alter the structure and dynamics of subtidal assemblages (Rogers 1990, Airoldi & Cinelli 1997, Wulff 1997, Bell & Barnes 2000). There is also correlative evidence that community structure can change along sedimentation gradients (Naranjo et al. 1996, Bell & Barnes 2000). Sewage plumes may increase rates of siltation (Baker et al. 1995, Bickford 1996), resulting in the burial or smothering of encrusting species. Notably, an increased cover of silt has been documented close to sewage outfalls (see Roberts et al. 1998). Field and flume studies of the morphology of some invertebrates indicate sensitivity to high rates of sedimentation (Riegl et al. 1996), leading to the occlusion of inhalant pores of filter feeders (Gerrodette & Flechsig 1979, Cerrano et al. 1999).

Due to its very nature, sewage contains significant concentrations of nutrients that are required for plant growth, i.e. nitrogen and phosphorus. Nitrogen is generally the limiting nutrient for algal growth in marine waters (Rhyther & Dunstan 1971). Several studies have shown that if growth-limiting factors such as light and temperature are optimal, then 'blooms' of macro-algae can occur in the presence of sewage effluent (Borowitzka 1972, Bellgrove et al. 1997). Increases in the availability of nutrients to shallow subtidal and intertidal habitats may also act to modify the structure of assemblages of invertebrates (Morris & Keough 2003). This may be particularly true for those that depend on phototrophic symbionts (Roberts et al. 1999), which are thought to contribute significantly to nutrition in some species (Wilkinson et al. 1999).

Since sewage effluent is primarily freshwater, it has the potential to reduce the salinity at its point of discharge (Baker et al. 1995). The degree of mixing between fresh- and salt-water at this point will depend on the quantity of effluent, the depth at which it is discharged and the local wave energy and current regimes (Baker et al. 1995). Given that freshwater can be toxic to marine organisms, there are surprisingly few studies which examine or discuss the role of reduced salinity in causing stress at either the community or population level (but see Hoegh-Guldberg & Smith 1989, Roberts & Scanes 2000).

Roberts et al. (1998) used a 'Beyond BACI' design (Underwood 1994) to assess the impact of a newly commissioned sewage outfall on a shallow (~20 m) temperate reef. The discharge of sewage onto the reef led to rapid change from a sponge-/algae-dominated assemblage to one that was dominated by silt and ascidians (Roberts et al. 1998). Prior to the discharge of sewage, the dominant sponge on the reef was *Cymbastela concentrica* (Lendenfeld), which decreased in abundance within 3 mo of the outfall being commissioned. *C. concentrica* is an erect, cup-shaped, lamellate sponge common on coral reefs to 30 m (Hooper & Bergquist 1992) and temperate reefs to depths of 8 to 60 m (Roberts & Davis 1996). Several studies have found that it is a phototrophic sponge (Cheshire et al. 1995, Roberts et al. 1999), which possesses symbiotic microalgae within its peripheral skeleton (Hooper & Bergquist 1992). A number of competing models were proposed to account for the decline of *C. concentrica* and other sponges (Roberts et al. 1998), but in the temperate regions of Australia, there is no research on processes by which sewage can potentially cause these changes (Hindell & Quinn 2000).

The major aims of this study were to examine whether the general models of increased shade, siltation, concentrations of key nutrients, or reduced salinity could account for observed declines in abundances of the sponge *Cymbastela concentrica*. Specifically, a series of manipulative field experiments were used to test the hypotheses that the rate of growth and reproductive status (number of larvae, eggs and sperm) of *C. concentrica* and the abundance of its symbiotic algae (measured as the concentration of chl *a*) would be adversely affected if light, siltation, nutrients and salinity were manipulated.

MATERIALS AND METHODS

Study locations and manipulation of sponges. Experiments to examine the effects of shade, siltation and nutrients were established in Hardys Bay near the entrance to Brisbane Water on the Central Coast, New South Wales, Australia (Fig. 1). This location was chosen to protect the experiments from the effects of storms. For the experiment examining the effects of reduced salinity, 5 locations were chosen along a salinity gradient in the Brisbane Water Estuary (Fig. 1). The 4 experiments were established for a period of at least 90 d. The shade and siltation experiments began in October 1998, whilst the nutrient and salinity experiments began approximately 1 mo later.

Divers using SCUBA collected individuals of a similar size from a natural population of *Cymbastela concentrica* on a nearby reef (depth 12 to 15 m) at Lyon Island (Fig. 1). On the surface, each sponge was blotted dry with a towel and any epibiota removed. Individuals were weighed (to the nearest gram using a field balance), tagged and randomly allocated to 1 of the 4 manipulative experiments. To determine the reproductive and symbiont status of the natural population of *C. concentrica*, reference sponges were also collected from the reef at Lyon Island (see Fig. 1) at the beginning, during and at the end of the experiments.

