

One Rational Strategy for Restoration of Coral Reefs: Application of Molecular Biological Tools to Select Sites for Rehabilitation by Asexual Recruits [☆]

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Experiments for reef rehabilitation were performed at two selected sites near Hurghada (Red Sea, Egypt) the reef close to the Marine Biological Station (with a high sedimentation rate from landfilling) and El-Fanadir Reef (a clear water site). Since only little is known about the influence of the physical environmental conditions, novel molecular biological approaches have been introduced to assess the metabolic status of corals. In order to avoid possible interference with symbionts the molecular studies have been performed with the octocoral *Dendronephthya klunzingeri*; this species does not contain zooxanthellae. The metabolic enzymes fructose-1,6-bisphosphatase and the succinat-dehydrogenase served as markers for the assessment of the health status of the corals. The cDNAs for both enzymes were isolated and their levels of expression were found to be correlated with the degree of environmental stress. High expression was found at the El-Fanadir Reef, while only low levels were measured at the Marine Biological Station, which is characterized by high sedimentation rates. From this it is concluded that the health state of *D. klunzingeri* from El-Fanadir is superior to the one from the Marine Biological Station. Six reef-building corals have therefore been selected from

El-Fanadir for the transplantation studies. We applied fixation of coral nubbins in plastic meshes with narrow openings. The asexual recruits remained either unfixed or had been glued to the mesh with epoxy resin. A total of 236 coral fragments were transplanted at the Marine Biological Station Reef and 108 fragments at El-Fanadir Reef. The referred technique was successful and the survival rates were higher for samples fixed with epoxy resin than for those without epoxy resin. The survival and growth rates of the coral transplants were found to be higher at the windward side of El-Fanadir Reef than on the leeward side of the same reef. Furthermore, the mortality rates at the leeward side of El-Fanadir Reef were still lower compared to the Marine Biological Station. The coral species *Pocillopora damicornis* grew well in the clearer water and hard rocky substrate but it did not grow at all in turbid water and sandy substrate; however, the species *Acropora humilis* grew well in both environments but with higher rates in the clearer water. After one year of transplantation, the massive coral *Favia stelligera* recorded the highest survival rate of all coral species at the Marine Biological Station Reef; but among branching corals, *A. humilis* had the highest value and *P. damicornis* the lowest. Contrary to this result, *P. damicornis* recorded the highest value at El-Fanadir Reef. It is concluded that asexual recruits of corals, taken from a site (*D. klunzingeri* from El-Fanadir) physiologically favourable for them, are suitable for a coral restoration strategy. © 2000 Elsevier Science Ltd. All rights reserved.

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[☆]The sequences reported here are deposited in the EMBL/GenBank database (Accession no. AJ251054 for the *Dendronephthya klunzingeri* fructose-1,6-bisphosphatase and AJ251055 for the *D. klunzingeri* succinat-dehydrogenase).

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Introduction

Unfortunately, destruction of corals by recreational activities is not precluded by just proclaiming an area as a coral reef preserve even if it is supported by varying legislation, rules, monitoring and management programmes since those conventional management plans increase the accessibility to those areas and enhance dramatically the impacts of tourism on reef habitats (reviewed in: Rinkevich, 1995). Therefore, the search for a suitable strategy to be integrated with the proper management similar to that of already established re-forestation in terrestrial habitats, gained the attention of many scientists during the last three decades (reviewed in: Pickering *et al.*, 1998). The primary objectives of coral transplantation are to improve (i) reef quality in terms of live coral cover, (ii) biodiversity and (iii) topographic complexity (reviewed in: Edwards, 1998).

Maragos (1974) and Birkeland *et al.* (1979) were among the first to demonstrate the ability of coral transplants to rehabilitate reefs damaged by elevated eutrophication (Hawaii) and by heated effluents produced by a power station (Guam), respectively. Recruitment is one important component of coral life history and factors that have been shown to influence recruitment include: the biotic composition of communities, local oceanography, substratum complexity and herbivore grazing intensity (Baggett and Bright, 1985; Carlton and Sammarco, 1987). The rate of stabilization of fragments is related to substrate type and distance from a patch of mature colonies, suggesting that standing colonies may protect regenerating fragments removed from the reef (Lirman and Fong, 1997). Recruitment pattern varies between fore-reef and back-reef study sites (Harriot and Fisk, 1987). Recovery periods for damaged reefs are highly variable and depend largely on the nature of the disturbance and how recovery is defined. However, recovery may be accelerated by stabilizing cracked reefs with cement, removing loose sand or rubble (Miller *et al.*, 1993), or consolidating rubble using sponges (Wulff, 1984), deploying artificial structures to serve as areas for coral settlement or stable sites for transplantation (Clark and Edwards, 1994), and transplantation of corals to damaged areas (Yap *et al.*, 1990).

