

## A floating mid-water coral nursery as larval dispersion hub: testing an idea

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**Abstract** The global decline in reef health has prompted the need for effective management methodologies, including the development of active restoration measures. One such approach is the ‘gardening concept’ that involves use of underwater nurseries where coral fragments are farmed before their transplantation into denuded reefs. Here we document enhanced sexual reproduction in colonies of the coral *Stylophora pistillata* cultured in mid-water floating nursery situated in nutrient enriched water, near the fish farms in Eilat, Red Sea. We found that after 2 years of nursery, the average number of oocytes per polyp in farmed colonies was ca. 35% higher than in corresponding naturally growing colonies. Small branches in the nursery developed gravid colonies that released equal (or more) numbers of planula larvae as compared to similar size, 5-year old naturally growing colonies. These nursery-borne planulae possessed more zooxanthellae and contained more chlorophyll per larva. While settled and metamorphosed in equal rates compared to planulae originated from reef-grown colonies, the nursery borne planulae developed faster growing young colonies. We estimate that a coral nursery could generate, during the reproductive season, tens of millions of planula larvae and therefore should be regarded as a ‘larval dispersion

hub’ that can be used as a management tool for natural recruitment enhancement.

### Introduction

The use of interconnections among marine protected areas (MPAs) has long been considered as an important management tool for marine reserves (Ogden 1997; Roberts 1997; Lockwood et al. 2002; Sala et al. 2002; Gerber et al. 2003). However, little has actually been done to enhance biodiversity through connectivity. Most studies have just attempted to map connectivity patterns or understand the processes occurring outside reserve boundaries that may affect local populations through connectivity. In some marine ecosystems, such as the Caribbean coral reefs system (Roberts 1997), reef sites are able to draw larvae from very large catchment basin and thus be affected by events occurring hundreds of kilometers away. However, evidence is mounting that although larval imports may attest to great distances of dispersal, the quantities necessary to replenish annual losses are substantially restricted in space and that larvae fail to achieve their dispersal potential (Cowen et al. 2006). Even coral reef larvae were found to settle close to home (Jones et al. 2005). Natural recruitment of coral reefs may be further restricted or prevented because of adverse shifts in protected areas’ communities and changes in environmental conditions (Risk 1999; Wilkinson 1999). This situation has stirred discussions on developing novel supplementary management acts, including active restoration measures (Rinkevich 2005, 2006).

One of the strategies suggested for enhancing coral reef rehabilitation is the ‘gardening concept’ (Rinkevich 1995, 2000; Epstein et al. 2001). This is a two-step resto-

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ration measure, in which the first step is mariculture of coral recruits in underwater nurseries and the second, when corals reach adequate size, their transplantation onto degraded reefs. Recently, an improved approach involving the use of a floating mid-water coral nursery has been successfully tested in the northern Gulf of Eilat, Red Sea (Rinkevich 2006; Shafir et al. 2006a, b). Whereas the above nursery prototype fulfilled the major restoration needs, such as mass production of coral colonies at low cost, with notable high survivorship and fast growth rates of farmed coral colonies, the preliminary results further showed improved reproductive patterns of nursery-farmed corals (Bongiorni et al. 2003). These results have encouraged us to test the feasibility of using corals farmed in mid-water floating coral nursery as potential larval production source for boosting coral restocking in small and isolated reef reserves, such as the coral reef reserve in Eilat, Red Sea (Epstein et al. 2005).

