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Nutrient enrichment caused by in situ fish farms at Eilat, Red Sea is detrimental to coral reproduction

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Abstract

Recent studies report conflicting results concerning the effects of eutrophication on coral reproduction. The present study examines reproductive effort in the brooding coral *Stylophora pistillata* exposed to chronic eutrophication caused by in situ fish cages (FC) in the northern Gulf of Eilat (Aqaba). Histological studies of 20 *S. pistillata* colonies transplanted to each of two study sites, one close to the nutrient enriched FC site and the other at a reference site (IUI), 8 km southwest of the FC site, show that, overall, corals from the FC site have a significantly higher percentage of polyps containing oocytes and testes than corals from the IUI site. However, average oocyte size and the percentage of oocytes reaching the size at which fertilization occurs (i.e., >200 μm) were both significantly greater in colonies at the IUI site compared to the FC site. As the reproductive season progressed, colonies at the IUI site exhibited a decrease in the percentage of polyps containing oocytes, concomitant with an increase in the number of polyps containing planulae, indicating successful development of oocytes into planulae. In contrast, in colonies at the FC site oocyte numbers were greatest at the end of the reproductive season, and overall, numbers of planulae were significantly lower compared with the IUI colonies, suggesting relative failure of oocyte maturation, fertilization and ensuing larval development. The significantly higher lipid content found during the reproduction season in IUI colonies compared with FC colonies corroborates this assertion. This data strongly suggest that nutrients released from the fish farms have adverse effects on successful production of larvae of *S. pistillata*. In view of the recent severe deterioration of the coral reefs of Eilat and their present critical state of health, the only chance for their renewal is the use of immediate, prudent and rational protection measures against all man-made perturbations.

Keywords: *Stylophora pistillata*; Eutrophication; Fish farms; Reproductive effort; Red Sea

1. Introduction

Coral reefs generally thrive in tropical seas characterized by oligotrophic waters (D'Elia and Wiebe, 1990). For many reef types, clear, nutrient-poor waters are considered a prerequisite for the development of healthy coral communities. On a local scale, human economic exploitation and various types of pollution, mainly eutrophication and siltation have exposed many fringing and offshore reefs to severe stresses (Brown, 1997). Recent increases in eutrophication along coastal regions due to increased land-use, agricultural effluent introduced through river runoff and discharge of untreated sewage have been shown to negatively impact coral community structure (Walker and Ormond, 1982; Pastorok and Bilyad, 1985; Grigg and Dollar, 1990; Stambler et al., 1991; Dubinsky and Stambler, 1996; Loya, 2004).

Reefs that are exposed to chronic nutrient enrichment show an increase in primary productivity, mainly due to development of macro-algae (Smith et al., 1981; Hatcher et al., 1989; Bell, 1992; Done, 1992; Hughes, 1994; Lapointe, 1997). These macro-algae, which rapidly occupy the hard substrate, may often overgrow, smother and competitively exclude underlying corals (Smith et al., 1981; Genin et al., 1995) and markedly reduce

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coral recruitment (Richmond, 1997; Abelson et al., 2000). Laboratory and field experiments have shown that nutrient enrichment of reef building corals significantly suppressed their rates of calcification (Kinsey and Davies, 1979; Marubini and Davies, 1996; Ferrier-Pages et al., 2000). Furthermore, nutrient enrichment increases turbidity (i.e., deteriorates water quality), reduces light necessary for coral growth, and may trigger epizootics or shifts in dominance to deposit and filter feeders (Grigg, 1995; Harvell et al., 1999). The potential for synergisms among impacts related to increased turbidity, changes in salinity and the presence of heavy metals that often accompany eutrophication, means that the direct effects of nutrient enrichment on coral physiology are still unclear. Moreover, because eutrophication is also usually associated with a rise in suspended particulate matter, difficulties in distinguishing the effects of dissolved and particulate matter from nutrient enrichment on coral physiology further complicate interpretations (Bongiorni et al., 2003b). It is not surprising therefore that there have been conflicting outcomes from studies investigating the direct impacts of nutrient increases on coral biology (Tomascik and Sander, 1985; Stambler et al., 1991; Ferrier-Pages et al., 2001; Koop et al., 2001; Szmant, 2002).