Two different strategies have been employed to study the potential use of coral recruits for restoration of damaged reefs. First, asexual recruits: Concrete or metal structures were recommended for artificial reef construction (Fitzhardinge and Bailey-Brock, 1989). Clark and Edwards (1994) transplanted 530 coral pieces by cementing them into the bottom. It was found that over a 28-month period most losses of transplants were due to the wave action; furthermore within 16 months most colonies had accreted naturally to the concrete mats. Van-Treek and Schuhmacher (1998) proposed to deposit calcium minerals from the seawater *in situ* by electrolysis on a template of any desired shape for transplantation of living coral fragments.

Second, sexual recruits: Several groups tried to transplant sexual recruits by collecting the reproductive products either in the laboratory by transplanting gravid colonies into aquaria just before they spawn gametes or shed planula larvae (Yates and Carlson, 1993) or in the field using plankton nets (Rinkevich and Loya, 1979). The use of sexual recruits is a longer and more complex process than using the asexual ones. Willis and Oliver (1988) found that coral planulae on the Great Barrier Reef were transplanted from one reef to another, 26 km down current within two days of spawning which indicates that planulae of broadcast spawners could be transplanted hundreds of kilometres. However, if damage of the area to which the larvae are transferred is extensive and the surface not suitable for settlement, the use of sexual recruits may be an inappropriate option.

One major hurdle for a successful transplantation is the selection of the appropriate matching sites; donor sites and transplantation, rehabilitation sites. As pointed out by Edwards (1998) there is no point in transplanting coral colonies to areas where water quality is poor as they will tend to die. Two different rational ways exist to assess the quality of the environment, either by using physical and chemical analysis of the milieu or by applying biomarkers. Since it is known that invertebrates living in the sea, inclusive of corals, can adapt to unfavourable milieu by inducing protecting scavenger proteins it appears justified to use one selected animal as a marker for the quality of the environment. Recently, it was shown that the sponge *Suberites domuncula* expresses a cysteine-rich metallothionein-like protein after exposure to cadmium (Schröder *et al.*, in press); subsequently it can cope with such unfavorable conditions. Therefore, biomarkers appear to be more suitable for the estimation of the actual health status of specimens from a given species than the assessment of physical or chemical stressors which reflect only the actual level of them. However, biomarkers can hardly be used alone for an assessment of ecological risks in pro- and/or retrospective manner (Suter, 1990). Applying this assumption to restoration strategies of coral recruits, biochemical and/or molecular parameters have been applied to assess the health state of corals which can serve as selection criteria for asexual recruits to be transplanted (reviewed in: Wiens *et al.*, in press). It is the purpose of the present study to elaborate molecular markers for the assessment of the physiological status of corals which will be used as source for asexual recruits. In the second step, these data will be implemented in a restoration strategy for coral reefs in Hurghada (Red Sea; Egypt). As a substrate, plastic meshes with narrow openings were used; the coral nubbins were inserted into the mesh and – where indicated – fixed with epoxy resin. The mesh was attached to the bottom.

For the molecular studies we have selected the octocoral *Dendronephthya klunzingeri* which is abundantly found in coral reefs in the Red Sea; this species does not contain zooxanthellae. As metabolic enzymes, selected

for the assessment of the health status of the corals, the fructose-1,6-bisphosphatase [Fru-1,6-Pase] and the succinat-dehydrogenase [succinat-DH] served as markers. The Fru-1,6-Pase is one key enzyme in the pathway of gluconeogenesis which maintains the glucose level in the metazoan organisms, while the succinat-DH, a member of the citric acid cycle, links this pathway with the respiratory chain. The latter enzyme is composed of a 27-kDa subunit, an iron protein, and a 70-kDa subunit, a flavoprotein. Both the Fru-1,6-Pase and the 27 kDa succinat-DH were cloned and used in the expression studies.

In order not to disturb the coral community, such species had been selected for transplantation which are most abundant at the selected collection site; at El-Fanadir-leeward side.

