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Costa Rican coral reefs under siltation stress

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Executive Summary

The major aim of this collaborative work (Project C14-015) is to evaluate the effects of siltation stress on Costa Rican coral reefs, to determine stress phenomena, to restore denuded reef areas and to train young Costa Rican scientists in reef management and restoration. In addition, one of the major goals of the project is to transfer modern techniques and knowledge in coral reef biology and in the biology of reef organisms to Costa Rican scientists.

During the 2nd fiscal year, former lines of research have been continued in the field and in the laboratory. In the field, both groups, (the Costa Rican group is headed by Dr. J. Cortez, and the Israeli group is headed by Dr. B. Rinkevich) continued the collaborative monitoring studies in the reef on the Pacific Coast of Costa Rica (once a month) and on the Caribbean Coast (every 3-4 months). Long-term monitoring observations of specific selected stations are continued at the same pace as before, in addition to field studies on variety of stress phenomena, such as the appearance of tumors in coral tissues and skeletons, bleaching events in Costa Rican reefs, interspecific and intraspecific competitions, and more. This year, we analysed fragments (ramets) and nubbins as a source material for restoring denuded coral reefs. Fragmentation in scleractinian corals has been recognized as an important facet in the life history portrait of many species. Ramets isolated from existing genets may establish new colonies; a phenomenon that is widely used in a variety of management measures. An analysis of regenerating branches of the coral *Stylophora pistillata* reveals that the architectural complexity of isolated branches may have a significant impact on the initiation of the regeneration process towards the typical structure and complexity of an intact colony. It is suggested that the 3-D structure complexity of isolated ramets should be taken into consideration when applying reef restoration practices. In addition, the approach of the development of inland broodstocks from nubbins (small size fragments, 1-3 mm long) was also evaluated. We also tested several strategies for gardening denuded reefs. The gardening concept consists of *in situ* or *ex situ* mariculture of coral recruits, followed by their transplantation into degraded reef sites. *In situ* nurseries were established in shallow waters, sheltering three types of coral materials taken from branching coral species (small colonies, branch fragments, and spat) that were monitored for up to two years. Pruning more than 10% of donor colonies' branches increased mortality, and surviving colonies displayed reduced reproductive activity. Maricultured isolated branches, however, exceeded donor colony life span and reproductive activity and added 0.5-45% skeletal mass per year. Nursery periods of 2 years, 4-5 years and more than >5 years have been estimated for small colonies, spat, and isolated branches, respectively. We conclude that *in situ* coral mariculture

is an improved practice to the common but potentially harmful protocol of direct coral transplantation. It is suggested that reef gardening may be used as a key management tool in conservation and restoration of denuded reef areas. The gardening concept may be applicable for coral reefs worldwide through site-specific considerations and the use of different local coral species.

As in previous years, both groups mutually carried out the study. To further facilitate the work, two Israeli scientists (Dr. D. Gateno and Dr. Y. Barki) are continuing to reside in Costa Rica, participating in all field trips and other scientific activities involved in the project. The Israeli PI visited Costa Rica once during this period for tight collaboration and discussions with the Costa Rican group. Funds were also used for participation of the Costa Rican partner, Jorge Cortés, in the IX Congreso Latinoamreicano sobre Ciencias del Mar (COLACMAR), San Andrés, 16-20 September, 2001 (Latin American Congress on Marine Sciences). The project results were presented in this symposium by two oral presentations.

Section I

A - B Published Data

Below are the detailed results of 3 manuscripts that were published during the fiscal year of 2001 and summary of major monitoring activities performed in Costa Rica (replacing Sections A-B). The work described in the manuscripts were supported by the AID-CDR project. Reprints of two papers are also appended (the third manuscript was published Dec. 2001 and we do not yet have its reprints):

Epstein, N. and B. Rinkevich. From isolated ramets to coral colonies: The significance of colony pattern formation in reef restoration practices. Basic Appl. Ecol. 2, 219-222, 2001.

Shafir, S., J. Van Rijn and B. Rinkevich. Nubbing of coral colonies: A novel approach for the development of island broodstocks. Aquarium Sci. Conserv. 3, 183-190, 2001.

Epstein, N., R.P.M. Bak and B. Rinkevich. Strategies for gardening denuded coral reef areas: The applicability of using different types of coral material for reef restoration. Restoration Ecol. (in press).

Four subjects conclude the material presented in Parts A-B.

SUBJECT 1 – RESTORATION AND COLONY PATTERN FORMATION

Fission (Hughes and Jackson 1980), budding and physical as well as biological forces that cause breakage in scleractinian corals (Highsmith 1982) are common agents for the formation of ramets *in-situ*. Coral fragments, as small as a single branch or a small nubbin (Rinkevich and Shafir 2000), initiate after their separation a series of developmental processes aiming to regenerate lost parts and to regain the 3-D colony structure. This also occurs in natal colonies; Loya (1976) has suggested that a broken colony of *Stylophora pistillata,* an Indo-Pacific branching coral, will allocate resources to the damaged parts in order to restore its former shape. An isolated ramet, however, reconstitutes a whole colony configuration from even a minute portion. This mode of regeneration, known as epimorphosis is characterized by growth of new, correctly patterned branches.

A branching coral colony may be considered as an integrated three-dimensional structural entity, where physiological parameters such as reproduction and growth patterns are

synchronized between different parts (Rinkevich in press). Following this tenet, regeneration of isolated ramets should reflect not only superficial linear growth patterns, but also coordinated, 3-D pattern formation processes aiming to restore the typical, species-specific colony shape. An isolated single tip branch, thus, should commence a sequence of pattern formation processes that promote apical ramification as well as lateral growth. These concepts of pattern formation may also assist in applied disciplines such as restoration of degraded coral reefs.

The measure of coral transplantation is probably the most common management practice used to facilitate natural recovery of damaged reef sites (Edwards and Clark 1998). The rational that ramets will grow and replace lost colonies inspires the use of coral fragments as the best material for transplantation (Harriot and Fisk 1988; Rinkevich 1995). An improved approach to reef restoration is the measure of 'gardening' (Rinkevich 1995). The gardening concept does not leave the ramet's regeneration to the leniency of local reef conditions and adds, as an intermediate phase, the issue of coral mariculture in underwater nurseries before transplanting them into denuded reef sites. Like other transplantation practices, the gardening concept focuses on the criteria of ramet survivorship and growth rates as measures for success. The idea that structural complexity may significantly influence ramet's growth and regenerative ability received little attention and, when discussed, it was attributed to ecological rather than to developmental concepts. One example is Highsmith (1982) who briefly remarked that 3-D ramet's shape could prevent branch burial under sediment. Here, we raise the idea that an architectural-complex threshold of the isolated ramet is required for faster regeneration to a full colony structure. We suggest that characteristics for colony pattern formation should be considered as an additional criterion (to the well discussed criteria of survivorship and growth) when employing transplantation methodologies for reef restoration. This is particularly relevant to branching forms.

We have found that the original architectural complexity of isolated ramets may influence the ability to commence a 3-D growth pattern. An example is illustrated in Fig. 1 (a-d) where 4 fragments taken from a single *Stylophora pistillata* colony (labeled by alizarin Red S), were cultured *in-situ* side by side on concrete plates at 6m' depth (Rinkevich 2000). These ramets, a small fragment of 2 cm long (Fig. 1a), a branch fragment of 4 cm long (Fig, 1b), a fragment of a bifurcated tip (about 3.5 cm long, Fig. 1c) and a ramet of about 5 cm long with two major branches (Fig. 1d) were cultured for 8 months after isolation. Subsequently, the branches were

brought to the lab, their tissue was removed and skeletal growth above the alizarin mark was examined. It is evident that the type of fragment influenced growth rate as well as architectural complexity. While in simple, linear coral fragments (Fig. 1a, b) only apical growth was recorded, within the same time course, fragments like the ramet in Fig. 1c displayed additional morphometric growth patterns, such as side branching (seen at its left fork) and a fission that formed a new double tip at its right fork. In a more complex morphology, (Fig. 1d), many new side-growing knobs appeared below the area of apical growth, along the main ramet axis. These protrusions will develop into side-growing branches, indicating an advanced 3-D pattern formation process (Fig. 1d; arrows beneath the alizarin line). In comparison to fragments 'a', 'b' and also 'c', ramet 'd' (Fig. 1) will most likely be able to regain the typical spherical colony shape (Loya 1976) in the shortest period of time. Fragment 'd' probably possessed the minimal complexity required to initiate not only apical extension but also 3- D growth patterns. Growth rate alone is not, therefore, the sole parameter for consideration when analyzing regeneration of isolated branch fragments.

During this experiment we followed the regeneration of additional 37 branches (20 single tip and 17 ramified ramets). Of the single tip branches, 17 displayed only linear, apical extension and 3 cases developed, each, an additional new tip through fission. Conversely, of the 17 ramified ramets, only 2 displayed just linear extension while 15 ramets developed new tips and branches on at least one of the forks, in addition to lateral protrusions on the main axis. The fragments in Fig. 1 represent, therefore the typical growth of many similar size fragments and illustrate the early stages of a multi-dimensional regenerative process (Rinkevich in press).

The 3-D growth patterns in regenerating *S. pistillata* ramets are probably 'canalized' (Waddington 1942) in accordance with the species' 'developmental rules' that denote the recreation of its iterated branching system and the typical colony shape. This architectural design may be the expression of the coral structural blueprint (such as homeobox genes) that encodes for specific biochemical processes (Gellon and McGinnis 1998) or can be the outcome of secreted waterborne bioactive chemical signals (isomones) that regulate and control branch growth rates and positions within the internal volume of the colony (Rinkevich and Loya 1985). Therefore the constraint of minimal structural complexity, imposed on ramets as a critical factor for fast 3-D regeneration, should be considered along with the growth rates and the survivorship criteria in reef restoration activities.

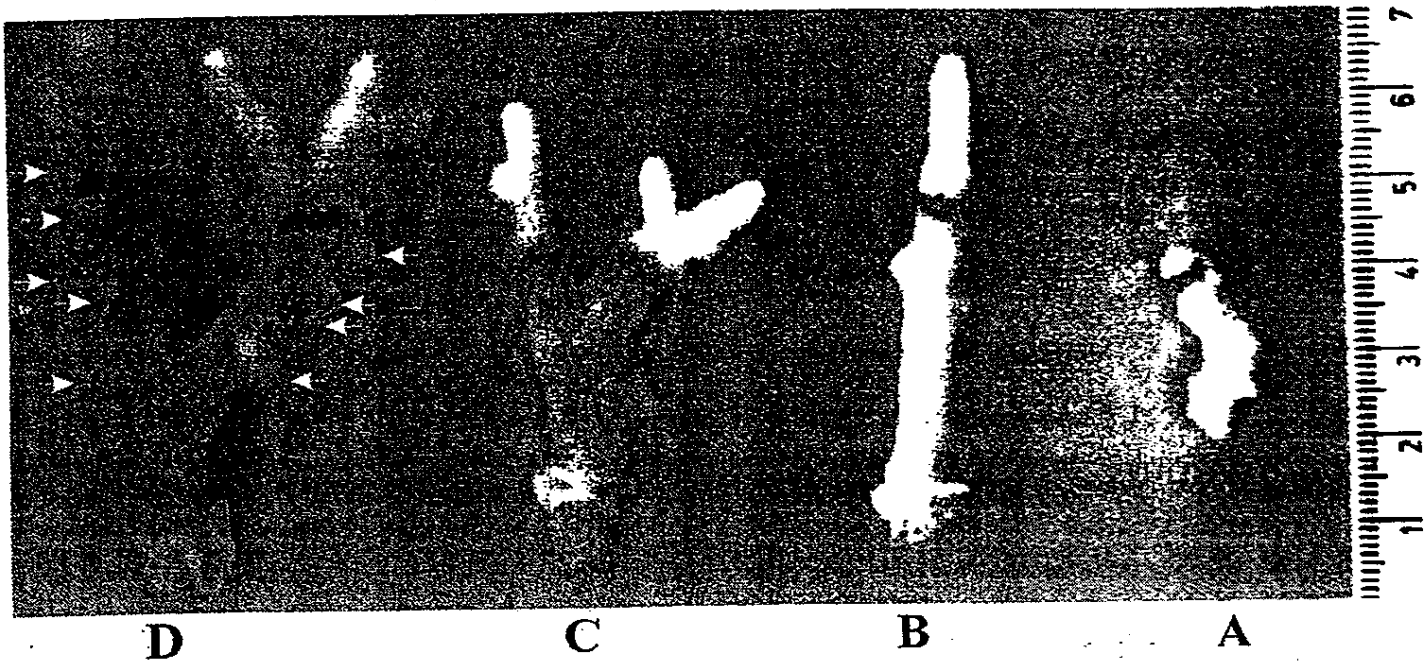

Fig. 1(a–d). Four fragments (a–d) with different architectural complexities taken from a single *S. pistillata* genet (from Rinkevich 2000). Black lines refer to alizarin marks. Photographs were taken at the end of the experiment. Alizarin marks reveal sizes at the beginning of the experiment. Scale on right in cm.