Eutrophication has been reported to cause subtle physiological changes in parameters such as coral growth, skeletal tensile strength, reproduction and the coral's ability to withstand disease (Stambler et al., 1991; Ferrier-Pages et al., 2000; Bucher and Harrison, 2002; Cox and Ward, 2003; Bruno et al., 2003). However, coral species react differently to increased nutrient enrichment (e.g. Tomascik and Sander, 1987; Ward and Harrison, 2000; Harrison and Ward, 2001; Bongiorni et al., 2003a), making it difficult to generalize trends. The mixed outcomes of studies investigating the impacts of nutrient enrichment on corals have consequently become a focus of debate among coral biologists (e.g. Szmant, 2002). The generally sublethal and long term nature of eutrophication impacts has undoubtedly contributed to the complexity of identifying specific effects, particularly because such stresses may not directly and immediately alter large scale parameters of coral biodiversity or coral cover. However, accumulation of sublethal effects may eventually have an overall effect on the reef ecosystem. Indeed Edinger et al. (2000) point out that eutrophication may cause an uncoupling of coral colony growth and reef growth due to increased net erosion on polluted reefs. Lapointe (1997) concludes that eutrophication may gradually increase the benthic autotroph population relative to frame builders (trophic phase shift). Such a trophic phase shift may be reflected in the reef's metabolic performance i.e., an increase in community net production (the difference between gross production and respiration), an increase in the ratio of gross production to respiration and a decrease in calcification (Silverman et al., 2003). This change may be accompanied by higher grazing pressure leading to increased bioerosion (Hallock, 1988; McCook, 1999 and literature therein), thus upsetting the delicate balance between growth and erosion of the reef framework.

Maintenance and renewal of coral reef communities depends on successful reproduction of the major reef framework builders (Harrison and Wallace, 1990). A reduction in reproductive effort, larval survivorship, settlement and metamorphosis, or survivorship of young coral colonies will reduce the probability for reef renewal in both the short and long term. Assessments of reproductive parameters therefore provide a key for ascertaining the propensity of a reef for stability and its ability to recover following catastrophes. There have been differing and at times conflicting reports on the effects of nutrient loading on coral reproduction (Tomascik and Sander, 1987; Harrison and Ward, 2001; Cox and Ward, 2003; Bongiorni et al., 2003a). For example, Tomascik and Sander (1987) reported that in *Porites porites*, a brooding coral, colonies at more heavily eutrophied sites produced fewer larvae, showed decreased gonad index and a higher rate of hermaphroditism than colonies at clean sites. Cox and Ward (2003) found effects of elevated nitrate loading on planula production in the brooder *Pocillopora damicornis,* but not on the broadcasting species *Montipora capitata*. Harrison and Ward (2001) found differing impacts on fertilization success (depending on the combination of nutrients used in the experiments) in two broadcasting species of *Acropora*. The recent elevated nutrients on coral reefs experiments (ENCORE) carried out on the Great Barrier Reef, showed that a variety of reproductive characteristics in corals maintained under different high nutrient regimes were negatively impacted (Ward and Harrison, 1997, 2000; Harrison and Ward, 2001; Koop et al., 2001). Results ranged from decreased fertilization in the broadcasting corals *Acropora longicyathus* and *Goniastrea aspera* to increasing numbers of irregular embryos and/or a reduction in planula settlement in *A. longicyathus* (Ward and Harrison, 1997; Koop et al., 2001). Although it is clear that the effects of nutrient enrichment may vary with coral species, type of eutrophication, as well as with the natural environmental parameters to which the corals were adapted prior to experimentation, there is general agreement that eutrophication affects corals at some stage of their reproductive cycle.

In contrast, Bongiorni et al. (2003a) reported no deleterious effect of nutrient enrichment associated with net-pen mariculture on early stages of oogenesis of *Stylophora pistillata* in the northern Gulf of Eilat (Aqaba). However they failed to study the effects of nutrient enrichment on larval development. Their interpretations and conclusions have been questioned, but either overlooked or inadequately responded

(see Loya and Kramarsky-Winter, 2003; Rinkevich et al., 2003; and Discussion of this paper).

In recent years the reefs in the northern Gulf of Eilat have undergone severe deterioration in terms of coral cover and biodiversity (Ben-Tzvi et al., 2004; review in Loya, 2004). Concurrently, there has been a rise in nutrients in this area. In the past, eutrophication was due to untreated sewage outflow from the city of Eilat at the northwestern part of the Gulf of Eilat (Aqaba). The building of a sewage treatment plant (in 1995) and diversion of the sewage outflow have largely stopped eutrophication from urban sources. However, the recent deployment of intensive in situ net-pen mariculture facilities at the northern tip of the Gulf has "replaced" urban sewage as a source of nutrient enrichment. These facilities yield more than 2400 tons of fish annually (primarily gilthead sea bream *Sparus aurata*, a non-indigenous species in the Gulf of Eilat). They discharge both dissolved organic matter (240 tons of nitrates and 40 tons of phosphates; Gordin, 2000) and particulate matter, resulting in an increase in nitrates and phosphates in the Gulf as well as an increase in particulate matter in the water column. In order to ascertain the long-term effects of these effluents on coral populations in the northern Gulf of Eilat, an integrated study on coral reproduction and health is currently being carried out. One focus of this study is to ascertain the effects of the net-pen mariculture effluent on the reproduction of *S. pistillata* colonies transplanted to a site close to these facilities compared with colonies transplanted to a site 8 km away. Here we report the results of the first stage of this study, a two-year analysis comparing the reproductive output of colonies at the two sites.