Materials and Methods

Chemicals and enzymes

DIG [digoxigenin] DNA labelling kit, DIG-11-dUTP, anti-DIG AP Fab fragments, CDP [disodium 2-chloro-5-(4-methoxyspiro{1,2-dioxetane-3,2'-(5'-chloro)-tricyclo[3.3.1.1.3,7]decan}-4-yl)phenyl phosphate] and CSPD [disodium 3-(4-methoxyspiro{1,2-dioxetane-3,2'-(5'-chloro)-tricyclo[3.3.1.1]decan-4-yl)phenyl phosphate] were from Boehringer Mannheim (Mannheim; Germany). The sources of chemicals and enzymes used were given previously (Kruse *et al.*, 1997; Wimmer *et al.*, 1999).

Corals

Specimens of *D. klunzingeri* (Studer) [Coelenterata: Anthozoa: Alcyonaria (Octocorallia)] were collected near Hurghada (Red Sea; Egypt). They were sampled from a depth of approximately 5 m at 25°C from their habitat, the hard bottom, and were either used immediately for experimental analysis or were kept at 22°C for three weeks after collection to adapt to this temperature.

For the transplantation studies the reef-building corals *Acropora humilis*, *Acropora verweyi* and *Acropora hemprichii* [Scleractinia: Acroporidae], *Stylophora pistillata* and *Pocillopora damicornis* [Scleractinia: Pocilloporidae], and *Favia stelligera* [Scleractinia: Faviidae] have been selected.

Preparation of the cDNA library from *D. klunzingeri*

Total RNA was extracted from coral tissue and polyadenylated mRNA was isolated from total RNA (Wiens *et al.*, in press). cDNA was prepared by using the ZAP Express™ cDNA synthesis kit. The cDNA library of *D. klunzingeri* was prepared in Hybri ZAPII (Stratagene) and packaged *in vitro* using MaxPlax™ Packaging Extract (Epicentre Technologies).

Isolation of the cDNA encoding the Fru-1,6-Pase

The partial coral *DEKLF16P* cDNA was cloned by polymerase chain reaction (PCR) from the *D. klunzingeri* cDNA library. The degenerate forward prim-

er 5'-TAT/CGCITTA/GTAT/CGGIAGT/CGCIAC/TATG-3', (where I = inosine) designed against the deduced nucleotide (nt) sequence of the conserved amino acid (aa) stretch aa₁₆₄ to aa₁₇₂ within the mammalian Fru-1,6-Pase cDNAs (e.g. the rat enzyme F16P_RAT (accession number CAA06313; Eschrich, 1998) was used in conjunction with the vector-specific reverse primer. The PCR reaction was carried out using a GeneAmp 9600 thermal cycler (Perkin-Elmer). An initial denaturation at 95°C for 3 min was used, followed by 35 amplification cycles of 95°C for 30 s, 58°C – 45 s, 74°C – 1.5 min, and a final extension step at 74°C for 10 min. The reaction mixture was as described earlier (Kruse *et al.*, 1997). A DNA fragment of ≈600 bp was obtained. This fragment was used to obtain the partial Fru-1,6-Pase clone (Ausubel *et al.*, 1995). The longest insert obtained had a size of 903 nt (excluding the poly(A) tail). The clone was termed *DEKLF16P* and was sequenced using an automatic DNA sequencer [Li-Cor 4200].

Isolation of the cDNA encoding the succinat-DH

The same strategy as described for the isolation of the Fru-1,6-Pase cDNA was chosen. The forward primer 5'-ATGGTICTIGATGCITTA/GATIAAA/GATIAA-3' was designed against the conserved region of mammalian succinat-DH cDNA aa₇₁ to aa₈₀. The annealing temperature was 52°C. The complete cDNA, *DEKLDHSB*, was obtained following standard procedures (Ausubel *et al.*, 1995).

Sequence analysis

The sequences were analysed using computer program Blast (Altschul *et al.*, 1997). Multiple alignment was performed with CLUSTAL W Ver. 1.6 (Thompson *et al.*, 1994), and the graphic presentation was prepared with GeneDoc (Nicholas and Nicholas, 1997). The graphical output of the bootstrap figure was produced by program 'Treeview' (Roderic D.M. Page, University of Glasgow, UK; [HTTP://http://taxonomy.zoology.gla.ac.uk/software.html#Treeviewing](http://taxonomy.zoology.gla.ac.uk/software.html#Treeviewing)).

