Attachment 9

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P.L. Harrison · S. Ward

Elevated levels of nitrogen and phosphorus reduce fertilisation success of gametes from scleractinian reef corals

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Abstract Spawned gametes were collected from colonies of *Acropora longicyathus* at One Tree Island and *Goniastrea aspera* at Magnetic Island, Great Barrier Reef, Australia, for use in fertilisation trials. Mean fertilisation rates were significantly reduced compared with controls ($P < 0.003$), when gametes from the branching coral *A. longicyathus* were exposed to elevated ammonium concentrations at 1 μM and above in one cross (60–64% reduction), and at 100 μM in another cross (16% reduction). Mean fertilisation success of *A. longicyathus* gametes was also significantly reduced compared with controls in both crosses ($P = 0.000$) at concentrations of 1 μM phosphate and above (35–75% reduction), and at 1 μM ammonium plus 1 μM phosphate and all higher concentrations (68–74% reduction). Similarly, the mean percentage of regular embryos that were developing normally was significantly reduced in most nutrient treatments compared with controls ($P = 0.000$). Fertilisation trials using gametes from the brain coral *G. aspera* resulted in a significantly lower percentage of regular embryos ($P = 0.001$) and a significantly higher percentage of deformed embryos ($P = 0.001$) developing after exposure to elevated nutrient treatments compared with controls. Mean fertilisation rates for this species were only significantly reduced ($P = 0.034$) in the 50 μM ammonium plus phosphate treatment in one cross (8% reduction), compared with the control. Therefore, ammonium and phosphate enrichment significantly impairs fertilisation success and embryo development in scleractinian reef corals.

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P.L. Harrison (✉) · S. Ward
Centre for Coastal Management,
Southern Cross University,
P.O. Box 157, Lismore, NSW 2480, Australia
E-mail: pharriso@scu.edu.au
Tel.: +61-66-203774
Fax: +61-66-212669

Introduction

Although coral reefs can exist in a moderate range of nutrient concentrations (Kinsey 1988), coral reefs normally flourish in tropical seas characterised by low levels of dissolved inorganic nutrients (D'Elia and Wiebe 1990). Therefore, scleractinian reef corals would appear to be generally adapted to oligotrophic conditions. A substantial and growing body of evidence from scientific studies world-wide indicates that eutrophication resulting from anthropogenic activities has the potential to seriously degrade or modify coral reef ecosystems (reviewed by e.g. Pastorok and Bilyard 1985; Grigg and Dollar 1990; Bell 1992; McCook 1999). Elevated concentrations of the nutrients nitrogen and phosphorus can cause stress in scleractinian reef corals, resulting in altered coral calcification and growth rates, and reduced reef calcification (e.g. Kinsey and Davies 1979; Walker and Ormond 1982; Tomascik and Sander 1985; Stambler et al. 1991).

Excessive nutrient inputs usually lead to enhanced growth of phytoplankton and benthic algae (e.g. Pastorok and Bilyard 1985; Schaffelke and Klumpp 1998), deterioration in water quality and changes in community composition. In Kaneohe Bay and Mamala Bay, Hawaii, chronic eutrophication caused by sewage pollution radically altered the structure of benthic communities and reef ecosystems (Smith et al. 1981; D'Elia and Wiebe 1990; Grigg 1995). Nutrient enrichment enhanced the growth of benthic macroalgae, promoting algal overgrowth of corals and exclusion of reef corals, and increased phytoplankton productivity and turbidity thereby shading benthos; where nutrient enrichment was severe, calcification decreased, and increased deposition of organic material resulted in a dominance of deposit and filter feeders (Smith et al. 1981; Maragos et al. 1985; Kinsey 1988; Grigg 1995). Recovery of some of these reef communities has been reported to occur only after the diversion of sewage effluent to deep-water outfalls (Maragos et al. 1985; Grigg and Dollar 1990; Grigg

1995). Similar community responses and phase shifts from coral to algal dominance have been reported on coral reefs subjected to chronic eutrophication and other perturbations in the Red Sea (Walker and Ormond 1982), Reunion Island (Naim 1993), Jakarta Bay (Tomascik et al. 1993), American Samoa (Green et al. 1997), Barbados (Tomascik and Sander 1987a), Belize (Lapointe et al. 1992), Martinique (Littler et al. 1992), Jamaica (Lapointe et al. 1997) and other regions.

Successful reproduction by scleractinian corals is essential for the maintenance and renewal of reef coral communities that form the basis of coral reef ecosystems (Harrison and Wallace 1990). However, reproduction appears to have a narrower tolerance to stress than other life functions (Harrison and Wallace 1990). Previous studies have shown that sublethal stress causes corals to divert resources away from reproductive activities to other life functions, including growth, maintenance and repair (reviewed in Harrison and Wallace 1990). Therefore, detailed studies of coral reproductive success can provide sensitive indicators of the effects of sublethal stressors on coral reefs.

