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DAPI-based vital staining reveals entry of heterologous zooxanthellae into primary polyps of a vertically-transmitting soft coral

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Abstract

Many cnidarians form an obligatory mutualism with photosynthetic algae (zooxanthellae) of the genus *Symbiodinium*. Break-down of this symbiosis leads to bleaching, and often death, of the host. Coral hosts may survive such break-down of symbiosis by exchanging their algal types, provided they can host multiple symbiont types. The soft coral *Litophyton crosslandi* (Octocorallia, family Nephtheidae) transmits its symbionts vertically to its sexual progeny during oogenesis. This has been considered a "closed symbiosis", in which the host retains the same single zooxanthellae type throughout its entire life span. In this work we examined the possible entry of heterologous zooxanthellae into primary polyps of a vertically transmitting host. Primary polyps were challenged with DAPI-stained heterologous clade C zooxanthellae, allowing their visualization and distinction from the homologous clade A ones. DAPI-stained zooxanthellae were found in the epidermis, and in some cases even in the gasterodermis, of the polyps. The entry of heterologous symbionts and their survival inside host tissue suggests that the latter may indeed possess the ability to host different algae. These findings also suggest an entry pathway via the epidermis that, prior to this study, has not been described for vertical transmitters. These results further contribute to the understanding of coral ability to cope with stress-induced bleaching.

Keywords: DAPI, symbiosis, zooxanthellae, soft corals, primary polyps, Red Sea

1. Introduction

Many stony and soft coral species rely on their dinoflagellate symbiotic algae (zooxanthellae) for survival (Falkowski et al., 1984; Muscatine et al., 1984; Porter et al., 1984; Baker, 2003). Under stressful conditions these zooxanthellae may be expelled, resulting in bleaching, which can often lead to death of the host (e.g., Hoegh-Guldberg, 1999; Baker, 2001; Douglas, 2003). Buddemeier and Fautin (1993) introduced the adaptive bleaching hypothesis, arguing that bleaching represents a mechanism by which the host may survive climate change, such as a rise in seawater temperature, by replacing its dominant symbionts with others that are more suitable for the new conditions, as has been found for example in the stony corals *Montastrea annularis* (see Toller et al., 2001) and *Pocillopora damicornis* (see Glynn et al., 2001).

Such ability is probably related to whether a coral species can host multiple clades of zooxanthellae, either concurrently within the same colony or in different colonies of the same species (reviewed by Goulet, 2006). It has not yet been established whether bleaching-induced changes in the dominant symbiont clade is a result of a "switching" process, in which the adult colony acquires a new algal clade from the surrounding water, or of a "shuffling" process, in which those algae previously existing in small numbers in the host now become dominant following expulsion of the previously dominant symbionts (reviewed by Coffroth and Santos, 2005). Though recent evidence of "background symbionts" residing in hosts previously believed to harbor a single clade (Mieog et al., 2007), and the increase in thermal tolerance due to a change in the dominant symbiont type, as was demonstrated for the hard coral *Acropora millepora* (Berkelmans and van Oppen, 2006) suggests that the "symbiont shuffling" scenario may be a common one in cnidarian hosts.

Trench (1987) suggested two modes of acquisition of the symbiotic algae by the sexually-produced offspring of

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host species: either by direct transmission to the egg or the brooded larvae (vertical, maternal inheritance, closed system), or from the ambient environment (horizontal, open system) by the post-larval stages. Several studies have raised the possible implications of the mode of symbiont acquisition (horizontal *vs.* vertical) on symbiont diversity and flexibility of the symbioses (e.g., Douglas, 1994; Rowan, 1998; Weis et al., 2001; Barneah et al., 2004; van Oppen, 2004; La Jeunesse et al., 2004; Rodriguez-Lanetty et al., 2004). Although many coral species host only one clade of symbionts (Baker, 2003; LaJeunesse, 2002; Goulet, 2006), their early developmental stages are not necessarily selective as to the zooxanthellae genotype they acquire, and they may form a symbiosis with both homologous and heterologous algae (e.g., Coffroth et al., 2001; Coffroth and Santos, 2005; Adler, 2006). "Shuffling" may thus occur in hosts whose juveniles have the ability to acquire and maintain symbiosis with different algal clades simultaneously (Baker, 2003; Little et al., 2004).