Northern blotting

RNA was extracted from liquid-nitrogen pulverized coral tissue with TRIzol Reagent. An amount of 5 µg of total RNA was electrophoresed through 1% formaldehyde/agarose gel and blotted onto Hybond N⁺ membrane following the manufacturer's instructions (Amersham; Little Chalfont, Buckinghamshire; UK) (Wiens *et al.*, 1998). Hybridization was performed with the probe for (i) the Fru-1,6-Pase, *DEKLF16P*, (ii) the succinat-DH, *DEKLDHSB* and as a control (iii) the complete sequence (1.5 kb) of the sponge *Suberites domuncula* β-tubulin (*SDBTUB*; to be published).

For the quantification of the Northern blot signals the chemiluminescence procedure was applied (Stanley and Kricka, 1990); CDP-Star was used as substrate. The screen was scanned with the GS-525 Molecular Imager (Bio-Rad; Hercules, CA; USA).

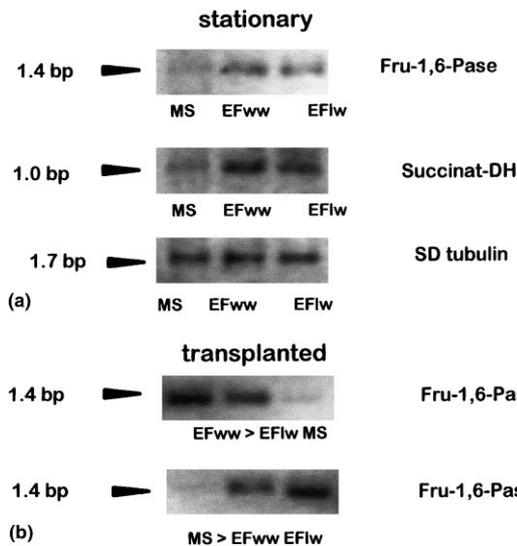


Fig. 2 Expression of the two enzymes, Fru-1,6-Pase and succinat-DH in the coral *D. klunzingeri* at different sites in the vicinity of Hurghada. (a) Level of expression of Fru-1,6-Pase and of succinat-DH in specimens collected at the following stationary sites: Marine Biological Station [MS], El-Fanadir-windward site [EFww] and El-Fanadir-leeward side [EFlw]. Tissue samples were collected, RNA extracted which was subjected to Northern blot analysis, using *DEKLF16P* (encoding for the Fru-1,6-Pase) or *DEKLDHSB* (encoding for the succinat-DH) as. 5 μ g of total RNA each was used for size separation. In parallel, the expression of β -tubulin (parallel samples of the expression of *DEKLF16P* in coral tissue) is measured to demonstrate that the same amount of RNA was applied per gel; the sponge probe from *S. domuncula* was used as a probe (SD tubulin). Further details are given under 'Materials and Methods'. (b) Experiments with specimens transplanted from the two sites (i) El-Fanadir-windward site [EFww], to El-Fanadir-leeward side [EFlw] or to the reef close to the Marine Biological Station [MS] or (ii) from the Marine Biological Station to the two reefs at the island El-Fanadir. The determinations of the level of expression, Northern blots, have been performed with tissue, taken from the specimens 10 days after transplantation.

Cloning of the coral succinat-DH

Sequence. Applying one degenerate primer, designed against one conserved region of the mammalian succinat-DHs the complete cDNA, *DEKLDHSB*, 1061 nt in length, encoding the coral succinat-DH was isolated by PCR technique. The potential open reading frame encodes a 282 aa long polypeptide DHSB_DEKL; Fig. 3. The deduced protein sequence of the coral Fru-1,6-Pase has a putative size (M_r) of 32,057 and an estimated isoelectric point (pI) of 8.46. The size of the transcript was 1 kb (Fig. 2), indicating that the complete cDNA was obtained.

The succinat-DH sequence comprises two characteristic sites present in the small subunit, the 2Fe-2S ferredoxin (Harayama *et al.*, 1991) as well as 4Fe-4S ferredoxin (the iron-sulphur binding region) (Otake and Ooi, 1987) signature at aa₈₆ to aa₁₀₄ and aa₁₇₉ to aa₂₀₁, respectively; (Fig. 3a).

