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Strategies for Mitigating Anthropogenic Stress Effects on Gulf of Aqaba Reefs

M26-010

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FINAL REPORT

Covering the period from *July 1, 2007 to April 31, 2010.*

Submitted to the US Agency for International Development, Bureau for Global Programs, Field Support and Research; Center for Economic growth and Agriculture Development.

TITLE OF PROJECT: Strategies for Mitigating Anthropogenic Stress Effects on Gulf of Aqaba Reefs

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2) Table of Contents

Cover letter	*1*
Cover Sheet	*2*
Table of Contents	*3*
Executive Summary	*4*
Research Objectives	*5-7*
Methods and Results	*7-27*
Impact Relevance and Technology Transfer	*28-29*
Project Activities/Outputs	*29-30*
Project Productivity	*30*
Future Work	*30*
References cited	*30-31*

3) Executive Summary:

The coral reefs in the northern Gulf of Aqaba/Eilat (Jordan/Israel) were of the most diverse reefs known, and are at the center of the economical development of the cities of Aqaba and Eilat that are centers for tourist activities. Yet, the coral reefs along the Gulf's shores are under severe human pressure, degrading rapidly. Ironically, the tourist industry serves as the major culprit for the reef decline in the Gulf. The overall aim of the project, entitled: 'Strategies for Mitigating Anthropogenic Stress Effects on Gulf of Eilat/Aqaba Reefs' targets to respond this threat. The two teams of scientists put three major aims to the research: 1. Quantify and rank the effects of the main anthropogenic stressors on reproduction, growth, and survival of corals in the Gulf of Aqaba/Eilat reefs: (i) Artificial illumination from marinas, vessels, and seaside facilities; (ii) Eutrophication. 2. Studying the impact of "humanly-preventable" stressors combined with temperature and UV on the metabolism of corals in the laboratory and in the field using two branching corals *Stylophora pistillata* and *Acropora eurystoma* and one massive coral, *Favia favus*. 3. Training young scientists by holding a joint workshop for all students to assure quality control and inter-calibration of methods, result reporting and analyses formats. Our results showed enhanced growth in all corals' genotypes when fed to repletion and reduced growth at the UV- treatment. Chlorophyll per fragment surface area over time has been raised in all tested genotypes under high feeding regimen. When fed to repletion all genotypes showed enhanced growth in zooxanthellae per surface area from the first to the second time point, but this growth in zooxanthellae vanished at the third time point. In addition, the surprising results for enhanced growth rates of illuminated coral colonies, the negative impacts of artificial lights on coral growth and survivorship and the non-significant measured rates of net photosynthesis and dark respiration – all may open new directions for using improved methodologies in the Gulf, in a way that tourism activities will not harm corals' livelihood. Results also revealed that the use of Halogen commercial artificial light is most deleterious to corals and that nighttime artificial light may cause misdirection of swimming coral larvae and therefore may have negative effect on recruitment of corals in the illuminated areas. Along the scientific activities, the partners trained an Israeli M.Sc. student and a Jordanian Ph.D. student (in addition to 8 international students) in various, up-to-date methodologies available to biological oceanographers in basic and applied research (areal chlorophyll concentration, zooxanthellae density, and pigment concentration per a zooxanthella cell, PAM and Fluorometer measurements, capture of planulae, etc.). While the project results are preliminary, their importance is in providing authorities, reserve managers and environmentalist NGOs clear answers regarding the severity and threshold levels of incipient damages caused by eutrophication and artificial lighting under various global climate change scenarios. This will allow formulating contingency plans for legislation needed to deal with foreseeable future developments.

4) Research Objectives:

Coral reefs provide a wide array of goods and services to human societies (Moberg and Folke, 1999; Spurgeon, 1992). Due to their economic importance and aesthetic attributes, there is an increasing interest in using reef services for the benefit of local populations. However, coral reefs worldwide face anthropogenic damages and many are affected by the same economic activities they sustain (Wilkinson et al., 1999; White et al., 2000). Human activities that cause reef degradation generate economic costs by interfering with the flow of goods and services provided by coral reefs (Wielgus et al., 2002). One of the fastest growing uses of coral reefs is recreation (Zakai and Chadwick-Furman, 2002), which constitutes a substantial part of the economy of many reef regions (Tratalos and Austin, 2001; White et al., 2000). Coral-reef biodiversity (Wilkinson, 1996), live coral cover (Pendleton, 1994) and water clarity (Wilkinson, 1996) are valuable attributes to the provision of recreational services of coral reefs. Correspondingly, coral diversity, coral species richness, living coral area and coral growth rates are vital parameters for the system's structure and function (DeVantier et al., 1998). Both trends are intermingled. For example, in Jamaica and Thailand, diving reef-tourism has sharply decreased because of coral reef degradation (Wilkinson, 1996). Cesar (2000) estimated the current and future financial losses to coral-reef tourism caused by the destruction of 1 km^2 of reef in Indonesia to be between US $27,900 and $100,800. Berg et al. (1998) valued attribute loss to represent a cost of between $5,500 and $368,000 per square kilometer for coral reefs in Sri Lanka. Correspondingly, Hodgson and Dixon (1998) predicted gross financial losses in the order of $40 million over a 10-year period because of tourism and fishery declines in the coastal area of Palawan, in the Philippines, resulting from increased sedimentation produced by logging.

Coral reefs contribute to the economies of at least 100 nation states and the livelihoods of over 100 million people. Regions like the Great Barrier Reef and the Caribbean reef systems contribute billions of dollars to their local economies (Koop et al., 2001). No such data are available for the reefs of the Gulf of Aqaba/Eilat, but they are in the hundreds of millions US$.

Reef resilience, environmental changes and the effects of elevated anthropogenic activities on coral reefs have become a major concern worldwide. However, the impacts of each type of the list of human activities have been difficult to extract from the synergistic impacts of other types of activities (Rinkevich, 2005). Although studies on different attributes of reef health can help to discern the different types and rates of reef degradation, this is not always a simple task (Edinger et al., 2000). This is also true for the coral reef at Eilat, as this reef reacts to disturbances in an increasingly human-impacted environment (Rinkevich, 2005).

The coral reefs in the northern Gulf of Aqaba/Eilat (Jordan/Israel) were of the most diverse reefs on earth. The reefs, which are of prime human interest, have been at the center of the economical development of the cities of Aqaba (Jordan) and Eilat (Israel). Both cities are major destinations for European tourists, because of their relative geographical proximity. Aqaba and Eilat are also main recreational hotspots for the citizens of both countries. Yet, coral reefs along the Gulf's shores are under severe human pressure, degrading rapidly. In Israel, the reefs have been considered as being at critical stage, and the reefs in Aqaba, Jordan are suffering as well. Further deterioration of the reefs may literally endanger the livelihood of the two cities by undermining the very base of the tourist industry and fishing. Ironically, it is the tourist industry that serves as the major culprit for the reef decline in the Gulf by excess SCUBA diving and snorkeling, which exceed its recovery rate. Responding tompart of the stressors involved with increased tourism and anticipated global changes, this project, entitles as: 'Strategies for Mitigating Anthropogenic Stress Effects on Gulf of Eilat/Aqaba Reefs', carries three major aims:

1. Quantify and rank the effects of the main anthropogenic stressors on reproduction, growth, and survival of corals in the Gulf of Aqaba reefs: (i) Artificial illumination from marinas, vessels, and seaside facilities; (ii) Eutrophication.
2. Studying the impact of "humanly-preventable" stressors combined with temperature and UV on the metabolism of corals in the laboratory and in the field using two branching coral

species *Stylophora pistillata* and *Acropora eurystoma* and one massive coral species, *Favia favus*.

3. Training young scientists to assure quality control and inter-calibration of methods, result reporting and analyses formats.

Above suggested study may provide significant information both from an economic and ecological perspective. Clearly, the beneficiaries are both countries, through the economic and cultural value of healthy coral reefs and their sustained conservation for future generations. Moreover, the aim of this study is also to provide environmental protection ministries, nature reserve authorities and NGOs with tools to use in planning their strategies for the protection of Red Sea coral reefs. These include reef zoning and regulation of touristic activities.

5) Methods and Results:

The three specific aims of the research were divided between the two collaborating laboratories, as follows:

i. IOLR team performed the study revealing the connections between eutrophication and temperature increase, acidification and UV.

ii. MSS team performed the study revealing the impacts of nighttime illumination on coral reproduction (along the Jordanian shore, next to the MSS).

iii. The two partners, following discussions performed by both PIs, executed the training of young scientists, simultaneously.

