2.2 Materials and Methods

Preparation of fragments

Groups of fragments (~15 cm long) were removed with scissors from colonies of *Clathraria rubrinodis* growing on the reef adjacent to the underwater observatory at Eilat (5-20 m). They were placed underwater in zip-lock bags and immediately transported to the Interuniversity Institute for Marine Sciences in Eilat (IUI) and maintained in outdoor running seawater tanks. After 24 hours, transplants of the desired size (see ahead) were produced, each comprised mostly of a single branch. The transplants were then individually attached to holes (2 mm in diameter) drilled in recycled PVC plates (20x10x1 cm), by metal-free Epoxy glue (Aqua Mend). In order to avoid contact between the glue and the live tissue of the transplant, its basal tissue was carefully removed, thus exposing ~0.5 cm of its axis and this was then glued into the holes. Each plate contained 8-12 transplants that were derived from the same parent colony and labeled accordingly (Fig. 2.1). The plates were attached to a PVC rack (70x100x1 cm) placed on the reef (see ahead). The transplants were photographed immediately following placement and later at several time intervals (see ahead) using a digital Olympus C-5060 WZ camera in an underwater housing. In order to eliminate distortion effect and to ensure repeated photography at a perpendicular angle, a frame was designed to hold the plate with the transplants (Fig. 2.2). Survival rates and growth patterns of the transplants were calculated from the digital photographs using the image analysis software Image Tool (Ver. 3.0).

Fig. 2.1 Recycled PVC plate with transplants of *Clathraria rubrinodis* derived from the same parent colony.

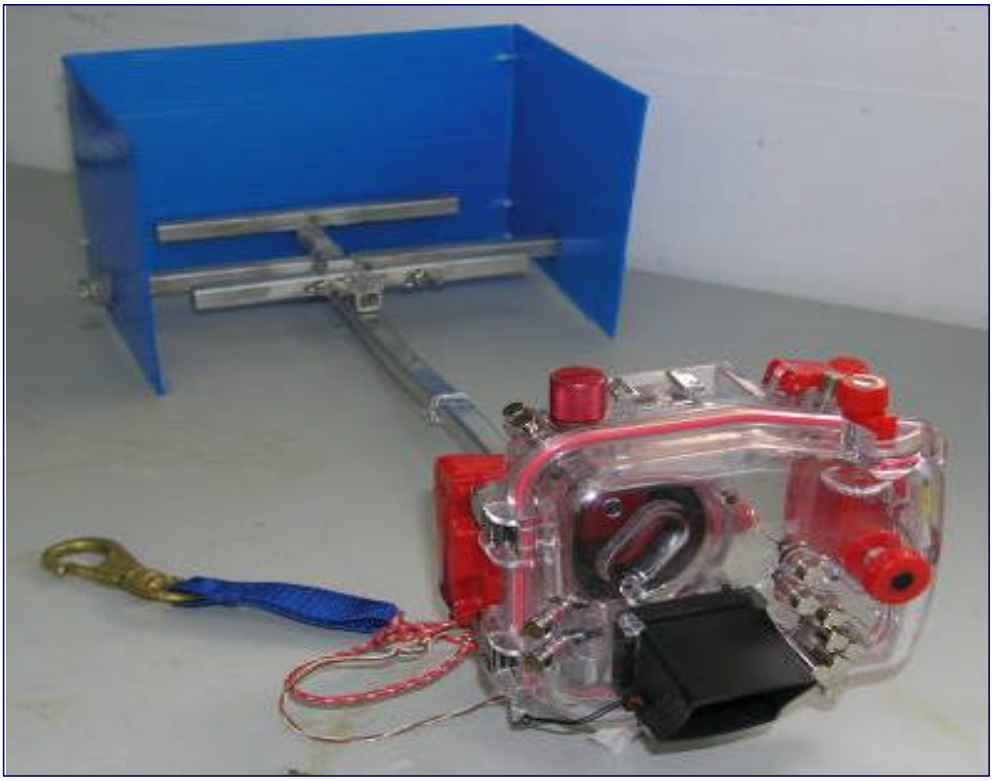

Fig. 2.2 Frame designed to hold plates with transplants of *Clathraria rubrinodis*, for photographing with a digital Olympus C-5060 WZ camera.

Combined transplantation experiments

In order to examine possible relationships between the initial size of the transplants, their survivorship and growth rates in running seawater tanks and later in field conditions, three size classes of transplants were prepared in August 2004: (1) small 2-3 cm (n=71); (2) intermediate 4-5 cm (n=72); and (3) large 6-7 cm (n=67). Batches of 8-12 transplants of similar size class were obtained from the same parent colony and then glued onto individual plates. Transplants of each size class were derived from the four colonies that were used in order to produce transplants of all three size classes. The transplants were reared for three months in running seawater tanks. The tanks were placed in the shade and grazers of the family Trochidae were introduced into the tanks in order to decrease algal development. On November 2004 the plates with the transplants were placed on the reef off the Oil Jetty, Eilat (15 m), adjacent to naturally-growing *C. rubrinodis* colonies. The rack with the plates was placed 20 cm above the bottom, in order to minimize accumulation of sediment or grazing by sea urchins or other organisms. The transplants were photographed monthly during the initial 3 month period (September, November and December 2004), and then in month 9 (May 2005) and month 12 (August 2005) post-transplantation.

Transplantation experiments under field conditions

Based on the results obtained in the above experiment (see Results ahead), in January 2004 large transplants (6-7 cm) were prepared as described above (n=64 transplants derived from 3 parent colonies, 8 plates). The transplants were photographed and then

placed on a rack on the IUI reef (9 m) besides natural-growing colonies of *C. rubrinodis*. At this experimental site the rack was covered with a PVC net (eye size 2x2 cm) in order to avoid establishment of damselfish territories that could lead to detachment of the transplants from their transplantation plates. In June 2005, a similar experiment was also conducted with large transplants (n=51 transplants derived from 3 parent colonies glued to 7 plates), placed on the Oil Jetty reef. In both experiments the transplants were monitored monthly for 3 successive months.

Computation of survivorship rate and growth

During the monitoring dates each transplant was scored as either alive or dead. Transplants that exhibited breakage, detachment or partial mortality were not included in survivorship and growth analysis. Data of survivorship were subjected to the non-parametric Long Rank Statistic test and $?^2$ test (SPSS, Ver. 12.0, Zar, 1999) in order to determine differences in survivorship related to size, source of transplants (among four parent colonies represented in the combined experiment) and type of transplantation experiment (running seawater tanks *vs*. field).

Growth data included both linear extension of the transplants and formation of new branches. The linear length of the transplants was determined by measuring the digitized images (±1 mm) from base to tip. When they also comprised side branches, the total length was calculated as the sum of the lengths of all branches. In order to detect changes in growth of the transplant between two successive measurement dates the following parameters were examined; (1) growth rate, (2) branching rate and (3) growth rate in

relation to the number of branches per transplant. Growth rate was computed for each measurement date as the ratio between the linear length increment of each transplant and its total length on the preceding date. When comparing the results obtained in the two transplantation experiments (running seawater tanks *vs.* field) the numeric values were square root transformed prior to the ANOVA test in order to meet the test requirements. Correlation between growth rate and number of branches of the transplants was examined by Spearman correlation test. Branching rate was calculated as the ratio between the number of branches per transplant between two successive measurement dates. Data were subjected to the non-parametric Friedman ANOVA test in order to detect differences in relation to size and type transplantation experiments (running seawater tanks *vs*. field).