Phylogenetic analyses. The deduced aa sequence DHSB_DEKL for the coral Fru-1,6-Pase shares $\approx 20\%$ of identical aa and $\approx 40\%$ similar aa (including identical aa) with the vertebrate sequences. A rooted phylogenetic

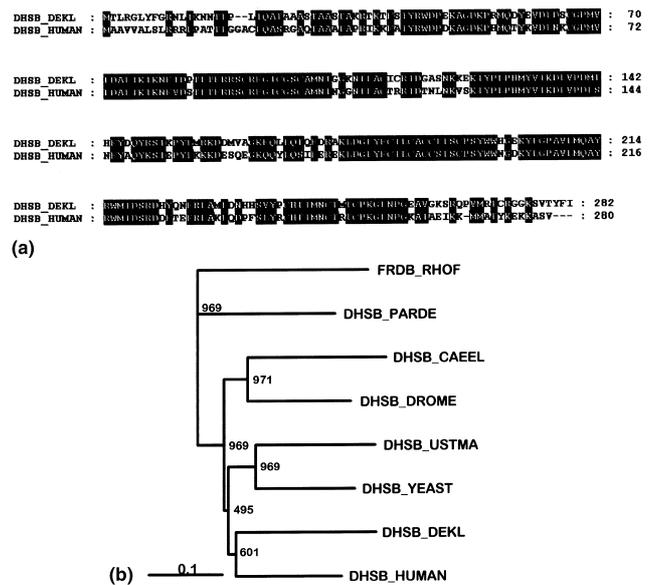


Fig. 3 *D. klunzingeri* succinat-DH polypeptide. (a) Alignment of the partial coral sequence DHSB_DEKL with the human succinat-DH IP subunit of complex II (DHSB_HUMAN, P21912 (Kita *et al.*, 1990)). The characteristic sites, the 2Fe-2S ferredoxin [2Fe-2S] as well as 4Fe-4S ferredoxin [4Fe-4S] one are indicated. In addition, the corresponding region on the nt level towards which the primer was designed [---] is marked. (b) Rooted phylogenetic tree, constructed after alignment of these sequences with the related sequence from *D. melanogaster* (DHSB_DROME, P21914 [Au and Scheffler, 1994]), *C. elegans* (DHSB_CAEEL, Q09545, [Matthews, 1995]), the yeast *S. cerevisiae* (DHSB_YEAST, P21801 [Lombardo *et al.*, 1990]) and the bacterium *P. denitrificans* (DHSB_PARDE, Q59662 [Dickins *et al.*, 1995]). The sequence fumarate reductase [iron-sulphur protein subunit] from the bacterium *R. fermentans* (FRDB_RHOF, BAA31216 [Miyadera and Kita, 1998]) was used as outgroup.

tree was constructed which revealed that the coral sequence forms again one branch with the mammalian succinat-DH, with an identity (similarity) of 63% (75%); (Fig. 3b). Lower are the identity (similarity) values for the invertebrates *Caenorhabditis elegans* [54% (68%)] and *Drosophila melanogaster* [56% (67%)]. Only distantly related are the sequences from the bacterium *Paracoccus denitrificans* [51% (64%)].

Expression of Fru-1,6-Pase and succinat-DH in *D. klunzingeri* from different sites

The technique of Northern blotting was applied to determine semiquantitatively the level of expression of the *DEKLF16P* gene in *D. klunzingeri* from different locations. As shown in Fig. 2a the level of expression of Fru-1,6-Pase is low at the Marine Biological Station and substantially higher at the two sites at the island El-Fanadir; at El-Fanadir-ww and El-Fanadir-lw. A similar pattern is seen for the expression of the gene encoding the succinat-DH, *DEKLDHSB*. The expression of tubulin was used to demonstrate that the same amounts of RNA have been applied per gel (Fig. 2a).

In order to establish that the levels of expression of the two genes encoding the enzymes Fru-1,6-Pase and

succinat-DH are dependent on the environmental conditions, specimens from *D. klunzingeri* were exchanged among the three locations selected. The determinations of the levels of expression have been performed 10 days after transplantation. It becomes evident that the expression of the Fru-1,6-Pase gene is downregulated if *D. klunzingeri* is transplanted from the El-Fanadir-ww site to Marine Biological Station (Fig. 2b); no considerable change is seen if the specimens are transferred from El-Fanadir-ww to El-Fanadir-lw. In contrast, if *D. klunzingeri* is transferred from the Marine Biological Station to the El-Fanadir-ww-or the El-Fanadir-lw site a strong up-regulation is seen (Fig. 2b).