All studies were performed in accordance with nationals' regulations and specific permits that were granted to both PIs by national authorities.

Methods performed by the IOLR team

Coral collection, experimental set-up and fragment preparation

Coral colonies were collected by SCUBA diving from the 10 meters depth reef in front of the Interuniversity institute for Marine Sciences in Eilat, Israel (IUI), located at the northern Red Sea (29° 30' N, 34° 55' E), Gulf of Aqaba-Eilat. Three species of branching corals, *Stylophora pistillata, Acropora sp., Pocillopora damicornis* and one species of a massive coral, *Favia sp* were used. The collected colonies ranged in size from 15-20 cm diameter (all potentially sexually reproducing colonies). Collected colonies were first acclimated at the IUI underwater tables in order

to provide a baseline level of physiological parameters for the experiments. Each colony was photographed and its morphological parameters were recorded. Than the branching species were cut into fragments of ~ 2-3cm, each, glued to substrates labeled by numbered tags and marked by alizarin Red S. Fragments from each colony were divided randomly into water tables (troughs), 250 fragments per trough. All specimens were grown at natural sun light under 80% shade nets (Fig. 1) and then subjected to four treatment combinations (Table 1). Fragments were fed by *Artemia* brine shrimps (600,000 *Artemia* brine shrimps, 3 times a week). Fragments' positions in the troughs were rotated and weighed each week. The initial wet weight (ANOVA, $F = 1.789$ and $p = 0.112$) of individual fragments did not vary between the treatments. The computer program SPSS version 15.0 (Norusis 1999) was used for statistical analyses.

Figure 1. Coral fragments glued on plastic pins (attached to rigid netting), each labeled by a numbered tag. The fragments were acclimated for ambient *ex-situ* conditions before inserting them into the different experimental manipulations.

Five fragments from each one of the sampled colonies were taken, prior to alizarin labeling, for evaluating the following biomass parameters: aerial chlorophyll concentration, zooxanthellae density, pigment concentration per zooxanthellae cell and photosynthetic quantum yield and light saturated photosynthetic rate P_{max}.

Table 1. Experimental procedures

Temperature	UV dose	Nutrition
Ambient	Low	Ambient
3°C above ambient	Low	Ambient
3°C above ambient	Low	high
3°C above ambient	Ambient	high
Ambient	Ambient	Ambient

Chlorophyll fluorescence measurements

In order to examine the fluorescentic behavior of the zooxanthellae two kinds of instruments were used: Pulse Amplitude Modulated fluorometer (Dual PAM-100, Walz, Effeltrich Germany) and Fluorescence Induction and Relaxation (FIRe fluorometer system, Satlantic, Canada). The PAM was also used as an actinic light source (red LED's, 720 nm). The measurement setup was based on basic light curve of six light intensities (22 μE; 126 μE; 339 μE; 531 μE; 825 μE; 1594 μE); each light intensity last for 5 minutes. Before starting the measurement, 10 minutes of dark-adapting periods were applied for every sample. After the light curve ended, the samples recovered at dark for 20-30 min, being a second measurement phase was taken in order to evaluate the rate of recovery and possible damages, if occurred. Fluorescence measurements were done during mid-day (10 AM until 3 PM) in 30ml light sealed plastic chamber for intact corals and in 3 ml chambers for isolated zooxanthellae and algae coulters. Dual PAM data was analyzed using MATLAB (R2008a) script.

The effect of coral metabolism on the zooxanthellae

Corals were grown in 40L glass aquariums under constant seawater flow in an open system of outdoor water tables under shade nets of 80%. An extra power head pump was placed in each aquarium to increase water flow. Fragments were divided into 2 treatments, fed and filtered water. The "fed" corals were fed 2-3 times a week with one-day-old live nauplii of *Artemia salina* that were freshly hatched on sight in separate aquarium. The water flow was stopped for 3 hours during the feeding time in order to allow the polyps to catch food. Temperature was controlled by a constant flow of seawater around the aquariums. The "filtered water" corals grew in freshly filtered water. The filter was made of wide plastic bottle with 60 μm plankton net and over flow tube in order to prevent flow of unfiltered water in case of clogged filter. The filter was cleaned every week and the net was replaced every month. Corals were taken for fluorescence measurements every 3-6 month. Oxygen and fluorescence measurement together were done at the end of the experiment after a period of 18 month. Control fragments were held under seawater at 5 m depth.

The effect of coral extract on the zooxanthellae

Intact fragments-holobiont of *Stylophora pistillata* were measured with the Dual PAM using the standard light curve. After fluorescence measurements coral tissue was removed using high pressure Air-Brush along with FSW (0.2 μm) and collected into 50 ml Falcon tubes. The air-brushed samples were homogenized using a Miccra D-9 electric homogenizer. Samples were moved to 15ml Falcons and then centrifuged with a Sigma 4K15 cooling centrifuge for 5 min, at 5000rpm in 4°C. The supernatant containing the host tissue extract was moved to another 15ml Falcon and was pH adjusted to sea water (pH 8.1, Sutton and Hoegh-Goldberg, 1990) using 0.1*N* NaOH and then stored at 4°C until used (no more than 20 min. later). The pellet containing the zooxanthella cells was washed with 6ml of FSW (0.2 μm) and centrifuged again for another 5 min, at 5000 rpm, at 25 °C to prevent extra stress on the algae cells. This process was repeated until the soup was clear. The freshly isolated zooxanthellae-FIZ were moved to three-25ml Erlenmeyer bottles. Each Erlenmeyer was filled with 3ml of FIZ and 2ml of Host-Extract and left for incubation on a linear shaker. Three samples were taken, one sample for each hour, for standard light curve fluorescence measurements using the Dual PAM.

Physiology tests

Protein concentrations of host and total samples were measured using a Bradford assay kit (Bio-Rad) (Bradford protocol-Bradford, 1976) with BSA as a standard. Protein readings were done with Thermo Scientific Multiscan Spectrum 1600 plate reader using 96 well plates. Each total protein sample was sonicated with Branson Sonifier B-12 for 10 seconds on ice to brake open the zooxanthellae. Z2 coulter counter (Beckman Coulter) was used to count the number of zooxanthellae cells for every sample. In order to prepare the sample for cell counting with the Z2 15μl of FSW containing zooxanthellae cells were taken from the original sample and 1000 times diluted in 15ml of FSW. For Chlorophyll measurements zooxanthellae-containing samples were centrifuged at 5000 rpm for 5 min. at 4°C. Supernatant was removed, one ml of 95% acetone was added to each sample and samples were incubated at 0°C in dark over night. Chlorophyll was then red in the following day by Thermo Scientific Multiscan Spectrum 1600 plate reader in 2ml glass cuvettes. The samples

were analyzed at wavelengths of 630, 664 and 750 nm. Dilutions with 95% acetone were performed if the peak of optical density (OD) at 664nm exceeded a value of 1. Chlorophyll was calculated using the following equation:

Chlorophyll (μg) = 11.43*(OD (664)-OD (750))-0.4*(OD (630)-OD (750))

Methods performed by the MSS team

Samples collection and preparations

Mature colonies of *Acropora eurystoma, Stylophora pistillata* and *Favia sp.* were first collected from the Jordanian coast of the Gulf of Aqaba from 5m to 17m depths. In a second set of experiments, *Galaxea fascicularis* colonies were used as a replacement for the *Favia* species. This is because of the limited number of available *Favia* samples. The collected coral colonies were left in a flow through aquaria in the laboratory for some time to acclimatize for the laboratory conditions. The healthy colonies were then fragmented, developing sets of nubbins to be used in the laboratory and field experiments. Coral fragments were glued to PVC pieces and/or to small plastic tubes by epo-putty adhesive. After few days of incubation in the laboratory, the healthy fragments were fixed to Aluminum Racks specifically prepared for this purpose and returned back to the sea for acclimatization (Fig. 2) and were then used in laboratory and field experiments.

