

Table 5 *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 phosphate. Other details as for Table 4

Factor	Cross	<i>F</i> (<i>df</i>)	<i>P</i>	SNK multiple comparisons
Percent fertilisation	1	1.953 (4,20)	0.141	nsd
	2	1.105 (4,20)	0.382	nsd
Percent regular embryos	1 (tr)	11.600 (4,20)	<0.001	0, 0.5 > 1, 5, 50 μM phosphate
	2	8.044 (4,20)	<0.001	0 > 0.5, 1, 5, 50 μM phosphate
Percent irregular embryos	1	7.311 (4,20)	0.001	0, 0.5 < 1, 5, 50 μM phosphate
	2	7.355 (4,20)	0.001	0 < 0.5, 1, 5, 50 μM phosphate

cross (Figs. 1, 2). As eutrophication usually involves increased loadings of both nitrogen and phosphorus, the results of the combined nutrient treatments are probably most relevant to predicting the effects of eutrophication on coral fertilisation success on reefs.

Mean fertilisation rates and the mean percentage of regular *A. longicyathus* embryos were higher in cross 1 compared with cross 2 in all three nutrient experiments (Figs. 1, 2). The lower fertilisation rates in the cross 2 controls compared with cross 1 controls suggests that sperm motility or the density of viable sperm, or sperm compatibility with eggs from colony 1, were reduced in sperm from colony 3 (used in cross 2) compared with sperm from colony 2 (used in cross 1). Similar differences in sperm viability or gamete compatibility between different coral colonies have been observed in other studies of fertilisation in reef corals (Willis et al. 1997; R. Miller, personal communication). Furthermore, fertilisation rates in broadcast-spawning *Acropora* spp. corals are relatively sensitive to changes in viable sperm concentrations, and fertilisation rates decrease rapidly below sperm concentrations of 10^5 sperm ml^{-1} (Willis et al. 1997; Harrison, unpublished data). This may explain why the reduction in fertilisation success in cross 2 compared with cross 1 was generally more pronounced in the nutrient treatments than in the controls. If the initial density of viable sperm was lower in cross 2 compared with cross 1, as indicated by the results in the controls, and nutrient enrichment impaired sperm activity or fertilisation processes, as indicated by the reduced fertilisation success in the nutrient treatments, a proportionally greater reduction in fertilisation success would be expected in response to nutrients in crosses having lower concentrations of viable sperm.

Relatively low and variable numbers of embryos at the first cleavage stage and irregular or deformed embryos occurred in the experiments with *A. longicyathus*

gametes (Figs. 3, 4). Embryo development in broadcast-spawning corals is usually fairly synchronous (Harrison and Wallace 1990; Harrison, unpublished data), and most of the embryos in the controls and lower nutrient concentration treatments were at an advanced stage of development. Therefore, the embryos at the first cleavage stage had probably stopped development some time prior to the end of the experiments, and would not have developed further. Most of the irregular embryos recorded in the controls and low nutrient concentration treatments were slightly irregular in form and may have developed normally, whereas the irregular embryos in the higher nutrient concentrations tended to be more obviously deformed. It is likely that the majority of these deformed and first cleavage stage embryos were not going to develop into normal viable coral planula larvae; hence, these embryos probably represent failed reproduction, despite being fertilised and initiating development.

A slightly different pattern of response to nutrient enrichment occurred in the experiments with *Goniastrea aspera* gametes. Mean fertilisation rates were generally lower in the elevated nutrient treatments compared with controls, but were only significantly reduced in one cross at the highest (50 μM) ammonium plus phosphate dose tested, and very few embryos at the first cleavage stage were present (Fig. 5). However, significant decreases in the mean percentage of regular embryos and significant increases in the mean percentage of irregular embryos occurred at low nutrient levels in all three experiments, at or above 0.5–1 μM nutrient concentrations (Figs. 6, 7). It seems likely that this increased incidence of irregular embryos in *G. aspera* would be as significant as the reduction in fertilisation rates in *A. longicyathus*, because most of these irregular embryos were seriously deformed and would therefore be unlikely to develop into viable planulae. Gulko (1995) found that exposure