A similar adaptation of the expression is seen for the enzyme succinat-DH as determined by Northern blotting. Again, the level of expression is low at the Marine Biological Station and is upregulated if the specimens are transferred to the two sites of the El-Fanadir Island (Fig. 2b). A downregulation occurs if the specimens are transplanted from the island to the Marine Biological Station (Fig. 2b).

From these data we conclude that the specimens living at the location close to the Marine Biological Station are characterized by a lower metabolic activity than those present at the two sites of the El-Fanadir Island.

Transplantation studies with reef-building corals

The reef-building corals *A. humilis*, *A. verweyi*, *A. hemprichii*, *S. pistillata*, *P. damicornis* and *F. stelligera* (Scleractinia: Faviidae) have been used to determine which species might be used preferentially for a restoration of the reef at the sites Marine Biological Station or El-Fanadir.

Asexual recruits were obtained from El-Fanadir. The coral nubbins were placed into plastic meshes and remained either unfixed or were fixed with epoxy resin prior to the transfer to the bottom of the sea (Fig. 4a). In Fig. 4b recruits from the coral *P. damicornis*, six months after transplantation at Marine Biological Station Reef are shown. It is obvious that algae started to overgrow the corals. Recruits from *S. pistillata*, six months after transplantation at Marine Biological Station Reef are shown in Fig. 4c. Fig. 4d shows the recruits from *A. hemprichii* which remained one year after transplantation at El-Fanadir Reef.

Health status of the recruits at the Marine Biological Station Reef

The determinations have been performed 6 months or 12 months after transplantation. The following parameters have been recorded: Percent of survival, percent of mortality, growth rate (in cm) and percent of attachment to the substratum. Based on periodical observations, no losses could be attributed to divers, storm or fish.

After six months. The results of coral transplantation after six months at the Marine Biological Station Reef are shown in Table 1. It is evident that those samples



Fig. 4 Use of asexual recruits for transplantation studies at two different locations close to Hurghada. (a) Coral nubbins at the beginning, fixed with epoxy resin to the plastic meshes just before taking them to be transplanted at the bottom of the sea. (b) The coral *P. damicornis*, six months after transplantation at Marine Biological Station Reef, algae starts to overgrow the corals. (c) *S. pistillata*, six months after transplantation at El-Fanadir Reef. (d) *A. hemprichii*, one year after transplantation at El-Fanadir Reef to the El-Fanadir-lw site.

which had been fixed with epoxy resin showed a higher degree of survival (85.7% and 90.5%, respectively) than recruits that had been attached to the mesh without epoxy resin (48.11–13.5%). *S. pistillata* showed the highest survival rate among samples fixed without epoxy resin (48.1%). *A. humilis* recorded a higher survival rate than *P. damicornis*, irrespective of the fixation method.

The massive coral *F. stelligera* had a higher survival rate (63.6%) than any of the branching corals which were fixed without epoxy resin. Among the specimens tested only the species *A. humilis* showed any growth (rate of 0.1–0.2 cm/6 months when fixed without epoxy resin and 0.1–0.3 cm/6 months when fixed with epoxy resin to the mesh).

After one year. Inspection after one year gave in some cases a different picture. Among all the corals *A. humilis* showed the highest survival rate after one year (Table 1). Again the species *P. damicornis* and *A. humilis* displayed an even higher survival in the assays fixed with epoxy (10.7% and 21.4%, respectively); lower is the rate if the recruits remained in the mesh without fixation with epoxy resin (8.1%, 13.8%).

From these studies it is evident that, among the branching corals fixed to the mesh without epoxy, the species *A. humilis* recorded the highest survival rate after one year irrespective of the fixation method applied. The massive coral *F. stelligera* is the only species which stabilised after six months showing no increase in mortality from six months to one year.

The growth rate of the species *A. humilis* when fixed with epoxy resin was 0.7 cm/yr compared with 0.3 cm/yr when fixed without epoxy resin. In addition, it was observed that the species *S. pistillata* has achieved a low growth rate (0.3 cm/yr) while neither of the two

TABLE 1

Extent of survival, growth rate and attachment of asexual recruits transplanted at the leeward side of Marine Biological Station Reef, Hurghada (Red Sea). The recruits have been attached to the mesh either in the absence or presence of epoxy resin. The corals were examined 6 and 12 months after transplantation as described under 'Materials and Methods'.