Little information is available on the effects of nutrient enrichment on reproductive processes in scleractinian corals. Field studies on reefs along a gradient of eutrophication and other pollution in Barbados, West Indies, showed that colonies of *Porites porites* from two polluted reefs produced fewer larvae than colonies from a less polluted reef (Tomascik and Sander 1987b). The reproductive season of *P. porites* populations began 2 months earlier at the polluted reefs, and a skewed 2:1 male to female sex ratio was recorded at the most polluted reef, possibly resulting from increased asexual reproduction from fragmentation (Tomascik and Sander 1987b). Coral settlement and recruitment studies at these sites showed that the number of juvenile coral recruits and the number of recruiting coral species decreased with increasing eutrophication of the reefs (Tomascik 1991; Hunte and Wittenberg 1992). Juvenile coral abundance was lower and mortality rates of juvenile corals were higher on eutrophic reefs with high sediment loads, compared with less eutrophic reefs with low sediment loads (Wittenberg and Hunte 1992). Although these field studies indicate that eutrophication reduces fecundity and recruitment of corals, other factors, including increased sedimentation and turbidity, competition with algae and other colonising organisms, and toxic effects of pollutants associated with the release of sewage and industrial and urban runoff at these sites, may have influenced these results. However, recent studies on the effects of experimentally elevated nutrient levels on coral reproduction at One Tree Reef on the Great Barrier Reef (GBR) during the ENCORE experiment support the findings from these studies at Barbados. Slightly elevated nutrient levels reduced coral larval settlement and recruitment rates (Ward and Harrison 1997, unpublished data), and significantly affected the fecundity and the volume of gametes produced by acroporid corals (Ward and Harrison 2000).

The majority of scleractinian corals are broadcast spawners (Harrison and Wallace 1990), and therefore their unprotected gametes may be exposed to pollutants and other stressors following spawning (reviewed in Harrison and Jamieson 1999). Fertilisation is a sensitive process, and bioassays using sea urchin sperm have been developed for biomonitoring of aquatic pollution (e.g. Kobayashi 1980; Zuniga et al. 1995). Recent studies have shown that fertilisation success in broadcast-spawning corals is reduced by exposure of gametes to oil pollutants (Harrison 1993, 1994, 1999), trace metals (Heyward 1988; Reichelt-Brushett and Harrison 1998), soft coral diterpenes (Aceret et al. 1995), UV radiation (Gulko 1995), low salinity (Harrison 1995) and coastal runoff from urban areas (Richmond 1993). Thus, fertilisation trials can provide a highly sensitive indicator of sublethal stresses in corals. There are no published data on the effects of nutrient enrichment on fertilisation success or embryo development in scleractinian corals. Therefore, the aims of the present study were to determine the effects of experimentally elevated concentrations of ammonium, phosphate and ammonium plus phosphate on the fertilisation success of gametes and early stages of embryogenesis in two common reef coral species.

Materials and methods

Experimental design

The effects of nutrient enrichment on fertilisation success and early embryo development were determined using spawned gametes from colonies of the branching coral *Acropora longicyathus* at One Tree Reef (southern GBR) and of the brain coral *Goniastrea aspera* at Magnetic Island (central GBR), during mass coral spawning periods (Harrison et al. 1984). The experimental design was based on methods developed for studies of oil pollutants on coral fertilisation success (Harrison 1994, 1999). Briefly, the experiments involved collecting spawned egg and sperm bundles from different coral colonies of selected species, separating the eggs and sperm to prevent fertilisation prior to experimental treatments, exposing replicate groups of eggs and separate replicate groups of sperm to normal seawater (for controls) or a range of nutrient treatments for 30 min, combining the eggs and sperm during a 5 h development period, then determining the percentage fertilisation and embryo development responses. For both coral species, three experiments were completed using normal seawater controls and a range of three or four elevated concentrations of the following nutrients: ammonium chloride (ammonium treatment), potassium dihydrogen phosphorus (phosphate treatment) and a combination of both nutrients (ammonium plus phosphate treatment). For each of the experiments, two crosses of eggs and sperm from different colonies were used, and, for each cross at each treatment concentration, five replicate fertilisation trials were done. Therefore, a total of 270 experimental fertilisation trials were completed during this study.