The general notion regarding the evolution of invertebrate-dinoflagellate symbiosis is that, with the possible exception of taxa that have vertical inheritance (Hunter et al., 1997), a lack of correlation between host and symbiont genotype is to be expected (van Oppen et al., 2001). It is expected that corals with vertical symbiont transmission will show higher levels of phylogenetic congruence with their symbionts, and that this might remain constant over time (Loh et al., 2001). Although most sexual progeny of host-species with vertical transmission symbiotic systems acquire the symbionts directly from the parent (Douglas, 1994; Coffroth and Santos, 2005), there have been some exceptions to this generalization. For example, early embryonic and planulae stages of the tropical scyphozoan *Linuche unguiculata* demonstrate a "semi-closed" system, in which most of the sexually-produced offspring are infected with maternal (homologous) algal symbionts during early development, but some may also acquire additional ones at later stages of their development (Montgomery and Kremer, 1995). For example, in the sea anemone *Cereus pedunculatus* acquisition of symbionts takes place during oogenesis (Davy et al., 1997); however, anemones that were subjected to a three-year dark treatment, rendering them aposymbiotic, acquired both homologous and heterologous zooxanthellae following stress-imposed bleaching. Interestingly, van Oppen (2004) has demonstrated that in the Great Barrier Reef (Australia) stony corals with vertical symbiont transmission, which are expected to have low symbiont diversity, were found to have similar symbiont diversity to those with horizontal transmission, thus suggesting a degree of flexibility in the closed system symbiosis.

The soft coral *Litophyton arboreum* (=*crosslandi,* emendation following Ofwegen, pers. comm.) is a common inhabitant on Eilat's reefs (Gulf of Aqaba, northern Red Sea). Its oocytes acquire zooxanthellae via vertical transmission from the parent colony (Benayahu et al., 1992). Recently, we found that colonies of *L. crosslandi* in Eilat host a different ITS2 genotype of clade A zooxanthellae to that found in colonies from the Dahlak Archipelago (Eritrea) (Zurel, 2006; GenBank accession numbers EF378615, EF378618, respectively), suggesting that this species is able to host multiple algal genotypes. These intriguing findings prompted testing the nature of the closed vertical transmission system of the species and to further determine the ability of its early developmental stages to acquire heterologous symbionts (Maoz, 2005; this study). Attempts to challenge primary polyps of *L. crosslandi* with heterologous zooxanthellae did not reveal their acquisition when using Rowan and Powers' (1992) PCR-RFLP based method (Maoz, 2005).

Additionally, when studying symbiosis the use of an *in situ* method is necessary in order to define the location of the symbiont within the host. PCR-based methods do not discriminate between internal symbionts and those aggregated on the outer surface, and thus may lead to false positive results (Moss et al., 2003). Attempts to challenge *L. crosslandi* using heterologous algae labeled with the cellulose-specific dye Fungi-fluor (Montgomery and Kremer, 1995) failed (Maoz, 2005), as they could not be distinguished from the homologous symbionts due to leaking of the dye from the stained algae into the surroundings.

In the current study we utilized an intra-cellular staining method for the symbiotic algae, using the compound 4'-6-Diamidino-2-phenylindole (DAPI). DAPI has been previously used for staining of live protists (Lessard et al., 1996) and for assessing zooxanthellae genome size, by adopting the flow cytometric analysis of its fluorescence (LaJeunesse et al., 2005). We examined for the first time the feasibility of using DAPI as a vital stain in order to detect the possible entry of heterologous zooxanthellae and to distinguish them from maternally-derived ones in primary polyps of a host with maternal transmission of symbionts, in order to better understand the nature of such a symbiotic system, especially in relation to the likelihood to acquire the former.

2. Materials and Methods

Isolation of zooxanthellae

DAPI-stained heterologous clade C zooxanthellae were used in infection experiments of primary polyps of *L. crosslandi*. Clade C zooxanthellae were isolated from polyps of the soft coral *Heteroxenia fuscescens* (see Yacobovich et al., 2004; Barneah et al., 2004) obtained from the reef across from the Inter-University Institute for

Marine Science (IUI) in Eilat. All experiments were conducted during August–September 2006. The coral samples were separately homogenized with a tissue grinder and transferred into 15 ml test tubes filled with Millipore-filtered (0.22 μm) seawater (FSW). The tubes were then centrifuged at 1,500 RPM for 10 min; the pellet was resuspended in fresh FSW, followed by two rounds of 5 min centrifugation at 1,000 RPM. Between centrifugation rounds, all visible host debris was removed from the tubes, thus acquiring clean solutions of symbiotic algae. Clade C zooxanthellae were isolated from five polyps removed at random from five different colonies of *H. fuscescens* and placed separately in five 50 ml plastic tubes containing 0.22 μm FSW, in order to prepare algal stocks. 0.22 μm FSW was added to each of the five stocks, yielding a final concentration of ca. 100,000 algal cells × 1 ml^{-1} (Yacobovitch et al., 2003). The algal stocks were placed in an incubator (LE-509) under a 12h light: 12h dark regime at 25°C for 24–48 hours until algal motility appeared (Yacobovitch et al., 2004).