Species	Number of transplanted recruits	Survival (%)	Growth rate (cm)	Attachment (%)
After six months branching corals				
Without epoxy				
<i>P. damicornis</i>	37	13.5	0.0	0.0
<i>A. humilis</i>	36	30.6	0.1–0.2	0.0
<i>S. pistillata</i>	54	48.1	0	0.0
With epoxy				
<i>P. damicornis</i>	56	85.7	0.0	0.0
<i>A. humilis</i>	42	90.5	0.1–0.3	0.0
Massive coral				
<i>F. stelligera</i>	11	63.6	0.0	0.0
(Total)	236	55.3 ± 1.3		
After one year Branching corals				
Without epoxy				
<i>P. damicornis</i>	37	8.1	0.0	0.0
<i>A. humilis</i>	36	13.8	0.3	0.0
<i>S. pistillata</i>	54	11.1	0.3	0.0
With Epoxy				
<i>P. damicornis</i>	56	10.7	0.0	0.0
<i>A. humilis</i>	42	21.4	0.7	0.0
Massive corals				
<i>F. stelligera</i>	11	63.6	0.0	0.0
(Total)	236	21.4 ± 7.8		

species *P. damicornis* and *F. stelligera* showed any growth at all (Table 1). None of the specimens transplanted to the Marine Biological Station were attached to the bottom.

Health status of the recruits at the El-Fanadir Reef

After six months. The results of the coral transplantation studies at El-Fanadir Reef after six months are shown in Table 2. The survival rates of samples at this

TABLE 2

Survival, growth rate and attachment of asexual recruits transplanted at two different sites at the El-Fanadir Reef (Hurghada). The measurements have been performed 6 and 12 months after transplantation.

Species	Number of transplanted recruits	Survival (%)	Growth rate (cm)	Attachment (%)
After six months				
Leeward side				
<i>A. humilis</i>	16	81.2	0.1	0.0
<i>A. verweyi</i>	15	80	0.1–0.2	0.0
<i>A. hemperichi</i>	18	77.8	0.0	0.0
<i>S. pistillata</i>	15	93.3	0.0	0.0
<i>P. damicornis</i>	14	78.8	0.0	0.0
Windward side				
<i>A. humilis</i>	16	87.5	0.4	37.5
<i>P. damicornis</i>	14	85.7	0.5	42.8
(Total)	108	83.4 ± 1.9		
After one year				
Leeward side				
<i>A. humilis</i>	16	68.7	0.5	75
<i>A. verweyi</i>	15	66.6	0.3	0.0
<i>A. hemprichii</i>	18	55.5	0.0	0.0
<i>S. pistillata</i>	15	60	0.5	66.6
<i>P. damicornis</i>	14	71.4	0.4	85.7
Windward side				
<i>A. humilis</i>	16	75	0.6	87.5
<i>P. damicornis</i>	14	78.5	0.7	92.8
(Total)	108	68 ± 2.8		

site (a clear water site) ranged from 93.3% (*S. pistillata*) to 77.8% (*A. hemprichi*). All samples transplanted to El-Fanadir had in general a higher survival rate than those fixed without epoxy resin at the Marine Biological Station (a site with high sedimentation rate). The survival rates of the two species *A. humilis* and *P. damicornis* at the windward side (87.5% and 85.7%, respectively) were higher than those at the leeward side (81.2% and 78.8%). On the leeward side only the two species *A. humilis* and *A. verweyi* recorded growth rates of 0.1 cm/6 months and 0.1–0.2 cm/6 months, respectively, while on the windward side *A. humilis* and *P. damicornis* recorded growth rates of 0.4 cm/6 months and 0.5 cm/6 months, respectively.

After one year. Among the species transplanted at the leeward side of the reef, it was measured (Table 2) that the species *P. damicornis* achieved the highest survival rate (71.4%) in contrast to the species *A. hemprichii* with the lowest value (55.5%). *A. humilis* also had a better survival rate at El-Fanadir Reef (68.7%) than at the Marine Biological Station Reef. The highest growth rate at the windward side was achieved by the species *P. damicornis* (0.7 cm/yr) while in the leeward side the highest values were recorded for the two species *A. humilis* and *S. pistillata* (0.5 cm/yr). The species *A. hemprichii* did not show any growth rate after one year of transplantation. It is important to note that the specimens, especially of the species *A. humilis* and *P. damicornis* and also *S. pistillata* were attached to the bottom to a large extent (Table 2).