Table 6 *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 plus 0, 0.5, 1, 5, or 50 μM phosphate. Other details as for Table 4

Factor	Cross	<i>F</i> (<i>df</i>)	<i>P</i>	SNK multiple comparisons
Percent fertilisation	1	3.238 (4,20)	0.034	0 > 50 μM ammonium + P
	2	0.898 (4,20)	0.484	nsd
Percent regular embryos	1	17.971 (4,20)	<0.001	0 > 0.5, 1, 5, 50 μM ammonium + P; 0.5 > 1, 5, 50 μM ammonium + P
	2	13.008 (4,20)	<0.001	0 > 0.5, 1, 5, 50 μM ammonium + P
Percent irregular embryos	1	16.648 (4,20)	<0.001	0 < 0.5, 1, 5, 50 μM ammonium + P; 0.5 < 1, 5, 50 μM ammonium + P
	2	18.765 (4,20)	<0.001	0 < 0.5, 1, 5, 50 μM ammonium + P

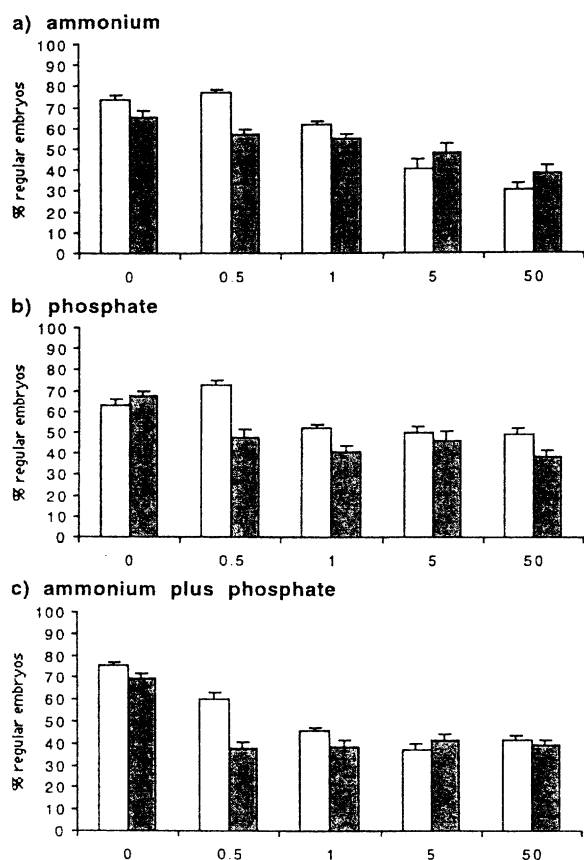


Fig. 6a-c *Goniastrea aspera*. Mean percentage of regular embryos in fertilisation trials using eggs and sperm of *G. aspera* at Magnetic Island. Nutrient exposures, shading and error bars as for Fig. 5

of *Montipora verrucosa* and *Fungia scutaria* sperm to UV-B radiation caused malformation of the resultant planulae, and a significant decrease in viable planulae.

Thus, in *A. longicyathus* and *G. aspera*, relatively small increases in the concentration of ammonium, phosphate, or both nutrients combined resulted in very significant decreases in reproductive success; fertilisation was more obviously inhibited in *A. longicyathus* gametes, whereas embryo development was disrupted in *G. aspera*. Overall, the impact of slightly increased nutrient concentrations was severe in both coral species, and resulted in significant reproductive failure. The concentrations at which fertilisation rates or embryo development were impaired (mostly at, or below, 0.5 or 1.0 μM ammonium and/or phosphate) are within, or below, the range of nutrient concentrations reported from eutrophic reefs in Hawaii (Smith et al. 1981; Kinsey 1988), Belize (Lapointe et al. 1992), Jamaica (Lapointe et al. 1997), Barbados (Tomascik and Sander 1985; Wittenberg and Hunte 1992) and Reunion Island (Naim 1993). Therefore, these experimental results are based on ecologically meaningful values, and indicate that relatively small increases in nutrient concentrations are likely to significantly impair coral reproductive success in nature.