2. Methods

2.1. Study sites

The fish cage site (FC site) was located on a sandy bottom at 19 m depth, 150 m west of the Ardag net-pen mariculture facility near the North Shore of the northern Gulf of Eilat (Aqaba) Red Sea (29° 30.23′ N, 34°55.14′ E). The facility is comprised of three parallel pontoons supporting fish cages yielding over 2400 tons of fish annually. The reference site had a similar sandy bottom devoid of reef structure and was located at the same depth (19 m) in front of the Interuniversity Institute (IUI), 8 km southwest of the FC site and adjacent to the Eilat Coral Nature Reserve (ECNR). The average monthly levels of nutrients in the FC vs. IUI sites during 2001 were 0.095 vs. 0.075 μM nitrite, 0.385 vs. 0.284 μM nitrate, 0.123 vs. 0.045 μM orthophosphate and 1.016 vs. 0.057 μM ammonium, respectively (I. David, B. Lazar, and A. Post unpublished data cited in Bongiorni et al., 2003a).

2.2. Experimental design

At the beginning of the gametogenic cycle (early December 2000), 40 mature and similarly sized *S. pistallata* colonies were collected from a depth of 10–15 m from a reef site midway between the impacted FC site and IUI reference site. They were randomly divided into two groups each containing 20 colonies, attached to tiles secured to the underside of plastic crates by underwater putty, and then deployed to either one of the sites (Fig. 1). Ten to 14 of the healthiest-looking corals (i.e. with full tissue integrity and no overgrowth of sponges or tunicates) were selected for gametogenic monitoring after a four-month conditioning period. Samples were collected in March and May of 2001 and March and June of 2002. At each sampling time, a single branch, 5–6 cm in length was sampled to ensure collection from gravid portions of colonies (Rinkevich and Loya, 1979a,b). To avoid possible planulae abortion due to the sampling procedure, the sampled branches were fixed in 4% seawater formalin immediately after collection. Prior to fixation each sampling container was checked for aborted planulae. To avoid repetitive damage to the colonies, 5–6 of the transplanted colonies were sampled alternately within each reproductive season.

The basal 1–2 cm portion of each fixed branch was processed for histological study according to the protocol Rinkevich and Loya (1979a,b) and the upper part of the branch was used for lipid extraction. Histological samples were sectioned serially (6 μm in thickness) and stained in haematoxylin/eosin. Serial cross sections from the oral, middle and aboral (close to the skeleton) regions of polyps, each containing approximately 50–60 polyps, were examined to determine the presence of gonads. The number, size and condition of gonads, as

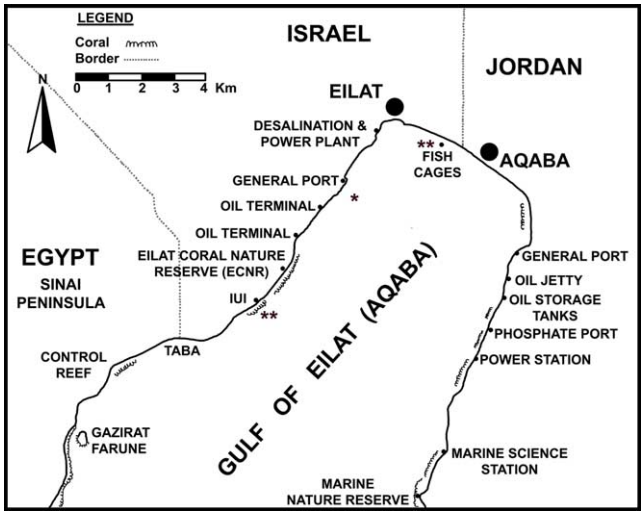

Fig. 1. Map of northern Gulf of Eilat (Aqaba). Single asterisk denotes the collection site. The fish cages (FC) site and Inter-university Institute (IUI) reference site are denoted by double asterisks.

well as the presence of planulae were recorded for 440–840 polyps per sampling time.

Lipid content of samples collected in May 2001 was determined by decalcifying fixed samples in formic acid sodium citrate solution (1:1 V/V), placing the tissue in embedding cassettes and drying in an oven for 24 h at 55 °C. Samples were then weighed to ±0.0001 g and placed in a solution of chloroform–methanol (2:1 V/V) for 24 h. The tissues were then re-dried (for 3 h) and reweighed. The percent lipid content in each sample was calculated as: $(\text{weight}_1 - \text{weight}_2)/\text{weight}_1 \times 100\%$.

2.3. Statistical analysis

All statistical analyses were carried out using Statistica 6.1. Normality and homogeneity of variance were tested using the Kolmorogov–Smirnov statistical test. Possible differences in reproductive effort between sites and sampling dates were tested using two-way analysis of variance with repeated measures followed by Fisher's least significant difference (LSD) test. When required, arcsine transformations were carried out. Data regarding lipids in coral tissues was analyzed using a non-parametric Kolmorogov–Smirnov test. Results are presented as averages ± standard deviations unless denoted otherwise.