Acropora longicyathus

Branches from five gravid colonies of *A. longicyathus* with mature, coloured gametes (Harrison et al. 1984) were collected from the lagoon at One Tree Reef just prior to the predicted time of spawning in November 1994. These colonies had not been exposed to experimentally elevated nutrient concentrations during gametogenesis. Corals were carefully transported to One Tree Island

Research Station, and branches from different colonies were isolated in large plastic aquaria until they spawned. After spawning, egg and spermatozoa bundles were collected from each colony and were kept isolated from gametes of other colonies to prevent fertilisation prior to the experimental treatments. Preliminary fertilisation test crosses were done to determine which colonies bred most successfully, and would therefore be used in the experiments. Preliminary test crosses involved combining groups of three to four egg–sperm bundles from each colony with three to four bundles from another colony, in separate 20 ml glass vials. After 1.5–2 h, the percentage fertilisation of the eggs in each of the test crosses was determined using a dissecting microscope, and three compatible colonies were chosen for the experiments. Cross 1 used eggs from colony 1 with sperm from colony 2, while cross 2 used eggs from colony 1 with sperm from colony 3.

Shortly after the preliminary test crosses were initiated, large numbers of spawned egg–sperm bundles were collected from the aquaria and placed into labelled glass beakers, and care was taken to ensure that there was no contamination of gametes from different colonies to prevent fertilisation prior to exposure to the experimental treatments. When the egg–sperm bundles had dissociated, eggs and sperm were separated by pouring the gametes through a 150 μm plankton mesh filter, and the concentrated sperm were placed in a glass beaker. The eggs were retained in the plankton mesh filter and were washed gently with ten changes of sperm-free seawater (SFSW) collected earlier in the afternoon from One Tree Reef lagoon. Glassware was rinsed with fresh water and then SFSW between washes to remove sperm. After the final wash to remove any residual sperm, eggs were placed into a large glass bowl to allow them to spread out over a wide surface area to prevent lysing. The separate containers of eggs and sperm were gently agitated to maintain the gametes in a healthy condition.

The initial spermatozoa concentration in the beakers of sperm from each colony was determined using a haemocytometer viewed under 400× magnification with a compound microscope. Two drops of formalin were added to 5 ml of concentrated sperm in a glass vial to immobilise the sperm, then the mean sperm concentration was calculated from counts of sperm in five haemocytometer grid squares. A sperm concentration of approximately 2.5×10^6 ml^{-1} has been shown to maximise fertilisation rates in other reef corals (Willis et al. 1997). Therefore, the initial sperm concentration was diluted with SFSW to achieve a final working concentration of $\sim2\times10^6$ sperm ml^{-1} in the experimental vials. This slightly suboptimal sperm concentration was used in order to quantify any potential increase or decrease in fertilisation rates in the experimental treatments.

The experimental treatments consisted of normal seawater controls with no added nutrients, and nutrient treatment concentrations of background nutrient levels (average 0.65 ± 0.69 μM ammonium, 0.2 ± 0.06 μM phosphate at One Tree Reef; Koop et al. 2001) plus 1, 10, or 100 μM ammonium chloride (ammonium treatment), or 1, 10, or 100 μM potassium dihydrogen phosphorus (phosphate treatment), or 1, 10, or 100 μM of both nutrients combined. Stock solutions of each nutrient were serially diluted with SFSW to twice the final concentration required in the 20-ml experimental vials, to allow for dilution by seawater associated with the gametes.

Groups of approximately 100 eggs were placed into separate wells in plastic trays using fine glass pipettes to facilitate counting. For each control or nutrient treatment concentration, five groups of ~100 eggs were added to a set of five 20 ml glass vials each containing 5 ml of SFSW, and 5 ml of sperm was added to another set of five vials. Each set of ten vials containing either eggs or sperm were then dosed with 5 ml of the appropriate nutrient treatment solution, or 5 ml of SFSW for the controls. Gametes were exposed to the nutrient solutions or seawater controls for 30 min before each set of 10 ml of dosed sperm was combined with the appropriate set of dosed eggs. This resulted in each of the five replicate vials for each treatment containing approximately 100 eggs in 20 ml of sperm and treatment solution. Vials were sealed and then placed in a mesh bag on a mooring line in the lagoon for 5 h, to maintain natural conditions of agitation and temperature during fertilisation and embryo development. After a 5 h incubation period, the vials were retrieved, and the contents of each vial were preserved by removing 5 ml of solution from each vial and replacing it with 5 ml of Bouin's fixative.

The contents of the vials were examined under a dissecting microscope, and the number of undivided eggs, regular embryos, irregular embryos and embryos at the first cleavage stage were recorded. Percentage fertilisation was determined from the total number of dividing eggs and regular and irregular embryos present; undivided eggs that showed no sign of cleavage were counted as unfertilised. These data were analysed using one-way analyses of variance, with dose as the treatment. Homogeneity of variances were tested using Levene's or Cochran's tests, and data were arcsine transformed when required. Student–Newman–Keuls' tests or Tamhane's *T2* tests were used to identify treatments that were significantly different.

Goniastrea aspera

The same experimental and analytical procedures were used to examine the effects of nutrient treatments on fertilisation success of *G. aspera* gametes at Magnetic Island in October 1995, except that the following concentrations of nutrients were used in each experiment: normal seawater controls, and seawater plus 0.5, 1, 5 and 50 μM nutrient concentrations. These reduced nutrient concentrations were selected based on the results of the first experiment at One Tree Reef with *A. longicyathus* gametes. The number of embryos at the first cleavage stage was not analysed separately for *G. aspera* because there were only nine of these embryos present in the three experiments.