DAPI-staining of zooxanthellae

DAPI (Promega) from a stock solution of 0.1 mg × 1 ml^{-1} in double distilled water was added to 1 ml taken from the 50 ml stock of motile clade C zooxanthellae and incubated once more overnight at the same temperature for staining by DAPI (see: Lessard et al., 1996). As DAPI is light-sensitive (Manzini et al., 1985), all incubations with DAPI in the present study were conducted in the dark. Preliminary tests revealed that optimal staining of zooxanthellae was achieved after a 1:500 dilution of the DAPI stock with FSW to a final concentration of 2 × 10^{-4} mg × 1 ml^{-1} (data not shown).

After overnight incubation of the algal cells, excess DAPI was washed with 0.22 μm FSW by centrifugation at 8,000 RPM for 6 min, the FSW was discarded and the algal pellet was resuspended in 1 ml fresh FSW. This step was repeated for each replicate in a series of 10 washes. In order to test the quality of this wash treatment, the FSW from the final wash was filtered with a 0.22 μm Millipore filter, thus ensuring that no stained zooxanthellae were present, and added to freshly-isolated clade C zooxanthellae. After overnight incubation at 25°C these zooxanthellae were scanned using a Leica manufactured DMRB upright microscope with a MAGNAFIRE 12-bit color CCD camera, using DAPI excitation filter BP 340–380 nm for detecting DAPI, thus eliminating any chance of excess DAPI occuring in the water in later experiments (see ahead).

This treatment was considered as a negative control when challenging primary polyps of *L. crosslandi* with DAPI-stained heterologous algae, thereby excluding the possibility that stained zooxanthellae, if observed within the polyp after the challenging experiments, were preexisting homologous zooxanthellae stained by excess DAPI in the surrounding seawater.

Collection of planulae and rearing of primary polyps

In order to obtain planulae, branches (10–15 cm in length) of *L. crosslandi* and colonies of *H. fuscescens* were collected from the IUI reef. They were placed underwater in seawater-containing zip-lock bags, immediately brought to the IUI and placed in 15 l aquaria supplied with running seawater. Two hours prior to sunset, each branch or colony was placed in a 2–5 l aerated aquarium in the laboratory and examined the following morning for the presence of released planulae. Planulae were collected from the aquaria using a Pasteur pipette and were washed twice with 0.45 μm FSW (see also Ben-David-Zaslow and Benayahu, 1998). The planulae were air transported to Tel Aviv University and placed in 50 ml containers, 10 planulae per container, filled with 0.45 μm FSW. The containers were placed in an incubator under a fixed 12h light: 12h dark regime at 25°C and the planulae underwent successful metamorphosis (Ben-David-Zaslow and Benayahu, 1998; Yacobovich et al., 2003).

Challenging primary polyps of H. fuscescens *and* L. crosslandi *with DAPI-treated zooxanthellae*

In order to determine whether DAPI-stained homologous clade C zooxanthellae isolated from *H. fuscescens* can be acquired by conspecifc primary polyps (2–3 weeks old), the latter were challenged for four successive days with 1 ml FSW containing ca. 100,000 DAPI-treated algal cells and maintained at 25°C in the dark. In order to test whether primary polyps (2–3 weeks old) of *L. crosslandi* allow the entry of heterologous algae into their tissue, they were similarly challenged with the DAPI-treated clade C zooxanthellae and maintained as above. Since this algal clade has been shown to successfully infect primary polyps of *H. fuscescens* (see Yacobovich et al., 2003), the former experiment was considered as a positive control for the latter, which was aimed at revealing whether any unsuccessful acquisition by the primary polyps was due to the DAPI treatment.