3. Results

3.1. Reproductive synchrony

Histological studies showed that, in general, the percentage of transplanted colonies containing female gonads did not differ significantly between the FC and IUI sites over the two years of the study (Fisher Exact test $p > 0.05$, Table 1).

3.2. Testes and oocytes

Testes were abundant in transplanted *S. pistillata* colonies at both sites, although the overall percentage of polyps containing testes was significantly higher in 2001 than in 2002 (two-way ANOVA, $p < 0.05$, Fig. 2). There were significantly more polyps with testes in colonies at the FC site than at the IUI site in the first year (Fisher LSD post-hoc, $p < 0.05$, Fig. 2) but not in the second year (two-way ANOVA, $p > 0.05$). As the reproductive period progressed, the average number of testes per polyp decreased significantly in colonies at both sites in the first year (Fisher LSD post-hoc; $p < 0.05$, Fig. 2), but only at the IUI site in the second year (Fisher LSD post-hoc; $p < 0.05$; Fig. 2).

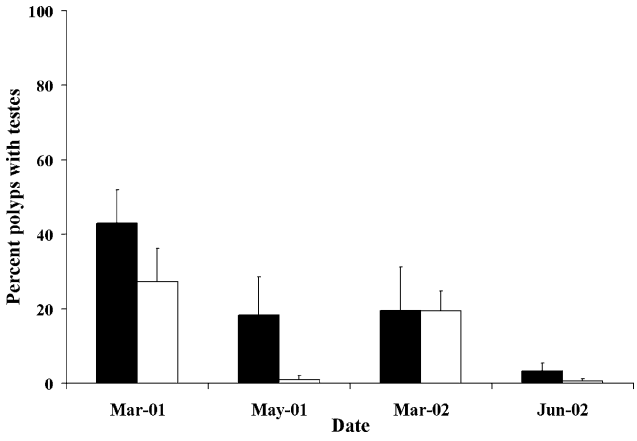

Fig. 2. Average percentage of polyps with testes (±SE) during 2001 and 2002 in colonies of *S. pistillata* transplanted to each of the experimental sites. Solid bars = colonies from the FC site; open bars = colonies from the IUI site.

The proportion of oocytes reaching a mature size (⩾ 200 μm) in transplanted *S. pistillata* colonies was significantly higher in the IUI site compared to the FC site, with <4% of oocytes reaching mature size at the FC site, compared to >13% of oocytes at the IUI site (ANOVA, $p < 0.05$). The average oocyte sizes recorded in the transplanted *S. pistillata* colonies at the two sites was significantly different (ANOVA, $p < 0.05$, Fig. 3a): the average oocyte size at the IUI site was 2.4 times greater than at the FC site in March 2001 (135 ± 55.8 μm at the IUI site compared to 50 ± 43.8 μm at the FC site) and 1.5 times greater in March 2002 (138 ± 54.7 μm at the IUI site compared to 89.7 ± 45.2 μm at the FC site; Fig. 3a). As oocytes were fertilized and progressed through embryogenesis, very few mature (>200 μm) oocytes remained in polyps of IUI colonies by May 2001

Table 1
Percentage of sterile (S), male (M) and hermaphroditic (H) colonies, of *S. pistillata* found for each sampling period at the two sites

Date	Fish cages (FC) site					IUI—reference site				
	N	*n*	S	M	H	*N*	*n*	S	M	H
March 2001	5	218	0	80	20	6	316	17	0	83
May 2001	5	209	40	0	60	6	230	17	16	67
March 2002	5	210	20	0	80	9	630	11	0	89
June 2002	5	283	20	0	80	5	294	20	0	80

Each set of colonies was sampled only once a year in order to prevent re-sampling of a colony that had been injured by the sampling procedure. *N* = No. of colonies sampled; *n* = number of polyps examined.