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SUBJECT 2 – NUBBINS FROM CORAL COLONIES

INTRODUCTION

Improved *ex situ* cultivation methodologies of reef corals are emerging issues in the fast developing industrial sector of trading marine ornamental organisms (summarized in Rinkevich and Shafir, 2000) and in the scientific attempts to use corals in controlled ecotoxicological and physiological studies (Davies, 1995; Tambutté et al., 1995; Reynaud-Vaganay et al., 1997; Ritchie et al., 1997; Rinkevich and Shafir, 2000; and literature therein). Important prerequisites for above approaches are the need for upgrading husbandry performances and the lack of available large numbers of ramets from specific coral genets (literature in Rinkevich and Shafir, 2000). These prerequisites are further illuminated by numeral documented studies during the last three decades which indicate that coral colonies of any studied species represent naturally high variations in physiological characteristics studied (e.g., Buddemeier and Kinzie, 1976; Rinkevich and Shafir, 2000).

Variety of studies (literature in Highsmith, 1982; Rinkevich, 1995; Tambutté et al., 1995; Carlson, 1999) evaluated the benefits of fragmenting coral colonies into several or many subclones (ramets, nubbins, microcolonies, etc.). These studies were carried out under *in situ* or *ex situ* conditions and revealed that isolated portions of coral colonies may survive and grow independently from original genets, even from species of which natural fragmentation is not part of their life history portrait.

The attempt to develop numerous genetically identical colonies from a single genet by isolating minute coral fragments, measuring as small as the size of a single isolated polyp (Al-Moghrabi et al., 1993; Ritchie et al., 1997; Rinkevich and Shafir, 2000), is a valuable and novel approach. Subcloning of minute fragments from a coral colony may, on the one hand reduce the stress inflicted on the colony and on the other, may yield a high number of ramets, available for variety of needs. These isolated minute fragments will be coined hereby as "nubbins"; a term used

earlier to characterize isolated whole coral branch tips. In the coral biology field, this term was introduced for the first time by Birkeland (1976), when attaching small pieces of *Pocillopora damicornis in situ* on artificial substrates and was employed later on to characterize a variety of branch segments in field and laboratory studies (literature in Davies, 1995; Reynaud-Vaganay et al., 1999). In these studies, nubbins were referred sometimes to the upper 3 cm long branch tips as well as to isolated single corallites (Davies, 1995), while in other cases, the 0.6-1 cm long branch tips were coined as microcolonies (Tambutté et al., 1995). We suggest to use the term "nubbins" literally (according to the Merriam-Webster Dictionary, a nubbin is "something that is small of its kind"), to characterize minute portions of coral colonies (a single isolated polyp or a coral fragment containing only a few polyps) and to term larger subclones of coral colonies as microcolonies, fragments or ramets.

We present here, for the first time, the rationale and the applicability of a novel *ex situ* approach for rearing large numbers of coral nubbins, isolated from the Red Sea coral *Stylophora pistillata*. These nubbins, when developed, can be easily used for a variety of scientific and applied needs, including physiological/ecotoxicological tests and as a suitable source for the aquarium industry trade.

MATERIALS AND METHODS

The branching hermatypic coral *Stylophora pistillata* (Esper, 1787) serves as a model coral species for a variety of purposes including physiological evaluations (literature cited in Rinkevich and Loya, 1987; Muscatine et al., 1989; Reynaud-Vaganay et al., 1999), restoration activities (Rinkevich, 1995) and as a candidate for the trade of ornamental marine organisms (Rinkevich and Shafir, 2000). Colonies typically form branching structures by an axial, rod-like growth pattern. New branches are usually added by a dichotomous fission of branch tips. In addition to the up-growing branches, lateral (inside and outside oriented) branches are also added (Rinkevich and

Loya, 1985). All together, the resulting symmetry of a typical *S. pistillata* colony approaches a sphere (Loya, 1976). Each polyp is ca. 2 mm in diameter.

The experiments conducted here were carried out on 3 *S. pistillata* colonies that were collected in shallow water (4-6 m depth) in front of the H. Steinitz Marine Biology Laboratory, Eilat, the Red Sea. The colonies were transferred immersed in seawater within insulated containers to the National Institute of Oceanography, Haifa, where they were maintained in a temperature-controlled running seawater table (Mediterranean water, 25 ± 1°C) illuminated by a combination of 3 different fluoresce bulbs: Cool White, Floura and Blue-Blue (Osram, Germany; 12:12 L:D regimen). Before introduction to the system, seawater (5 l/h) was mechanically filtered (10 μm, sand filtration) to reduce particles and then was biologically filtered by algae (*Ulva lactuca*, 1 m^3 tank, conditioning period of 3-4 hours) to reduce nutrient levels.

Nubbins, 2-15 polyps each, taken from branch tips and mid branches, were pruned from the colonies by an electrician cutter. Traumas were minimized by limited air exposure for less than 5 min., and by washing each nubbin with filtered seawater upon isolation, a procedure that also helped in removing the skeletal debris. Exposed cut skeleton surfaces were then soaked with small pieces of paper towel. A small drop of cyanoacrylate glue (super glue) was added to the exposed dead skeleton surface area and the nubbins were positioned on dry glass slides with cut surface areas contacting the substrate. Nubbins were affixed at this position for 10 seconds, and were then carefully brought back to the water table. No single sign of toxicity produced by the glue was recorded, confirming earlier studies (Davies, 1995; our unpublished results).

Observations were made once a week. The number of polyps per nubbin was directly counted microscopically. Glass slides were cleaned during the observations to remove debris and fouling organisms by using soft, small brushes and by small pieces of razor blades attached to firm handles.

RESULTS

Three sets of experiments were conducted to test the applicability of this *ex situ* novel approach. In the first set of experiments, 20 nubbins (approximately 5 polyps each) were subcloned from a single *S. pistillata* colony (8 from the tips; 12 from mid branches approximately 3 cm below the tip) and were observed for up to 90 days. All nubbins were cultured in a single 16 l running seawater aquarium (5 $l.h^{-1}$; 25±1°C).

During the first 14-21 days, coral tissues started to regenerate lost parts by overgrowing exposed skeletons. This regeneration process enabled nubbins to firmly attach with tissue to the glass substrate or to the ring of cured glue which sometimes developed around the nubbin. Following this regenerative phase, nubbins grew horizontally on the glass substrate (a detailed example case is outlined in Fig. 1). An encrusted sheet of only coral tissue was first formed, often developing a flat ring of tissue without polyps or calcified material, around the rim of the excess glue or around the nubbin. After a few (7-10) days, calcium carbonate deposition began from the centre towards the periphery of each ring. In most cases, a thin (2-3 mm in diameter) clear tissue without calcification was always seen. Polyps appeared within 6-8 days after formation of the encrusted layer of tissue. Some of the polyp's layout appeared before major calcification took place by formation of a polyp's silhouette through a tissue rearrangement into 6 major septae and central mouths. Following calcification, the whole typical polyp was formed, including the tentacles and the axial columella.

The rate of production of polyp (mainly within the flattened areas) was impressive. After 3 months, all 20 nubbins survived, revealing on the average, 44.2±20.8 and 47.4±13.4 polyps.$nubbin^{-1}$ for branch tips and branch mids, respectively ($p>0.05$; t test; Fig. 2). Within this period, therefore, the number of polyps increased by a factor of 5-6 times, providing an average growth rate of 0.42 polyps.day^{-1}.

In the second set of experiments, 192 nubbins were pruned from tips and mid branches of the 3 *S. pistillata* colonies (64 nubbins from each colony) and were distributed evenly between 4 16 l aquaria subjected to 4 different light regimen. Three bulbs per aquarium (Blue-Blue alone, Cool-white alone, Floura alone and a combination of the above different lights) provided ample light (450 Lux.). Results (Fig. 3) indicate the superiority of the combination treatment over the uniform sources of illumination ($p<0.05$, ANOVA). After 107 days, the average sizes of nubbins in each aquarium were 33.6 in the light combination, 30.9 in the Floura treatment, 23.6 and 19.5 in the Cool-White and the Blue-Blue respectively, revealing a growth rate of 195%, 160%, 128%, 116%, respectively.

The third set of experiments was designed to compare survivorship and growth of similar size nubbins between *ex situ* and *in situ* conditions. Total of six hundred nubbins (5-15 polyps.nubbins^{-1}), were taken from 3 different *S. pistillata* colonies, and were distributed equally between inland maintained aquaria (under conditions as described in Materials and Methods) and the field, in front of the Marine Biological Laboratory at Eilat (at 5 m depth on plastic tiles, 1m above the natural reef substrate). After 40 days a complete *in situ* loss of the nubbins was recorded as compared to 95% survival in the *ex situ* treatment (Table 1). Over a 3½ month period, 60% of these nubbins survived (high survival even after several unexpected mechanical failures) of which, 65% spread over the substrate.

DISCUSSION

A successful production of coral clones by nubbing minute portions from coral colonies (at a size of a single or several few polyps each) may serve as a preferred methodology for the establishment of large numbers of genetically identical colonies (ramets) from any individual genet. These ramets can be reared *ex situ* under identical conditions and may be used as highly valuable source in studies engaged with coral physiology and ecotoxicology (Al-Moghrabi et al., 1993; Davies, 1995; Tambutté et al., 1995; Reynaud-Vaganay et al., 1997; Ritchie et al., 1997; Rinkevich

and Shafir, 2000) or as important material in the marine ornamental coral trade industry (Rinkevich and Shafir, 2000). This is further emphasized by the fact that the lack of genetically identical replicates has often hampered coral physiological studies (Ritchie et al., 1997) and that the trade industry is heavily dependent on field collected material due to the of lack of established inland broodstocks (Rinkevich and Shafir, 2000). A successful *ex situ* mass production of coral colonies will also be of help in decreasing the pressure on natural populations (Ritchie et al., 1997; Rinkevich and Shafir, 2000).

It is also well documented that marine invertebrates are characterized by a high variation in almost any aspect of their life history patterns (Kinne, 1977). This may imply that it is a difficult task to maintain these organisms *ex situ* for longer periods. However, highly improved maintenance protocols are easily available and are frequently practiced by aquarium hobbyists as well as by the industry (Adey and Loveland, 1991; Delbeck and Sprung, 1994; Sprung and Delbeck, 1997). It is also evident that different coral species require different maintenance conditions and protocols (Gateño et al., 2000) and that even different genotypes within a specific coral species may request genet-related variations when reared under identical conditions (unpublished). It is therefore important to adjust the best rearing conditions for any specific genotype. The use of high numbers of nubbins from specific coral genets and the numerous data already gathered in the literature may partially eliminate the above technical obstacle.

The results of the present study, the first which analyzes the applicability of raising nubbins *ex situ*, revealed a high survival of nubbins under inland conditions (as compared to their poor survival *in situ*) and a fast growth rate of minute coral subclones possessing only a few polyps each. Different culture conditions (such as light quality, etc.) directly affect growth rates and it is evident that the optimal conditions for maintaining and rearing large numbers of nubbins *ex situ* are not yet established. Further experiments are required to elucidate such optimal conditions.