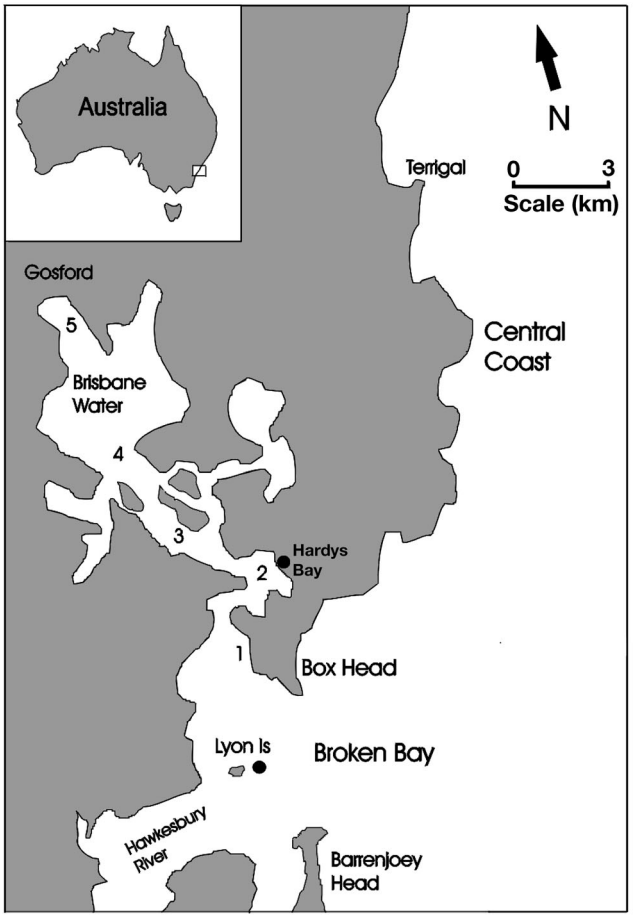

Fig. 1. *Cymbastela concentrica.* Study locations at Lyon Island (natural populations of sponges) and in the Brisbane Water estuary, New South Wales, Australia. 1–5: locations where sponges were deployed

Once the experiments had been set up, weekly visits were made to remove any epibiota or silt that may have settled on the sponges for a period of up to 13 wk, depending on the experiment. At the end of each experiment, the sponges were collected, blotted dry, weighed in the field (nearest gram) and appropriately preserved (see below) for further analysis.

Experimental design. Each experiment was designed to examine the effects on growth, reproductive status and symbionts in the sponge *Cymbastela concentrica*, using 3 replicate sponges for each treatment. For the experiment examining the effects of reduction of light, hereafter referred to as the shade experiment, rectangular metal frames (0.8 × 0.6 × 0.8 m) were placed on the substratum at a depth of approximately 3 m in each of 3 random sites. Sites were approximately 15 × 15 m and separated by up to 100 m. Each frame had a wire basket in which a single sponge was held (sponges were oriented naturally), suspended at least 0.3 m off the substratum, as the experiment was done over soft-sediments. The experiment required 3 treatments: (1) shade treatment, where black perspex was placed on the top and along the sides of the metal frame, to half way down the frame, which allowed water to circulate through the unit, whilst reducing the amount of available light; (2) perspex control, where the metal frame was covered with clear perspex in the same manner as the shade treatment, which allowed light to enter; and (3) frame control, which consisted of the metal frame with no perspex, to determine whether perspex caused artefacts. The amount of light under each experimental frame was measured *in situ*, using a Li-Cor (LI-185) light sensor to establish whether the shade treatment significantly reduced the light reaching the sponges. The amount of light (measured in micro-Einsteins: µE) reaching the sponges in each of the shade, perspex control and frame control treatments was 12.8 ± 0.2 (mean ± SE), 265.0 ± 3.5 and 293.3 ± 4.1 µE, respectively. This represented a reduction of over 90% in available light under the shade treatment, which is typical of the amount of shading that may be produced by a sewage outfall plume (Coade 1995).

The 'silt-addition' experiment required 3 treatments: (1) addition of silt; (2) no silt; and (3) cage control, at each of 3 random sites. The sponges were placed into small wire cages, which were welded to the top of a 0.8 m length of aluminium pipe. The cage control consisted of a wire mesh base to which the sponge was secured using a small cable tie. The pipes were pushed 0.3 m into the substratum, at a depth of approximately 3 m, keeping the cages at least 0.5 m off the bottom. Using a small plastic shovel, approximately 5 g of fine silt was added to the 'silt treatment' sponges at least once per week. The amount of silt to be applied was estimated from rates of deposition to the seafloor by suspended particulate matter associated with the discharge of sewage (Bickford 1996). The no-silt treatment was exposed to ambient levels of siltation, while the control sponges were carefully fanned to free any silt that may have settled since the last site visit.

The 'nutrient-addition' experiment included 4 treatments: (1) addition of nitrogen; (2) addition of phosphorus; (3) addition of nitrogen and phosphorus; and (4) a control (where no nutrients were applied), which were placed at 3 random sites. The same type of cage was used as described in the siltation experiment. The sponges were placed into the cages with a small hessian bag containing 50 g of 'slow-release' fertilizer (Osmocote™), i.e. nitrogen (11.5% NH_4^+ and 11.5% NO_3^-) or phosphorus (18% PO_4^{-3}), or a mixture of both (Udy & Dennison 1997). A hessian bag containing no nutrients was placed in the control treatment cages.

Finally, for the experiment examining the effects of salinity, experimental sponges were deployed at 5

locations, at increasing distances from the entrance to the Brisbane Water Estuary (Fig. 1). A gradient was determined along the estuary by collecting 3 replicate measurements of salinity using a YEOKAL 611 submersible data logger at each of the 5 locations. The collection of salinity readings was continued each week to establish the average salinity for each location over the period of the experiment. The salinity for locations 1 to 5 was 34.5 ± 0.4 (mean ± SE), 34.0 ± 0.4, 33.7 ± 0.5, 32.5 ± 0.6, 30.6 ± 1.2, respectively. Sponges were placed in cages as described in the previous experiments and 3 replicate cages were placed at each of 3 sites nested within each location.