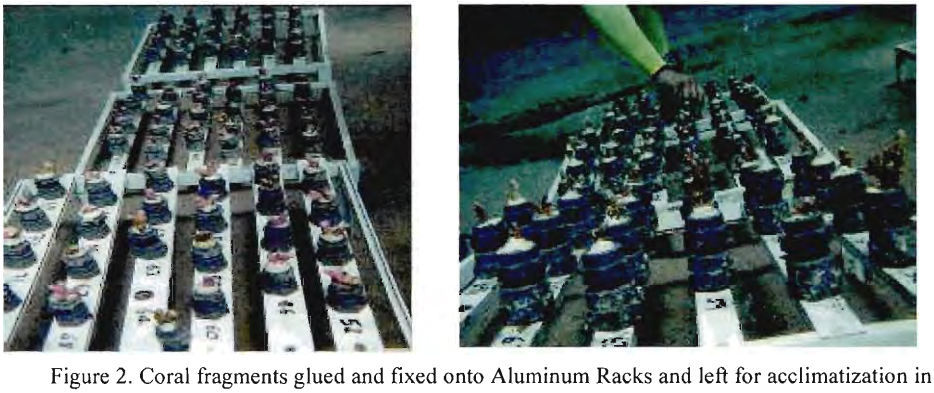

Figure 2. Coral fragments glued and fixed onto Aluminum Racks and left for acclimatization in the sea

Laboratory incubations

Fragments of the three selected coral species were prepared and left for acclimatization in the sea for two months. The samples were then brought back to the laboratory and weighed using digital balance for buoyant weight record. Four commercial light bulbs (Halogen, Sodium, Metal

Halide and mixed Argon and Nitrogen Lights) were used as illumination sources in four flow-through aquaria. Flow rates and light intensities were adjusted to become equal among the four aquaria (Fig. 3). Equal numbers of fragments from the three selected coral species were subdivided among the four incubation tanks.

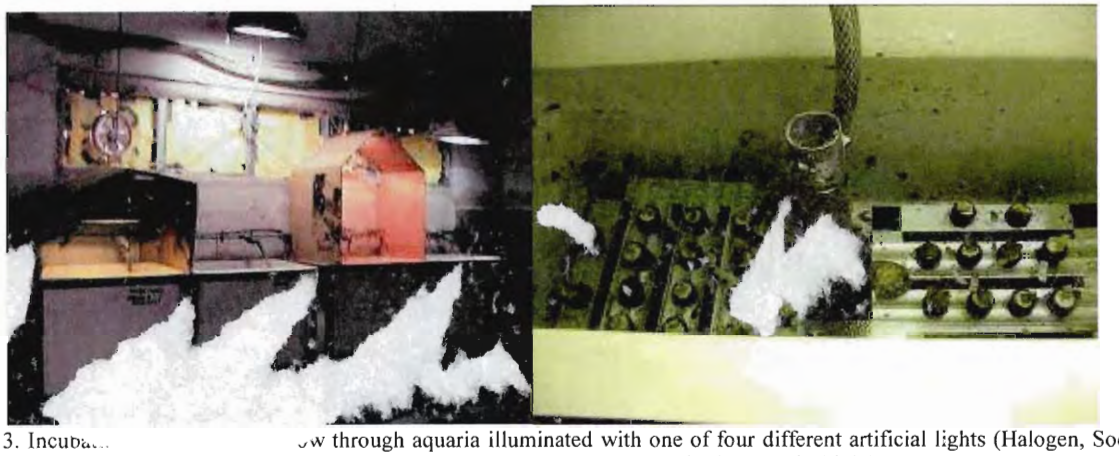

Figure 3. Incuba... ...w through aquaria illuminated with one of four different artificial lights (Halogen, Sodium, Metal Halide and mixe... ...Nitrogen Lights; left) and samples incubated in the aquaria (right).

Field Experiments

In the field, three racks having 15 samples from each species were incubated in an area exposed to a nighttime artificial light coming from a neighboring port facility (Fig. 4). Three other racks having the same number of samples were incubated in the same area at the same depth but in a shaded area relative to the nighttime artificial light source. The survival rates were followed with time of incubation for each species. Also, physiological parameters and growth rate changes were followed with time for all samples incubated.

Figure 4. The field site selected for *ex situ* incubation of coral samples. The site is enlightened during the night with a surface measured light intensity of about 1000 μmole $m^{-2}s^{-1}$.

Wet Weight

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Wet-weight method was employed for measuring the weight changes with time for the field and the laboratory incubations. The coral growth rate was calculated in g/day for all samples. In this method, an analytic balance was used to measure the coral weight every month to calculate the weight change (Fig. 5).

Figure 5. Wet weight methodology for determining the weight change of corals incubated in the field and the laboratory.

Zooxanthellae Counting

Zooxanthellae counting was carried at a weekly basis and later at a monthly basis. In this method, small coral fragments were taken from the colonies that were incubated under the different artificial night time lights as follows;

1. *Acropora eurystoma* and *Galaxea facsicularis* fragments were fixed in a 4% formalin before decalcification for half an hour, then they were further decalcified in a 50% formic acid solution.
2. *Stylophora pistillata* fragments were decalcified in a 25% formic acid solution.
3. When coral tissues were completely decalcified, tissues were drained throughout the night.
4. The weight of the dry tissue was measured by analytical balance.
5. The dry tissue was then homogenized in 1ml distilled water (Fig. 6).
6. Using hematocytometer, the zooxanthellae cells were counted under light microscope and recorded for each coral sample.

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Figure 6. Isolation and counting of Zooxanthellae in the coral samples.

Study of coral settlement

Coral settlement *in situ* was studied by using tiles fixed on specially designed racks (Fig. 7). Two racks carrying the tiles were submerged in shallow water. One rack was deployed in an area exposed to excess artificial night time light, while the other was deployed nearby, but in sheltered area with respect to night time artificial light. Every two months one tile was brought from each rack, read under stereomicroscope in zigzag lines to record the recruited organisms.

Figure 7. Recruitment of planula larvae studied by using settlement tiles fixed onto especially designated aluminum racks in order to elucidate the influence of nighttime artificial light in the field.

Dark Respiration and net photosynthesis

Strathkelvin Respirometer model 928 was used to measure the rates of dark respiration and net photosynthesis in coral samples incubated in the laboratory under different artificial lights. Oxygen rates were calculated as μmol/min O_2.

Histological study

An attempt was made to study the influence of artificial light on the cellular level of coral tissues. Unfortunately, this did not work for some technical problems that we could not overcome. We plan to try this again in the near future, as we believe that artificial light must have an influence on the gonads of the corals.

IOLR Results

No significant difference in growth was found between different genotypes at the same treatment (Fig. 8). All genotypes showed enhanced growth when fed to repletion (N+) and reduced growth at the UV- treatment. While no significantly different ($p>0.05$), variation in growth between treatments for genotype S-19 is lower as compared to genotypes S-43 and S-47. Cumulative species-specific growth per sampling period showed highest growth for fragments fed to repletion (N+) while UV- treatments showed the lowest growth (Fig. 9). Chlorophyll per fragment surface area over time was raised in all 3 genotypes under high feeding regimen. In the absence of food and UV no changes in chlorophyll per fragment surface area were found (Figs. 10 and 11).

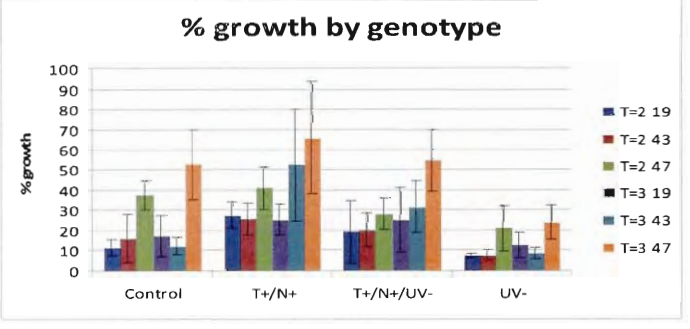

Figure 8. *Stylophora pistillata*. Variation in growth rate (in percentage) between different genotypes. 19, 43 and 47 are three different genotype. T=2 (5 months from T=0) and T=3 (7 months from T=0) are sampling time points.

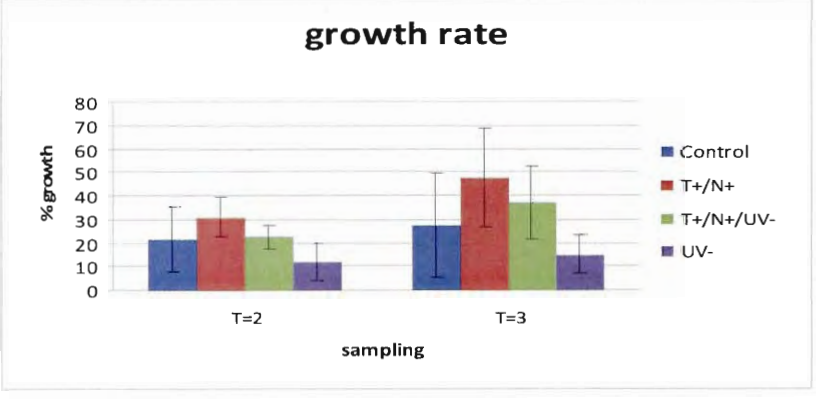

Figure 9. *Stylophora pistillata*. Cumulative species-specific growth at different treatments along the two sampling periods T=2 (5 months from T=0) and T=3 (7 months from T=0).