Results

Experiments with *Acropora longicyathus*

Mean fertilisation rates of *A. longicyathus* gametes were significantly reduced by exposure to elevated nutrient concentrations compared with controls, in all three nutrient experiments at One Tree Island (Fig. 1; Tables 1, 2, 3). Similarly, the mean percentage of regular embryos that were developing normally was significantly reduced in most nutrient treatments compared with controls (Fig. 2; Tables 1, 2, 3).

Ammonium

The response of *A. longicyathus* gametes to elevated levels of ammonium varied between the two crosses. In cross 1, mean fertilisation rates were high (~94%) for the control, 1 and 10 μM ammonium treatments, but were significantly reduced when gametes were exposed to 100 μM of ammonium (Fig. 1a; Table 1). In the second cross, mean fertilisation rates were significantly reduced for all concentrations of ammonium compared with the control, with a >60% reduction in mean percentage fertilisation in the ammonium treatments relative to the control (Fig. 1a; Table 1). Similar trends were evident in the percentage of regular embryos that developed. In cross 1, means of ~93% regular embryos were recorded in the control, 1 and 10 μM ammonium treatments, while a mean of 68% regular embryos occurred in the

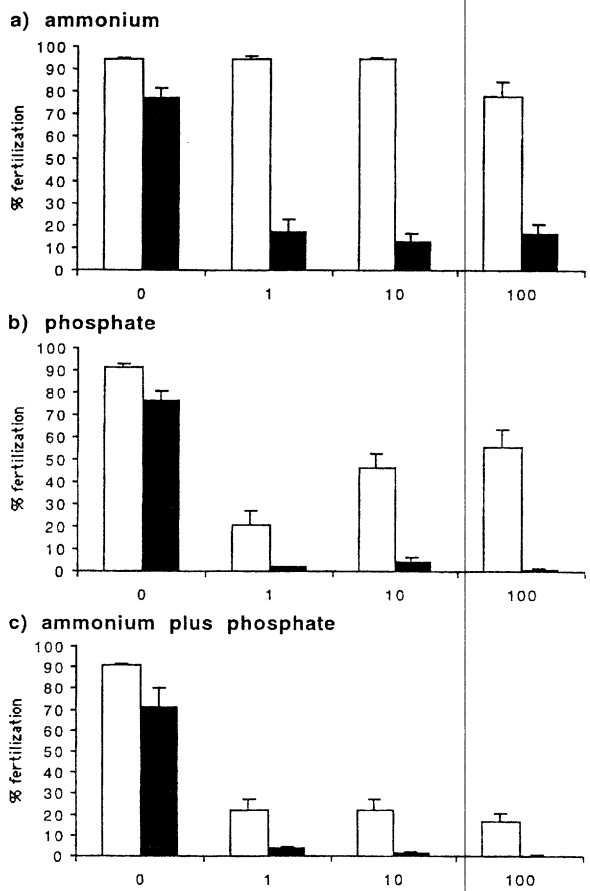

Fig. 1a–c *Acropora longicyathus*. Mean percentage fertilisation recorded in fertilisation trials using eggs and sperm of *A. longicyathus* at One Tree Island. Gametes were exposed to: **a** ammonium, **b** phosphate, or **c** ammonium plus phosphate, at concentrations of background nutrient levels plus 0 (seawater control), 1, 10, or 100 μM ammonium, phosphate, or ammonium plus phosphate. *Open columns* represent cross 1; *filled columns* represent cross 2. Error bars are standard errors

100 μM ammonium treatment (Fig. 2a). However, the means for these treatments did not differ significantly according to the conservative Tamhane's *T2* test for unequal variance multiple comparisons (Table 1). In cross 2, the mean percentage of regular embryos present was significantly reduced in all ammonium treatments compared with the control (Fig. 2a; Table 1).

The mean percentage of embryos at the first cleavage stage was significantly higher in the 100 μM ammonium treatment compared with the control, 1 and 10 μM ammonium treatments in cross 1 (Fig. 3a; Table 1). In the second cross, the mean percentage of embryos at the first cleavage stage was higher in all ammonium treatments compared with the control (Fig. 3a); however, these differences were not significant (Table 1). Variable numbers of irregular embryos were present in some of the fertilisation trials (Fig. 4), and the degree to which these irregular embryos were deformed tended to increase with increasing ammonium concentration. In cross 1, the mean percentage of irregular embryos was significantly higher in the 100 μM ammonium treatment compared with the control, 1 and 10 μM ammonium treatments (Fig. 4a; Table 1). In cross 2, the mean percentage of irregular embryos was significantly higher in the control compared with the ammonium treatments (Fig. 4a; Table 1).