Sexual and asexual derived colonies are the two major strategies for the establishment of *ex situ* broodstocks and to circumvent the need for wild harvested colonies (Rinkevich and Shafir, 2000). Each of these alternate approaches has its own benefits and pitfalls, but they are complementary to each other. We are therefore at the beginning of a long-term, scientific study. In order to make progress, there is now a clear need for further studies on a variety of coral species using different methodological approaches.

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Table 1. *Stylophora pistillata*: nubbin's survivorship and substrate encrusting under field and laboratory conditions.

Tested parameters	Conditions	Observational period					
		7 days		40 days		103 days	
		No. of nubbins	%	No. of nubbins	%	No. of nubbins	%
Survivorship	*In situ*	242	82%	0	0%		-
	Ex situ	288	96%	285	95%	180	60%
Substrate encrusting	*Ex situ*	0	0%	153	51%	117	39%

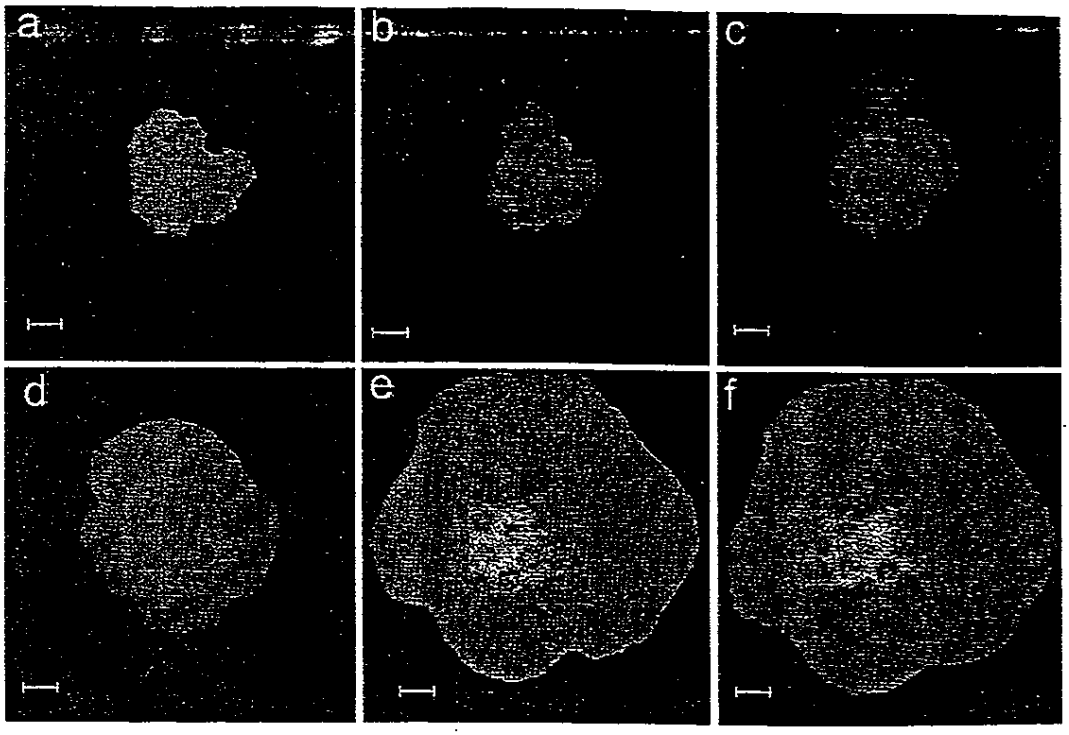

Figure 1. Surface spreading of a small *Stylophora pistillata* nubbin. Areas measured from top projection image of the nubbin. a = 10 polyps, 2 days after isolation, 14.5 mm^2; b = 16 polyps, 19 days, 15.6 mm^2; c = 17 polyps, 26 days, 26.0 mm^2 (at this stage the nubbin began to spread over the substrate); d = 22 polyps, 33 days, 29.1 mm^2; e = 23 polyps, 40 days, 74.4 mm^2; f = 28 polyps, 47 days, 86.1 mm^2. Scale bars = 1 mm.

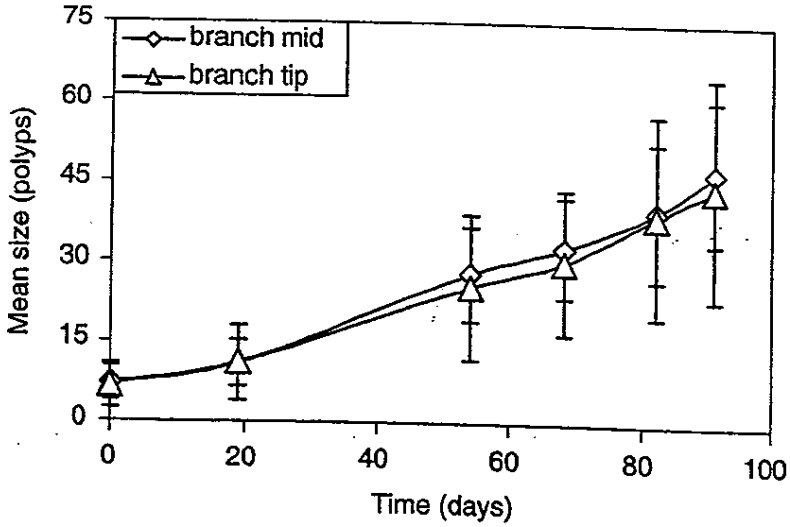

Figure 2. Growth of *S. pistillata* nubbins isolated from tip and mid branch.

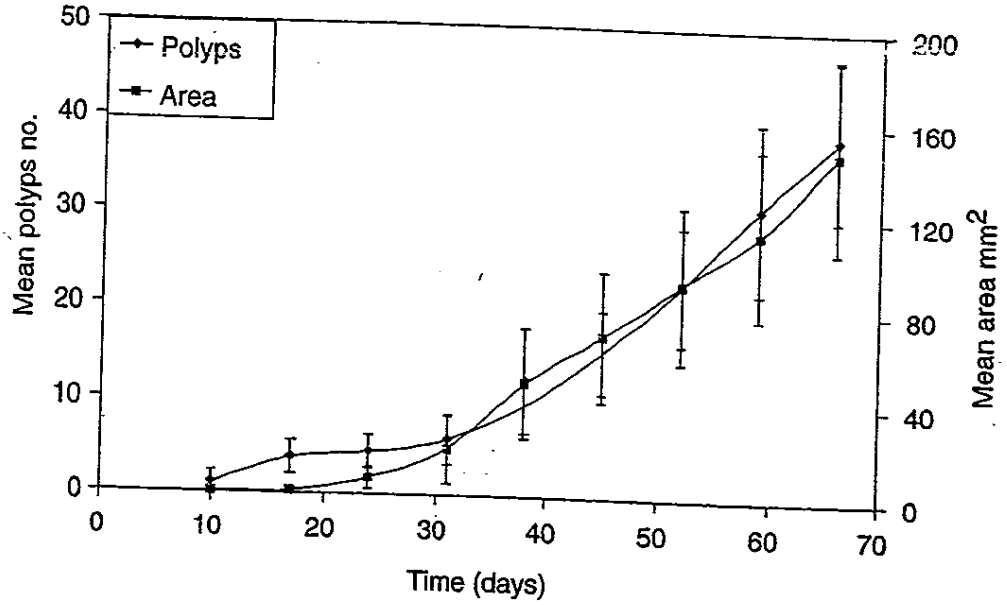

Figure 3. Growth measurements by polyp numbers and by surface area of *S. pistillata* nubbins.

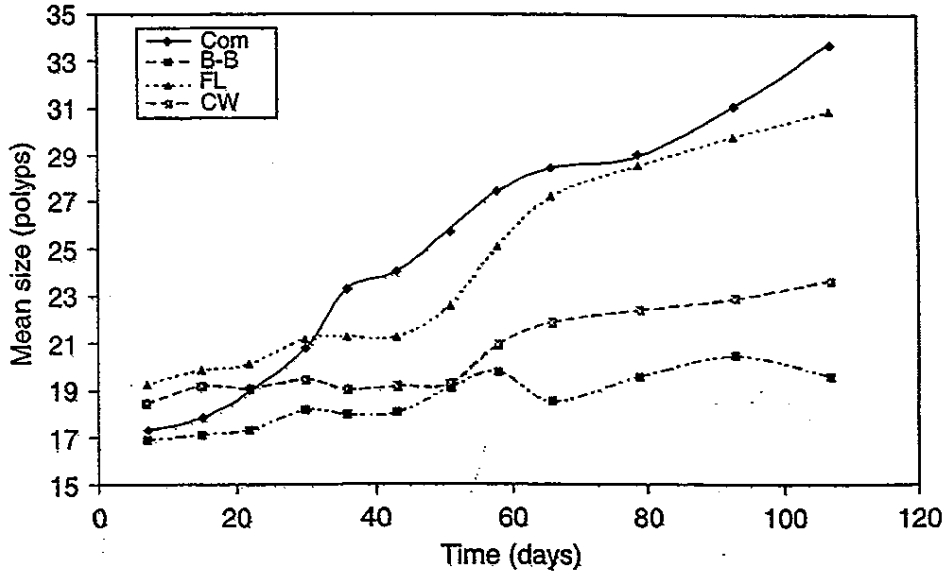

Figure 4. Growth of *S. pistillata* nubbins under four different light regimens. B-B = Blue-Blue, Fl = Floura, CW = Cool-White, Com = a combination of the above three light sources.

SUBJECT 3 – GARDENING THE CORAL REEFS

Coral reefs are among the ecosystems that suffer intense abuse by man (McClanahan 1999). Their worldwide decline (e.g. Wilkinson 1993, 1999; Sebens 1994; Rinkevich 1995; Hodgson 1999) has raised the need for urgent development of adequate restoration methodologies. In recent years, impacts from recreational activities have been classified among the most prominent devastating agents to coral reefs (Rinkevich 1995; Chadwick-Furman 1997; van Treeck & Schuhmacher 1999). Moreover, efforts to conserve degrading reefs have failed to produce significant results and rehabilitation measures did not successfully compensate for the fast degradation (Rinkevich 1995; Risk 1999). Small-size tourist-popular reefs are particularly susceptible to fast degradation through intense recreational abuse. In large reef areas, these harmful impacts may still be balanced or are compensated by the size and resilience.

The small Coral Nature Reserve at Eilat, Northern Red Sea, may serve as a test case. This easily accessed fringing reef (Loya & Slobodkin 1971) is confined into a 4.0 km long 'marine protected belt' (Ortal & Nemtzov 1997). Hundred of thousands of registered scuba dives per year are performed there on a limited number of dive sites (Meshi & Ortal 1995). High levels of colony breakage and alterations in population structure of *Stylophora pistillata,* a key coral species were documented in Eilat (Epstein et al. 1999). This contradicts the expectations for reef conservation resulting from the tight legislation and management measures that have been employed in Eilat for years. Reef recovery therefore, does not sufficiently compensate the intense destruction by recreational activities.

This has led to the development of concepts and techniques for reef rehabilitation, including the submersion of artificial reef structures (Jensen 1997; Pickering et al. 1998; van Treeck & Schuhmacher 1999), coral transplantation (e.g. Harriott & Fisk 1988; Guzman 1991; Smith and Hughes 1999) and the establishment of low-profile underwater nurseries (Rinkevich 1995, 2000).

Rehabilitation of reef ecosystems may progress through implementation of both preventive as well as active restoration measures. Rinkevich (1995, 2000) has proposed a two-step restoration strategy termed *gardening of denuded coral reefs*. The central concept of this strategy is the mariculture of coral recruits in nurseries. In the first step, a large *in-situ* pool of farmed corals is established on low profile artificial substrates. These nurseries are installed in sheltered zones and the different types of coral recruits are maricultured for several years. In the second step, nursery-grown coral colonies are transplanted to degraded reef sites. This strategy is theoretically linked to terrestrial forest plantation ideas. Tree-silviculture is a core strategy in forest restoration programs (Christensen et al. 1996). Tree cultivation has been practiced successfully for years with forest trees (Anon 1988; Vowell 1994; Berg 1995) and with mangroves (Teas 1980; Chan et al. 1988; Khoon et al. 1995).