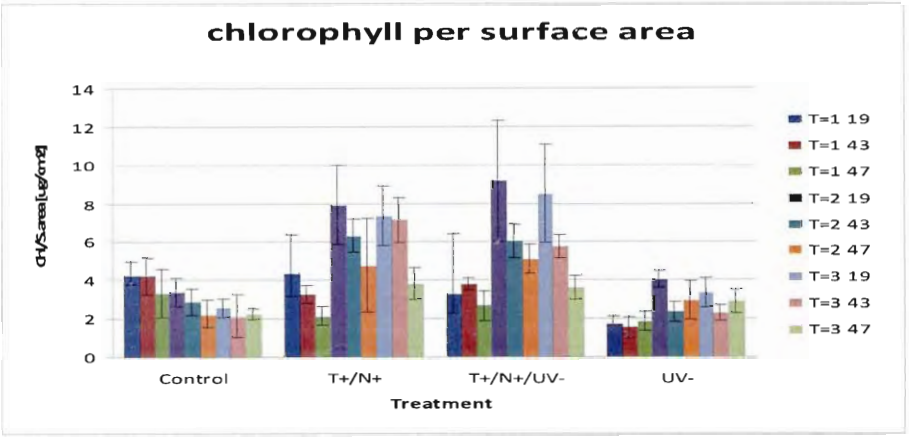

Figure 10. *Stylophora pistillata*. Genotype specific chlorophyll per fragment surface area measurements along three sampling periods T1 (2 months from T=0), T=2 (5 months from T=0) and T=3 (7 months from T=0).

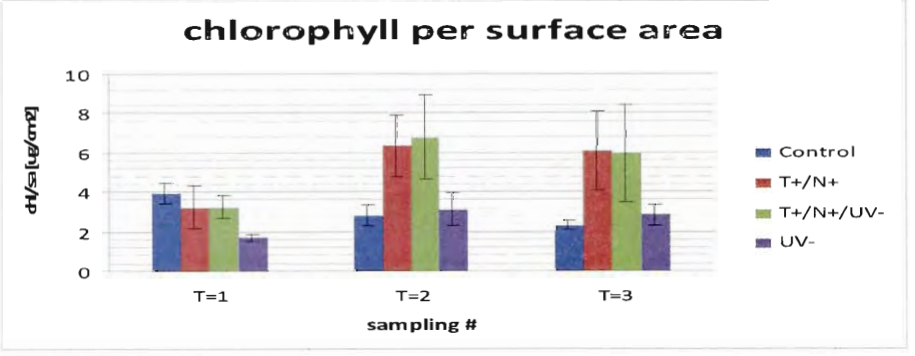

Figure 11. *Stylophora pistillata*. Chlorophyll per fragment surface area along the three sampling dates T1 (2 months from T=0), T=2 (5 months from T=0) and T=3 (7 months from T=0).

When fed to repletion (N+), genotype 19 was the only genotype with significant difference in zooxanthellae per fragment surface area (Fig. 12). When fed to repletion (N+) all genotypes show enhanced growth in zooxanthellae per surface area from the first to the second time point, but this growth in zooxanthellae vanished at the third time point. The control and the UV- treatment obtained the same number of zooxanthellae along the time scale (Fig. 13). All treatments showed no significant changes along time scale in chlorophyll concentration per zooxanthella. The reduction in chlorophyll concentration per zooxanthella at the third time point is probably due to season changes (Fig. 14). Chlorophyll concentration per zooxanthella in each genotype showed a small increase over time when fed. At the UV- irradiation treatment no increase in chlorophyll concentration per zooxanthella was found (Fig. 15). The averages of all genotypes along three sampling periods revealed no significant change in chlorophyll when compared with protein

concentrations over time and over treatments (Figs. 16, 17).

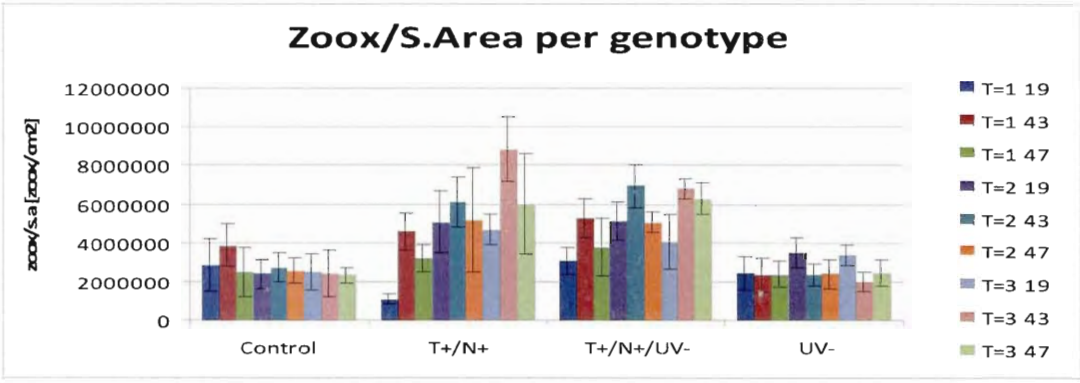

Figure 12. *Stylophora pistillata.* Variation in zooxanthellae per genotype surface area measurements along three sampling periods T1 (2 months from T=0), T=2 (5 months from T=0) and T=3 (7 months from T=0).

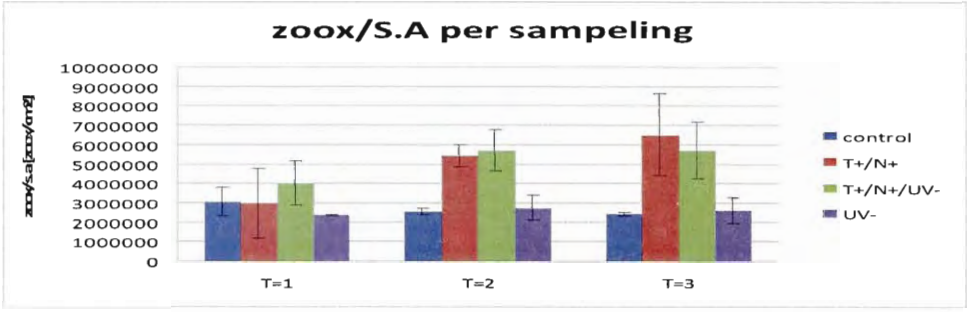

Figure 13. *Stylophora pistillata.* Variation in zooxanthellae per surface area measurements (average of all genotypes) along three sampling periods T1 (2 months from T=0), T=2 (5 months from T=0) and T=3 (7 months from T=0).

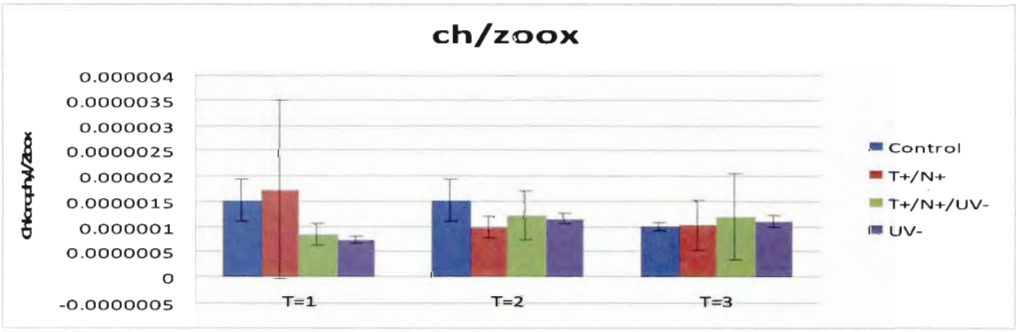

Figure 14. *Stylophora pistillata.* Variation in chlorophyll per zooxanthella measurements (average of all genotypes) along three sampling periods T1 (2 months from T=0), T=2 (5 months from T=0) and T=3 (7 months from T=0).