Phosphate

Phosphate had a more pronounced deleterious effect than ammonium on fertilisation rates of *A. longicyathus* gametes. There were significantly lower mean fertilisation rates in all concentrations of phosphate used in both cross 1 and 2, compared with the controls (Fig. 1b; Table 2). Mean fertilisation rates were < 5% in all concentrations of phosphate in cross 2, and were < 1% in the 100 μM treatment. In cross 1, the lowest mean fertilisation rate of 20.4% occurred in the 1 μM phosphate concentration, and significantly higher mean fertilisation rates occurred in the 10 and 100 μM phosphate treatments (Fig. 1b; Table 2). Similar trends were evident in the mean percentages of regular embryos formed, which were significantly higher in the controls compared with all of the phosphate treatments, in both crosses (Fig. 2b; Table 2). Significantly higher mean

Table 1 *Acropora longicyathus*. Comparison of fertilisation and embryo development responses of gametes exposed to background nutrient levels plus 0, 1, 10, or 100 μM ammonium: the percentage fertilisation of eggs, the percentage of regular embryos present, percentage of eggs that were at the first cleavage stage, and the percentage of irregular embryos present. One-way analyses of variance and Student–Newman–Keuls' (*SNK*) or Tamhane's *T2* (*T2*) tests were used (*nsd* no significant differences among treatments; *tr* data were arcsine transformed due to heterogeneity of variances)

Factor	Cross	*F* (*df*)	*P*	Pairwise multiple comparisons
Percent fertilisation	1 (tr)	6.976 (3,16)	0.003	SNK: 0, 1, 10 > 100 μM ammonium
	2	47.382 (3,16)	0.000	SNK: 0 > 1, 10, 100 μM ammonium
Percent regular embryos	1 (tr)	10.845 (3,16)	0.000	*T2*: nsd
	2	51.121 (3,16)	0.000	SNK: 0 > 1, 10 100 μM ammonium
Percent first cleavage	1 (tr)	10.061 (3,16)	0.001	SNK: 100 > 10, 1, 0 μM ammonium
	2	1.110 (3,16)	0.374	SNK: nsd
Percent irregular embryos	1 (tr)	4.947 (3,16)	0.013	SNK: 100 > 10, 1, 0 μM ammonium
	2	8.413 (3,16)	0.001	SNK: 0 > 1, 10, 100 μM ammonium

Table 2 *Acropora longicyathus*. Comparison of fertilisation and embryo development responses of gametes exposed to background nutrient levels plus 0, 1, 10, or 100 μM phosphate. Other details as for Table 1

Factor	Cross	*F* (*df*)	*P*	Pairwise multiple comparisons
Percent fertilisation	1	23.868 (3,14)	0.000	SNK: 0 > 1, 10, 100 μM phosphate; 1 < 10, 100 μM phosphate
	2 (tr)	132.862 (3,16)	0.000	SNK: 0 > 1, 10, 100 μM phosphate
Percent regular embryos	1	37.453 (3,14)	0.000	SNK: 0 > 1, 10, 100 μM phosphate; 1 < 10, 100 μM phosphate
	2 (tr)	137.559 (3,16)	0.000	SNK: 0 > 1, 10, 100 μM phosphate
Percent first cleavage	1	6.282 (3,14)	0.006	SNK: 0 < 1, 10, 100 μM phosphate
	2	2.372 (3,16)	0.109	SNK: nsd
Percent irregular embryos	1	0.083 (3, 14)	0.968	SNK: nsd
	2	12.966 (3,16)	0.000	*T*2: 0 > 1, 100 μM phosphate

percentages of embryos at the first cleavage stage were recorded in the phosphate treatments compared with the control in cross 1, while there were no significant differences among the treatments in cross 2 (Fig. 3b; Table 2). There were no significant differences in the mean percentages of irregularly formed embryos among the treatments in cross 1, whereas a significantly higher mean percentage of irregular embryos was recorded in the control compared with the phosphate treatments in cross 2 (Fig. 4b; Table 2).

Ammonium plus phosphate

The combination of ammonium plus phosphate had the greatest deleterious effects on fertilisation rates and development of regular embryos in *A. longicyathus*. Mean fertilisation rates were significantly reduced by > 67% compared with controls, in all concentrations of ammonium plus phosphate in both crosses (Fig. 1c; Table 3). The mean percentage of regular embryos formed was also significantly higher in the controls than in all nutrient treatments in both crosses, and no regular embryos developed in the 10 and 100 μM nutrient treatments in cross 2 (Fig. 2c; Table 3). There were no significant differences in the mean percentages of embryos at the first cleavage stage or irregular embryos among the treatments in cross 1 (Figs. 3c, 4c; Table 3). In cross 2, in which the rate of fertilisation was extremely low in the nutrient treatments, a significantly higher mean percentage of embryos at the first cleavage stage occurred in the control compared with the nutrient treatments, while there were no significant differences in the mean percentage of irregular embryos among the treatments (Figs. 3c, 4c; Table 3).