Histology and microscopy

After four consecutive days of challenging primary polyps of *H. fuscescens* and *L. crosslandi* with DAPI-stained zooxanthellae, they were fixed in 4% formaldehyde in FSW and left overnight in the dark at room temperature. Excess formaldehyde and free zooxanthellae were removed by two washes with 0.45 μm FSW. The polyps were then embedded in 1% agarose gel (GIBCO, Invitrogen Corporation) and transferred to 70% EtOH. Agarose was used in order to maintain the natural orientation of the primary polyps while sectioning them (Fig. 1a), thus enabling later examination for the presence of algae in the tentacles, pharynx or gastrovascular cavity of the primary polyps (Yacobovitch, 2001). Following dehydration through a graded series of EtOH (40–100%), the polyps were embedded in paraplast (Monojet Scientific), and 8 µm thick sections were prepared and placed on StarFrost 76 × 26 mm microscope slides (Resy, Germany) (see also Adler 2006). The slides were mounted using a water-based aquamount as this had been found suitable for the purpose of the present study, whereas the xylen-based glue CONSUL-MOUNT™ (Thermo Sandon, Pittsburg, USA) disturbed the DAPI fluorescence. All histological preparations were kept in the dark at 4°C.

Slides were scanned for DAPI-stained zooxanthellae using a Leica manufactured DMRB upright microscope with a MAGNAFIRE12-bit color CCD camera, using DAPI excitation filter BP 340-380 nm. DAPI-stained nuclei of the zooxanthellae were distinguished at X 400 magnification.

3. Results

Algal staining

Though a 100% staining efficiency was not achieved, DAPI succeeded in staining up to 81.82% (n=110 algal cells) of the clade C zooxanthellae extracted from *Heteroxenia fuscescens* colonies in preliminary trials. The stained algae maintained their motility and underwent mitotic divisions (data not shown). Clade C algae freshly isolated from *H. fuscescens* and incubated in seawater taken from the final excess-DAPI washing treatment as negative control were not stained. The latter two sets of results, which were used by us as controls, indicated that the DAPI-stained algae found in the sectioned primary polyps (see ahead) were of the treated clade C motile algae used to challenge the primary polyps, and not clade A symbionts stained by excess DAPI in the water.

Challenging primary polyps of H. fuscescens *with DAPI-treated homologous zooxanthellae and primary polyps of* L. crosslandi *with DAPI-treated heterologous zooxanthellae*

Twenty primary polyps of *H. fuscescens* out of the 25 tested ones survived the 4-day period of incubation in the dark. The sections revealed DAPI-stained homologous algae within the gastrodermis of 12 of them (data not shown) and inside the gastrovascular cavity of 16 (in 10 polyps stained algae were found at both sites).

Twenty-two primary polyps of *L. crosslandi* out of the 25 tested ones survived the 4-day period of incubation in the dark. In nine polyps fluorescent microscopy of the sections revealed aggregation of the heterologous *H. fuscesens* clade C zooxanthellae both on the outer surface of their epidermal cell layer (Fig. 1b) and localized in these cells (Fig. 1c and d). In three polyps DAPI-stained algae resided in the gastrodermal cells amongst the homologous clade A zooxanthellae, which did not become stained (Fig. 1e). Notably, two primary polyps harbored DAPI-stained heterologous clade C algae within the gastrovascular cavity (Fig. 1f).

4. Discussion

Primary polyps of *L. crosslandi* allowed the entry of DAPI-stained heterologous motile clade C zooxanthellae into their tissues, a finding that contradicts the previously held notion about the closed nature of this symbiotic system (Benayahu et al., 1992; Barneah et al., 2004; Maoz, 2005). DAPI-stained symbionts were observed mainly in the ectoderm of the primary polyps (Fig. 1c and d), thus indicating their entrance through these cells. Notably, the acquisition of algae through ectodermal cells has previously been demonstrated for embryonic stages of the horizontally-acquiring soft corals, *Xenia umbellate* and *Anthelia glauca* (see Benayahu and Schleyer, 1998), and the stony corals *Pocillopora meandrina*, as well as in embryos of *Fungia scutaria* (see Marlow and Martindale, 2007). Transfer of zooxanthellae from the ectoderm to the gastroderm was also observed in planulae of the soft corals *Xenia macrospiculata* (see Achituv et al., 1992) and *L. crosslandi* (see Benayahu, 1997). All these examples indicate that the ectoderm may play a role in the algal entrance process. Intake of algae though the mouth opening of the polyp may lead to their digestion by the host (Widdig and Schlichter, 2001) and therefore, acquisition via the ectoderm may avoid their loss. Our findings thus suggest that the residence of algal symbionts in epidermal cells of the primary polyps is far more common than previously considered, and indicates a transitional stage until their gastrodermal residence is achieved (see also Benayahu and Schleyer, 1998). Stained algae were also observed in the gastrovascular cavity of some of the challenged primary polyps of *L. crosslandi* (Fig. 1e), suggesting that algal entry may also occur via this more common pathway, whereby the symbionts enter through the mouth opening into this cavity and are then endocytosed there (e.g., Kinzie, 1974; Yacobovich, 2003; Pasternak et al., 2004).