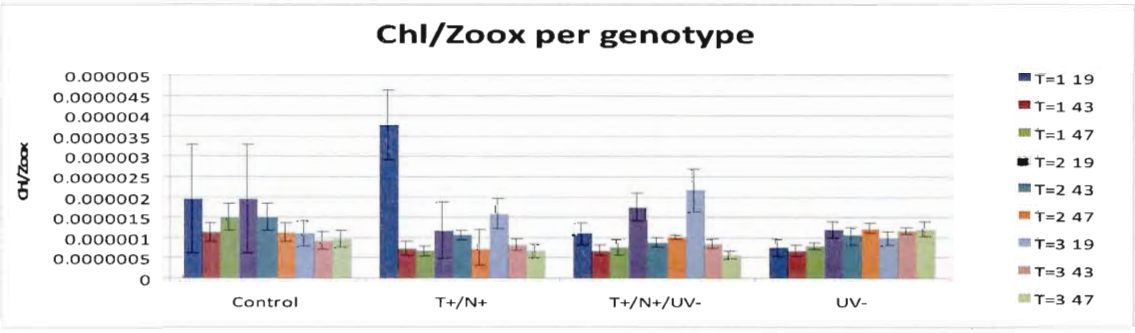

Figure 15. *Stylophora pistillata.* Variation in chlorophyll per zooxanthella measurements along three sampling periods T1 (2 months from T=0), T=2 (5 months from T=0) and T=3 (7 months from T=0).

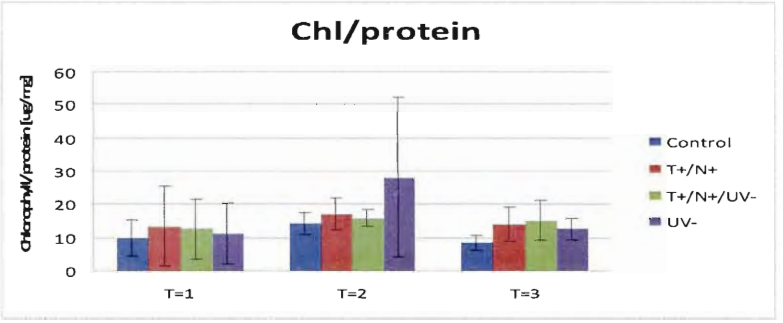

Figure 16. *Stylophora pistillata.* Variation in chlorophyll changing regarding protein concentrations over time and treatments (average of all genotypes) along three sampling periods T1 (2 months from T=0), T=2 (5 m from T=0) and T=3 (7 m from T=0).

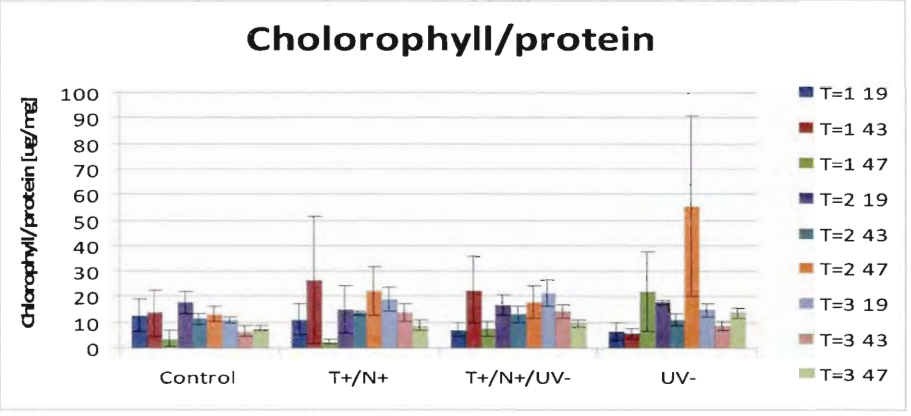

Figure 17. *Stylophora pistillata.* Variation in genotype chlorophyll changes with regard to protein concentrations over time and treatments along three sampling periods T1 (2 months from T=0), T=2 (5 m from T=0) and T=3 (7 m from T=0).

Measurements for photosynthetic quantum yield (ϕ) from *Stylophora pistillata* fragments reveled, at the beginning of the experiment, no difference between fragments from different genotypes at the same treatment (Fig. 18) or between fragments from the same genotype at different treatments (Fig. 19). Variation in Non Photochemical Quenching-NPQ between fragments from the same genotype at the same treatment (control) revealed differences between genotypes, especially when comparing *Stylophora pistillata* genotype 38 (collected from 15m depth) with all other genotypes (Figs. 20, 21). Same implied to the variation in Fv/Fm across treatments, in the various genotypes that were studied (Fig. 22).

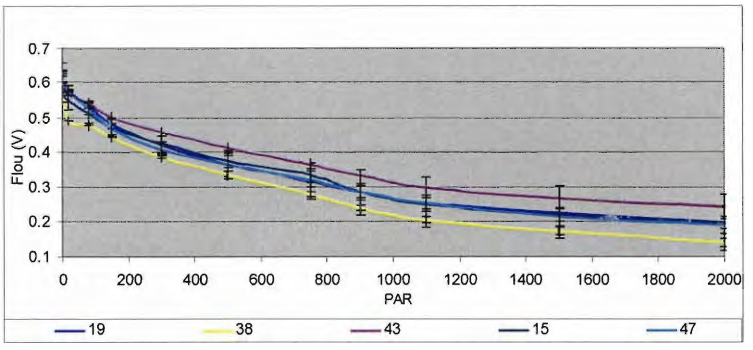

Figure 18. *S. pistillata*. Variation in photosynthetic quantum yield (ϕ) between different genotypes at the same treatment (control).

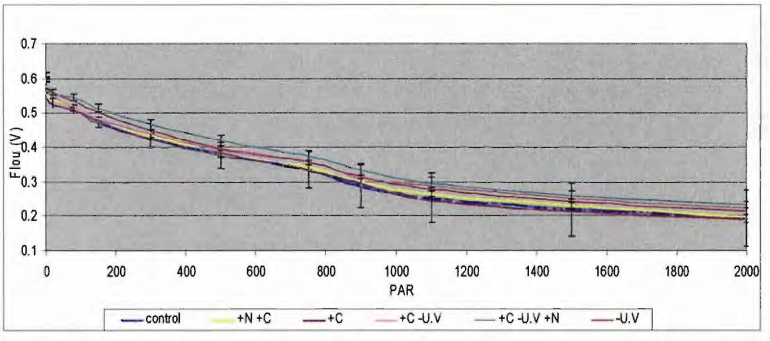

Figure 19. *S. pistillata*. Variation in photosynthetic quantum yield (ϕ) between fragments from the same genotype at different treatments.

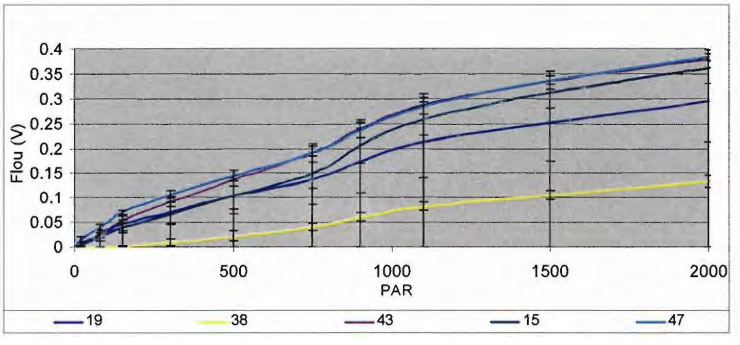

Figure 20. *Stylophora pistillata*. Variation in *Non Photochemical Quenching-NPQ* between fragments from the same genotype at the same treatment (control).

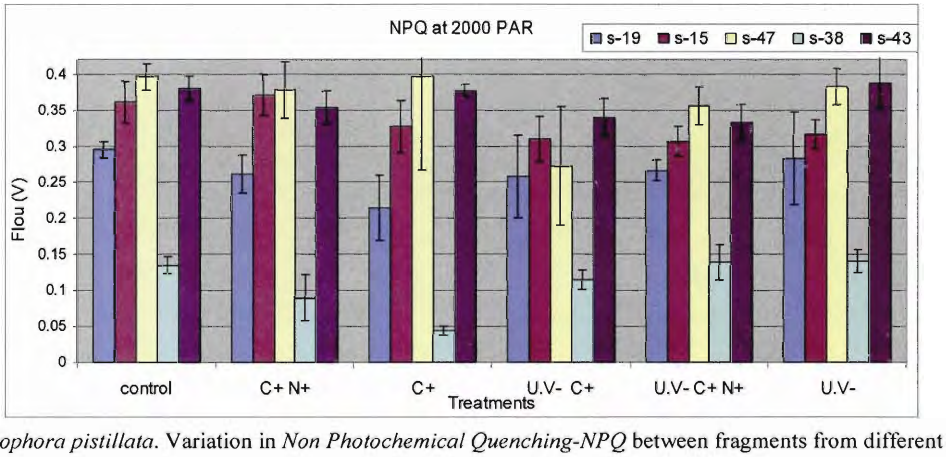

Figure 21. *Stylophora pistillata*. Variation in *Non Photochemical Quenching-NPQ* between fragments from different genotypes at each treatment (the result of s-38 genotype is significantly different).