### Materials and methods

Planulae of *Stylophora pistillata* were collected from colonies growing in two sites: (1) Eilat's mid-water coral nursery (10–12 m depth, 10 m away from the Ardag fish farm; FF) located at the northern shore of the Gulf of Eilat, Red Sea (29°32.4'N, 34°58.40'E). The intensive net-cage fish farming of gilthead seabream (*Sparus aurata*) promotes eutrophication near the farm (Bongiorni et al. 2003). Recently measured yearly average of nutrient levels (June 2004–May 2005) were 0.110  $\mu\text{M}$  nitrite, 0.437  $\mu\text{M}$  nitrate, 0.105  $\mu\text{M}$  phosphate and 0.192  $\mu\text{M}$  ammonia (Israel National Monitoring of the Gulf of Eilat, <http://www.iui-eilat.ac.il/NMP/index.htm>). (2) colonies growing naturally in front of Eilat's Inter-University Institute (IUI), 8 km southwest to Ardag FF. This reef is a low profile fringing reef, dominated by hermatypic corals, commonly used for field studies. The average nutrient levels at the IUI site measured during the same period were 0.0104  $\mu\text{M}$  nitrite, 0.428  $\mu\text{M}$  nitrate, 0.046  $\mu\text{M}$  phosphate and 0.054  $\mu\text{M}$  ammonia.

*Stylophora pistillata* is a hermaphroditic brooding species with a long reproductive season (January–July; Rinkevich and Loya 1979b, 1983). Planula larvae were collected in both sites in situ (May, June 2005 and April 2006) by plankton nets placed over gravid colonies (10–15 cm in diameter) before sunset and removed the following morning. Colonies of this size at IUI are at least 5 years old (Loya 1976). Colonies of this size at FF were the product of fast growth of small fragments (about 1–3 cm long branches) that reached 10–15 cm diameter colony size within 2 years (Shafir et al. 2006b). Within 2 days from collection, planulae, in groups of 30, were

shipped to the laboratory in Haifa in 50 ml plastic tubes, containing filtered seawater. In the laboratory, larvae were transferred to 60 mm Petri dishes, lined by underwater transparencies (Mailer's) that were preconditioned in flow-through aquaria for a week. Settled planulae were numbered individually by lead pencil, transferred with the transparency substrates and glued to 5.0  $\times$  7.5 cm glass slides. Larvae also metamorphosed upside down on the water tension on the surface of the Petri dishes. These polyps were gently picked up with the tip of a fine paintbrush and placed in a humidified chamber (15 min) on pieces of transparencies, preglued to the glass slides. Slides containing the attached polyps were transferred to flow-through aquaria at 23–25°C under a 12–12 h light–dark regimen. Young colonies were fed every other day with fresh *Artemia* and observed under a Nikon SMZ800 stereomicroscope once a week for up to 3 months. Since at this early astogenic stage, colonies grew at 2D only, growth was measured in terms of added polyps or added surface area per unit of time. Photographs were taken using Color View 2 Soft Imagin System camera with a millimetric grid for scale bar. Growth rates were calculated using the image analysis package TINA 2.0. Slides and animals were cleaned from debris and fouling organisms, using small pieces of razor blades and fine paintbrushes.

Samples from single branches, representing the reproductive activity of the colonies (Rinkevich and Loya 1979a), were taken from each of eight *S. pistillata* colonies (four from each site, 12 m depth, 10–15 cm diameter) during May 2005. Serial cross sections (5  $\mu\text{m}$  thick) were prepared, as described (Rinkevich and Loya 1979a). Twelve polyps from each coral sample were carefully examined (Olympus BX50 upright microscope) in consecutive serial sections for the presence of male gonads, the number of oocytes or eggs per polyp and oocyte size.

Chlorophyll *a* and chlorophyll *c*<sub>2</sub> were extracted from batches of five planulae each by immersing in 90% acetone for 24 h at 4°C; each batch of planulae originated from either one of six IUI colonies or eight FF colonies. Spectroscopic measurements of pigments were calculated at 630, 663 and 750 nm based on the spectroscopic equations of Jeffery and Humphrey (1975). To determine the number of zooxanthellae per planula, cells were extracted from batch of five planulae of seven different colonies at IUI ( $n = 35$  planulae) and eight different colonies at FF ( $n = 40$  planulae). The planulae were incubated for 10 min at room temperature, in an eppendorf tube containing  $\text{Ca}^{2+}$ – $\text{Mg}^{2+}$ -free artificial seawater (ASW) with ethylenediamine-tetracetic acid (EDTA), as described in Rinkevich et al. (2005). Tissue dissociation was

achieved by multiple pipettations. Cell suspensions were diluted with sterile seawater and centrifuged (4,300 rpm) for 10 min at room temperature. Pellets were re-suspended in FSW and cells counted under microscope by hemocytometer.