Laboratory procedures. The growth rate (fresh weight [FW] g^{-1} d^{-1}) of each sponge was calculated by subtracting its initial weight from its final weight and dividing by the number of days it was deployed (this varied slightly for each experiment). Sub-samples of sponges used for analysis of reproductive status were fixed in a gonad fixative, FAACC (100 ml: 37 to 40% formaldehyde solution; 5 ml glacial acetic acid; 1.3 g calcium chloride dihydrate; 85 ml distilled water) for 48 h, and then transferred to 75% ethanol (Fromont 1999). Thin sections (8 µm) from each replicate sponge were cut using a microtome and stained with haematoxylin and eosin, mounted and examined under the light microscope for the presence of larvae, eggs (oocytes) and sperm (spermatocytes). The mean number of larvae and oocytes in each sponge was estimated from 3 'fields of view' (400× magnification) that were haphazardly placed over each section. Spermatocytes were estimated using 3 randomly placed 4 × 3 µm sub-quadrats. The concentration of chl *a* was determined for each sponge by homogenising sub-samples using a mortar and pestle and extracting the chlorophyll using 90% acetone for 30 min on ice and in the dark. A Shimadzu UV1601 spectrophotometer was used to measure the concentration of chl *a* within each sponge (Clesceri et al. 1985).

Data analysis. For each experiment, differences among treatments in the rate of growth of each individual, the number of spermatocytes, and the concentration of chl *a* were analysed using a 2-factor ANOVA. In the experiments on shading, siltation and nutrients, sites were considered to be random and treatments were fixed. In the experiment examining a salinity gradient, locations were considered to be fixed and sites were nested within location. Post-hoc pooling procedures were used when the Site × Treatment interaction was found to be non-significant at p = 0.25; this allowed appropriate *F*-tests to be constructed for main effects (Winer 1971). Prior to analysis, the data were examined for homogeneity of variance using Cochran's test (Winer 1971). Where variances were heterogeneous, data were transformed to ln(x + 1) (Winer 1971). Where transformations did not result in homogeneous variances, analyses were done on the untransformed data (Underwood 1981). Where significant differences were found in the ANOVA, Student-Newman-Keuls (SNK) multiple comparisons were done at the appropriate alpha level to determine differences among means (Winer 1971). Examination of the data for the number of oocytes revealed that there were many zeros among replicates within a treatment; therefore, chi-square analyses (Winer 1971) were used to formally examine these data. The data for larvae were excluded from further statistical analyses because several of the treatment means were equal to zero.

RESULTS

Sponge growth

There was a significant loss of weight in *Cymbastela concentrica* (after 90 d) under the shade treatments, whereas there was growth in the sponges in the perspex and frame controls at each of the 3 sites (Table 1, Fig. 2a). Specimens in both controls appeared 'healthy' and were similar in colour and consistency to reference sponges collected from Lyon Island at the end of the experiment.

In the silt-addition experiment, there was a significant loss of weight in *Cymbastela concentrica* (after 90 d) in the treatment where silt was added compared with the controls where growth of the sponges occurred (Table 2, Fig. 2b). There was no significant difference in the growth of sponges between the control treatments or among sites.

Table 1. *Cymbastela concentrica*. Effects of shade on the rate of growth, reproductive status (spermatocytes) and symbiotic algae (chl *a*) in sponges. *F*-ratios in **bold** have been calculated after pooling. ns: not significant, p > 0.05; *p < 0.05; **p < 0.01. S: shade; P: clear perspex; C: control; SNK: Student-Newman-Keuls test

Source of variation	df	Growth MS	Growth *F*	Spermatocytes MS	Spermatocytes *F*	Chl *a* MS	Chl *a* *F*
Site	2	0.0014	1.5ns	0.164	0.75ns	1.788	6.8**
Treatment	2	0.0106	15.0**	2.38	9.95*	8.836	**35.8****
Site × Treatment	4	0.0007	0.7ns	0.239	1.1ns	0.182	0.70ns
Residual	18	0.0009		0.218		0.262	
Cochran's test		**		ns		ns	
Transformation		**		ln(x + 1)		ln(x + 1)	
SNK		S < P = C		S < P = C		S < C = P	