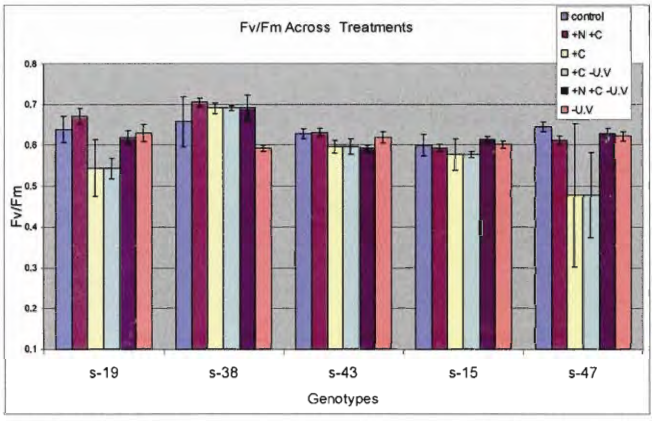

Figure 22. *Stylophora pistillata.* Variation in Fv/Fm across treatments.

MSS Results

Field results on growth and mortality of coral samples revealed that the exposed to light coral fragments had higher rates of growth compared with the sheltered coral samples (Figs. 23, 24). This unexpected difference in growth rates might be due to the localized environmental conditions such as the local currents and suspended organic matter. It is also possible that the nighttime artificial light attracted more zooplanktons during nighttime as compared with the sheltered area. Since corals are known to feed on suspended zooplanktons during the night, it is therefore possible that the corals incubated in the exposed area had more food than the shaded corals, which resulted in higher growth rates. The results obtained have also shown species differences in terms of the growth rates, with the *Acropora* sp. having the highest growth rates. The mortality rates were nil in *Stylophora* and *Galaxea* and high (up to 60%) in the *Acropora* sp. fragments, which indicates that this species is more sensitive than the other two studied species (Fig. 25).

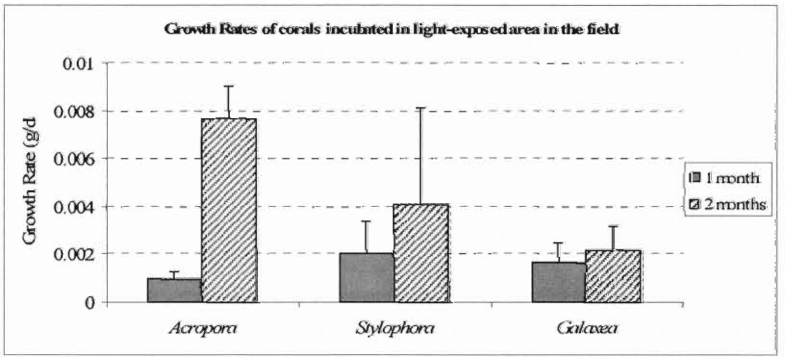

Figure 23. Growth rates of the three coral species incubated at light-exposed area in the field.

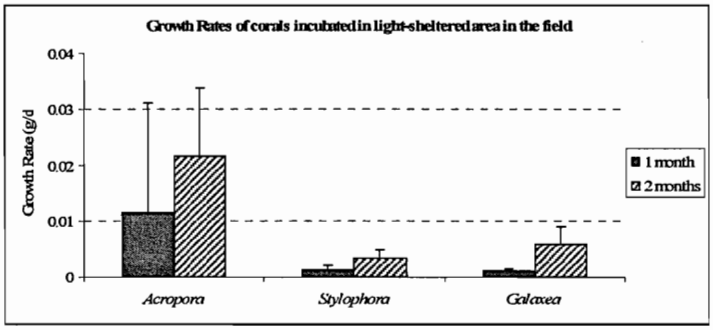

Figure 24. Growth rates of the three coral species incubated sheltered area in the field.

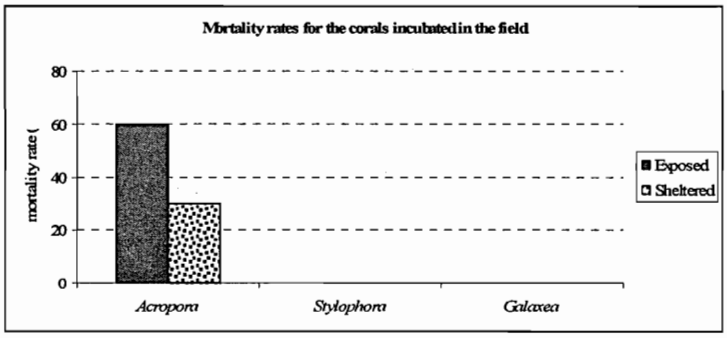

Figure 25. Mortality rates for corals incubated in the field in light-exposed area and sheltered area.

The results obtained from the laboratory incubations further revealed that the different artificial lights directly influenced the growth rates of the coral species, in different species specific patterns. Under the Halide light, the rate of growth for *Acropora* was not significant during the 4 months of incubation, while fragments of *Stylophora* had decreasing growth rate with the time of incubation. Contrary to the *Stylophora*, *Galaxea* fragments showed significant increase of growth rate with time of incubation under the same incubating conditions (Fig. 26). The differences in growth rates might be due to species differences among the corals used.

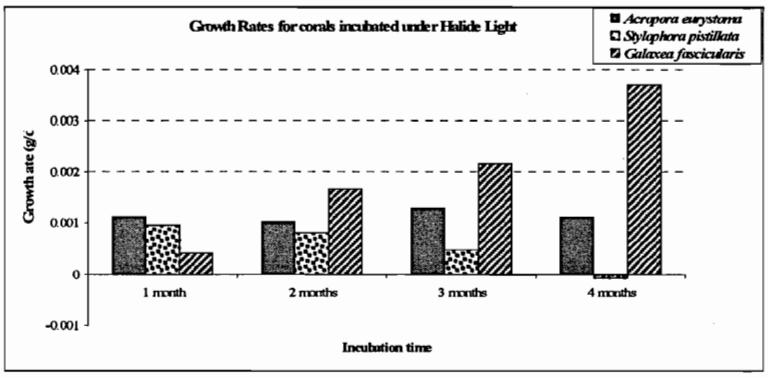

Figure 26. Weight changes of the three coral species incubated under the Halide light.

Incubations of the corals under the mixed light have shown that both *Acropora* and *Stylophora* responded by decreasing rates of growth and then re-increase again after 4 months of incubation (Fig. 27). This might indicates for long-term acclimatization of the two coral species to the conditions provided by the artificial illumination. On the other hand, *Galaxea* colonies had a growth rate that increased gradually in the first two months of incubation and then increased at a much higher rate after 4 months of incubation. Similar trend was observed upon incubating the coral fragments from the three coral species under the Sodium light (Fig. 28). The incubation of corals under the Halogen light has led to the death of all coral fragments from the three species studied within short periods and therefore, the growth rate could not be measured.

The mortality rates for the corals incubated under the four commercial lights are shown in Figure 29. A 100% mortality was obtained when the corals were illuminated by Halogen light (Fig. 30; started with bleaching of coral fragments), while 20% mortality of *Acropora* fragments was obtained upon incubation of the corals under the sodium light and 50% mortality of *Stylophora* was recorded, when the corals were incubated under the Mixed light. The illumination by the Halide light produced no mortality for any of the corals incubated, which indicates that this light type is probably the best for use.

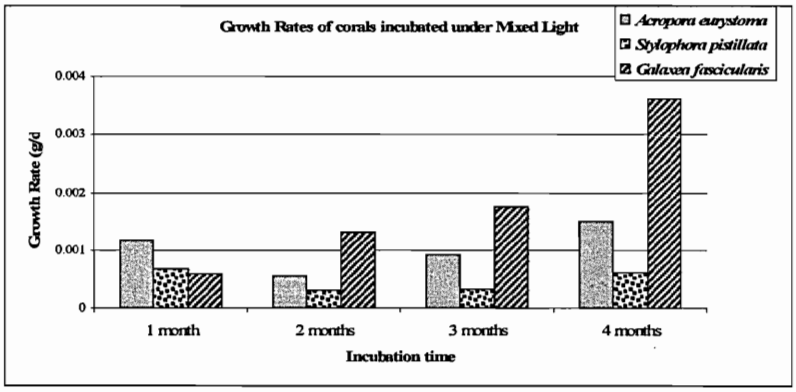

Figure 27. Weight changes of the three coral species incubated under the Mixed light.