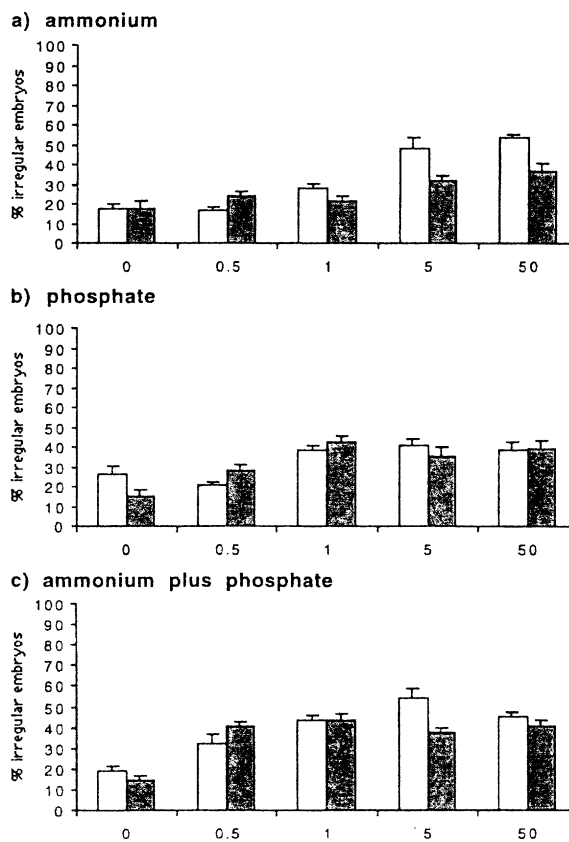


Fig. 7a-c *Goniastrea aspera*. Mean percentage of irregular embryos in fertilisation trials using eggs and sperm of *G. aspera* at Magnetic Island. Nutrient exposures, shading and error bars as for Fig. 5

It is possible that the difference in responses of gametes and embryos of these two species reflects a difference in the tolerance to eutrophication stress by these different coral taxa, as the methods used for the fertilisation trials were the same in both sets of experiments. Previous fertilisation experiments using these methods have also shown that fertilisation rates of acroporid coral gametes were more severely affected than faviid gametes by oil pollutants (Harrison 1994, 1999, unpublished data). Therefore, it is possible that faviid gametes are more resistant to these types of pollutant stresses than acroporid gametes. Faviidae coral colonies are usually more stress tolerant than acroporids (Harrison, personal observations); hence, the observed differences may reflect different systematic trends with respect to stress tolerance. Alternatively, the corals used in these experiments may have been adapted to different nutrient regimes, as inshore reefs on the GBR, such as reefs at Magnetic Island, are typically exposed to higher nutrient loadings than offshore reefs, such as One Tree Reef (e.g. Bell 1992; Furnas et al. 1997; Koop et al. 2001). In addition, the fertilisation experiments were run in different years, and with slightly different nutrient concentration ranges. Therefore, further comparative studies of acroporid and faviid species are needed to determine

whether any systematic or cross-shelf patterns occur in fertilisation responses to nutrient pollution.

A number of other recent studies have shown that fertilisation in broadcast-spawning coral gametes is highly sensitive to water quality and pollutants (reviewed in Harrison and Jamieson 1999). Low concentrations of oil hydrocarbons and dispersants have been shown to reduce or block fertilisation in *Acropora tenuis* gametes (Harrison 1993, 1994, 1999, unpublished data). Fertilisation success of *Goniastrea aspera* gametes was significantly reduced to 41% mean fertilisation by exposure to $20 \mu\text{g l}^{-1}$ copper, and fertilisation was almost completely blocked at $200 \mu\text{g l}^{-1}$ copper (Reichelt-Brushett and Harrison 1998). Heyward (1988) found that fertilisation rates of two faviid coral species were greatly reduced or blocked by exposure to $100\text{--}500 \mu\text{g l}^{-1}$ copper and $1000 \mu\text{g l}^{-1}$ zinc. Aceret et al. (1995) examined the effects of diterpenes derived from the soft coral *Sinularia flexibilis* on the eggs and sperm of the corals *Acropora tenuis* and *Montipora digitata*. Exposure of coral gametes to 5 ppm diterpene resulted in reduced fertilisation rates, sperm ceased swimming after 1 h exposure, and eggs lost cellular integrity and burst a few hours after treatment. Stormwater runoff has also been shown to inhibit or reduce fertilisation in broadcast-spawning reef corals (Richmond 1993).