Experiments with *Goniastrea aspera*

Exposure to elevated nutrient concentrations resulted in a slightly different pattern of fertilisation and development responses in *G. aspera* gametes compared with *A. longicyathus* gametes. Mean fertilisation rates were not significantly affected by elevated concentrations of ammonium or phosphate alone, but were significantly reduced by the highest concentration (50 μM) of ammonium plus phosphate in one cross (Fig. 5; Tables 4, 5, 6). However, the mean percentage of regular embryos decreased significantly, and the mean percentage of irregular and deformed embryos increased significantly, in most of the ammonium or phosphate treatments, and in all of the ammonium plus phosphate treatments (Figs. 6, 7; Tables 4, 5, 6).

Ammonium

There were no significant differences in the mean percentage fertilisation rates of *G. aspera* gametes among the four ammonium treatments and the controls, in either of the crosses (Fig. 5a; Table 4). However, in both crosses, the mean percentage of regular embryos was significantly higher in the controls, 0.5 and 1 μM ammonium treatments compared with the 50 μM ammonium treatment, and in the controls compared to the 5 μM ammonium treatment (Fig. 6a; Table 4).

Table 3 *Acropora longicyathus*. Comparison of fertilisation and embryo development responses of gametes exposed to background nutrient levels plus 0, 1, 10, or 100 μM ammonium plus 0, 1, 10, or 100 μM phosphate. Other details as for Table 1

Factor	Cross	*F* (*df*)	*P*	Pairwise multiple comparisons
Percent fertilisation	1	72.430 (3,16)	0.000	SNK: 0 > 1, 10, 100 μM ammonium + P
	2	59.111 (3,16)	0.000	*T*2: 0 > 1, 10, 100 μM ammonium + P
Percent regular embryos	1	145.670 (3,16)	0.000	SNK: 0 > 1, 10, 100 μM ammonium + P
	2 (tr)	69.902 (3,16)	0.000	*T*2: 0 > 1, 10, 100 μM ammonium + P
Percent first cleavage	1	1.380 (3,16)	0.285	SNK: nsd
	2 (tr)	3.773 (3,16)	0.032	SNK: 0 > 1, 10, 100 μM ammonium + P; 0, 1, 10 > 100 μM ammonium + P
Percent irregular embryos	1	1.259 (3,16)	0.322	SNK: nsd
	2	3.634 (3,16)	0.036	*T*2: nsd

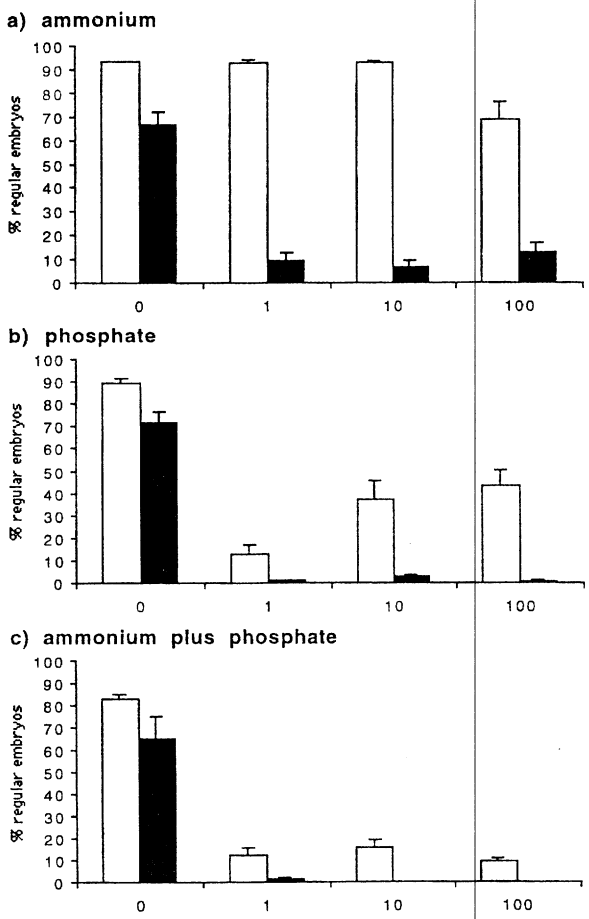

Fig. 2a–c *Acropora longicyathus*. Mean percentage of regular embryos in fertilisation trials using eggs and sperm of *A. longicyathus* at One Tree Island. Nutrient exposures, shading and error bars as for Fig. 1