In the present study DAPI-staining has proven to be an efficient method for detecting the entry and intracellular residence of even a very low number of heterologous symbionts, which is beyond the detection ability of PCR-based methods, such as used by Maoz (2005). It should be noted, however, that the DAPI-staining technique did not yield an absolute staining efficiency and, consequently, is not suitable for quantifying the true number of heterologous algal cells that entered the polyp tissue, since the unstained

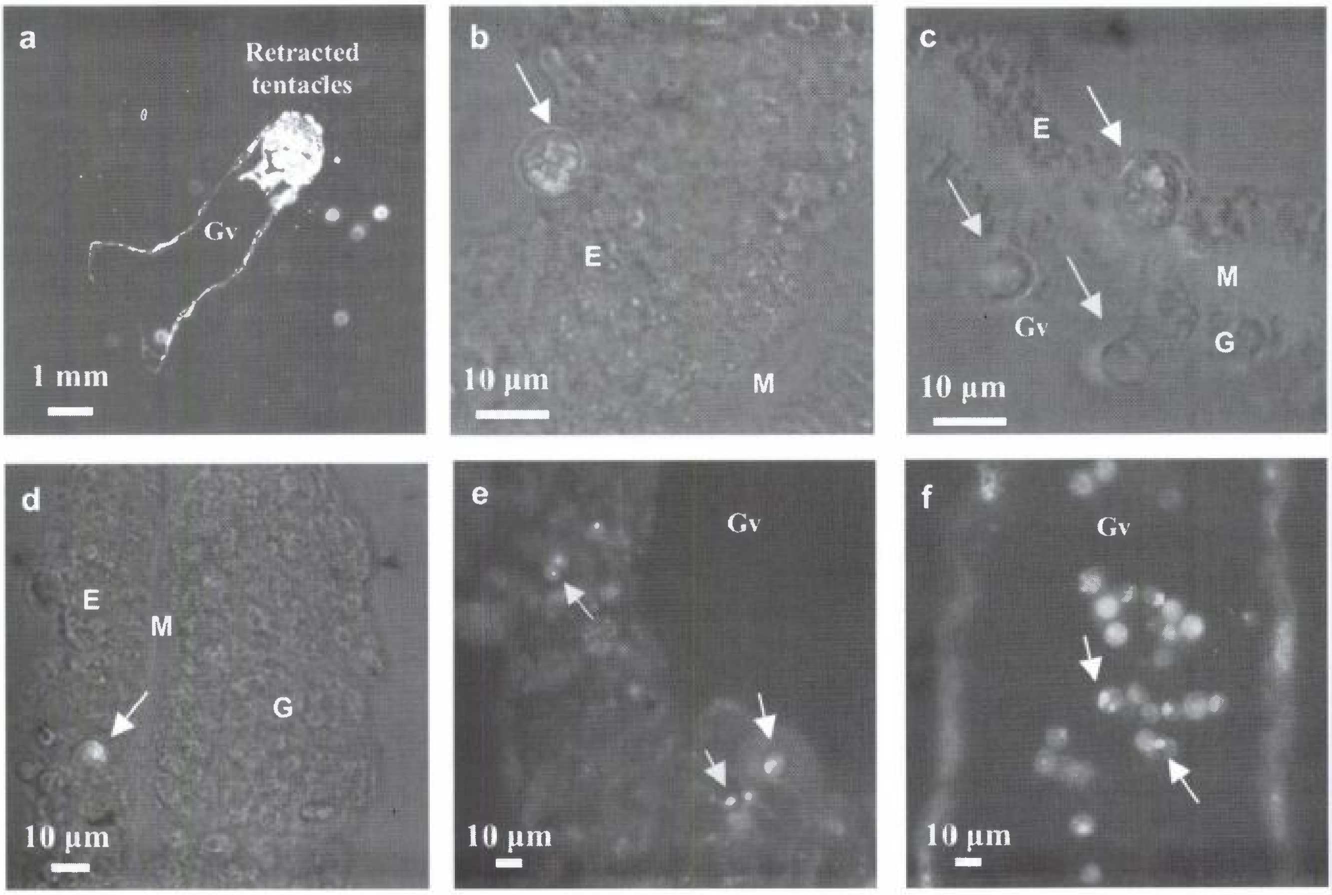

Figure 1. DAPI-stained heterologous clade C zooxanthellae from *Heteroxenia fuscecsence* acquired by primary polyps of *Litophyton crosslandi*. a) Longitudinal section of polyp (Bright Field photography); b) heterologous alga (white arrow) attached to the outer surface of the polyp; c,d) heterologous algae in the ectoderm (Bright Field and fluorescent photography); e) DAPI-stained heterologous (white arrows) and homologous algae (yellow arrows) within the gastrodermis; and f) DAPI-stained heterologous algae within the gastrovascular cavity. E = epidermis, M = mesoglea, G = gastrodermis, Gv = gastrovascular cavity. *See cover illustrations.*

ones can not be recognized. This method is also not suitable for long-term monitoring of the newly-acquired algae since constant darkness is required in order to maintain DAPI fluorescence, which would eventually lead to the death of both algae and polyps (Davy et al., 1997). The question of whether the short-term residence of these algae represents a formation of a new symbiosis requires further study.

Our findings demonstrate that cnidarian hosts previously considered as true closed system symbioses, may in fact possess the ability for entry and possible residence of heterologous symbionts also from the environment. Therefore, they further support earlier findings by van Oppen (2004), suggesting a certain degree of flexibility in the closed system symbiosis. Such ability as demonstrated in our study may be beneficial to these hosts under stressful conditions, including bleaching events (see Buddemeier and Fautin, 1993). Further studies are undoubtedly needed in order to determine whether entry of the DAPI-stained symbionts indeed represents formation of a successful symbiosis by vertically-transmitting hosts such as *L. crosslandi*.