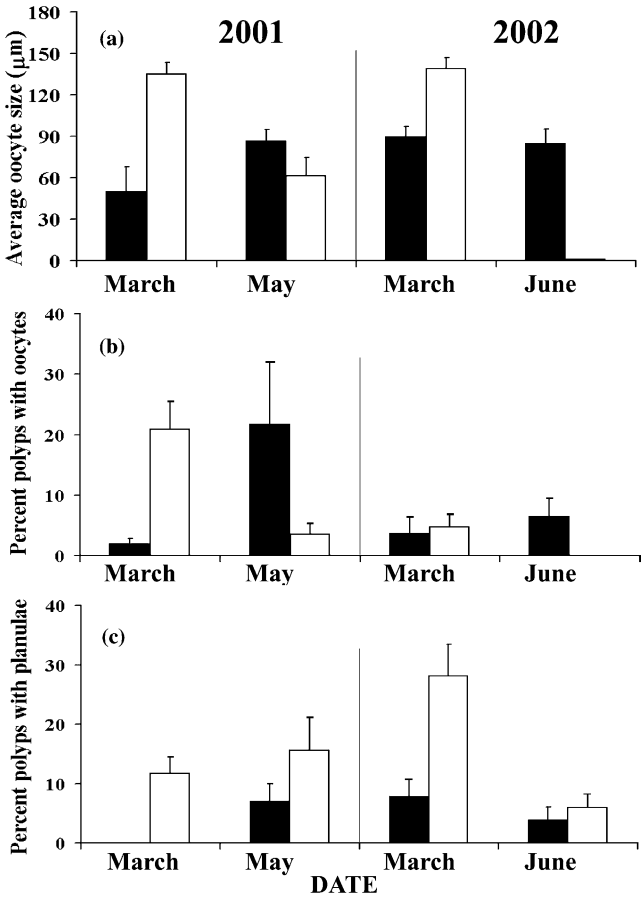

Fig. 3. Reproductive dynamics in colonies of *S. pistillata* transplanted to each of the experimental sites during 2001 and 2002. (a) Average oocyte size in polyps of *S. pistillata* colonies. (b) Average percentage of polyps containing oocytes (one or more). (c) Average percentage of polyps containing planulae. Solid bars = colonies from the FC site; open bars = colonies from the IUI site. Error bars denote standard errors.

(Fig. 3a and b). In 2002, no oocytes remained in polyps at the IUI site by June and only a few small oocytes remained in colonies at the FC site (see Fig. 3a and b). Towards the end of the reproduction cycle (May 2001 and June 2002), average oocyte size at the IUI site was significantly lower compared to oocyte size in March of both years (Fisher LSD post-hoc; $p < 0.05$, Fig. 3a and b). In contrast, there was an increase in average oocyte size between March and May at the FC site in 2001, but very few oocytes reached the mature size of ≈200 μm. During 2002, average oocyte size in colonies from the FC site did not change much with the progression of the reproductive season remaining at ≈90 μm (Fig. 3a) and the few remaining oocytes did not reach mature size (Fig. 3b).

Overall, corals from the FC site had a significantly higher percentage of polyps containing oocytes than corals from the IUI site (two-way ANOVA; $p < 0.05$ Fig. 3b). In March 2001 (four months after transplantation), the percentage of polyps with oocytes in colonies at the FC site was significantly lower than at the IUI site (Fisher LSD post-hoc; $p < 0.05$, Fig. 3b). In May 2001 (six months after transplantation) this trend was reversed and there were significantly more polyps with oocytes in colonies at the FC site than at the IUI site (Fisher LSD post-hoc; $p < 0.05$). In March 2002 (the consecutive reproductive cycle), although there were more polyps with oocytes at the IUI site, the differences between the two sites were not significant (Fisher LSD post-hoc; $p > 0.05$). In June 2002, no polyps containing oocytes were found at the IUI site (Fig. 3b), while at the FC site there were still few polyps with oocytes. No significant difference was found between the proportion of polyps with oocytes in June 2002 and March 2002 in the FC site (Fisher LSD post-hoc; $p > 0.05$; Fig. 3b). In general, as the reproductive season advanced, fewer polyps contained oocytes in IUI site colonies, while the opposite trend was evident in colonies at the FC site (Fig. 3b).

3.3. Planulae

Significantly more planulae were found in tissues of IUI colonies of *S. pistillata* than in FC colonies in March of both years (Fisher LSD post-hoc; $p < 0.05$ Fig. 3c). From March to May 2001 (peak planulation months for *S. pistillata*; see Rinkevich and Loya, 1979b), there was an increase in the number of polyps containing planulae at both sites, but these increases were not significant (Fisher LSD post-hoc; $p > 0.05$; Fig. 3c). Towards the end of the reproductive season in 2002, there was a decrease in the percentage of polyps with planulae at both sites, but this was only significant at the IUI site (Fisher LSD post-hoc; $p < 0.05$; Fig. 3c). Overall, during both 2001 and 2002, there were significantly higher percentages of *S. pistillata* polyps with planulae at the IUI site than at the FC site (two-way ANOVA; $p < 0.01$ Fig. 3c, Fig. 4). In both sites, more planulae were produced in 2002 than in 2001 (Fig. 4).

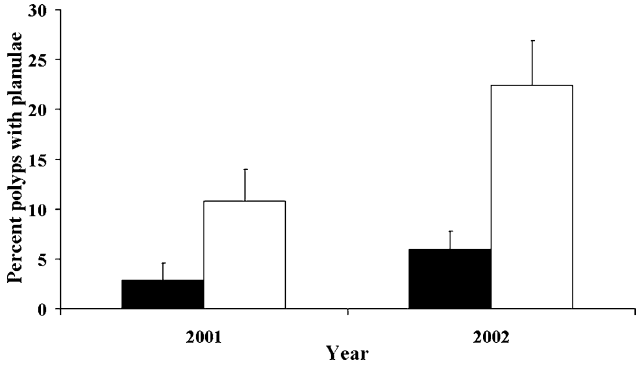

Fig. 4. Annual reproductive effort (average percentage of polyps with planulae ±SE) in colonies of *S. pistillata* transplanted to each of the experimental sites. Solid bars = colonies from the FC site; open bars = colonies from the IUI site.