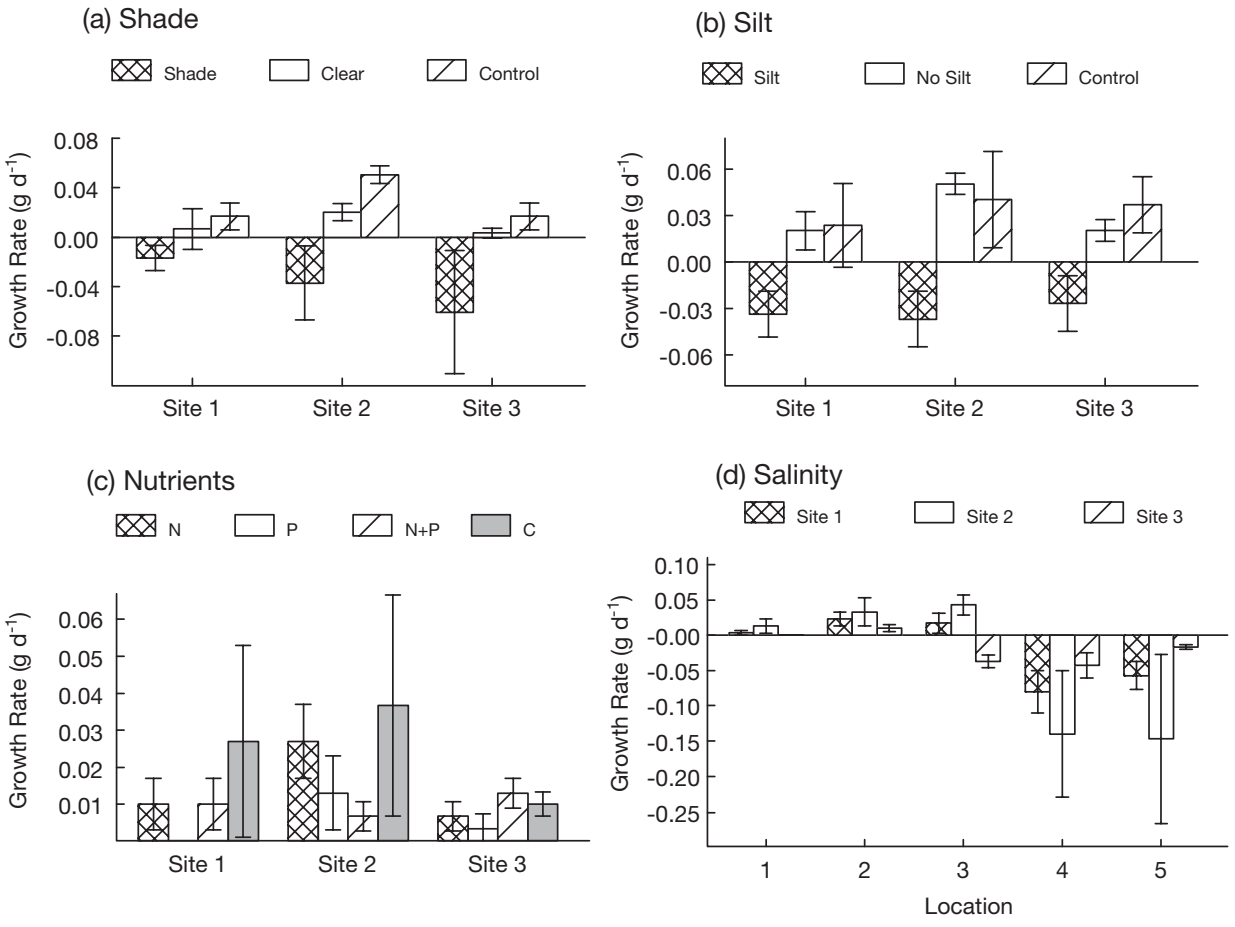

Fig. 2. *Cymbastela concentrica.* Mean (±SE) rate of growth in (a) shade, (b) silt, (c) nutrients (N: nitrogen; P: phosphorus; C: control), and (d) salinity experiments at locations in Brisbane Water

Addition of nitrogen, phosphorus or a combination of both did not affect the growth of *Cymbastela concentrica*. There were no significant differences in growth among treatments at each of the 3 sites (Table 3, Fig. 2c). All sponges in the nutrient-addition experiment appeared to be similar in colour and consistency to reference sponges collected from the reef at Lyon Island at the end of the experiment.

Low salinity had a negative effect on the growth of *Cymbastela concentrica* (Table 4, Fig. 2d) after 90 d. There were no significant differences among the rates of growth at Locations 1, 2 and 3; however, there was a reduction in sponge weight at Locations 4 and 5 (Table 4), where the salinity was on average 3 to 5 ppt lower than the locations closer to the mouth of the estuary. There was no significant difference between sites nested within locations and generally the sponges at all locations appeared healthy.

Table 2. *Cymbastela concentrica.* Effects of siltation on the rate of growth, reproductive status (spermatocytes) and symbiotic algae (chl *a*) in sponges. *F*-ratios in **bold** have been calculated after pooling. ns: not significant ($p > 0.05$); $^{*}p < 0.05$; $^{**}p < 0.01$. S: silt; N: no silt; C: cage control

Source of variation	df	Growth MS	Growth *F*	Spermatocytes MS	Spermatocytes *F*	Chl *a* MS	Chl *a* *F*
Site	2	0.00047	0.7ns	35.14	**5.2****	2.18	2.8ns
Treatment	2	0.013	**20.83****	59.12	**8.7****	7.28	**9.9****
Site × Treatment	4	0.00038	0.5ns	8.36	1.3ns	0.50	0.64ns
Residual	18	0.00069		6.44		0.78	
Cochran's test		ns		ns		ns	
Transformation		None		None		ln(x + 1)	
SNK		S < N = C		S < N = C		S < N = C	

Reproductive status

Cymbastela concentrica collected randomly from the natural populations

near Lyon Island (reference sponges) all possessed larvae, oocytes and spermatocytes at the start of the experiments (i.e. they were reproductively active). For each of these variables, means were lower in sponges collected at the end of the experiment compared with those collected at the start (Table 5). At the end of the experiment, the mean number of larvae, oocytes and spermatocytes was generally greater in the natural population (reference sponges) compared with those in the experimental control treatments (Table 5).

Significantly fewer spermatocytes were found within sponges kept in the shaded treatment (Table 1, Fig. 3a) compared with both control treatments. Thus, sponges that were placed under the shade treatment were not as reproductively active as those within the control treatments.

There were significantly fewer spermatocytes within sponges to which silt had been added (Table 2, Fig. 3b) compared with controls. However, we did not detect significant differences in the number of spermatocytes following the addition of nutrients (Table 3, Fig. 3c).

For sponges deployed along the salinity gradient, analyses revealed significant differences in the number of spermatocytes among locations (Table 4, Fig. 3d). Numbers were greater at Location 1 (~35 ppt) and fewer in sponges at Location 5 (~31 ppt) compared with all other locations (Fig. 3d, Table 4).

For the number of oocytes, it was assumed that there were no Site × Treatment interactions; therefore, the data for each site could be pooled for each treatment. For each treatment, chi-square analysis confirmed that the expected values differed significantly from the observed values (Table 6). Examination of the observed values showed that for the shade experiment fewer oocytes were recorded from sponges exposed to shade compared with sponges under the clear perspex or frame controls (Table 6). Fewer oocytes were recorded from sponges exposed to the addition of silt compared to 'no-silt' or cage control sponges (Table 6). Fewer oocytes were also found in sponges exposed to a combination of both nitrogen and phosphorous compared to control sponges or sponges exposed to nitrogen or phosphorous (Table 6). Finally, for the experiment examining the effects of salinity, more oocytes were recorded from sponges deployed at Locations 2 and 3 compared to Locations 1, 4 and 5 (Table 6).