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Field collection of animals complied with a permit issued by the Israel Nature and National Parks Protection Authority.

REFERENCES

Achituv, Y., Benayahu, Y., and Hanania, J. 1992. Planulae brooding and acquisition of zooxanthellae in *Xenia-macrospiculata* (Cnidaria, Octocorallia). *Helgoländer Wissenschaftliche Meeresuntersuchungen* **46**: 301–310.

Adler, I. 2006. Infection of early developmental stages of soft corals with homologous and heterologous symbiotic algae. MSc Thesis under supervision of Prof. Yehuda Benayahu, George S. Wise Faculty of Life Sciences, Tel-Aviv University, Israel.

Baker, A.C. 2001. Ecosystems – Reef corals bleach to survive change. *Nature* **411**: 765–766.

Baker, A.C. 2003. Flexibility and specificity in coral-algal symbiosis: Diversity, ecology, and biogeography of *Symbiodinium*. *Annual Review of Ecology Evolution and Systematics* **34**: 661–689.

Barneah, O., Weis, V.M., Perez, S., and Benayahu, Y. 2004. Diversity of dinoflagellate symbionts in Red Sea soft corals: mode of symbiont acquisition matters. *Marine Ecology Progress Series* **275**: 89–95.

Ben-David-Zaslow, R. and Benayahu, Y. 1998. Competence and longevity in planulae of several soft corals. *Marine Ecology Progress Series* **163**: 235–243.

Benayahu, Y., Weil, D., and Malik, Z. 1992. Entry of algal symbionts into oocytes of the Coral *Litophyton arboreum*. *Tissue and Cell* **24**: 473–482.

Benayahu, Y. 1997. Developmental episodes in reef soft corals: Ecological and cellular determinants. *Proceedings of the 8th International Coral Reefs Symposium, Smithsonian Tropical Research Institute, Balboa, Panama* **2**: 1213–1218.

Benayahu, Y. and Schleyer, M.H. 1998. Reproduction in *Anthelia glauca* (Octocorallia, Xeniidae). II. Transmission of algal symbionts during planular brooding. *Marine Biology* **131**: 433–442.

Berkelmans, R. and van Oppen, M.J.H. 2006. The role of zooxanthellae in the thermal tolerance of corals: a "nugget of hope" for coral reefs in an era of climate change. *Proceedings of the Royal Society of London B, Biological Sciences* **273**: 2305–2312.