There are apparently no other published studies on the effects of nutrients alone on fertilisation processes in other marine invertebrates. However, fertilisation rates in sea urchins have been used as a bioassay to compare water quality in polluted and unpolluted areas. Fertilisation trials using exposure of sperm of the sea urchin *Arbacia spatuligera* to serially diluted effluent and seawater were used to compare two polluted areas with two pristine sites (Zuniga et al. 1995). The two polluted sites received municipal wastes and effluents from chemical, steel and fishmeal facilities, and significant reductions in fertilisation rates were found at both polluted sites.

Fertilisation is a process fraught with difficulty in broadcast-spawning corals. Gametes are rapidly diluted following spawning of corals (Oliver and Babcock 1992), or other invertebrates (Pennington 1985), and gamete dilution can therefore play a major role in limiting reproductive success in marine invertebrates (Leviton and Sewell 1992). Predation on spawned egg and sperm bundles may be a significant selective pressure maintaining synchronous mass coral spawning on the Great Barrier Reef (Harrison et al. 1984; Harrison and Wallace 1990). Mass coral spawning events also result in enormous quantities of gametes from participating coral species being mixed with gametes from other species, families and even phyla, resulting in increased potential for hybridisation (Willis et al. 1997) and potential problems with gamete interaction for fertilisation (Harrison et al. 1984). For broadcast-spawning reef corals, any additional reduction in fertilisation success due to pollutants, such as nutrients, could result in very low numbers of viable larvae developing. Consequently, eutrophication could significantly reduce the number of

potential larval recruits that are essential for maintaining coral communities and for colonising new reef areas (Harrison and Wallace 1990). Furthermore, recent studies have shown that slightly elevated nutrient levels significantly affect the fecundity of reef corals (Tomascik and Sander 1987b; Ward and Harrison 2000) and significantly reduce larval settlement rates (Ward and Harrison 1997, unpublished data) and recruitment on eutrophic reefs (Tomascik 1991; Hunte and Wittenberg 1992). Therefore, eutrophication has the potential to impair or inhibit all of the major sexual reproductive processes in scleractinian reef corals.

The results of this study provoke some interesting questions for further research. Some differences were observed in the fertilisation responses of gametes from different *A. longicyathus* colonies to elevated nutrients, and similar variation in fertilisation success has been observed in hybridisation studies involving reciprocal crosses of eggs and sperm from different coral colonies (Willis et al. 1997). Therefore, further experiments using reciprocal crosses of gametes from more colonies of a range of acroporid, faviid and other dominant coral taxa are needed to quantify the effects of nutrients on coral fertilisation success in more detail, and to determine whether or not the patterns found in this study are typical of scleractinians in general. In studies on fertilisation rates in sea urchins, sperm are usually exposed to the potential stressor or pollutant (Zuniga et al. 1995), and Gulko (1995) found that sperm of the coral *Fungia scutaria* were more sensitive to UV damage than the eggs. To determine whether either the sperm or the eggs are more sensitive to nutrients, and to help elucidate the mechanism for reduced fertilisation in response to nutrient enrichment, fertilisation trials could be run in which only the eggs or the sperm are initially exposed to the nutrients. It would also be informative to examine a range of lower concentrations of nutrients on fertilisation success in reef corals, to better define the tolerance thresholds for nutrient enrichment on coral reefs.