3.4. Lipids in coral tissues

The average percent lipid found in coral tissues from colonies ($n = 6$) that had been transplanted to the IUI site was significantly higher ($28.7 \pm 4.8\%$) than that of colonies ($n = 5$) from the FC site in May 2001 ($20.0 \pm 5.8\%$; Kolmorogov–Smirnov, $p < 0.05$).

4. Discussion

4.1. Gametogenic dynamics and larval development

The present study examines in situ effects of nitrification caused by net-pen fish farms on the reproductive processes of experimentally transplanted coral colonies. Determining the effect of a stressor on reproductive effort requires an understanding of the dynamics of gametogenesis and larval development of the target species (Loya and Kramarsky-Winter, 2003). In the reef coral, *S. pistillata*, the subject of this study, oogenesis begins in early fall and peaks in winter when spermatogenesis begins. As oocytes mature, there is an increase in the number of brooded larvae concurrently with a decrease in the number of oocytes, which is consistent with fertilization and development of brooded planulae (Rinkevich and Loya, 1979a). Towards the end of the reproductive season in early summer, there is a decrease in mean oocyte size, oocyte number and testes number, reflecting fertilization. In addition, there is a decrease in the number of planulae, reflecting release of brooded larvae. Thus, to understand the effects of nutrification on reproduction of *S. pistillata*, it is critical to determine if eggs reach sizes that would enable fertilization and development into healthy larvae.

4.2. Impacts of nutrification on reproductive synchrony

Comparison of the percentage of colonies with female gonads between resident colonies (data from Bongiorni et al., 2003a) and transplanted colonies (our study) at the IUI site, demonstrated that there was no significant difference between the two groups (Fisher Exact test $p > 0.05$, Table 2). Hence, the transplantation procedure did not affect adversely female gonads in colonies at the IUI site. At the FC site, in contrast, a significantly higher percentage of resident colonies had female gonads when compared with transplanted colonies (Fisher Exact test $p < 0.05$, Table 2). It is possible that the impact of transplantation acting synergistically with stress induced by high eutrophication at the FC site resulted in the transplanted colonies a lower proportion of polyps containing female gonads in March 2001. However, reversal of this pattern by May 2001 suggests that such possible impact was short-lived. Furthermore, the percentage of transplanted colonies containing female gonads did not differ significantly between the FC and IUI sites over the two years of the study (Fisher Exact test $p > 0.05$, Table 1). The early loss of female function is similar to results of previous studies that showed a loss of female function due to stress in the gonochoric coral *Porites porites* (Tomascik and Sander, 1987) as well as in the hermaphroditic coral *S. pistillata* (Rinkevich and Loya, 1989).

In an earlier study, Rinkevich and Loya (1979b) noted that the percentage of colonies containing polyps with female gonads in resident *S. pistillata* at the IUI site was highly variable, suggesting some asynchrony in gametogenesis within the population. We found similar variability in the present study, whereas Bongiorni et al. (2003a) found that virtually all resident colonies at the FC site contained female gonads in 2001. The discrepancies in reproductive synchrony and reproductive activity within and between transplanted and resident colonies at the two sites may be explained, in part, by the results of a recent molecular study conducted by Zvuloni (2003). Zvuloni found that genetic variability was reduced in naturally occurring adult colonies at a site close to the FC site, compared to populations at three sites located at increasing distances further south. Moreover, genetic variation of recruits was significantly greater than for adult coral colonies, suggesting that post-recruitment mortality limits the number of genotypes able to survive at this site. This conclusion is supported by other studies, which show that the amount of genetic variability in benthic invertebrates is reduced in polluted environments (Patarnello et al., 1991). Thus, selection processes might be reflected in a much more

Table 2
Comparison between the percent of *S. pistillata* colonies with female gonads, in naturally occurring (resident) colonies (data for resident colonies from Bongiorni et al., 2003a) and transplanted colonies (this study) at each site during 2001

Percentage colonies with female gonads	Fish cages (FC) site		IUI—reference site	
	Resident colonies	Transplanted colonies	Resident colonies	Transplanted colonies
January (BSAR)	93	–	71	–
March (LLKW)	–	20	–	83
May (BSAR and LLKW)	100	60	67	67

BSAR = Bongiorni et al., 2003a; LLKW = Loya et al., this paper. Total number of colonies and polyps examined by BSAR = 28 and 485, respectively and by LLKW = 22 and 973, respectively.

synchronized reproductive season in the resident coral population at the FC site, when compared with both the transplanted and resident colonies at the IUI site.