Corals fulfill a central role in reef communities (Loya 1986, 1998; Bak & Meesters 1997). The replenishment of mechanically degraded reef sites with new coral colonies may support the reef's community structure and its biological functions. Sustainable *in-situ* coral mariculture may significantly relieve the pressure from donor reef sites that are currently the sole source for coral transplantation operations. The deployment of *in situ* coral mariculture has already resulted in significant outcomes (Bowden-Kerby 1997; Franklin et al. 1998; Rinkevich 2000), but its applicability on large-scale reef areas needs to be further evaluated (Edwards & Clark 1998).

Branching corals provide four potential types of coral material for nursery purposes. These include small colonies, branch fragments, larvae, and nubbins (minute size fragments). Young colonies settled in unstable reef areas can be collected, larvae are harvested using plankton nets, and branches are detached *in-situ* from donor colonies with cutting pliers (Rinkevich 1995). Similarly, one can harvest nubbins *ex-situ* by pruning a branch into large numbers of single or few polyp size units (Rinkevich & Shafir 2000; Shafir et al. 2001). The benefits and costs for mariculture of each one of these types of recruits and their applicability for the *in-situ* nurseries should be further

considered. Here, we studied the pertinence of *Stylophora pistillata,* one of the most abundant coral species in the Northern Gulf of Eilat (Loya 1972). This r-strategist species is characterized by rapid growth and a high reproductive rate (Loya 1976a; Rinkevich & Loya 1984). Its colonies form highly complex spatial configurations that provide habitat and shelter for many species of crabs, fishes and cryptic organisms, while the dead skeletons are inhabited by a variety of encrusting and boring organisms (Rinkevich & Loya 1979a). It is therefore a species of focal importance in Eilat's reef ecosystem that is used as a representative species to test the gardening concept.

MATERIAL AND METHODS

Donor colonies

This study was carried out in front of the H. Steinitz Marine Biology Laboratory (MBL) at Eilat, Northern Red Sea. Twenty-seven adult *S. pistillata* colonies, averaging 6.5 cm in geometric mean radius ($\bar{r}$; Loya 1976b) were chosen in depth of 5-10 m. Colonies of this group size possess about 90 major branches each (Epstein, pers. obs). All colonies were labeled *in situ* by alizarin Red S solution (10-15 mg/L) for 24 hours in transparent plastic bags as described by Rinkevich and Loya (1984). The post labeling acclimation period was 48h. Then, the colonies were divided into four groups: A (16 colonies), B (3 colonies), C (3 colonies) and 5 control colonies. Using side-cutting pliers, 10 major branches were removed from each colony of group A (ca. 10% damage), 20 branches from group B colonies (ca. 20% damage), and 30 from group C colonies (ca. 30%). The 16 colonies of group A were further divided into two: colonies 1-8 (group A1) were pruned in April 1997 (period of planulae release) and colonies 9-16 (group A2) in October 1997 (period of gonad development; Rinkevich & Loya 1979a). Group B and C colonies were pruned in April 1997. We followed the colonies for up to two years. To detect pruning impacts on reproductive activity, female gonads were counted (10 polyps/branch) in histological sections (methodology in Rinkevich

& Loya 1979a) performed on tissue samples taken from pruned and undamaged colonies each April and November of the years following pruning (1997-1999).

Mariculture of asexual recruits

We followed *S. pistillata* isolated branches in two sets of experiments. Experiment no. 1 examined survivorship of isolated branches at two reef sites, 500m apart (Epstein et al. 1999): the highly visited reef area of the MBL (60 branches) and the restricted reef area of the Coral Nature Reserve, site NR (40 branches). Experiment no. 2 compared growth and survivorship between isolated branches and small colonies maricultured at the MBL site. Three hundred and ten branches (removed from the colonies of groups A, B and C) were placed at the nursery. In addition, 140 young *S. pistillata* colonies ($\bar{r}$ = 2.5cm ± 0.8) growing on small boulders at the MBL strolling zone (< 1.5m depth) were numbered by means of plastic tags. Seventy colonies were stained *in-situ* with alizarin Red S dye, than transported to the nursery at 10m depth (Fig. 1a). The remaining 70 colonies were left in their original settling site at the strolling zone. Colonies of both groups did not differ significantly in initial weight and size (t test, $p>0.05$).

Handling procedure and nursery type

Branches were pruned *in-situ* by side-cutting pliers and than carefully transferred to the nursery substrates where they were promptly held in upright position. In Exp. no. 1, we used cement tiles that were pre-glued with branch-holding plastic clips (10 branches/tile) as nursery substrates. The tiles were securely placed at 10-12m depths at both sites, ca. 0.5m above sandy bottom or directly attached to natural hard substrates. In Exp. no.2 we used as nursery substrate five crates (1x 0.5 x 0.4m in size, each), made of plastic net (mesh size 1cm^2) that were placed at the MBL site. About 60 branches were placed on each crate. The crates were fastened to the reef bottom within an area of ca. 30m^2, two at 5m and three at 10m depth. Crates were light in weight, easy to handle and transport underwater and the design proved durable in storms.

For growth measurements, colonies and branches were brought to the laboratory where coral tissue was removed by hydrogen peroxide solution (Rinkevich & Loya 1984). Skeletons were rinsed under tap water for several minutes and oven dried (60^0C, overnight). Growth was measured as new deposited skeleton above the alizarin marked area.

Mariculture of sexual recruits

Planula larvae of *S. pistillata* were collected *in-situ* during the 1998 reproductive season (January- June, Rinkevich & Loya 1979b). Planulae were placed in petri- dishes (total 83 plates, Greiner, 35mm diameter, up to 20 larvae per dish and 85mm diameter, >20 larvae per dish). Within 3-4 days, most of the larvae settled either on walls of the dishes or on the water surface tension. Planulae that settled on the water surface were gently lifted by a thin brush and carefully attached to bottom of dry dishes before covering them with seawater. All dishes were submerged in running seawater in shaded outdoor containers. After 3 months, 30 dishes containing 400 primary polyps were transferred to an underwater nursery (10m depth) at the MBL. The nursery construction was composed of a 2.5m long iron rod shuffled 1m deep into the sandy bottom (Fig. 1b). Fifteen dishes were positioned horizontally and 15 vertically to the substrate, 1.5 m above the sand.

RESULTS

Pruning effects on donor colonies

All 6 colonies of groups B and C (20 to 30% of branches removed) died within the first month following treatment. Extensive pruning of branches was detrimental to donor colonies.

Of the group A colonies (10% branches removed), 2 colonies of subgroup A1 and 2 of A2 died within 4 weeks following pruning. Three A1 colonies died within a year while the remaining 3 colonies survived for almost 2 years. Four A2 colonies died within a year and the remaining two died shortly before the end of the second year (Table 1). During the course of the study, neither one of the control colonies had died.

Colonies of subgroup A1 were pruned in April 1997, at the peak of the reproductive season. Developing oocytes of the next reproductive season were observed in all November 1997 tissue samples taken from the 6 surviving colonies (ranged 0.8±0.6 to 2.2±0.9 gonads/polyp; Table 1). Two of the three surviving colonies had gonads on April 1998, the following reproductive period (0.4±0.5 and 1.5±1.2 gonads/polyp), but none was reproductive on November 1998 (Table 1).

Colonies of subgroup A2 were pruned in October 1997 during gametogenesis. After one month, female gonads were recorded in tissue histological sections of only two colonies (0.5±0.7 and 1.0±0.8 gonads/polyp, Table 1). Mechanical damage during the period of gonad production appears to immediately reduce reproductive activity. However, 5 months later, at the peak of the reproductive season, 5 out of the 6 were sexual reproductive, with up to 3.2±1.3 gonads/polyp. On November 1998, the two surviving colonies of subgroup A2 had no gonads. On April 1999, all colonies of subgroups A1 and A2 were dead. During each of the 4 sampling dates, at least 4 out of the 5 control colonies contained gonads. Reproductive activity of control colonies was significantly higher than in damaged colonies (Nov 1997, Apr 1998, ANOVA, $p<0.05$). On November 1998, none of the remaining five group A colonies were reproductive as compared to 4/5 of control colonies.

Mariculture: Asexual recruits

The survivorship of the branches held on cement tiles at the MBL site sharply decreased to 25% within 6 months and differed significantly from that at the NR site where 82.5% of the branches were still alive (Fig. 2, Exp. no. 1; t test, $p< 0.05$). On the plastic crates, situated at the MBL site (Fig. 2, Exp. no. 2), however, branches showed 83% survivorship after 6 months and 61% after 18 months, significantly higher than the MBL cement tiles branches ($p<0.05$, t-test). Survivorship did not differ significantly among the crates, neither between the 5 and 10m depths branches (data not shown; Duncan grouping, $p>0.05$).

Isolated branches also showed high reproductive activity (Table 1). Between 1-3 branches were sacrificed on November 1998 from branch groups A1 and A2 respectively (1- and 1.5y subsequent to pruning) for histological examination and gonads were counted in 10 polyps of each branch. All isolated branches originating from group A1 colonies contained 0.8-1.5 eggs/polyp on Nov. 98, while none of the respective donor colonies was reproductive. More importantly, even branches derived from donor colonies that died later on (nos. 2, 5 and 6, Table 1) were reproductive. Isolated branches of four out of the 6 donor colonies of group A2 were reproductive on Nov. 98, containing 1.1-1.8 gonads/polyp, whereas corresponding colonies (nos. 10 and 14) had no gonads and nos. 11 and 13 were already dead. Nursery branches can exceed donor colony life span and reproductive activity.

Yearly increase in skeleton mass of isolated branches (average initial weight 4.9g ±2.0, n=112) ranged 0.5%-45% and averaged 8.2%±9.2.

With regard to the small colonies, 44% survived within 1.5 years of mariculture (Fig. 2; n=30). Of the 70 colonies left at the original settling place that receives the highest trampling and wave energy impacts, we were able to locate only 7 (10%).

The nursery colonies were clustered into 5 size classes according to initial weight (<9.9, 10.0-19.9, 20.0-29.9, 30.0-39.9, >40g, Fig. 3a, b). Average growth year^{-1} decreased with size increase. The smallest group (<9.9g) displayed the highest growth rate (119%±60) and added 8.8g/y ±6.7 on average, as compared to 35%±14 growth rate and 24.6g±11 weight addition of the largest group (>40g, Fig. 3a, b). Yearly increase in skeleton mass of the small colonies (average initial weight 32g±28, n=30) ranged 35%-119% and averaged 75%±32. We found a significant correlation ($y=3.1939x^{2.2957}$, $R^2=0.88$, $p<0.01$) between $\bar{r}$ (which reflects colony age; Loya 1976c; Muscatine et al. 1985) and weight of small colonies (Fig. 4) revealing iterative constraints of accretive growth.

Sexual recruits

Most of the larvae in all dishes settled shortly after release. In total, 85% (n=2035) of the collected larvae settled within 3-4 days. Within two months of *ex-situ* maintenance in outdoor containers, ca. 60% died and additional mortality of 10% was recorded in the third month, leaving 875 (41%) surviving spat. Four hundred primary polyps were transferred to *in-situ* nursery at the MBL site at 10m depth. After 1 month, survivorship of 5% (20 spat) was recorded. In an earlier set of experiments, 599 freshly settled polyps were transferred to *in-situ* conditions immediately after settlement and none survived after 1 month. This time, the 20 spat remained alive through the 18 months observational period and grew rapidly. At age of two weeks (still in *ex-situ* containers), flat spat were about 7-8 polyps and <5 mm in diameter (Fig. 1c). At the age of 6 months (after 3 months *ex-situ*), basal disk diameter reached 1.0 cm and the primary up-growing branch appeared (Fig. 1d). After 10 months, basal diameter approached 2.0 cm (Fig. 1e) and within 18 months, small colonies of about 3-4 cm in diameter had developed (Fig. 1f).