Table 3. *Cymbastela concentrica.* Effects of nutrients on the rate of growth, reproductive status (spermatocytes) and symbiotic algae (chl *a*) in sponges. *F*-ratios in **bold** have been calculated after pooling. ns: not significant ($p > 0.05$); $^{*}p < 0.05$; $^{**}p < 0.01$). –: no test required

Source of variation	df	Growth MS	Growth *F*	Spermatocytes MS	Spermatocytes *F*	Chl *a* MS	Chl *a* *F*
Site	2	0.0005	2.3ns	1.02	0.2ns	3.340	0.7ns
Treatment	2	0.0006	**3.0ns**	9.63	1.7ns	3.452	0.5ns
Site × Treatment	4	0.0002	0.85ns	5.71	1.1ns	6.503	1.4ns
Residual	18	0.0002		5.24		4.559	
Cochran's test		*		ns		ns	
Transformation		*		None		None	
SNK		–		–		–	

Table 4. *Cymbastela concentrica.* Effects of salinity on the rate of growth, reproductive status (spermatocytes) and symbiotic algae (chl *a*) in sponges. ns: not significant ($p > 0.05$); $^{*}p < 0.05$); $^{**}p < 0.01$); L1 to L5: Locations 1 to 5, respectively

Source of variation	df	Growth MS	Growth *F*	Spermatocytes MS	Spermatocytes *F*	Chl *a* MS	Chl *a* *F*
Location	4	0.024	4.55*	33.1	9.54**	64.43	1.5ns
Sites (Location)	10	0.0052	0.99ns	3.46	0.51ns	41.85	0.8ns
Residual	30	0.0053		3.66		49.66	
Cochran's test		**		ns		**	
Transformation		**		None		**	
SNK		L1 = L2 = L3 > L4 = L5		L1 > L2 = L3 = L4 > L5		–	

Table 5. *Cymbastela concentrica.* Mean number (±SE, per field of view) of larvae, oocytes and spermatocytes in natural populations (reference sponges) at Lyon Island (n = 9) and in the experimental controls for shade (n = 18), silt (n = 18), nutrients (n = 9) and salinity (n = 9) at the end of the experiments (shade and silt: January 1999; nutrients and salinity: February 1999; dates in table given as mo/yr)

Variable	Lyon Island (reference sponges) Start (10/98)	Lyon Island (reference sponges) End (02/99)	Experimental controls Shade	Silt	Nutrient	Salinity
Larvae	0.1 ± 0.1	0.04 ± 0.04	0.1 ± 0.03	0.04 ± 0.03	0 ± 0	0.1 ± 0.05
Oocytes	1.5 ± 0.8	0.7 ± 0.1	0.5 ± 0.1	0.3 ± 0.1	0.5 ± 0.2	0.2 ± 0.1
Spermatocytes	9.4 ± 0.6	6.7 ± 0.9	3.4 ± 0.5	5.24 ± 0.87	5.11 ± 0.61	9.7 ± 1.0

