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

Abstract Spawmed 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 \( \mu \text{M} \) and above in one cross (60–64% reduction), and at 100 \( \mu \text{M} \) 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 \( \mu \text{M} \) phosphate and above (35–75% reduction), and at 1 \( \mu \text{M} \) ammonium plus 1 \( \mu \text{M} \) 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 \( \mu \text{M} \) 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.

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...
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-spaying 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 stressors 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 phosphate (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