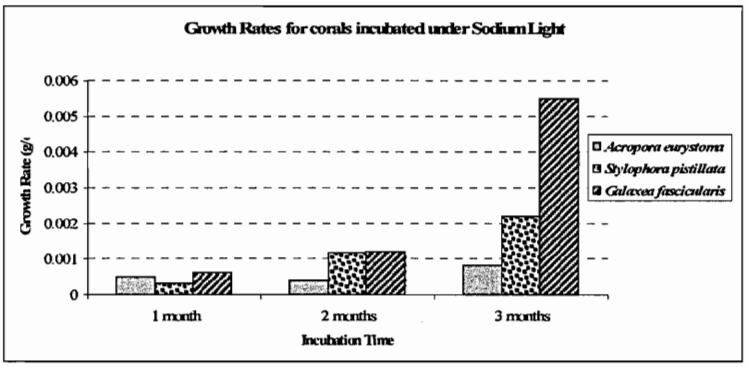

Figure 28. Weight changes of the three coral species incubated under the Sodium light.

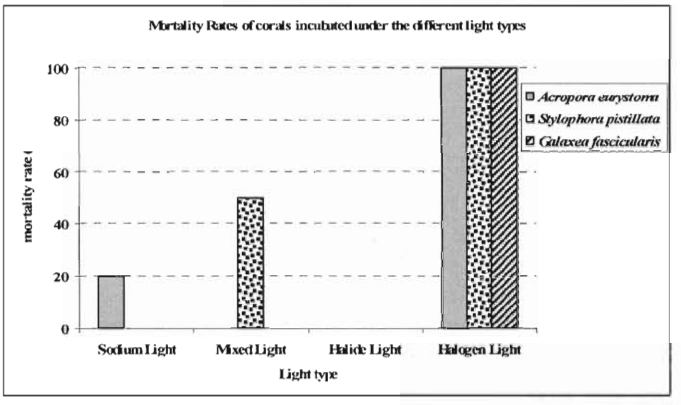

Figure 29. Mortality rates for the coral incubated under the four artificial light types.

Figure 30. Bleaching of the coral samples incubated in the Halogen Tank was obvious after less than 2w incubation.

The number of zooxanthellae per gram tissue weight was calculated for the three coral species incubated under the different light types. The results obtained showed that all three coral species responded to the artificial illuminations by gradual increase in their zooxanthellae contents (Fig. 31-33). Among the different artificial lights used, the sodium light had the least effect on corals in terms of the zooxanthellae contents.

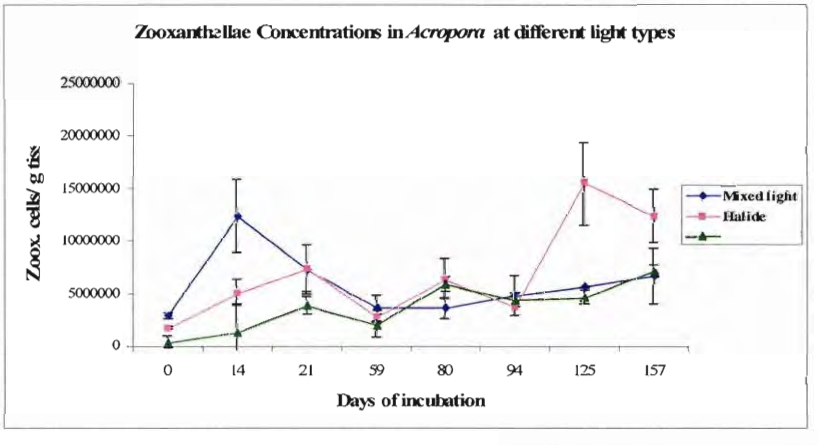

Figure 31. Zooxanthellae contents in *Acropora* fragments illuminated by the three commercial lights.

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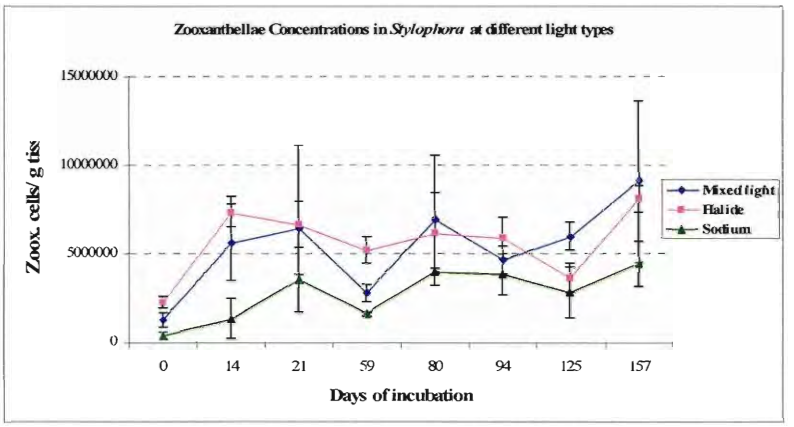

Figure 32. Zooxanthellae contents in *Stylophora* fragments illuminated by the three commercial lights.

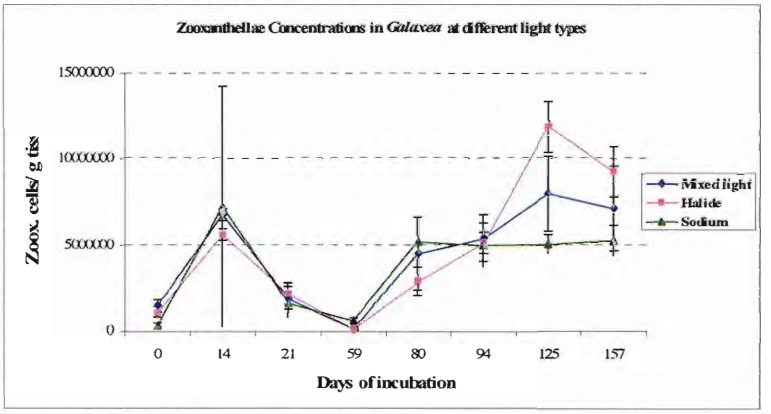

Figure 33. Zooxanthellae contents in *Galaxea* fragments illuminated by the three commercial lights.

The measured rates of net photosynthesis and dark respiration did not show any significant trend, as there were no significant differences among the different corals used or among the different incubation treatments (Fig. 34, 35). This might be due to the limited number of measurements that we could provide, since we faced some technical obstacles with instrument's failures. Additional work is planned in the near future after the instrument will be fixed.

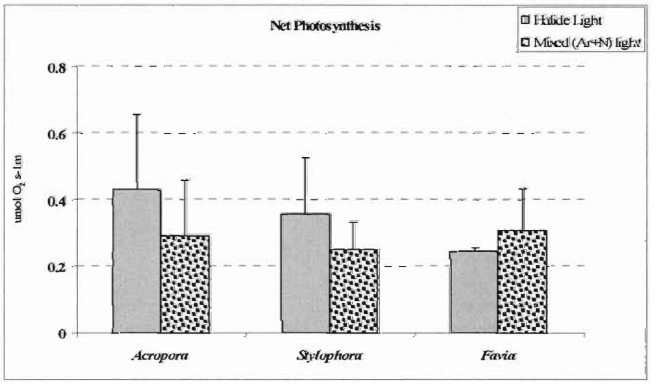

Figure 34. Net photosynthesis rates measured in corals incubated under the Halide and the Mixed lights.

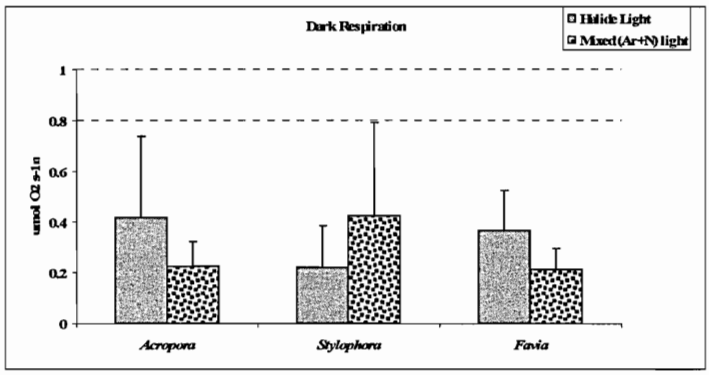

Figure 35. Dark respiration rates measured in corals incubated under the Halide and the Mixed lights.