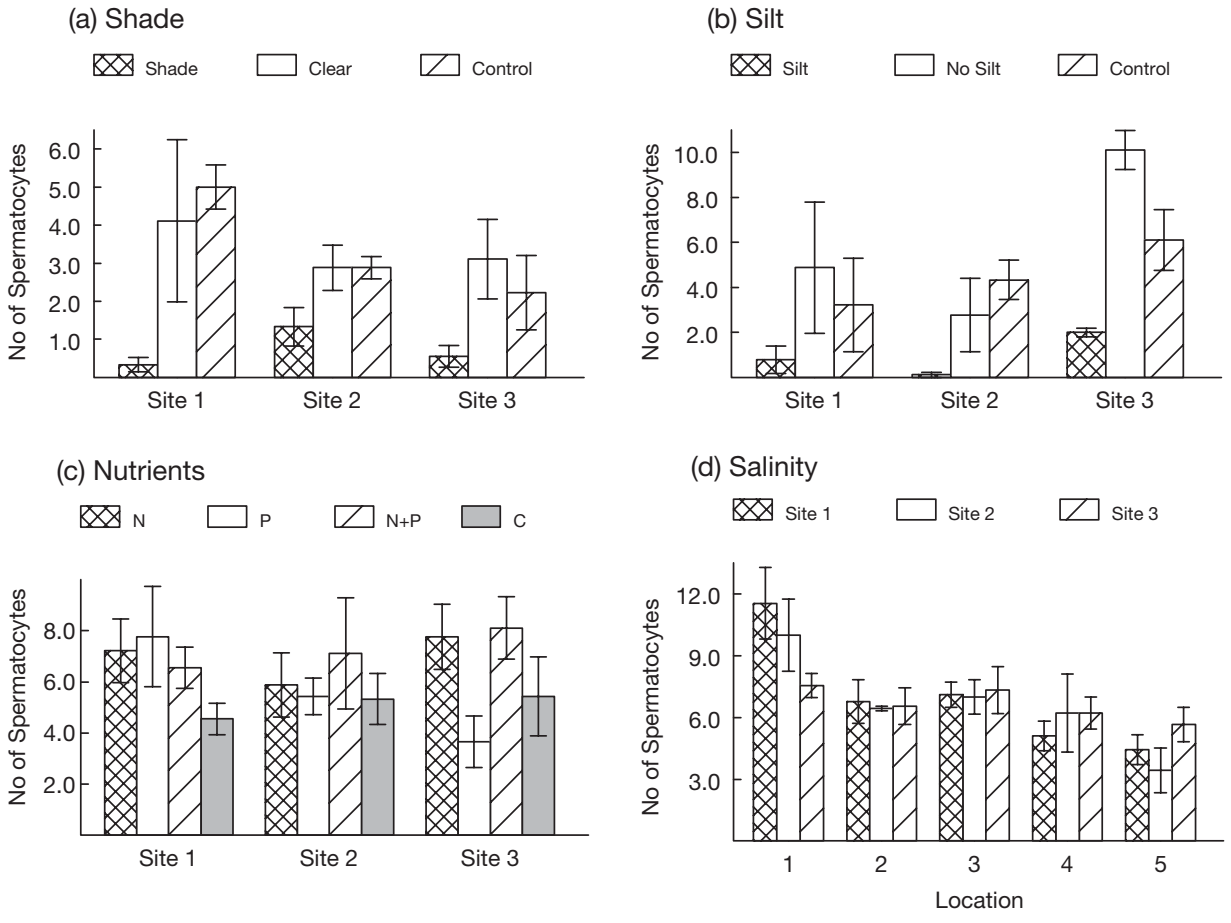

Fig. 3. *Cymbastela concentrica.* Mean number (±SE, per field of view) of spermatocytes in sponges in (a) shade, (b) silt, (c) nutrients (N: nitrogen; P: phosphorus; C: control), and (d) salinity experiments at locations in Brisbane Water

Symbiotic algae

Natural populations (reference sponges) of *Cymbastela concentrica* near Lyon Island generally had greater mean concentrations of chl *a* than those in the experimental controls at the conclusion of the experiment (Table 7). Concentrations of chl *a* within these reference sponges did not change over the course of the experiments (Table 7). The lower chl *a* concentrations in the experimental sponges could be due to handling or relocation stress. The major difference in the concentration of chl *a* was evident in the nutrient

Table 6. *Cymbastela concentrica.* Summary of chi-squared tests for the number of oocytes in sponges in the shade, silt, nutrient and salinity experiments (*p < 0.05; **p < 0.01). Obs.: observed; exp.: expected

Shade			Silt			Nutrient			Salinity		
Treatment	Obs.	Exp.	Treatment	Obs.	Exp.	Treatment	Obs.	Exp.	Treatment	Obs.	Exp.
Shade	2	9	Silt	1	4.67	N	13	10.75	L1	5	7
Clear	18	9	No silt	3	4.67	P	16	10.75	L2	12	7
Control	7	9	Control	10	4.67	N+P	2	10.75	L3	14	7
						Control	12	10.75	L4	4	7
									L5	0	7
Total	27			14			43			35	
df	2			2			3			4	
Chi-square	14.9**			9.6**			10.3*			19.4**	

Table 7. *Cymbastela concentrica.* Mean (±SE) chl *a* concentrations (µg g^{-1}) in sponges collected from the natural population (reference sponges) at Lyon Island (n = 9) and in the experimental controls for shade (n = 18), silt (n = 18), nutrients (n = 9) and salinity (n = 9) at the end of the experiment (shade and silt: January 1999; nutrients and salinity: February 1999; dates in table given as mo/yr)

Reference sponges (Lyon Island)			Experimental controls			
Start (10/98)	Mid (12/98)	End (02/99)	Shade	Silt	Nutrient	Salinity
145.1 ± 26.8	150.1 ± 13.9	146.1 ± 36.6	60.6 ± 8.8	49.3 ± 10.3	3.2 ± 0.9	8.5 ± 5.3

and salinity experiments, where all concentrations were considerably lower than in the other 2 experiments (Table 7).

There was a significantly lower concentration of chl *a* in sponges within the shade treatment compared with the controls (Table 1, Fig. 4a), and Site 1 had significantly greater concentrations than the other 2 sites (Table 1, Fig. 4a). Lower concentrations of chl *a* were also measured in sponges where silt was applied, compared with the controls (Table 2, Fig. 4b), but there were no significant differences between sites. No significant difference in concentrations of chl *a* was detected among treatments in experiments examining the effects of nutrients (Table 3, Fig. 4c), or changes in salinity (Table 4, Fig. 4d).

Sponges under the shade treatment and the silt-addition treatment had a bleached appearance and showed evidence of necrosis around the edges. Some of the replicate sponges in the silt-addition treatment also had a 'blackened' surface colour. The sponges in the control treatments for both of these experiments appeared to be similar in colour and consistency to reference sponges collected from the reef at Lyon Island at the end of the experiment.