DISCUSSION

Studies on coral reefs worldwide have documented the harmful physical effects of recreational activities such as SCUBA diving, snorkeling and reef trampling on corals (Talge 1992; Rinkevich 1995; Allison 1996; Chadwick- Furman 1997; Muthiga & McClanahan 1997; Rouphael & Inglis 1997). Habitat degradation has been estimated to affect coral populations through alteration of recruitment, growth, and colony (partial) mortality processes (Bak & Meesters 1999). A recent monitoring study at the coral reef of Eilat has further demonstrated that *S. pistillata* population structures differed significantly between a closed-to-the-public site and two adjacent highly visited reef sites. Results reflected differences in living coral coverage, maximal colony sizes, and colony breakage (Epstein et al. 1999). The conclusion of this monitoring program was that the 'no-use' zone policy, a management measure implemented successfully in large reef areas such as the Great

Barrier Reef of Australia (Marion 1994; Christensen et al. 1996), is not sufficient in the limited reef area of Eilat. The growing mechanical damage inflicted to corals during recreational activities, however, is not the sole illness of the Eilat's reef. As in other coastal areas with rapidly growing human populations, the presence of marine pollution agents of domestic and industrial origins has been documented for decades. The Eilat reef community has undergone numerous species extinctions and its resilience capacity has been sharply reduced (Loya 1976d, 1986; Fishelson 1995). Today, on top of pollution, the sharp conflicts between conservation and the tourist industry further decimate reef-building coral populations (Riegel and Velimirov 1991).

To alleviate mechanical degradation, Rinkevich (1995, 2000) has proposed a two-step restoration protocol termed as "gardening of denuded reefs", which is based on the *in-situ* mariculture of coral colonies in protected underwater 'nurseries'. The concept of nursery mariculture on the sea floor has already been applied on other reef invertebrates such as Tridacna juveniles grown within plastic cages (Jintana et al. 1996). Our protocol incorporates low-profile artificial substrates, as temporary coral nursery sites. First, coral material is relocated into the nurseries and maricultured there to an adequate size. Thereafter it is transplanted into degrading reef sites.

Branching species like *S. pistillata* may offer several types of coral material for gardening. In this basic study, we tested the mariculture of 3 types, *ex-situ* settled sexual recruits (spat) and two asexually derived materials, small colonies and isolated branches. The use of *S. pistillata* nubbins has also been discussed (Rinkevich & Shafir 2000; Shafir et al. 2001). Past studies that employed either one of the above types of coral materials for transplantation (Bowden-Kerby 1997; Franklin et al. 1998; Rinkevich 2000 and literature therein) did not evaluate the pros and cons for their use, nor for their potential of being appropriate to various circumstances in different reef areas. Our recent studies (this work; Rinkevich 2000; Rinkevich & Shafir 2000; Shafir et al. in press) provide for the first time analytical evaluations for the consideration of using different coral materials (Table 2).

The following discussion is mainly confined to *S. pistillata* at Eilat's reef. It is obvious that additional evaluation of other coral species at different locations will provide more complete strategic protocols for gardening of denuded reef areas.

Small colonies

The relocation of small colonies from unprotected sites subjected to frequent natural disasters (i.e. storms) and human activity (i.e. trampling) into protected nurseries successfully salvages genotypes from being lost and maintains high genetic heterogeneity (Table 2). This protocol does not involve any documented damage to transplants. Maricultured colonies maintained high survivorship and revealed high biomass addition through rapid growth. On the average, small colonies grew 75%±32 in dry skeleton weight per year, the fastest growth rates out of the three tested coral material. They require therefore the shortest mariculture phase. A colony of 2.0-3.0 cm $\bar{r}$ in size (between 20-40g in weight) that is placed in a nursery may reach, within a period of 2 years, $\bar{r}$ of 4.0-5.0 cm and a weight of up to 90g that we consider (Table 2) as a suitable size for transplantation. No *ex-situ* facilities are needed and operational costs are low. However, this coral material may not be available in all reef locations.

Isolated branches

The strategy of pruning branches from large colonies is recommended in cases where enough donor colonies are found in or near the impacted area. It is of the best use in areas where naturally fragmenting species like *Acropora* (Bowden- Kerby 1997) are common. The material retrieved from colonies may be limited and for some species this approach is not highly recommended. Pruning of more than 10% of *S. pistillata* colony branches may result in mortality and reduced reproductive activity. The reduction of a single colony into many ramets may also lead to the formation of reef-monocultures and decrease genetic heterogeneity (Table 2). The average growth rate of isolated branches was ca. 10- times lower than of small colonies. Isolated branches require therefore a longer *in-situ* mariculture period (>5y) to attain the 90g-weight class (Table 2).

Branch survivorship is highly variable, but ramets are available all year around and *in-situ* maintenance may reduce operational costs.

Sexually produced primary polyps

Ex-situ settled *S. pistillata* spat displayed fast growth rates *in-situ,* reaching a colony size of 3-4 cm diameter within 18 months. The mariculture period of these laboratory settled polyps to a $\bar{r}$ of 4.0-5.0cm is estimated as 4-5 years from day of settlement (Table 2). The *ex-situ* maintenance phase increases survivorship by several orders of magnitude as compared to settlement in nature. Large numbers of larvae can be obtained *in-situ* from a few gravid colonies without inflicting any physical damage. Surviving spat may increase the species' genetic heterogeneity. This approach is only applicable during the reproductive season and is particularly suitable for *S. pistillata*, a species that reproduces 6-7 months each year (Rinkevich & Loya 1979a, b). In order to obtain high numbers of recruits, the *ex-situ* maintenance requires laboratory facilities and higher operational costs. This strategy is highly recommended where funds are available and planulating species are abundant. It has not yet been tested on broadcasting species.

Coral nubbins

The fourth strategy is the *ex-situ* mariculture of huge numbers of nubbins (of a single or few polyps size) pruned from any single branch. It involves minimal damage to donor colonies and evidence is accumulating for fast growth rates and high survivorship under *ex-situ* conditions (Rinkevich & Shafir 2000; Shafir et al. 2001). Nubbins may need extended *ex-situ* maintenance periods (longer than 5y, Table 2). Major drawbacks are the reduction in genetic variability (mono-clones) and the high investment in manpower and laboratory facilities. This technique is especially applicable when there is an urgent need to preserve a few remaining genotypes in a demolished reef area and where other coral materials are limited.

A framework for reef restoration

Restoration ecology is emerging in recent years as an independent discipline. However, in contrast to conservation biology, restoration ecology is still lacking solid theoretical background and general guidelines (Hobbs & Norton 1996). Hobbs & Norton (1996) also emphasized the necessity to develop generalities and principles in order to form a conceptual framework for restoration protocols. With regard to coral reef restoration, the aim of the commonly used coral transplantation techniques is to speed up recovery of degrading reefs. These techniques have been criticized for not being sustainable biological tools for restoring degrading reef communities (Edwards & Clark 1998). One of their main drawbacks is the need to obtain coral material from unaffected donor reef areas for transplanting into impacted areas.

The rationale for *in-situ* coral mariculture stems from a different point of view. *In-situ* coral nurseries can supply transplantation operations with corals adapted to natural reef conditions, with minimal harm to existing colonies. The consideration of different coral species, depending on location, as well as the various coral materials for mariculture makes this approach flexible and applicable worldwide through site-specific adaptations. The basic idea of *in-situ* nurseries (Rinkevich 1995) has already been proven applicable by Bowden-Kerby (1997), who demonstrated the potential of a sheltered lagoon-like reef area to be used as a natural nursery for loosely scattered corals. Franklin et al. (1998) successfully cultured coral fragments *in-situ,* cemented into small plastic cups, and Rinkevich (2000) demonstrated the potential of low profile substrates as nurseries.

The current state of reef biodegradation worldwide results from a synergistic effect of multiple factors of pollution, overexploitation, and tourism, and requires a multifaceted approach towards sustainable ecological restoration. Unfortunately, this is impossible in many reef areas where human populations depend on coral reef resources. Therefore, heavily impacted reefs in general, and small popular reef sites in particular, require improved restoration techniques that can specifically compensate for the rapid loss.

We envisage *in-situ* nurseries as sustainable sources for coral recruits that will continuously supply coral colonies for transplantation within several years from establishment. This concept of coral mariculture may serve as a key management tool in the conservation and rehabilitation of small-scale denuded reef areas or reefs in danger of extinction.

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Table 1. Reproductive characteristics of group A (10% damaged donor colonies) and nursery branches up to 2 years after the pruning events. (D= the donor colony is found dead, 0= not reproductive).

Experimental group	Colony no.	Average number of female gonads/polyp at				
		Nov.97	Apr.98	Nov.98		Apr.99
		Colonies	Colonies	Colonies	Branches	
A1	1	1.6±0.8	1.5±1.2	0	1.3±1.1	D
	2	1.1±0.7	D	-	0.8±1.7	-
	3	1.1±0.6	0.4±0.5	0	1.1±1.3	D
	4	0.8±0.6	0	0	1.5±0.9	D
	5	1.6±0.9	D	-	1.3±2.1	-
	6	2.2±0.9	D	-	0.8±1.5	-
A2	9	0.5±0.7	0.1±0.4	D	-	-
	10	0	1.2±1.1	0	1.1±0.7	D
	11	0	0	D	1.8±0.8	-
	12	1.0±0.8	1.5±0.8	D	-	-
	13	0	3.2±1.3	D	1.5±0.5	-
	14	0	0.8±0.9	0	1.2±0.4	D
Controls	1	0	1.5±1.0	1.2±0.9	-	0
	2	2.3±0.9	2.3±1.4	0	-	0.9±0.7
	3	1.7±0.6	1.7±1.0	0.8±1.1	-	1.3±1.2
	4	2.4±1.0	2.1±1.2	1.6±1.3	-	2.2±0.8
	5	3.5±0.8	1.8±1.4	1.2±0.8	-	1.0±0.5

Table 2. Evaluation for the use of four different types of *S. pistillata* materials for reef restoration.

	Coral Materials			
Points for Consideration	Branches	Small Colonies	Coral Larvae	*Nubbins
General ecological impact	Negative- replacement of established genotypes with ramets	Positive- rescuing genotypes settled in areas subjected to frequent disasters	Highly positive- increasing survivorship of sexual recruits by several orders of magnitude	Negative- development of monocultures
Effect on survivorship	Negative- increasing colony mortality with pruning	Positive- survivor of genotypes that supposed to die in place of origin	No effect	Minimal negative impact resulting from limited pruning protocol used
Effect on reproductive activity	Negative effects on donor colonies, no effect on isolated branches	No documented effects	No documented effects	Unknown
Effect on colony pattern formation	Negative, takes considerable time for proper patterning of lost parts	No effect	No effect	Moderate impacts resulting from the limited pruning protocol used
Amount of material derived from donor colonies	Moderate, each donor colony supplies several units	Minimal, only a single unit by each genotype	Few gravid colonies may produce high numbers of larvae	Few branches from a donor colony may provide hundreds of nubbins
Availability of type material	Year round	Following the reproductive season	Only during reproductive season	Year round
Contribution of material to the species genetic pool	Reducing genetic heterogeneity	No effect	Increasing genetic heterogeneity	Highly reducing genetic heterogeneity
Potential biomass added to the reef	Moderate, few added colonies per genotype	Moderate to high, depending on number of rescued colonies	Significantly higher than natural recruitment rate	High, large numbers of added colonies per donor genotype

Table 2 (continued).