Buddemeier, R.W. and Fautin, D.G. 1993. Coral bleaching as an adaptive mechanism. *Bioscience* **43**: 320–326.

Coffroth, M.A., Santos, S.R., and Goulet, T.R. 2001. Early ontogenetic expression of specificity in a cnidarian-algal symbiosis. *Marine Ecology Progress Series* **222**: 85–96.

Coffroth, M.A. and Santos, S.R. 2005. Genetic diversity of symbiotic dinoflagellates in the genus *Symbiodinium*. *Protist* **156**: 19–34.

Davy, S.K., Lucas, I.A.N., and Turner, J.R. 1997. Uptake and persistence of homologous and heterologous zooxanthellae in the temperate sea anemone *Cereus pedunculatus* (Pennant). *Biological Bulletin* **192**: 208–216.

Douglas, A.E. 1994. *Symbiotic Interactions.* Oxford Scientific Publications. pp. 79–99.

Douglas, A.E. 2003. Coral bleaching – how and why? *Marine Pollution Bulletin* **46**: 385–392.

Falkowski, P.G., Dubinsky, Z., Muscatine, L., and Porter, J.W. 1984. Light and the bioenergetics of a symbiotic coral. *Bioscience* **34**: 705–709.

Glynn, P.W., Mate, J.L., Baker, A.C., and Calderon, M.O. 2001. Coral bleaching and mortality in Panama and Ecuador during the 1997–1998 El Nino-Southern oscillation event: Spatial/temporal patterns and comparisons with the 1982–1983 event. *Bulletin of Marine Science* **69**: 79–109.

Goulet, T.L. 2006. Most corals may not change their symbionts. *Marine Ecology Progress Series* **321**: 1–7.

Hoegh-Guldberg, O. 1999. Climate change, coral bleaching and the future of the world's coral reefs. *Marine and Freshwater Research* **50**: 839–866.

Hunter, C.L., Morden, C.W., and Smith, C.M. 1997. The utility of ITS sequences in assessing relationships among zooxanthellae and corals. *Proceedings of the 8th International Coral Reefs Symposium, Panama* **2**: 1599–1602.

Kinzie, R.A.,III. 1974. Experimental infection of aposymbiotic gorgonian polyps with zooxanthellae. *Journal of Experimental Marine Biology and Ecology* **15**: 335–345.

LaJeunesse, T.C. 2002. Diversity and community structure of symbiotic dinoflagellates from Caribbean coral reefs. *Marine Biology* **141**: 387–400.

LaJeunesse, T.C., Lambert, G., Andersen, R.A., Coffroth, M.A., and Galbraith, D.W. 2005. *Symbiodinium* (Pyrrhophyta) genome sizes (DNA content) are smallest among dinoflagellates. *Journal of Phycology* **41**: 880–886.

Lessard, E.J., Martin, M.P., and Montagnes, D.J.S. 1996. A new method for live-staining protists with DAPI and its application as a tracer of ingestion by walleye pollock (*Theragra chalcogramma* (Pallas)) larvae. *Journal of Experimental Marine Biology and Ecology* **204**: 43–57.

Little, A.F., van Oppen, M.J.H., and Willis, B.L. 2004. Flexibility in algal endosymbioses shapes growth in reef corals. *Science* **304**: 1492–1494.

Loh, W.K.W., Loi, T., Carter, D., and Hoegh-Guldberg, O. 2001. Genetic variability of the symbiotic dinoflagellates from the wide ranging coral species *Seriatopora hystrix* and *Acropora longicyathus* in the Indo-West Pacific. *Marine Ecology Progress Series* **222**: 97–107.

Maoz, A. 2005. Algal acquisition and specificity in soft corals (families Nephtheidae and Xeniidae). MSc Thesis under supervision of Prof. Yehuda Benayahu, George S. Wise Faculty of Life Sciences, Tel-Aviv University, Israel.

Manzini, G., Xodo, L., Barcellona, M. L., and Quadrifoglio, F. 1985. Interaction of DAPI with synthetic and natural deoxy- and ribonucleic acids. *Journal of Biological Sciences* **8**: 699–711.

Marlow, H.Q. and Martindale, M.Q. 2007. Embryonic development in two species of scleractinian coral embryos: *Symbiodinium* localization and mode of gastrulation. *Evolution & Development* **9**: 355–367.