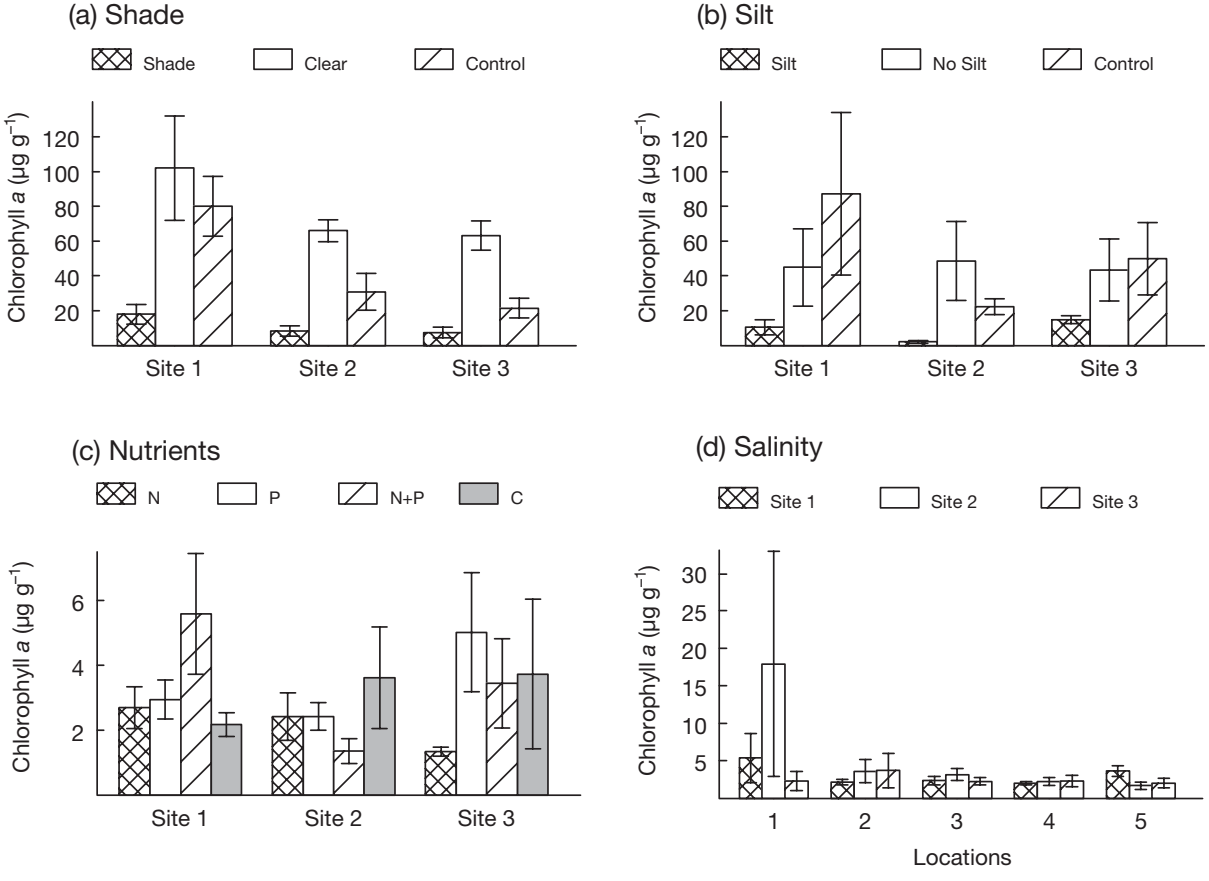

Fig. 4. *Cymbastela concentrica.* Mean (+SE) concentration of chl *a* (µg g^{-1}) in sponges in (a) shade, (b) silt, (c) nutrients (N: nitrogen; P: phosphorus; C: control), and (d) salinity experiments at locations in Brisbane Water

DISCUSSION

We found a significant reduction in the growth and reproductive status of the sponge *Cymbastela concentrica*, as well as significant changes in photosynthetic symbionts on exposure to shade and siltation. A decrease in salinity of around 2 ppt also affected the rate of growth and reproductive status of *C. concentrica*, but did not alter the concentrations of chl *a*. In contrast, we did not detect any significant change in the rate of growth, reproductive status or symbiotic relationship in *C. concentrica* following the addition of nutrients (nitrogen and phosphorus).

Shading

Studies of the effects of shading on marine assemblages have included those associated with seagrass meadows (Fitzpatrick & Kirkman 1995), kelp forests (Kennelly 1989), coral reefs (Rogers 1990), subtidal caves (Cinelli et al. 1977) and artificial structures (Glasby 1999). In this study we found a significant effect of shading on the growth and reproductive status of the sponge *Cymbastela concentrica*. Thacker (2005) also reported that the mass of the sponge *Lamellodysidea chlorea* de Laubenfels was significantly smaller after 2 wk of shading compared with controls, whilst its symbiotic association with *Oscillatoria spongeliae* (Schulze) Hauck was not affected by shading. Rogers (1990) also found that the net productivity in assemblages of corals was reduced when light was experimentally manipulated to mimic the shading associated with increased turbidity.

Deleterious effects of shading have also been observed at the assemblage level. After reducing light, Glasby (1999) found significant effects on the cover of sessile macrobenthic assemblages (e.g. algae, serpulid worms, bryozoans and ascidians) occurring on artificial structures, and his findings were consistent with those of other workers (Kennelly 1989, Rogers 1990, Fitzpatrick & Kirkman 1995). Glasby (1999), however, reported that the cover of sponges did not differ between shaded and unshaded treatments, and postulated that, given more time, greater abundances of sponges may have been observed. This prediction was based on earlier work that reported greater abundances of sponges associated with shading (Cinelli et al. 1977).

Plumes of sewage and suspended particulate matter can alter the intensity and spectral quality of light reaching organisms on subtidal reefs (Baker et al. 1995), which can, in turn, affect metabolic processes (Rogers 1990). Here, where light was manipulated to mimic the effects of shading by a plume of sewage, the photosynthetic symbionts (measured as the concentration of chl *a*) within the experimental sponges were significantly reduced. There is clear evidence that many tropical marine sponges are phototrophic, and that symbiotic algae provide the bulk of their carbon energy requirements (Wilkinson et al. 1999, Thacker 2005). Cheshire et al. (1995) demonstrated that *Cymbastela* sp. from reefs in southern temperate Australia were probably phototrophic, whilst Roberts et al. (1999) provided evidence that a surprisingly high proportion of temperate reef sponge species (including *Cymbastela concentrica*) also had this potential. Thus, it would appear that *C. concentrica* relies on its symbionts for at least some of its nutritional requirements, and this is likely to be compromised under conditions that limit the amount of light available for photosynthesis (Cheshire et al. 1997).

Siltation

In this study, increased siltation led to a reduction in weight and lower reproductive activity in the sponge *Cymbastela concentrica*. It also altered the symbiotic relationship between the sponge and its micro-algae. It is conceivable that burial would effectively stop any photosynthetic activity by micro-algae. Clogging would also reduce the flow of water and, therefore, nutrients. The sponge may therefore loose any nutritional benefit from its symbiotic micro-algae as a result of decreased or complete shutdown of normal pumping activities (Gerrodette & Flechsig 1979).