	Coral Materials			
Points for Consideration	Branches	Small Colonies	Coral Larvae	*Nubbins
Transplant survivorship	Variable, according to conditions	High	Low, but several orders of magnitude higher than under natural conditions	High
Transplant growth rate	Moderate	Fastest	Fast	Lowest
Estimated mariculture period	>5y	2y	4-5y	Longer
Working sites	All *in-situ*	All *in-situ*	*Ex-situ* followed by *in-situ*	*Ex-situ* followed by *in-situ*
Manpower	Low at pruning, transplantation and during nursery maintenance	Low at transplantation and during nursery maintenance	High at the stages of larval collection and *ex-situ* maintenance, low thereafter	High at all phases
Operational costs	Low	Low	High	High
Priority of use	Recommended for cases where coral fragments are already scattered on reef bottom with low recovery rates	Highly recommended for reefs with areas subjected to frequent disasters	Highly recommended where ex-situ facilities and manpower are available to support larval collection and maintenance protocols	Recommended where coral materials, especially branches are limited in quantities

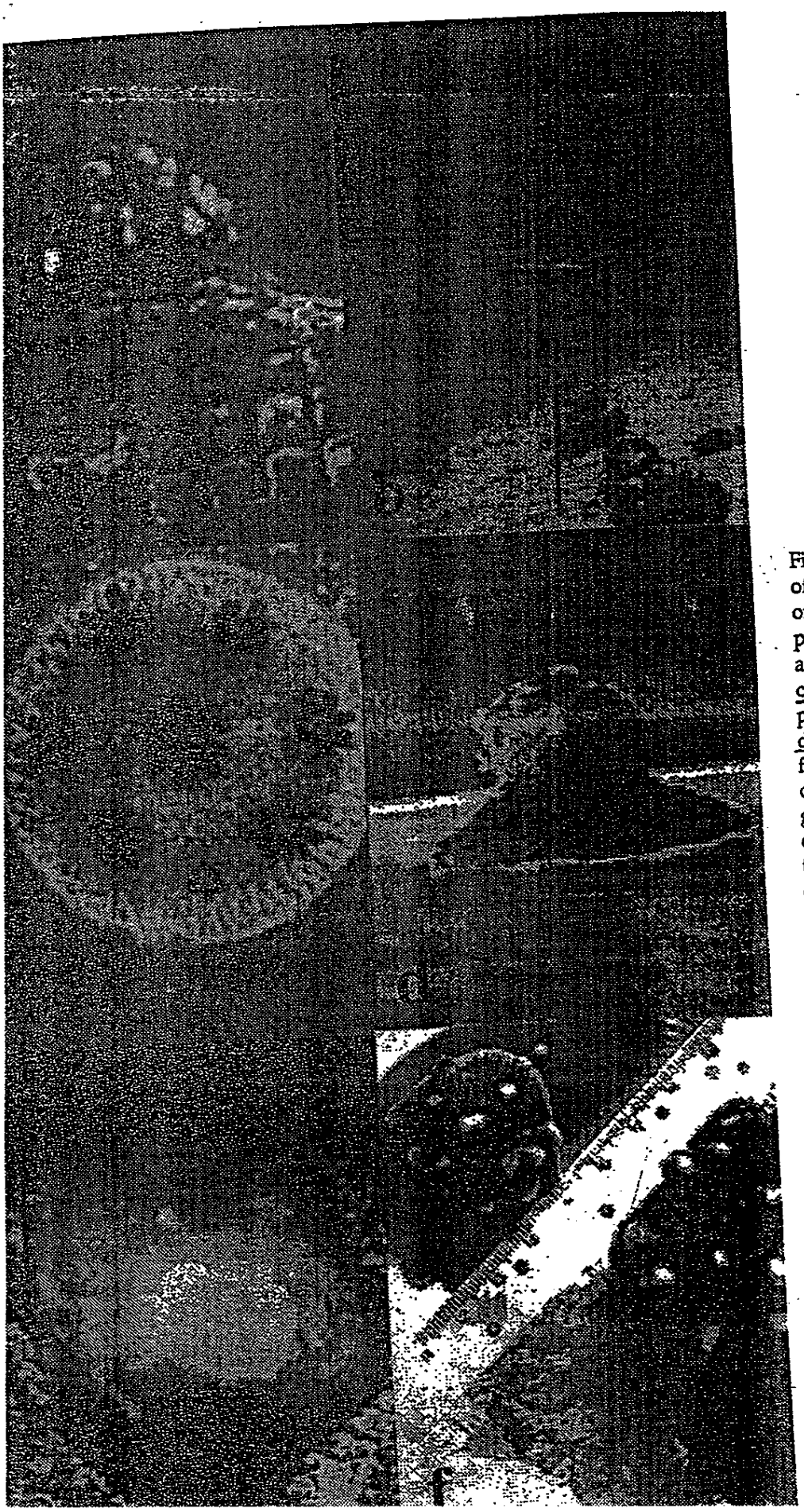

Figure 1. *S. pistillata*: *In situ* mariculture of sexual and asexual recruits: a. A colony attached *in situ* to a low-profile plastic crate. b. Spat (sexual recruits) on an *in situ* nursery, 1.5 m above bottom. c. Two-week old *S. pistillata* spat of 8 polyps size and about 5 mm diameter. d. Six-month old colony, developed from *ex situ* larval settlement. The basal disc is about 1.0 cm, supporting the upgrowing stem. e. Ten-month old colony, developed from *ex situ* larval settlement. The basal diameter is about 2.0 cm. f. Eighteen-month old, fully developed, young *S. pistillata* colonies from *ex situ* larval settlement. Colony diameter about 3–4 cm.

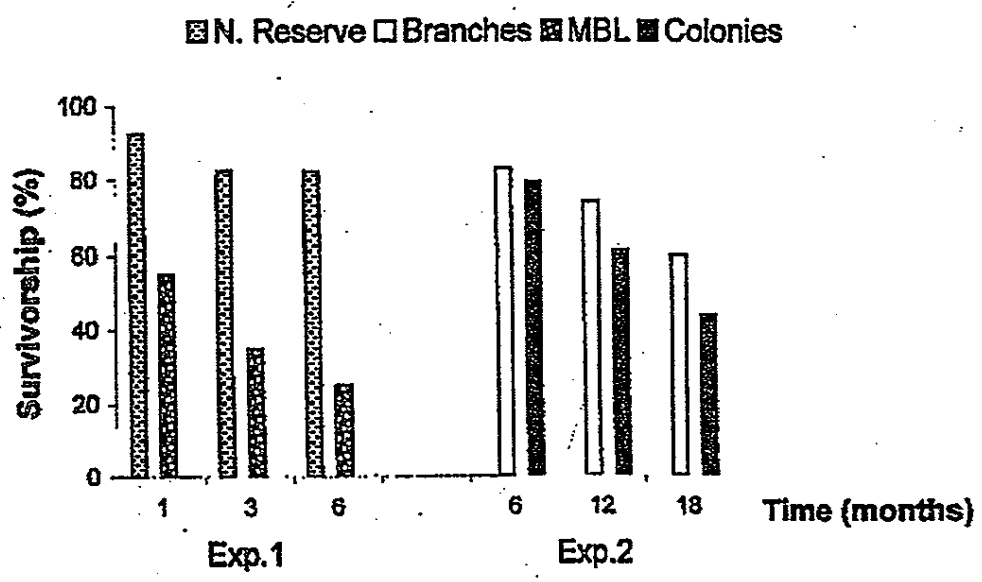

Figure 2. Survivorship of nursery-maintained, isolated *S. pistillata* branches (experiment 1, experiment 2) and small colonies (experiment 2). MBL = Marine Biology Laboratory, NR = Coral Nature Reserve.

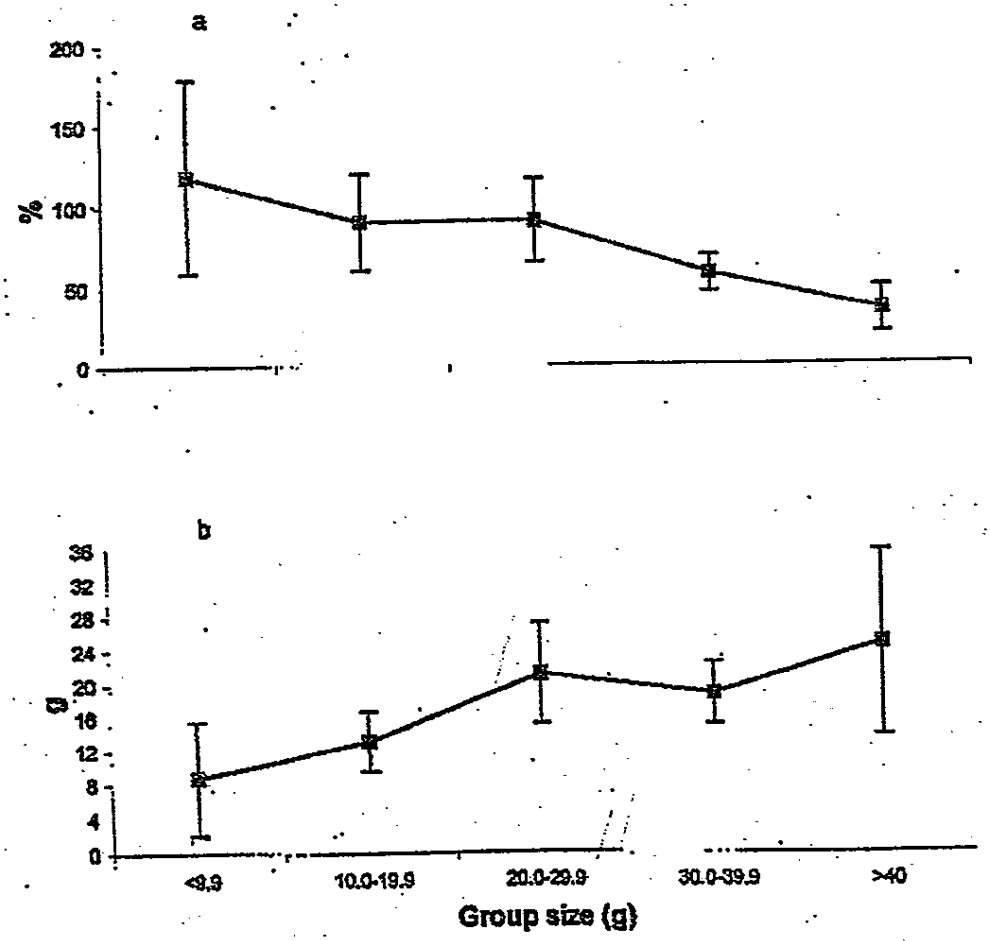

Figure 3. Small *S. pistillata* colonies at *in situ* nurseries: Yearly average growth rates (a = percentages; b = total weight added in skeleton mass) of different group sizes.

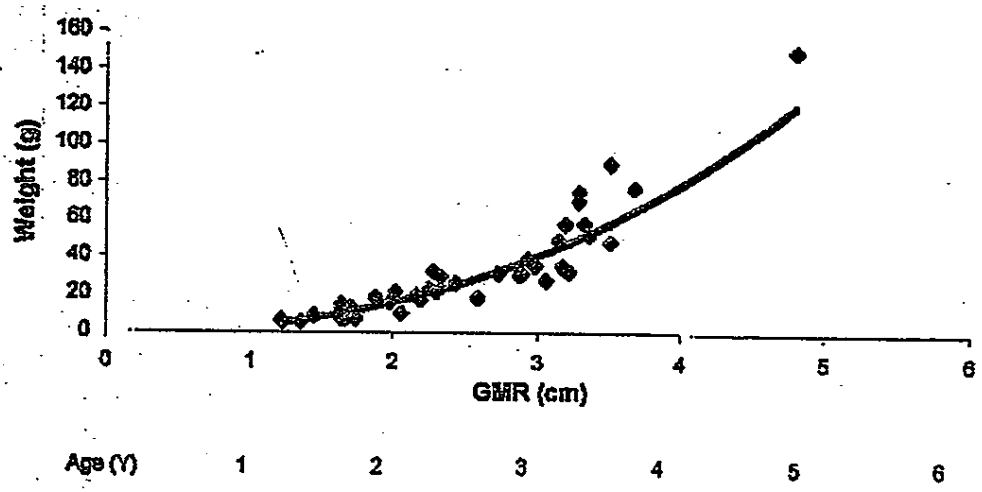

Figure 4. The correlation between geometric mean radius (GMR), weight and age of small *S. pistillata* colonies. The deduced age is calculated sensu Muscatine et al. (1985).