Data processing was performed with SPSS software for windows version 13.0.1. Normality and homogeneity of variance were tested by Kolmogorov–Smirnov and Levene's statistical tests, respectively. Two-way ANOVA, *t* tests and Mann–Whitney tests were carried out. When required, data was ln transformed. Results are presented as average  $\pm$  SD except where indicated.

## Results

Total of 1,315 planulae were collected from 11–14 colonies per site, in three collection dates (Table 1). High variation in number of planulae per coral colony (0–106) was recorded in both sites. Planulae collected from the FF site were significantly longer than those from the IUI site ( $2.1 \pm 0.4$ ,  $1.7 \pm 0.2$ , respectively;  $P < 0.05$ , *t* test) and highly pigmented (Fig. 1). Chlorophyll content per planula was significantly higher at the FF (Fig. 2;  $P < 0.05$ , *t* test), the outcome of higher number of zooxanthellae per planula (Fig. 2;  $P < 0.05$ , *t* test). We also observed that the FF collected planulae were,

under laboratory conditions, more active than the IUI collected larvae (data not shown).

In both year-2005 collections, no significant difference in the number of planulae per colony was recorded between the two sites (Table 1; Mann–Whitney,  $P > 0.05$ ), in contrast to year 2006 collection, that revealed significantly higher number of planulae per colony at the FF site (Table 1; Mann–Whitney  $P < 0.05$ ). No significant difference was recorded in IUI between 2005 and 2006 collections ( $14.7 \pm 13.9$ ,  $11.9 \pm 17.5$ , respectively, Mann–Whitney,  $P > 0.05$ ).

Year 2005 collected larvae were followed for settlement rates. Only 60–68% of the planulae survived during the first 2 weeks, out of which 27.1–36.1 metamorphosed and settled (Table 1). No significant difference ( $P > 0.05$ ) between the two sites was documented either in the average number of collected planulae per coral colony or in ex situ settlement rates (Table 1). More than 80% of the settled planulae survived the 3-month observation period under laboratory conditions (data not shown). No significant difference in survivorship rates ( $P > 0.05$ ) was observed between spatia originated from larvae collected from both sites.

Growth of 17 randomly selected primary polyps from each site was monitored. Data was collected at four time points during the first 3 months after settlement (Fig. 3). After 3 months of ex situ maintenance,

**Table 1** Results of planulae collections and settlement

Sampling month	Site	Number of colonies	Released planulae					Statistical difference ( $P < 0.05$ )
			Per coral colony	Maximum/colony	Minimum/colony	Survived (%) <sup>a</sup>	Settled (%) <sup>b</sup>	
May 2005	IUI	11	$15.5 \pm 24.3^c$	86	0	61	30.5	NS
	FF	12	$10.4 \pm 10.3$	38	0	68	27.1	
June 2005	IUI	13	$8.8 \pm 8.7$	34	0	65	28.0	NS
	FF	13	$10.6 \pm 7.3$	18	0	60	36.1	
April 2006	IUI	14	$14.7 \pm 13.9$	41	0	ND	ND	S
	FF	12	$46.7 \pm 39.3$	106	2	ND	ND	

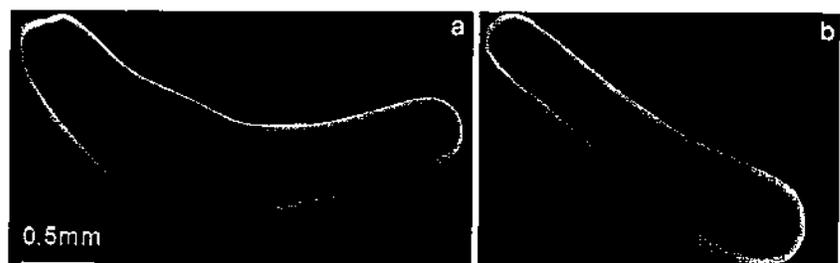
ND not done, NS not significant, S significant