Field and flume studies of the morphology of some invertebrates indicate sensitivity to high rates of sedimentation (Riegl et al. 1996). Heavy siltation has been shown to affect the ability of some sponges to pump water (Gerodette & Flechsig 1979), whereas some species are sensitive to burial beneath sediment (Wulff 1997). The ability to counteract this smothering and clogging is believed to be an important mechanism in many sponges, and those species that cannot tolerate increased sedimentation will be disadvantaged (Barthel & Gutt 1992). Coping with increased sedimentation is important for deep-water species where siltation during periods of low current velocity can occur or where there is the potential for excessive siltation from sewage outfalls (Barthel & Gutt 1992). Siltation has also been shown to alter the structure and dynamics of encrusting communities (Airoldi & Cinelli 1997), and there is correlative evidence that changes in the structure of sessile invertebrate assemblages occur along sedimentation gradients (Naranjo et al. 1996). In contrast, Bell & Barnes (2000) reported that the diversity of sponges was greater in areas that experienced higher rates of sedimentation compared with areas with lower rates of sedimentation.

Nutrients

The nutrient-addition experiment provided no evidence to support the model that nutrients enhanced symbiotic micro-algal growth, i.e. the symbiotic algae were not nutrient limited under the conditions of the experiment, and in turn we failed to detect an affect on sponge growth and reproduction. In contrast, increased concentrations of nutrients in sewage can alter the growth rates of macro-algae (Borowitzka 1972). Bayne et al. (1999) used slow-release fertilizers to examine the response of benthic macro-algae to the addition of nutrients in a similar experiment to ours. Whilst they were able to quantify the concentrations of nutrients being released from their treatments, they found that this release was rapid and difficult to control. In concluding that 'key' nutrients, nitrogen and phosphorus, did not enhance symbiotic algal growth or the growth of the sponge, we assumed that the slow-release fertilizers 'suffused' our treatments and that greater symbiotic activity would result in greater growth of the sponge. Although we did not test these assumptions, we are confident that slow-release fertilizers enhance nutrients beyond ambient conditions (e.g. Worm et al. 2000).

Salinity

A decrease in salinity by approximately 2 ppt resulted in negative growth rates and lower reproductive activity in *Cymbastela concentrica*. Marine sessile organisms are generally stenohaline, i.e. they have a low tolerance to changes in salinity, and reduced salinity has been shown to decrease the diversity and abundance of sponge-dominated assemblages (Witman & Grange 1998). Maldonado & Young (1998), using a transplant experiment, relocated keratose sponges to depths where there was a pycnocline of lower salinity, temperature and oxygen. This resulted in the death of their sponges, which they suggested was due to changes in temperature and loss of nutrient-producing symbionts. It is important to note that our experimental examination of salinity was correlative and there could be potential confounding effects from other water quality gradients in the estuary. The fact that concentrations of chl *a* did not vary significantly along the salinity gradient would suggest that turbidity, at least, was not a confounding factor during the time of the experiment.

The low chl *a* readings obtained in the salinity and nutrient experiments reflected an overall change in the relationship with photo-symbionts of the experimental control sponges compared with that of the natural population (reference sponges) collected from Lyon Island. These 2 experiments started and finished a month later than the experiments examining shading and siltation. The only factor that changed significantly within the estuary over this period was an increase in water temperature of ca. 2°C. Increased temperature and heat-stress has been correlated with changes to the photosystems of symbiotic algae in corals (Jones et al. 2000). The thermal sensitivity of photosynthetic symbionts is believed to be the cause of coral bleaching where the tissue whitens as symbiotic algae 'die-off' or are ejected during periods of elevated water temperature (Hoegh-Guldberg 1999). Interestingly, there were no differences in the rate of growth of *Cymbastela concentrica* in the earlier experiments compared with the later ones where reduced abundances of symbiotic algae were recorded. If the reduction in the symbiotic algae was caused by an increase in water temperature, then this must have occurred close to the end of the experiment. Thus, the rate of growth of the sponges was not significantly affected during that time. Nevertheless, it is advisable to interpret the results of the 2 sets of experiments in isolation because some uncontrolled factor, such as a change in water temperature, may have affected the symbiotic relationship between the sponge and its micro-algae.

Whilst the experiments reported here give us important information about the potential mechanisms for a single species, the results should not be extrapolated to an entire assemblage, and it would be constructive to repeat these types of experiments at that scale. It would also be instructive to examine the effects of these abiotic variables in concert. The simultaneous application of abiotic variables can have dramatic and unexpected results (e.g. Przeslawski et al. 2005). Such synergies may also be occurring among variables associated with sewage plumes. Finally, many millions of dollars are spent annually on monitoring the effects of sewage on the marine environment (Koop & Hutchings 1996). In our opinion, it is questionable whether much of this expenditure is justified, and greater emphasis should be placed into gaining a better understanding of the processes of sewage-related disturbance in marine assemblages, using an experimental framework.

Acknowledgements. We thank B. Roberts, S. Murray, K. Zimmerman, S. Fawcett, K. Casey and B. Diaz for assistance in the field. We are especially grateful to B. Roberts for constructing and G. Henry (NSW EPA) for supplying the materials for the experimental units. J. Turnbull (Wollongong University) assisted with the chlorophyll analyses and K. Wadwell (Veterinary Pathology Diagnostic Services, University of Sydney) prepared the slides for histology. We also thank T. Church for assessing and interpreting the reproductive histology. G. Chapman and A. Butler provided helpful comments on the draft manuscript. A. J. Underwood is thanked for comments and advice with the analyses of the reproductive data. This paper represents contribution No. 259 from the Ecology & Genetics Group, University of Wollongong.

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Editorial responsibility: Antony Underwood (Contributing Editor), Sydney, Australia

Submitted: February 1, 2005; Accepted: August 8, 2005
Proofs received from author(s): December 18, 2005