4.3. Impacts of nutrification on gametogenesis

We found that more than three times as many oocytes reached the normal fertilization size of $\geqslant 200$ μm in the transplanted colonies at the IUI site (13%) than in the FC site (4%) and developed normally into planulae larvae (Fig. 3a and c), indicating that nutrification associated with net-pen fish farming adversely affects oogenesis. In particular, most oocytes in colonies at the FC site remained small (Fig. 3a). It could be argued that increasing oocyte size between March and May/June at the FC site was an indication of a longer reproductive season period, it is unlikely for two reasons: (1) most of the 4, 8 or 16 primordial oocytes produced by polyps typically atrophy and/or are resorbed during normal gametogenesis to act as "nurse cells", and (2) only mature large oocytes (200–230 μm) are fertilized internally and develop into brooded planulae, the development of just a few large, mature oocytes signifies normal successful oogenesis (Rinkevich and Loya, 1979a).

Our results suggest that recent interpretations by Bongiorni et al. (2003a) of the impacts of net-pen fish farming on the reproduction of naturally growing (resident) *S. pistillata* colonies, at the same two sites that we have been working, are erroneous. They reported: (1) larger, but not significantly different, average oocyte size was recorded in colonies at the FC site compared with the IUI site, and (2) an increase in the average number of oocytes per polyp, as the reproductive season advanced in resident colonies at the FC site. Their interpretation of these results was that higher reproductive activity is evident in the FC site colonies compared with the IUI colonies because of their improved nutrition provided via fish excretion. In view of our knowledge on the dynamics of gametogenesis and larval development in *S. pistillata* (summarized in Section 4.1), we however interpret observation (2) above, as an abnormal pattern of development. Furthermore, we claim that the significant decrease in numbers of oocytes per polyp towards the end of the reproductive period at the IUI site (found in both studies), concomitantly with the increase in number of planulae found in our study (Fig. 3c) indicate that many of the mature eggs in the IUI colonies were fertilized and developed into planulae larvae. In contrast to Bongiorni et al.'s (2003a) interpretation of the results, we suggest that, in resident colonies, the increase in number of oocytes per polyp at the FC site coupled with the reduced numbers of oocytes reaching mature sizes, imply that fertilization and ensuing larval development was largely deterred in the FC site colonies. Indeed, following experimental nutrient loading, the production of large numbers of small oocytes has been previously reported in some broadcasting coral species (Ward and Harrison, 2000; Cox and Ward, 2003). Cox and Ward (2003) also showed that the brooding coral *Pocillopora damicornis* failed to produce planulae following exposure to ammonium enrichment. In addition, the higher proportion of polyps containing testes at the FC site is also consistent with studies that demonstrate development of a greater number of male gonads in colonies exposed to increased levels of nutrients (Tomascik and Sander, 1987; Ward and Harrison, 2000).

The question remains as to whether the smaller oocytes that predominated in the colonies at the FC site were successfully fertilized, and whether they then developed into viable planula larvae. The available information on reproduction of *S. pistillata* (Rinkevich and Loya, 1979a,b) together with results from the present study indicate that such development is unlikely. The relatively high percentage of polyps with oocytes found at the FC site, compared to the IUI site combined with the deficiency in large, mature oocytes, even as the reproductive season progressed (Fig. 3a and b), indicates that these oocytes were most likely not fertilized (i.e., "wasted oocytes"), particularly in view of the fact that the number of testes decreased with progression of the reproductive season (Fig. 2). Studies of other coral species exposed to elevated nutrient levels support this conclusion (Tomascik and Sander, 1987; Cox and Ward, 2003; Harrison and Ward, 2001). Furthermore, even if fertilization was not affected, it is possible that ensuing larval development may have been, as has been shown in previous studies of a number of coral species (Harrison and Ward, 2001; Bassim and Sammarco, 2002).

4.4. Impacts of nutrification on planulae development and reproductive effort

In *S. pistillata* successful fertilization and ensuring larval development usually results in one oocyte developing into one planula per polyp (Rinkevich and Loya, 1979a), hence, reproductive effort in this brooding coral should be assessed by the number of planulae produced as was carried out in the present study, (see also Loya and Kramarsky-Winter, 2003). Bongiorni et al.'s (2003a) criterion of comparing numbers of 'female oocytes per polyp' and average oocyte have sizes, (similar to Ward and Harrison, 2000, who worked on broadcasting species) rather than quantitatively recording the development of mature planula per polyp, is insufficient for drawing conclusions concerning reproductive effort in a brooding species like *S. pistillata*. Hence, their use of the ecological concept 'reproductive effort', in concluding that resident *S. pistillata* colonies exhibited higher reproductive activity in the FC site compared to the IUI site, is misleading, because they do not provide any indication if the oocytes developed

normally, reached maturity, were fertilized or if they have developed into healthy planulae.

The results of the present study indicate that, despite the overall higher number of oocytes present in tissues of colonies transplanted to the FC site (Fig. 3b), the percent of polyps with planulae was significantly lower at the FC site compared to the IUI site (Figs. 3c and 4). The fact that more planulae were present in colonies at the IUI site as the reproductive season advanced, indicates that fertilization and subsequent development to the planulae stage took place in more colonies at the IUI site than at the FC site.