The results of the present study demonstrate that relatively small increases in nutrient levels can significantly reduce fertilisation rates and successful embryo development in two species of reef corals. The concentrations at which deleterious effects on fertilisation success occurred were generally very low, and only slightly above background nutrient levels on coral reefs. This has important implications for the management of coral communities and water quality in coral reef areas. There is now substantial experimental evidence which demonstrates that nutrient enrichment has a deleterious effect on many critically important aspects of sexual reproduction in scleractinian reef corals. Slight nutrient enrichment significantly alters coral gametogenic patterns, reduces fertilisation success and impairs early embryo development, and reduces or inhibits coral larval settlement and recruitment. Therefore, nutrient pollution poses a substantial threat to the ecology of coral reef ecosystems, not only to existing coral communities, but also to coral reproductive processes. Chronic nutrient

pollution is likely to cause reproductive failure in at least some corals, thereby impairing or inhibiting recovery of coral reefs impacted by human disturbance or natural perturbations. Impaired reproductive success and larval recruitment probably explain the decline or deterioration observed in many coral communities that are impacted by sublethal levels of eutrophication. Together, these results clearly show that in order to maintain healthy coral reef ecosystems, nutrients will have to be managed carefully, not only to avoid gross eutrophic responses and phase-shifts in reef communities, but also in order to maintain the critically important reproductive and recruitment processes that renew reef coral populations.