SUBJECT 4 – MAJOR MONITORING ACTIVITIES

Environmental data was collected from April 2000 to October 2001 from five localities (Islas Palmitas, Güiri-güiri (shallow and deep), Islas Pelonas, Esmeralda and Punta Cacique) at Bahía Culebra. As observed previously, the temperature sensors show a drop in seawater temperature in December as a result of the intensification of the upwelling in the region, but the magnitude varies over different years (Fig. 1). Resuspended sediments have been collected from four of the five sites mentioned above. Suspended sediment samples were collected from four sites. Sediments in suspension data from one of the sites, Esmeralda is presented in Figure 2. The highest values correspond to months of heavy rain, with the exception of February 2001, which might be due to heavy surge. Salinity oscillates between 31 and 37 PSU, varying greatly during the year, with the highest and one of the lowest values in consecutive months (Fig. 3).

The growth rate (mm/year) of coral species from Bahía Culebra and Islas Murciélago have been determined, and compared to means and extreme values reported from other regions in the eastern Pacific.

	Bahía Culebra		Eastern Pacific	
	Mean	Range	Mean	Extreme Values
Species				
Pavona clavus	20.6	8.5-28.0	14.2	5.4-17.4
Pocillopra damicornis	47.8	28.0-75.6	38.6	17.3-43.7
Pocillopora elegans	44.5	29.0-67.2	34.8	19.3-38.6
Pocillopora eydouxi	30.8	21.0-39.0	No data available	
Pocillopora inflata	31.5	20.0-44.0	No data available	
Pocillopora meandrina	38.5	18.0-56.0	No data available	
Psammocora stellata	13.9	6.0-21.6	No data available	

This growth rate data is interesting since at Bahía Culebra the growth rates ??? are higher than at other localities. Being Bahía Culebra an upwelling region, it was initially expected that growth rates would be lower to non-upwelling regions. Further research is needed to explain this apparent paradox.

In August of this year, the two-year invertebrate recruitment study on ceramic plates and sponges at the two sites of Esmeralda and Palmitas was terminated. We are in the process of quantifying the species and spatial distribution of the animals settled on the plates, and the species composition of the animals settled into the sponges. We have some preliminary data on the plate study, which

involves the temporal pattern of barnacle settlement, and the usage of the plates by damselfish to deposit their eggs.

For the last six months we have also been setting up a vertical temperature logging array, consisting of continuous temperature sensors attached to a vertical rope in a column of water near Güiri-güiri. These sensors take temperature readings every five seconds for a period of a little over 24 hours. The data from them is being used to elucidate water movements induced by upwelling and the tides.

A project looking at coral tissue and skeleton rejuvenation and how it is affected by sedimentation and fish bites has been started. We are using photographs to monitor the monthly progress of the tissue rejuvenation in *Pavona* corals, and we are seeing if this coral has similar rates of rejuvenation in aquaria.

Measurement of resuspended sediment at our study sites continues. We have placed six more sediment tubes, for a total of nine, at the Güiri-güiri site, since there is a hotel being constructed on the beach there. There are three separate stations of three tubes each placed across the reef in that area. We are also taking sediment samples from this beach every six months to be able to monitor changes in the sediment composition. So far we have made two of these beach sampling runs.

We are commencing a project to census the fish population of the bay, and to measure its temporal variance. There was a fish census completed in the bay in 1999, and we are hoping to compare our results with this work as well as with other work done on the Pacific coast of Costa Rica. Also, in the coming year we are planning to work on damselfish aggression and it's relation to temperature.

These projects are important for the area since the entire locality Bahía Culebra is zoned for hotels. Having a data base of baseline ecological values for the area is important for proper management. Additionally the marine invertebrate fauna of the eastern tropical Pacific is very poorly studied, and the knowledge base of local species is very limited. The plate and sponge studies are helping to better this situation.

Tumorous formations were investigated in the massive coral *Pavona clavus* in a reef composed almost exclusively of this species in northern Pacific of Costa Rica between March 1998 to April 2001. Tumors are conspicuous and easily observed structures on the surface of corals. In some cases, tumors overgrow some colonies, covering all their surface areas. A survey of the incidence of tumors in the reef showed that more than half of the population presented this phenomenon and that incidence increased with depth. Tumors and normal skeletons and tissue were studied using a variety of techniques: scanning and transmission electron microscopy, histology, *in situ* alizarin marking for growth rates and x-ray radiographs of coral slides. Macroscopic and microscopic

analysis of the skeleton showed the presence of three kinds of tumors: Type I, with thin and disorganized septae growing in a chaotic manner, type II, with larger tan normal polyps, and type III with fewer septae but much ticker than the normal ones. *In situ* tissue to tissue contacts between normal and tumorous coral fragments showed no infection of the normal tissue. We reach to the conclusion that these so called tumors can be better described as hyperplasic formations due to the lack of significant differences between cell organization between normal and tumorous tissues.

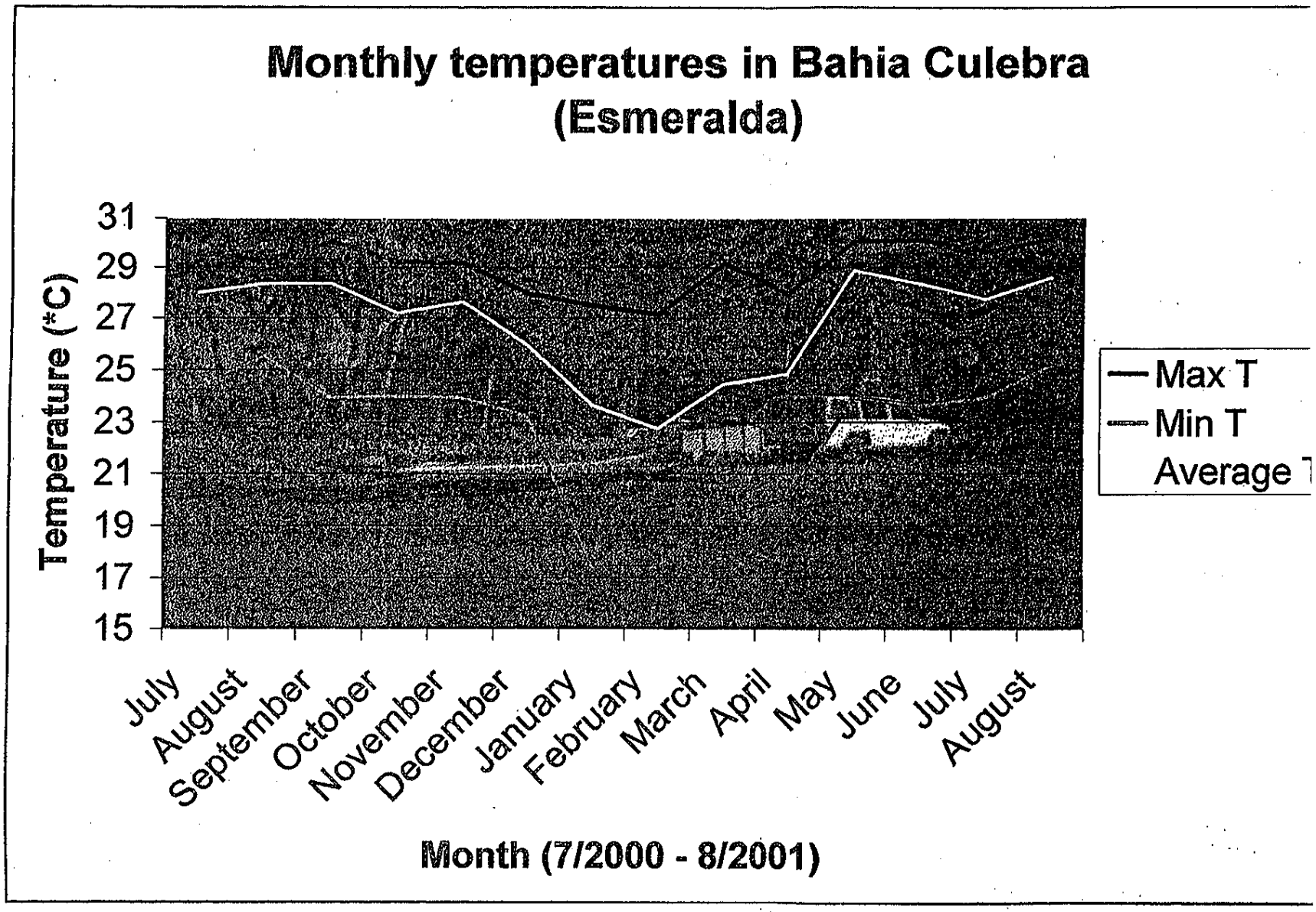
Monthly temperatures in Bahia Culebra (Esmeralda)
Temperature (*C)
31
29
27
25
23
21
19
17
15
July
August
September
October
November
December
January
February
March
April
May
June
July
August
Month (7/2000 - 8/2001)
Max T
Min T
Average T

Figure. 1

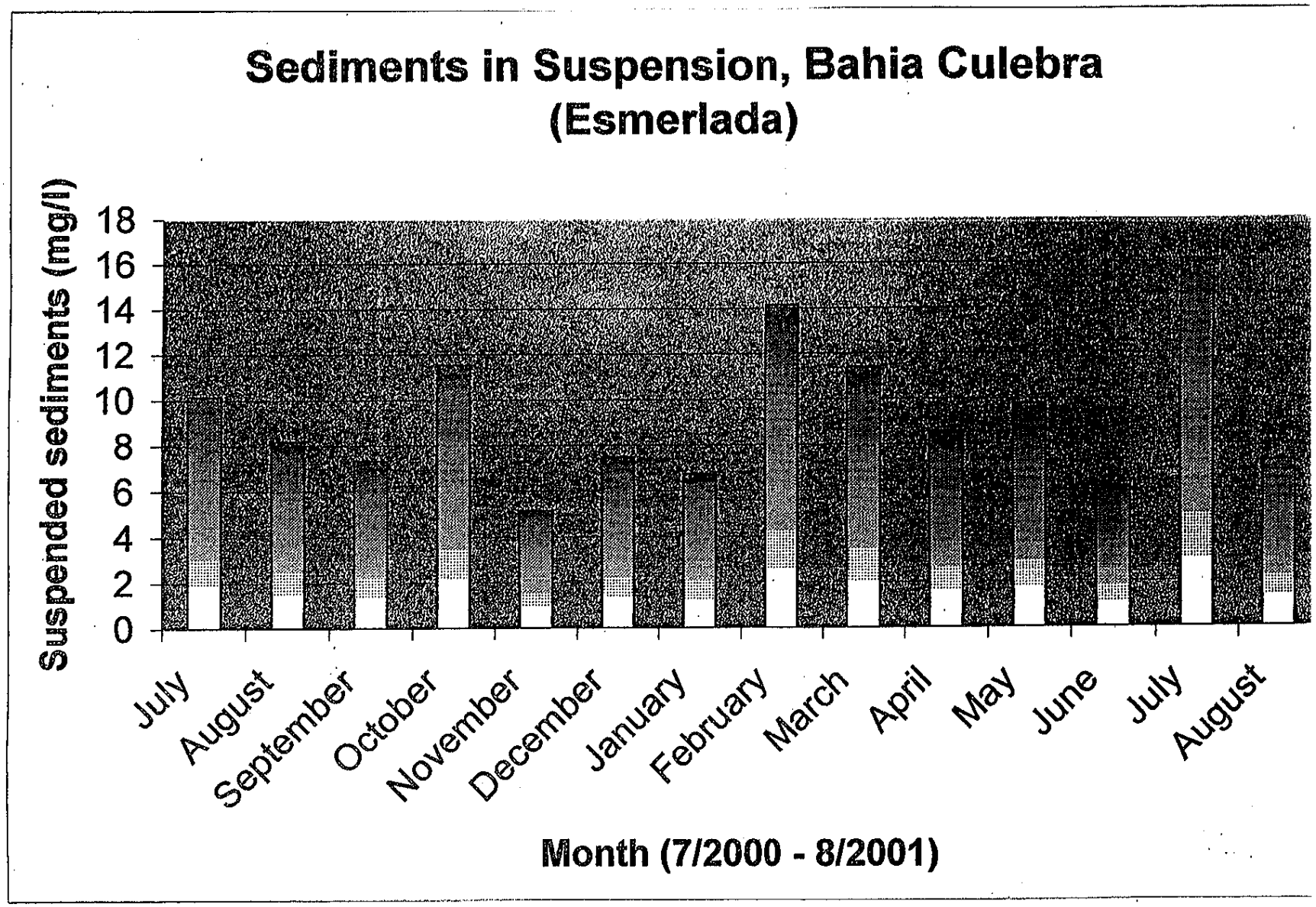
Sediments in Suspension, Bahia Culebra
(Esmerlada)
Suspended sediments (mg/l)
18
16
14
12
10
8
6
4
2
0
July
August
September
October
November
December
January
February
March
April
May
June
July
August
Month (7/2000 - 8/2001)