Recruitment-The recruitment treatments included two sets of tiles fixed on specialized racks made in angles, deployed in exposed area, and sheltered area as shown in the methods section. Tiles were removed from the racks after 2, 4, 7, 9, 11 and 13 months of incubation for recruitment analysis. There are 9 recruiting items that were studied on the tiles (Table 2).

The results obtained revealed an increased number of recruiting organisms with time of incubation (Tables 3-8). It was also noted that the sheltered tiles in both experiments had similar rates of recruitment, with slight increase in the sheltered rack. With respect to recruiting organisms, the highest numbers of organisms were found to be the ascidians and the tubeworms, with numbers that were uncountable. Furthermore, there were no hard corals recruited on the tiles even after more that a year of incubation. This might suggest a negative effect of nighttime artificial light on the coral larvae recruitment. Supporting to this idea came from the results on the tiles that were deployed away from the area with nighttime artificial light. These tiles had corals recruited on them (results not shown).

Table 2. Biological item codes used in the recording for the recruitment tiles.

Biological Item Code	
HC	hard coral
SC	soft coral
AS	ascidians
SP	sponge
BV	bivalve
FO	foraminifera
BR	Bryozoa
TW	tube worm
AG	algae

Table 3. Recruitment tiles readings after two months of deployment (R; rough surface of the tile, S; smooth surface of the tile).

	Exposed area		Sheltered area	
Item	R	S	R	S
HC				
SC				
AS	25	17	16	18
SP				
BV			11	
FO				
BR	2		2	
TW	10	13	9	5
AG				

Table 4. Recruitment tiles readings after four months of deployment (R; rough surface of the tile, S; smooth surface of the tile).

	Exposed area		Sheltered area	
Item	R	S	R	S
HC				
SC				
AS	20	22	6	14
SP				
BV				
FO				
BR	5	3	2	1
TW	15	13	11	10
AG				

Table 5. Recruitment tiles readings after 7 months of deployment (R; rough surface of the tile, S; smooth surface of the tile).

Item	Exposed area		Sheltered area	
	R	S	R	S
HC				
SC			1	
AS	25	21	16	2
SP				
BV			1	
FO				
PR	5	2	4	
TW	38	17	27	16
AG				

Table 6. Recruitment tiles readings after 9 months of deployment (R; rough surface of the tile, S; smooth surface of the tile).

Item	Exposed area		Sheltered area	
	R	S	R	S
HC				
SC				
AS	57	27	43	63
SP				
BV	2		4	1
FO	7	46	3	30
BR	7		5	
TW	51	33	74	96
AG				
SA				

Table 7. Recruitment tiles readings after 11 m of deployment (R; rough surface of the tile, S; smooth surface of the tile).

depth	Exposed area		Sheltered area	
	R	S	R	S
HC				
SC	3			
AS	57	27	43	63
SP				
BV	12			4
FO	9	24	11	28
BR	15			
TW	142	97	181	156
AG				
SA				

Table 8. Recruitment tiles readings after 13 m of deployment (R; rough surface of the tile, S; smooth surface of the tile).

depth	Exposed area		Sheltered area	
	R	S	R	S
HC				
SC	1		1	
AS	7	32	19	46
SP			1	
BV	2		4	
FO	4			20
BR			9	
TW	12	72		27
AG				
SA				

6) Impact Relevance and Technology Transfer:

Over the past 50 years, an increase in nutrient concentrations has been worldwide in reef sites closely situated to major human populations. However, scientific results do not reveal what degree of eutrophication affects coral reefs and by what measure. Consequently, experimental results are contradictory. Eutrophication may affect coral reefs through different scales and levels. At the ecosystem level, anthropogenic nutrient-enrichment may enhance algal growth (in cases and sites where no grazing pressure compensates algal growth), leading to competition for space between macro-algae and corals and increase in water turbidity because of stimulation of phytoplankton growth. At the cellular level, exposure of coral colonies to elevated nutrients may lead to up to five fold increases in zooxanthellae density and chlorophyll a content per algal cell. Carbon limitation reduces photosynthetic rate per algal cell and reduces the translocation of photosynthates from the algae symbionts to coral host. In such cases most of the photosynthetically acquired carbon is respired by the algae.

Our results show that all genotypes enhanced growth when fed to repletion (N+) and reduced growth at the UV- treatment. Cumulative species-specific growth per sampling period showed highest growth for fragments fed to repletion (N+) while UV- treatments showed the lowest growth. Chlorophyll per fragment surface area over time was raised in studied genotypes under high feeding regimen. In the absence of food and UV no changes in chlorophyll per fragment surface area were found. When fed to repletion (N+) all genotypes showed enhanced growth in zooxanthellae per surface area from the first to the second time point, but this growth in zooxanthellae vanished at the third time point. The control and the UV- treatment obtained the same number of zooxanthellae along the time scale. All treatments showed no significant changes along time scale in chlorophyll concentration per zooxanthella. Measurements for photosynthetic quantum yield from *Stylophora pistillata* fragments reveled, at the beginning of the experiment, no difference between fragments from different genotypes at the same treatment or between fragments from the same genotype at different treatments. Variation in Non Photochemical Quenching-NPQ between fragments from the same genotype at the same treatment (control)

revealed differences between genotypes, especially when comparing the *Stylophora pistillata* genotype collected from 15m depth with all other genotypes. Our experiment concludes no harm to the experimental coral when fed to repletion (N+). Contrary, we conform the results seem to favor the hypothesis that "fed corals are happy corals". Clearly, larger scale trials are warranted and more work is needed to further test this study conclusion. This will perhaps lead to a change in the prevailing dogma on nutrients impacts on Gulf's reefs, and to accepting the notion that eutrophication in the northern Gulf of Eilat/Aqaba does not harm the environment (pending an efficient grazing pressure). Such act may diversify job qualities in Aqaba and Eilat, providing new positions for both Israeli and Jordanian citizens, improving both sides' economic conditions. In addition, the surprising results for enhanced growth rates of illuminated coral colonies, the negative impacts of artificial lights on coral growth and survivorship and the non-significant measured rates of net photosynthesis and dark respiration – all may open new directions for using improved methodologies in the Gulf, in a way that tourism activities will not harm corals' livelihood. Results also revealed that the use of Halogen commercial artificial light is most deleterious to corals and that nighttime artificial light may cause misdirection of swimming coral larvae and therefore may have negative effects on recruitment of corals in the illuminated areas. It is therefore recommended to ban the use of this light type in shoreline facilities.

Now that the project is complete, the scientific capabilities of cooperating Jordanian-Israeli scientists has been clearly improved. In addition to the Israeli and the Jordanian students trained in the project, eight international students were trained in MSS: Justin Rezarri (USA), Semir Kuklo (Turkey), Carinne Hutchinson (Australia), Casey Hawkey (Australia), Laura Colgan (USA), Nicola Taylor (Britain), Hulya Keles (Turkey) and Chiara Franco (Italy).

7) Project Activities/Outputs:

As previously mentioned, we confronted along the project duration administrative obstacles resulted from the reluctant of officials to grant permission for traveling between Israel and Jordan, and no visa permission was granted to allow our Jordanian colleagues to visit Israel. A major obstacle was solved at the end of December 2008, but the war in Gaza that has started postponed all planned

mutual visits. Most of the knowledge transfer was than performed via mail, email, and telephone conversations. The Israeli and the Jordanian teams met at the Aqaba border crossing during June 30, 2009. The meeting day was devoted for the description of the experiments and the results, for bi-lateral knowledge transfer, for future planned experiments and for the analysis of results. No joint publication has yet been submitted.

8) Project Productivity:

When the project was first created, it was planned to last for at least three years. As the project budget and its length were severely reduced, we had to cut some of the activities planned and that proposed aims. However, within the limited time frame and reduced budget, this research reached significant outcomes (impacts were specified in item 6 above).

9) Future Work:

As described above, our project results challenged the dogma that leads to end of the fish farm industry, one of the largest economic sources at south Israel. More work has to be done to confirm the emerged conclusion that under proper bottom-up biological control, a modest eutrophication in the northern Gulf is not a significant threat to the coral reefs. In a similar way, more work should be devoted to elucidate the possible impacts of artificial illumination on corals and coral reefs. It is possible that much of the claims against artificial illumination (for touristic activities at night) are not substantial. On the other hand, some artificial lights, such as the Halogen bulbs, were found to be deleterious to corals. Clearly, more work is needed to elucidate the details of this phenomenon.

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