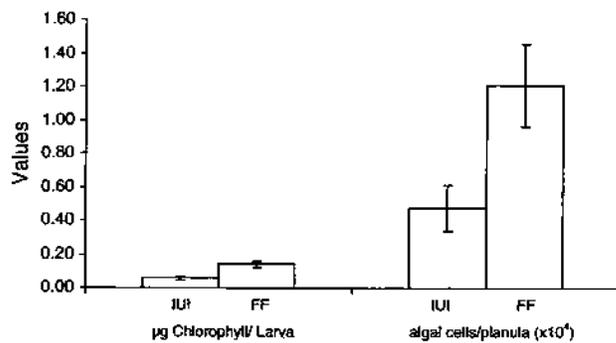
<sup>a</sup> Percentage of survived planulae out of released (within 2 weeks)

<sup>b</sup> Percentage settled of survived at the first 2 weeks

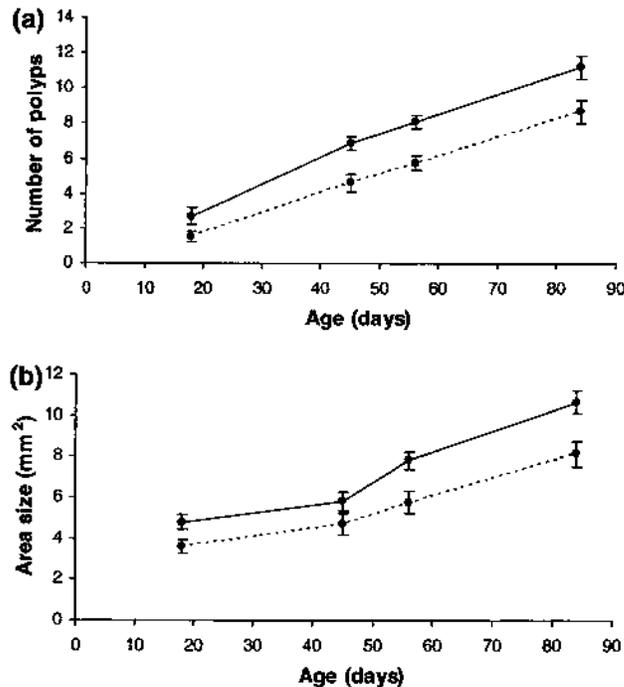
<sup>c</sup> When excluding a single extreme case of 86 planulae/one colony, the value for IUI at this sampling date is  $8.5 \pm 7$

**Fig. 1** Typical morphologies of planulae collected at a FF site, b IUI site





**Fig. 2** Average (mean  $\pm$  SE) zooxanthella numbers and chlorophyll contents per planula larva at both sampling sites



**Fig. 3** Growth of *Stylophora pistillata* spats originating from FF and IUI collected planulae: **a** number of polyps; **b** area size (mean  $\pm$  SE). Solid lines FF, dotted lines IUI

the young colonies from the FF site reached the size of  $11.2 \pm 2.7$  polyps and  $10.6 \pm 2.3$  mm<sup>2</sup> area size, whereas the colonies from IUI reached the size of  $8.7 \pm 2.7$  polyps and  $8.1 \pm 2.6$  mm<sup>2</sup> area size (about 30% larger young colonies from FF collected larvae; Fig. 3;  $P < 0.05$ ,  $t$  test). This outcome was consistent, as at all time points during the study, colony area size and number of polyps in the FF site were higher than in the IUI site, statistically significant ( $P < 0.05$ ,  $t$  test) at ages 18, 56 and 84 days. At age 45 days, statistical significance was documented only in number of polyps ( $P < 0.05$ ,  $t$  test).