4.5. Impacts of nutrification on lipids in coral tissue

Lipid content provides an additional indication of the reproductive state of corals during the reproductive season. Lipids are stored in the coral's oocytes and particularly in developing planulae. During the peak reproductive season, lipid content of coral tissues (tissue, oocytes and developing planulae) is expected to be higher than during non-reproductive periods (Rinkevich and Loya, 1979a; Harrison and Wallace, 1990; Leuzinger et al., 2003). Hence, our finding that lipid content was greater in IUI colonies than in FC colonies is indicative of greater reproductive effort in IUI colonies. Indeed, Bongiorni et al. (2003a) found the same result, but dismissed the implication of greater reproductive effort in IUI corals by saying that 'it is possible that higher physiological–biochemical levels are associated with environmental conditions at the fish farm'. In contrast, we argue that these results provide strong corroboration that IUI site colonies are reproductively more active than colonies at the nutrient-enriched FC site.

4.6. Trade-off between coral growth and reproduction

Past studies have suggested that trade-offs occur in energy allocation between growth and reproduction in corals (Loya, 1985; Harrison and Wallace, 1990). It is likely that nutrient enrichment, which is known to cause rapid linear extension of corals (Hoegh-Guldberg et al., 1977), will tilt this dynamic balance towards faster growth. The rapid extension (growth) of coral nubbins at the FC site reported by Bongiorni et al. (2003a) is therefore not surprising. Nevertheless, it is likely that rapid linear extension occurred at the expense of skeletal density. If rapid vertical extension of nubbins at the FC site results in lower skeletal density, compared with corals at the IUI site (see Bucher and Harrison, 2002), they will be vulnerable to higher rates of breakage and bioerosion. Thus, determining whether or not rapid linear extension in *S. pistillata* is beneficial to the coral reefs of Eilat (as Bongiorni et al., 2003a,b contend) also depends on studying how nutrification associated with in situ fish farming affects the infilling processes that control skeletal density. Our results suggest the likelihood that the faster vertical elongation of nubbins at the FC site occurs at the expense of their future reproductive output (i.e., a trade-off between coral growth and reproductive effort). Thus, in contrast to Bongiorni et al.'s (2003a) conclusions regarding the "beneficial" effect of fish cage nutrification on *S. pistillata* reproduction, we suggest that the results reported in both studies attest to the opposite to a severe reduction in the reproductive effort of *S. pistillata* colonies near the nutrient enriched FC site.

4.7. Conclusions, future perspectives, and a call for action

The present study shows unequivocally that reproductive effort of *S. pistillata* was detrimentally affected at a site close to the net-pen fish cages (FC site) compared with a reference site (IUI) 8 km further south.

Currently, we are carrying out a large scale integrated study on the population dynamics of two branching and two massive coral species in the northern Gulf of Eilat, in the vicinity of the FC site and at two reference sites 8–10 km further south (encompassing coral population dynamics, coral reproductive effort, occurrence of diseases and syndromes infecting corals, bioerosion and coral isotopic composition tracing possible anthropogenic stress). Our focus in the present study was to ascertain the effects of the net-pen mariculture effluent on the reproduction of *S. pistillata* colonies. The results reported in this paper are the preliminary findings of this large scale study. It is imperative to expand the experiments reported here and sample higher numbers of both transplanted and resident colonies, in order to better understand the effects of nutrification on both populations. This is important because nutrient loading is not locally restricted, but affects colonies further down the coast (see Abelson et al., 1999; Lazar et al., 2000; Loya, 2004; Silverman et al., 2004), particularly during deep water mixing events (Genin et al., 1995). Thus, Silverman et al. (2004) found a significant decrease in net $CaCO_3$ addition by frame builders (mainly hermatypic corals) relative to net growth of macroalgae in recent years in the Eilat Coral Nature Reserve (ECNR). They attributed this deterioration in the ECNR reef growth to the chronic eutrophication caused by the fish farms in the northern Gulf of Eilat. Such an acute reef community change may be accompanied by higher grazing pressure leading to increased bioerosion (Hallock, 1988; Dubinsky and Stambler, 1996; McCook, 1999; McCook et al., 2001 and literature therein), thus upsetting the delicate balance between growth and erosion of the reef framework.

At present, the coral reefs of Eilat are severely damaged and exist in a critical state. The alarming records of coral mortality in recent years (Loya, 2004), the decrease

in recruitment (Ben-Tzvi et al., 2004), the decrease in reef growth (Silverman et al., 2004), the increasing percentages of corals affected by pathogenic diseases, and the first accounts of sparse coral bleaching observed in the last two years at Eilat, are strong indications of the fragile state of health of the coral populations in Eilat (reviewed by Loya, 2004). The continuation of the present eutrophication rate originating from the fish farms constitutes one of the major causes of the continued deterioration of the unique coral reefs of Eilat and comprises a serious threat to their very existence. The only chance for their recovery is the use of immediate, prudent and rational protection measures against all man-induced perturbations.

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