Mieog, J.C., Van Oppen, M.J.H. et al. 2007. Real-time PCR reveals a high incidence of *Symbiodinium* clade D at low levels in four scleractinian corals across the Great Barrier Reef: Implications for symbiont shuffling. *Coral Reefs* **26**: 449–457.

Montgomery, M.K. and Kremer, P.M. 1995. Transmission of symbiotic dinoflagellates through the sexual cycle of the host scyphozoan *Linuche unguiculata*. *Marine Biology* **124**: 147–155.

Moss, C., Green, D.H., Pérez, B., Velasco, A., Henríquez, R., and McKenzie, J.D. 2003. Intracellular bacteria associated with the ascidian *Ecteinascidia turbinata*: phylogenetic and *in situ* hybridisation analysis. *Marine Biology* **143**: 99–110.

Muscatine, L., Falkowski, P.G., Porter, J.W., and Dubinsky, Z. 1984. Fate of photosynthetic fixed carbon in light-adapted and shade-adapted colonies of the symbiotic coral *Stylophora* pistillata. Proceedings of the Royal Society of London Series B Biological Sciences 268: [illegible].

Pasternak, Z., Bachar, A., Abelson, A., and Achituv, Y. 2004. Initiation of symbiosis between the soft coral *Heteroxenia fuscescens* and its zooxanthellae. *Marine Ecology Progress Series* 279: 113–116.

Porter, J.W., Muscatine, L., Dubinsky, Z., and Falkowski, P.G. 1984. Primary production and photoadaptation in light-adapted and shade-adapted colonies of the symbiotic coral, *Stylophora pistillata*. *Proceedings of the Royal Society of London Series B Biological Sciences* 222: 161–180.

Rodriguez-Lanetty, M., Krupp, D.A., and Weis, V.M. 2004. Distinct ITS types of *Symbiodinium* in Clade C correlate with cnidarian/dinoflagellate specificity during onset of symbiosis. *Marine Ecology Progress Series* 275: 97–102.

Rowan, R. and Powers, D.A. 1992. Ribosomal RNA sequences and the diversity of symbiotic dinoflagellates (zooxanthellae). *Proceedings of the National Academy of Sciences, USA* 89: 3639–3643.

Rowan, R. 1998. Diversity and ecology of zooxanthellae on coral reefs. *Journal of Phycology* 34: 407–417.

Trench, R.K. 1987. Dinoflagellates in non-parasitic symbioses. In: *The Biology of Dinoflagellates*. Taylor, F.J.R., ed. Blackwell, Oxford, pp. 530–570.

Toller, W.W., Rowan, R., and Knowlton, N. 2001. Repopulation of zooxanthellae in the Caribbean corals *Montastraea annularis* and *M. faveolata* following experimental and disease-associated bleaching. *Biological Bulletin* 201: 360–373.

Van Oppen, M.J.H. 2004. Mode of zooxanthella transmission does not affect zooxanthella diversity in acroporid corals. *Marine Biology* 144: 1–7.

Van Oppen, M.J.H., Palstra, F.P., Piquet, A.M.T., and Miller, D.J. 2001. Patterns of coral-dinoflagellate associations in *Acropora*: significance of local availability and physiology of *Symbiodinium* strains and host-symbiont selectivity. *Proceedings of the Royal Society of London Series B Biological Sciences* 268: 1759–1767.

Weis, V.M., Reynolds, W.S., deBoer, M.D., and Krupp, D.A. 2001. Host-symbiont specificity during onset of symbiosis between the dinoflagellates *Symbiodinium* spp. and planula larvae of the scleractinian coral *Fungia scutaria*. *Coral Reefs* 20: 301–308.

Yacobovitch, T. [illegible]. Acquisition of zooxanthellae by [illegible] offspring of the soft coral *Heteroxenia fuscescens*. MSc Thesis under supervision of Prof. Yehuda Benayahu, George S. Wise Faculty of Life Sciences, Tel Aviv University, Israel.

Yacobovitch, T., Benayahu, Y., and Weis, V.M. 2004. Motility of zooxanthellae isolated from the Red Sea soft coral *Heteroxenia fuscescens* (Cnidaria). *Journal of Experimental Marine Biology and Ecology* 298: 35–48.

[illegible] [illegible]. Distribution and specificity of algal symbionts in [illegible] associating with corals (family [illegible]) in the Red Sea. MSc Thesis under supervision of Prof. Yehuda Benayahu, George S. Wise Faculty of Life Sciences, Tel Aviv University, Israel.