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References

- Aceret TL, Sammarco PW, Coll JC (1995) Effects of terpenes derived from the soft coral *Simularia flexibilis* on the eggs, sperm and embryos of the scleractinian corals *Montipora digitata* and *Acropora tenuis*. *Mar Biol* 122:317–323
- Bell PRF (1992) Eutrophication and coral reefs – some examples from the Great Barrier Reef lagoon. *Water Res* 26:553–568
- D'Elia CF, Wiebe WJ (1990) Biogeochemical nutrient cycles in coral reef ecosystems. In: Dubinsky Z (ed) *Coral reef ecosystems: ecosystems of the World*, vol 25. Elsevier, Amsterdam, pp 49–74
- Furnas M, Mitchell A, Skuza M (1997) Shelf-scale nitrogen and phosphorus budgets for the Central Great Barrier Reef (16–19°). In: Lessios HA, MacIntyre IG (eds) *Proc 8th Int Coral Reef Symp*, vol 1. Smithsonian Tropical Research Institute, Balboa, Panama, pp 809–814
- Green AL, Birkeland RH, Randall RH, Smith BD, Wilkins S (1997) 78 years of coral reef degradation in Pago Pago Harbour: a quantitative record. In: Lessios HA, MacIntyre IG (eds) *Proc 8th Int Coral Reef Symp*, vol 2. Smithsonian Tropical Research Institute, Balboa, Panama, pp 1883–1888
- Grigg RW (1995) Coral reefs in an urban embayment in Hawaii: a complex case history controlled by natural and anthropogenic stress. *Coral Reefs* 14:253–266
- Grigg RW, Dollar SJ (1990) Natural and anthropogenic disturbance on coral reefs. In: Dubinsky Z (ed) *Coral reef ecosystems: ecosystems of the World*, vol 25. Elsevier, Amsterdam, pp 439–452
- Gulko DA (1995) Effects of ultraviolet radiation on fertilization and the production of planula larvae in the Hawaiian coral *Fungia scutaria*. In: Gulko D, Jokiel PL (eds) *Ultraviolet radiation and coral reefs*, HIMB technical report 41, University of Hawaii, Honolulu, pp 135–147
- Harrison PL (1993) Coral spawning on the Great Barrier Reef. *Search (Syd)* 24:45–48
- Harrison PL (1994) The effects of oil pollutants on fertilization rates in the scleractinian coral *Acropora tenuis*. Australian Coral Reef Society, Townsville
- Harrison PL (1995) Status of the coral reefs of Kuwait. Final report to the United Nations Industrial Development Organization and the United Nations Development Programme. United Nations Industrial Development Organization, Vienna
- Harrison PL (1999) Oil pollutants inhibit fertilization and larval settlement in the scleractinian reef coral *Acropora tenuis* from the Great Barrier Reef, Australia. Sources, fates and consequences of pollutants in the Great Barrier Reef and Torres Strait. Workshop series no. 25, Great Barrier Reef Marine Park Authority, Townsville, pp 8–9
- Harrison PL, Jamieson BGM (1999) Cnidaria and Ctenophora. In: Jamieson BGM (ed) *Reproductive biology of the invertebrates*, vol IX, part A. Progress in male gamete biology. IBH, Oxford, pp 21–95
- Harrison PL, Wallace CC (1990) Reproduction, dispersal and recruitment of scleractinian corals. In: Dubinsky Z (ed) *Coral reef ecosystems: ecosystems of the World*, vol 25. Elsevier, Amsterdam, pp 133–207
- Harrison PL, Babcock RC, Bull GD, Oliver JK, Wallace CC, Willis BL (1984) Mass spawning in tropical reef corals. *Science* 223:1186–1189
- Heyward AJ (1988) Inhibitory effects of copper and zinc sulphates on fertilization in corals. In: Choat JH, et al (eds) *Proc 6th Int Coral Reef Symp*, vol 2. Symposium Executive Committee, Townsville, pp 299–303
- Hunte W, Wittenberg M (1992) Effects of eutrophication and sedimentation on juvenile corals. II. Settlement. *Mar Biol* 114:625–631
- Kinsey DW (1988) Responses of coral reef systems to elevated nutrient levels. In: Baldwin CL (ed) *Nutrients in the Great Barrier Reef region*. Great Barrier Reef Marine Park Authority, Townsville, pp 55–65
- Kinsey DW, Davies PJ (1979) Effects of elevated nitrogen and phosphorus on coral reef growth. *Limnol Oceanogr* 24:935–940
- Kobayashi N (1980) Comparative sensitivity of various developmental stages of sea urchins to some chemicals. *Mar Biol* 58:163–171
- Koop K, Booth D, Broadbent A, Brodie J, Bucher D, Capone D, Coll J, Dennison W, Erdmann M, Harrison PL, Hoegh-Guldberg O, Hutchings P, Jones GB, Larkum AWD, O'Neil J, Steven A, Tentori E, Ward S, Williamson J, Yellowlees D (2001) ENCORE: the effect of nutrient enrichment on coral reefs. Synthesis of results and conclusions. *Mar Pollut Bull* 42:91–120
- Lapointe BE, Littler MM, Littler DS (1992) Modification of benthic community structure by natural eutrophication: the Belize Barrier Reef. In: Richmond RH (ed) *Proc 7th Int Coral Reef Symp*, vol 1. University of Guam, Mangilao, pp 323–334
- Lapointe BE, Littler MM, Littler DS (1997) Macroalgal overgrowth of fringing coral reefs at Discovery Bay, Jamaica: bottom-up versus top-down control. In: Lessios HA, MacIntyre IG (eds) *Proc 8th Int Coral Reef Symp*, vol 1. Smithsonian Tropical Research Institute, Balboa, Panama, pp 927–932
- Levitian DR, Sewell M (1992) How distribution and abundance influence fertilization success in the sea urchin *Strongylocentrotus franciscanus*. *Ecology* 73:248–254
- Littler MM, Littler DS, Lapointe BE (1992) Modification of tropical reef community structure due to cultural eutrophication: the southwest coast of Martinique. In: Richmond RH (ed) *Proc 7th Int Coral Reef Symp*, vol 1. University of Guam, Mangilao, pp 335–343
- Maragos JE, Evans C, Holthus P (1985) Reef corals in Kaneohe Bay six years before and after termination of sewage discharges (Oahu, Hawaiian Archipelago). In: Gabri e C, et al (eds) *Proc 5th Int Coral Reef Congr*, vol 4. Antenne Museum – EPHE, Moorea, French Polynesia, pp 189–194
- McCook LJ (1999) Macroalgae, nutrients and phase shifts on coral reefs: scientific issues and management consequences for the Great Barrier Reef. *Coral Reefs* 18:357–367
- Naim O (1993) Seasonal responses of a fringing reef community to eutrophication (Reunion Island, western Indian Ocean). *Mar Ecol Prog Ser* 99:137–151
- Oliver JK, Babcock RC (1992) Aspects of the fertilization ecology of broadcast spawning corals: sperm dilution effects and in situ measurements of fertilization. *Biol Bull (Woods Hole)* 183:409–417