Figure. 2

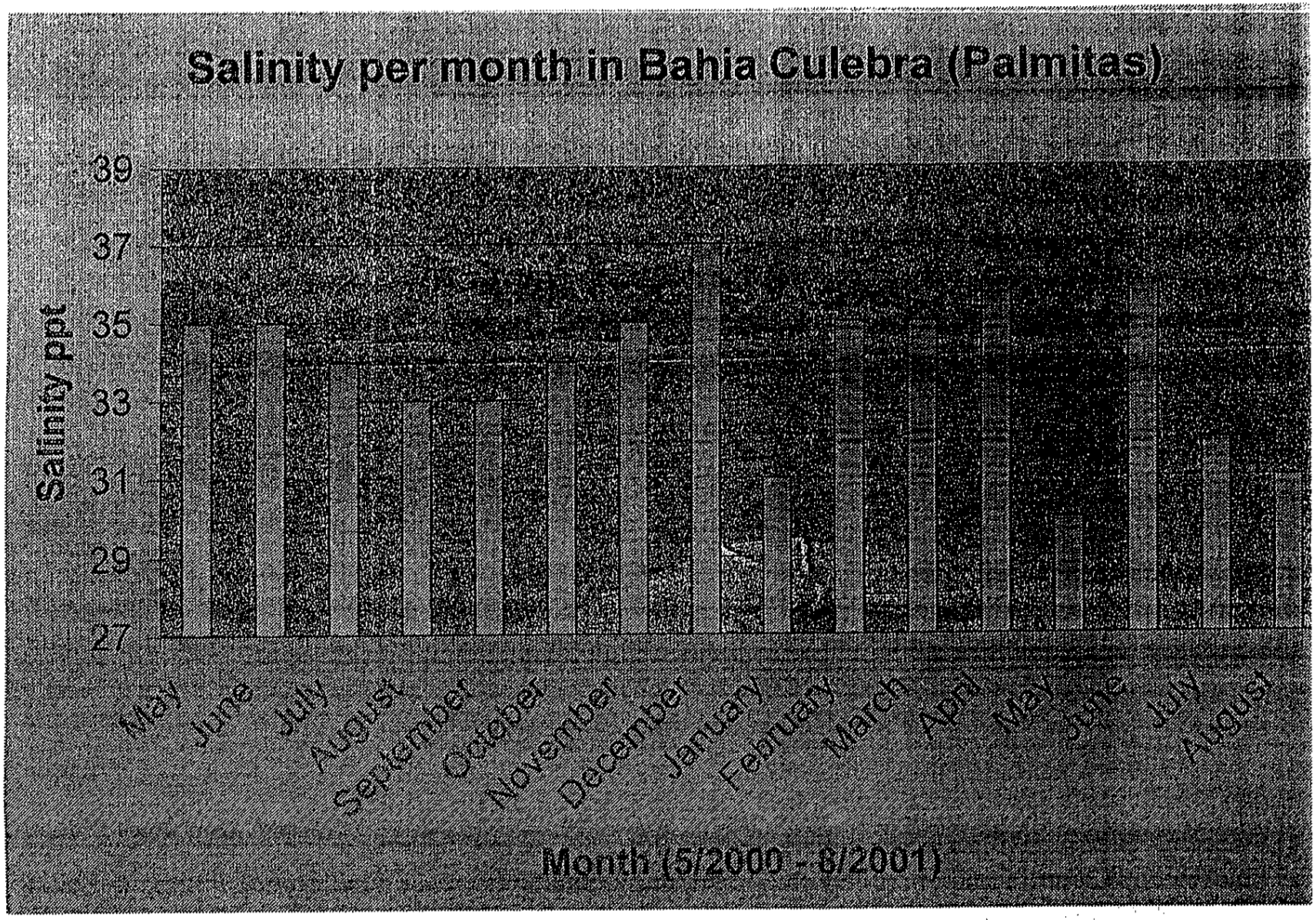
Salinity per month in Bahia Culebra (Palmitas)
Salinity ppt
39
37
35
33
31
29
27
May
June
July
August
September
October
November
December
January
February
March
April
May
June
July
August
Month (5/2000 - 8/2001)

Figure.3

C. Scientific Collaboration

Two Israeli postdoctoral fellows (Dr. D. Gateno and Dr. Y. Barki) continue to reside in Costa Rica and are working together with the Costa Rican team, as well as in direct collaboration contacts with the Costa Rican PI. An Israeli Ph.D. student (S. Shafir) is now working on the same questions in the Red Sea coral reefs and is analyzing some of the data collected from Costa Rican coral reefs.

During the fiscal year of 2001, one field trip was conducted by the Israeli principal investigator to Costa Rica's reefs (on both sides, the Pacific and the Caribbean coasts). This trip which was conducted collaboratively by both teams of scientists, continued the framework of mutual collaboration that was established in the specific areas studied.

We have selected areas in the Pacific (Bahia Culebra) and the Caribbean (Cahuita) coasts that will be studies over the next years. From the Costa Rican side, two students have been selected to develop their thesis within the framework of the project. A Costa Rican technician who visited Israel last year is collecting basic physical and biological data at the Pacific site. All studies/field trips will be mutually inspected by both PIs. In addition, Carlos Jiménez, a Costa Rican Ph.D. student has been helped in his research on paleoclimate of the northern section of the Pacific coast of Costa Rica. This research will help decipher the intensity of passed upwellings, whose intensity is apparently is regulated by El Niño and maybe La Niña. A German student, Tilman Alperman, did his Master's Thesis research at Bahía Culebra looking at the fisheries of ornamental fish. He found a decline in catch between 1994 and 2000. This decline may be due to a reduction in fishing effort because of the low profitableness of the activity. However, analysis of fish populations indicate that the fisheries can't expand any more because of possible over-fishing. His thesis:

Alperman, T.J. 2001. The fisheries of ornamental fishes in Guanacaste, Costa Rica, with special emphasis on the population dynamics of the Cortez Rainbow wrasse, *Thalassoma lucasanum* (Gill 1863). M.Sc. Thesis, ISATEC, University of Bremen, Germany. 83 pages.

D. Description of the Project impact

As mentioned above, (Parts A, B of Section I and in Part D of Section II), the results of the projects have already been published in the 10 manuscripts (2 during 1999, 4 during 2001). Detailed evaluation of "reef management policies", the concepts for reef domestication, reef restoration

policies as developed from the studies and the other results obtained from the collaborative studies will help the decision making authorities for choosing better protocols in reef management. We have also started to evaluate genetic structures of coral populations and geometric characteristics of isolated branches. This opens novel routes and opportunities for reef conservation. Detailed monitoring data received, will help us in understanding the year-to-year changes in Costa Rican coral reefs. This will establish a "data bank" of physical-chemical reef conditions. In addition, the data being generated has been used for several projects apart from this one: zooplankton distribution and seasonal changes in Bahía Culebra, the effects of El Niño in Bahía Culebra and paleoclimatic reconstructions.

E. Strengthening of developing country institution

The mutual work of the two Israeli scientists residing in Costa Rica with the local scientific group and the recent visit of the Costa Rican team to Israel further strengthen the CIMAR scientific activities. Close studies that are done provide the opportunity to transfer ideas, data, knowledge and specific scientific issues to local students. Several meetings with other scientists from CIMAR were already conducted, for the purpose of involving additional scientists in the projects and several close collaborative study projects have been estblished between CIMAR and Univ. Bremen, Germany.

F. Future work

No changes in future studies. The work will proceed as scheduled.

Section II

A. Managerial Issues

Not applicable

B. Budget

No major changes

C. Special concerns

none

D. Collaboration, publication, training, travel

Collaboration has been detailed in part C. of Section I. Ten manuscripts were published under the AID-CDR project:

1. Tom, M., Douek, J., Yankelevich, I., Bosch, T.C.G. and Rinkevich, B. Molecular characterization of the first heat shock protein 70 (HSP70) from a reef coral. Biochem. Biophys. Res. Commun. 262, 103-108, 1999.
2. Epstein, N., R.P.M. Bak and B. Rinkevich. Implementation of small scale "no-use zone" policy in a reef ecosystem: Eilat's reef-lagoon six years later. Coral Reefs 18, 327-332, 1999.
3. Barki, Y., J. Douek, D. Graur, D. Gateño and B. Rinkevich. Polymorphism in soft coral larvae revealed by amplified fragment-length polymorphism (AFLP) markers. Mar. Biol. 136, 37-41, 2000.
4. Gateño, D., Barki, Y. and B. Rinkevich. Aquarium maintenance of reef octocorals raised from field collected larvae. Aquarium Sci. Conservation 2, 227-236, 2000.
5. Rinkevich, B. and S. Shafir. *Ex situ* culture of colonial marine ornamental invertebrates: Concepts for domestication. Aquarium Sci. Conservation 2, 237-250, 2000.
6. Epstein, N., R.P.M. Bak and B. Rinkevich. Toxicity of 3rd generation dispersants and dispersed Egyptian crude oil on Red Sea coral larvae. Mar. Pollut. Bull. 40, 497-503, 2000.

7. Epstein, N. and B. Rinkevich. From isolated ramets to coral colonies: The significance of colony pattern formation in reef restoration practices. Basic Appl. Ecol. 2, 219-222, 2001.

8. Shafir, S., J. Van Rijn and B. Rinkevich. Nubbing of coral colonies: A novel approach for the development of island broodstocks. Aquarium Sci. Conserv. 3, 183-190, 2001.

9. Jiménez, C., J. Cortés, A. León and E. Ruiz. Coral bleaching and mortality associated with El Niño 1997/98 event in an upwelling environment in the eastern Pacific (Gulf of Papagayo, Costa Rica). Bulletin of Marine Science 69, 151-169, 2001.

10. Epstein, N., R.P.M. Bak and B. Rinkevich. Strategies for gardening denuded coral reef areas: The applicability of using different types of coral material for reef restoration. Restoration Ecol. (in press).

Manuscripts 1-6 were appended before. Manuscripts 7-9 are appended here.

Travel and training: A trip to and from Costa Rica was recently activated, where the Israeli PI visited the Costa Rican colleagues for 1 week, for discussions and trips to the field. As stated before, a tight collaboration has been established on a day-to-day basis, as the result of the two Israeli postdoctoral fellows residing in Costa Rica. This is in addition to the direct contacts through e-mail, faxes and fast mails between the two laboratories. Funds were also used for supporting Jorge Cortes at the IX Congreso Latinoamericano sobre Ciencias del Mar (COLACMAR), San Andres Isla, Columbia. Universidad Nacional de Colombia, Sede San Andres, 16-20 September, 2001. (Latin American Congress on Marine Sciences).

E. Request for American Embassy, Tel Aviv

None.