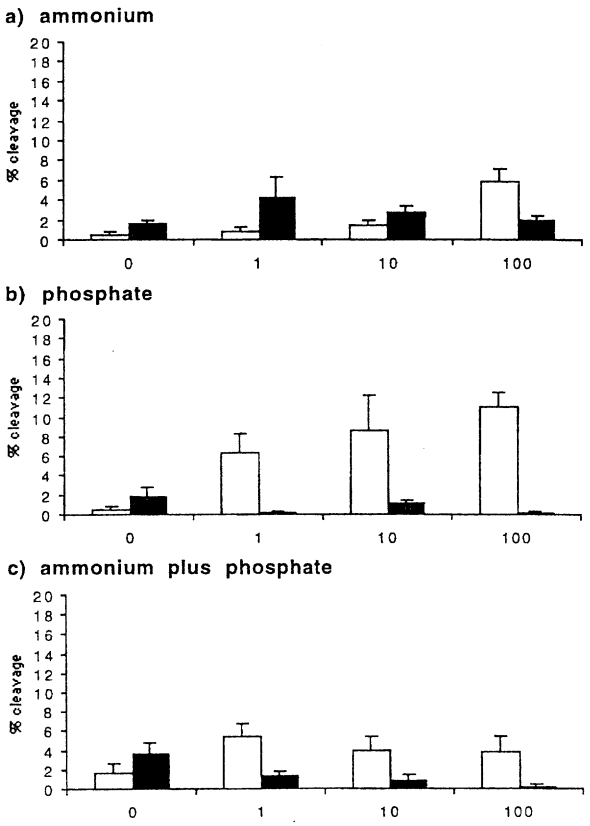

Fig. 3a–c *Acropora longicyathus*. Mean percentage of embryos that were at the first cleavage stage in fertilisation trials using eggs and sperm of *A. longicyathus* at One Tree Island. Nutrient exposures, shading and error bars as for Fig. 1

Conversely, the mean percentage of irregular deformed embryos formed was significantly higher in the 5 and 50 μM ammonium treatments, in both crosses, compared with the controls and lower ammonium concentration treatments (Fig. 7a; Table 4).

Phosphate

Responses of *G. aspera* gametes to phosphate treatments were similar to those in the ammonium experiment. The mean percentage fertilisation rates were not significantly affected by exposure to elevated concentrations of phosphate compared to the controls in either cross (Fig. 5b; Table 5). However, the development of embryos was significantly harmed by elevated phosphate concentrations. The mean percentage of regular embryos was significantly higher, and the mean percentage of irregular embryos was significantly lower, in the controls and 0.5 μM phosphate treatments compared with higher phosphate treatments in cross 1, and in the controls compared with all phosphate treatments in cross 2 (Figs. 6b, 7b; Table 5).

Ammonium plus phosphate

Mean fertilisation rates of *G. aspera* gametes were higher in the controls than in the nutrient treatments; however, mean percentage fertilisation was only significantly reduced in the 50 μM ammonium plus phosphate treatment compared with the control in cross 1 (Fig. 5c; Table 6). The mean percentage of regular embryos was significantly lower, and the mean percentage of irregular embryos was significantly higher, in all ammonium plus phosphate treatments in both crosses, compared with the controls (Figs. 6c, 7c; Table 6).

Discussion and conclusions

These are the first data on the effects of nutrient enrichment on fertilisation success of broadcast-spawning scleractinian reef corals. The results of these experiments demonstrate that slightly elevated concentrations of ammonium, phosphate and both nutrients combined can adversely affect or block fertilisation and embryo development processes in spawned gametes of the branching coral *Acropora longicyathus* and the faviid brain coral *Goniastrea aspera*. Although the overall trends in the experiments were similar, and showed

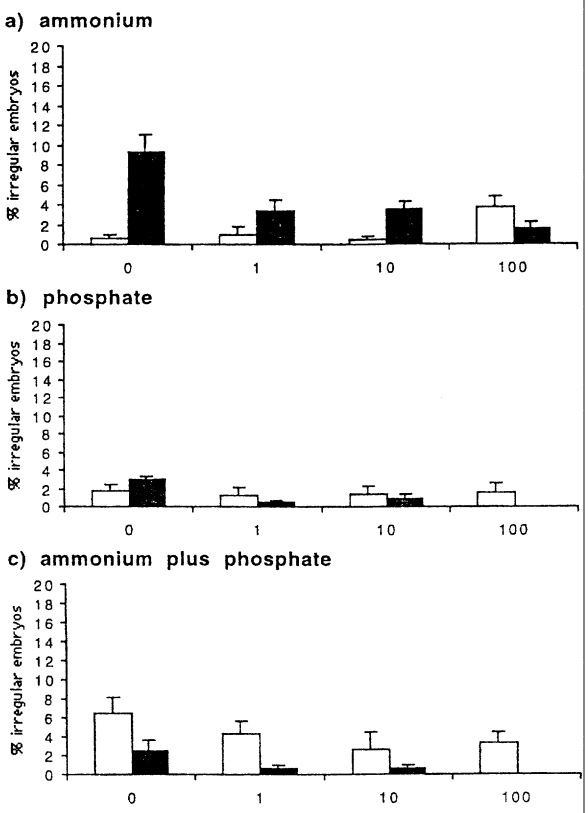

Fig. 4a–c *Acropora longicyathus*. Mean percentage of irregular embryos in fertilisation trials using eggs and sperm of *A. longicyathus* at One Tree Island. Nutrient exposures, shading and error bars as for Fig. 1