Total of 96 polyps in the eight sampled *S. pistillata* colonies were examined by serial sections. Whereas, the average number of oocytes per polyp in the FF

colonies was 34.9% higher than in the IUI ( $0.85 \pm 0.82$  vs.  $0.63 \pm 0.76$ , respectively), these numbers were not significantly different ( $P > 0.05$ , two way ANOVA), resulting from the recorded high variation in the number of oocytes per polyp. No significant difference was recorded between IUI and FF sites in the percentage of polyps that possessed male gonads (81 vs. 83%, respectively). Sizes of oocytes in *S. pistillata* colonies growing at IUI site did not differ from the FF site ( $146.0 \pm 52.3$  vs.  $127.3 \pm 58.5$   $\mu$ m diameter, respectively;  $P > 0.05$ ,  $t$  test).

## Discussion

The results of this study have not only re-confirmed our previous outcomes of enhanced sexual reproduction in nursery-farmed corals cultured in nutrient-enriched water (Bongiorni et al. 2003), but also revealed three new interesting facets: (1) after just 2 years under nursery conditions, small fragments of *S. pistillata* have developed female gonads and released viable planula larvae that settled and metamorphosed at quantities/rates equal (or more) to 5 years old colonies, residing in the natural reef; (2) nursery-borne larvae are larger, equipped with higher numbers of endosymbionts, revealing higher chlorophyll content per planula; (3) nursery-borne larvae have given rise to small colonies that showed improved growth rates compared to spats originating from naturally grown colonies. Farming about 10,000 colonies in our small scale nursery ( $36 \times 1$  m size; Shafir et al. 2006a, b), transformed this entity to a 'larval dispersion hub' that could release during the 7 months reproductive seasonality of *S. pistillata* (Rinkevich and Loya 1979b, 1987) over 20 million larvae (calculated from the actual average number of larvae harvested at the FF site; Table 1). We have estimated (unpublished) that a team of two workers could farm more than 70,000 coral colonies/year in a larger mid-water nursery; a small floating reef that may produce a pool of up to 150 million planulae per year. While the number of planulae developed may fluctuate between brooding versus broadcast species or even between different brooding species, the potential of such 'larval dispersion hub' for enhancing natural recruitment is significant. As such floating nursery can be relocated during the reproductive season to upstream sites, this may increase the number of planulae that will enter and settle into the target reefs, further improving recruitment rates.

There is an increasing demand for developing active reef restoration measures since many reef areas worldwide have lost their resilience and their ability to recover naturally (Rinkevich 2005). While restoration of terres-

trial ecosystems has been applied for nearly two centuries (Rinkevich 2006), the concept of active reef restoration is less than 2 decades old. The need is particularly urgent for remediation of small but highly visited reef sites such as the coral nature reserve in Eilat, where it was documented that the application of no-use-zone measure was not sufficient to compensate for the stress inflicted by anthropogenic activities (Epstein et al. 1999, 2005). These outcomes further act in concert with the suggestion (Cowen et al. 2006) that sustainable reef population requires larval import from outside the local area.

Restoration by sexually-produced entities (larvae, small colonies; Rinkevich 1995, 2005; Raymundo et al. 1999; Gleason et al. 2001; Petersen and Tollrian 2001; Heyward et al. 2002) may recreate improved coral populations. Coral population sizes and genetic parameters will certainly be enhanced in sites supplied copiously from nurseries nearby. This approach alleviates the stress caused by whole colony/fragments transplantation acts (Edwards and Clark 1998). Corollary of the above approach are reef sites characterized by enhanced reef resilience, minimizing obstacles like inbreeding (from depleted genetic heterogeneity), loss of genetic diversity and reduced genetic adaptation of restored populations (Frankham 1999; Jones 2003; Hufford and Mazer 2003; McKay et al. 2005). Most previous studies that tried to employ sexually produced propagules as source material for restoration were forced to use elaborate ex situ practices (Richmond 1995; Raymundo et al. 1999; Sammarco et al. 1999; Petersen and Tollrian 2001). This is not the case with the reproductive output of nursery-farmed coral colonies. Such floating nursery can be easily translocated into up-stream, catchments regions, near small MPAs, providing on-spot ample numbers of coral larvae for improved recruitment. Marine restoration ecology is an emerging scientific discipline that addresses unexplored realms and problems faced by practitioners. The floating 'larval dispersion hub', when approved as an effective management tool, may also have a dramatic influence on the design and implementation of regional network of MPAs.

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