- Pastorok RA, Bilyard GR (1985) Effects of sewage pollution on coral reef communities. *Mar Ecol Prog Ser* 21:175-189
- Pennington JT (1985) The ecology of fertilization of echinoid eggs: the consequences of sperm dilution, adult aggregation and synchronous spawning. *Biol Bull (Woods Hole)* 169:417-430
- Reichelt-Brushett AJ, Harrison PL (1998) The effect of copper, zinc and cadmium on fertilization success of gametes from the scleractinian reef coral *Goniastrea aspera*. *Mar Pollut Bull* 38:182-187
- Richmond RH (1993) Effects of coastal runoff on coral reproduction. In: Global aspects of coral reefs, health, hazards and history. University of Miami, Miami, pp 360-364
- Schaffelke B, Klumpp DW (1998) Short-term nutrient pulses enhance growth and photosynthesis of the coral reef macroalga *Sargassum baccularia*. *Mar Ecol Prog Ser* 170:95-105
- Smith SV, Kimmerer J, Laws EA, Brock RE, Walsh TW (1981) Kaneohe Bay sewage diversion experiment: perspectives on ecosystem responses to nutritional perturbation. *Pac Sci* 35:279-402
- Stambler N, Popper N, Dubinsky Z, Stimson J (1991) Effects of nutrient enrichment and water motion on the coral *Pocillopora damicornis*. *Pac Sci* 45:299-307
- Tomascik T (1991) Settlement patterns of Caribbean scleractinian corals on artificial substrata along a eutrophication gradient, Barbados, West Indies. *Mar Ecol Prog Ser* 77:261-269
- Tomascik T, Sander F (1985) Effects of eutrophication on reef-building corals. I. Growth rate of the reef-building coral *Montastrea annularis*. *Mar Biol* 87:143-155
- Tomascik T, Sander F (1987a) Effects of eutrophication on reef-building corals. II. Structure of scleractinian coral communities on fringing reefs, Barbados, West Indies. *Mar Biol* 94:53-75
- Tomascik T, Sander F (1987b) Effects of eutrophication on reef building corals. III. Reproduction of the reef building coral *Porites porites*. *Mar Biol* 94:77-94
- Tomascik T, Suharsono, Mah AJ (1993) Case histories: a historical perspective of the natural and anthropogenic impacts in the Indonesian Archipelago with a focus on the Kepulauan Sea, Java Sea. In: Global aspects of coral reefs, health, hazards and history. University of Miami, Miami, pp 304-310
- Walker DI, Ormond RFG (1982) Coral death from sewage and phosphate pollution at Aqaba, Red Sea. *Mar Pollut Bull* 13:21-25
- Ward S, Harrison PL (1997) The effects of elevated nutrient levels on settlement of coral larvae during the ENCORE experiment, Great Barrier Reef, Australia. In: Lessios HA, MacIntyre IG (eds) Proc 8th Int Coral Reef Symp, vol 1. Smithsonian Tropical Research Institute, Balboa, Panama, pp 891-896
- Ward S, Harrison PL (2000) Changes in gametogenesis and fecundity of acroporid corals that were exposed to elevated nitrogen and phosphorus during the ENCORE experiment. *J Exp Mar Biol Ecol* 246:179-221
- Willis BL, Babcock RC, Harrison PL, Wallace CC (1997) Hybridization among mass spawning reef corals. *Coral Reefs* 16[Suppl]:53-65
- Wittenberg M, Hunte W (1992) Effects of eutrophication and sedimentation on juvenile corals. I. Abundance, mortality and community structure. *Mar Biol* 112:131-138
- Zuniga M, Roa R, Larain A (1995) Sperm cell bioassays with the sea urchin *Arbacia spatuligera* on samples from two polluted Chilean coastal sites. *Mar Pollut Bull* 30:313-319