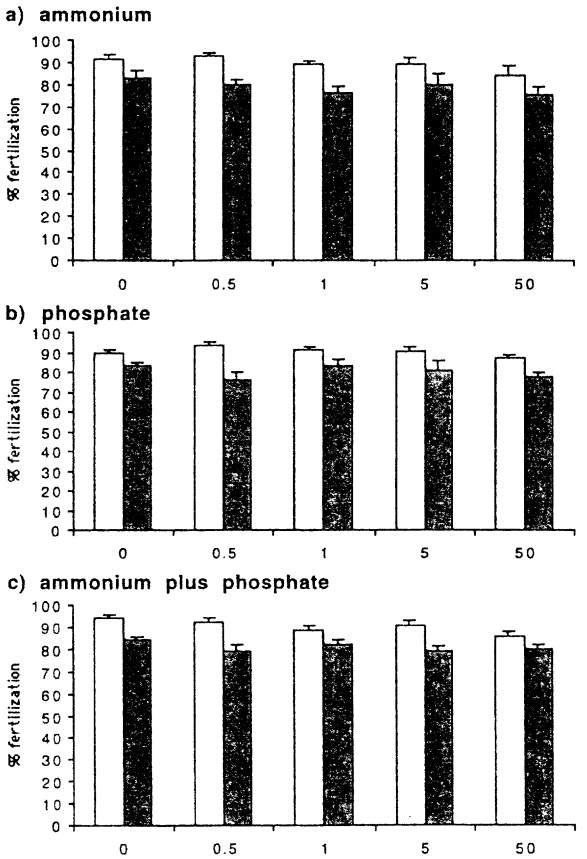

Fig. 5a–c *Goniastrea aspera*. Mean percentage fertilisation recorded in fertilisation trials using gametes of *G. aspera* at Magnetic Island. Gametes were exposed to: **a** ammonium, **b** phosphate, or **c** ammonium plus phosphate, at concentrations of background nutrient levels plus 0 (seawater control), 0.5, 1, 5, or 50 μM ammonium, phosphate, or ammonium plus phosphate. *Open columns* represent cross 1; *shaded columns* represent cross 2. Error bars are standard errors

reduced fertilisation success in response to elevated nutrient levels, the pattern of responses varied slightly between the two coral species, to some extent among the nutrient experiments, and in some cases between the two crosses (Figs. 1, 2, 5, 6).

In the experiments with *A. longicyathus* gametes, mean fertilisation rates were significantly reduced when gametes were exposed to elevated nutrient treatments compared to the fertilisation rates in the controls with normal seawater. In five of the six crosses, both the mean fertilisation rates and the mean percentage of regular embryos present were significantly reduced in the lowest (1 μM) nutrient treatment and all higher nutrient concentrations tested; in the other cross, mean fertilisation rates were significantly reduced in the 100 μM ammonium treatment, but not in the lower nutrient concentrations. The combination of ammonium plus phosphate enrichment caused the greatest reduction in fertilisation success in both crosses, while phosphate treatments had a greater deleterious effect on mean fertilisation success than ammonium treatments for each

Table 4 *Goniastrea aspera*. Comparison of fertilisation and embryo development responses of gametes exposed to background nutrient levels plus 0, 0.5, 1, 5, or 50 μM ammonium: the percentage fertilisation of eggs, the percentage of regular embryos present, and the percentage of irregular embryos present. One-way analyses of variance and Student–Newman–Keuls' (*SNK*) tests were used (*nsd* no significant differences among treatments; *tr* data were arcsine transformed due to heterogeneity of variances)

Factor	Cross	*F* (*df*)	*P*	SNK multiple comparisons
Percent fertilisation	1 (tr)	1.228 (4,20)	0.331	nsd
	2	0.839 (4,20)	0.517	nsd
Percent regular embryos	1	23.780 (4,20)	< 0.001	0, 0.5, 1 > 50 μM ammonium; 0 > 5 μM ammonium
	2	7.003 (4,20)	0.001	0, 0.5, 1 > 50 μM ammonium; 0 > 5 μM ammonium
Percent irregular embryos	1 (tr)	31.636 (4,20)	< 0.001	50, 5 > 1, 0.5, 0 μM ammonium
	2	3.889	0.001	50 > 1, 0.5, 0 μM ammonium; 5 > 0 μM ammonium