Comparison between El-Fanadir and Marine Biological Station

The two sites can be compared in the three species *P. damicornis*, *A. humilis* and *S. pistillata*; these species were successfully transplanted in the different biotopes. The study proved that the survival rates of these three species after one year in El-Fanadir Reef (a clear water site) are high (71.4%, 68.7%, 60%) compared with the lower values (8.1%, 13.8% and 11.1%) for the same three species that had been transplanted at the Marine Biological Station Reef. The growth rates of the three species in the leeward side of El-Fanadir Reef were 0.4, 0.5 and 0.5 cm/yr, respectively compared to the values for the same species in the same circumstances at Marine Biological Station Reef (0, 0.3 and 0.3 cm).

It can be concluded from the study that the species *P. damicornis* grew well in El-Fanadir Reef in contrast to the behaviour at the Marine Biological Station Reef. It is also notable that the species *A. humilis* grew in El-Fanadir Reef to a larger extent than at the Marine Biological Station Reef when fixed in the mesh without epoxy resin.

Conclusion

Using two novel biochemical/molecular biological markers for the metabolic activity in corals, the two

enzymes Fru-1,6-Pase and succinat-DH, it could be demonstrated that a high expression of these genes correlates with the survival rates of asexual transplants at two selected areas in Hurghada. For the expression studies the octocoral *D. klunzingeri* was chosen. The expression is highest at those sites which are non-stressed, the locations around the El-Fanadir Island. The expression of the two metabolic enzymes is low at sites which are characterized by a high sedimentation rate, close to the Marine Biological Station. This correlation, gene expression and physical stress, will be studied in future in more detail to clarify if the molecular biological parameter can be used as a powerful biomarker for the assessment of pollution in general. For the transplantation studies samples were taken from El-Fanadir since from there the most viable specimens can be expected.

In the present study, molecular biological parameters were chosen for the selection of the transplantation sites for the asexual recruits. It is certainly the best strategy to integrate biomarkers with data from physical and chemical analyses as a measure of the health state of recruits. However, this multidisciplinary approach is costly but not prone to misleading conclusions. The stress load – both chemical and physical adverse influences on the environment – in a touristic area such as the one of Hurghada is very complex and dynamic. Therefore, the quantification of the physical and chemical stresses reflects only a momentary situation. Based on this rationale it appears to be justified to use molecular markers as an alternative. A well-selected biomarker might include not only a momentary response of the animals but might reflect a longer-term adaptation to the environment. Under this premise the sedimentation rate has been found the most prominent physical parameter which appears to be important for the studies reported here (M.S.A. Ammar and A.H. Nawar; to be published); it is lowest at El-Fanadir-ww with 4.0 (mg/cm²/day), followed by El-Fanadir-lw with 6.1 and Marine Biological Station with 12.6.

The overall survival of transplanted coral colonies of approximately 70% after one year at El-Fanadir is close to the survival recorded by Auberson (1982). The higher survival rates of the two species *P. damicornis* and *A. humilis*, when fixed with epoxy resin compared to non-fixed recruits indicates the importance of attaching the colonies securely and strongly to avoid loss or detachment from the substrate in case of high or even moderate energy environment. In addition, epoxy resin may help to prevent loosening of the attachment of corals to the bottom by the effect of high sedimentation rate and to compensate for the difficulties of accretion on sandy bottoms. The lower mortality rate after one year at the clean site, El-Fanadir (clean site) compared to that measured at Marine Biological Station (a site polluted by sedimentation from landfilling) indicates the importance of clear water for artificial reef construction. High

sedimentation rate causes expulsion of zooxanthellae, cellular damage and after complete burial, death (Rogers, 1983).

The massive coral *F. stelligera* is an exception to this result; it stabilized after one year (only a 36.3% mortality was measured). The stability of the massive coral *F. stelligera* towards an unfavorable environment may be due to the broader surface area which enables it to trap larger amounts of light even at the turbid site; besides, it may have a high cleaning mechanism for sediments.

The considerable accretion among transplants at El-Fanadir Reef with rocky substrate and the complete absence at Marine Biological Station indicates that – as expected – hard substrates with clearer waters are more suitable for settlement and growth of transplanted corals in contrast to transplantation of corals in stressed areas.

We conclude;

- (i) Physical fixation of coral nubbins in plastic meshes of narrow openings and gluing them with epoxy resin is a successful means of coral transplantation as documented by a low mortality rate.
- (ii) The survival rate among the coral species transplanted in the leeward side of El-Fanadir Reef (clean site) is higher than that of the same species at the Marine Biological Station Reef
- (iii) The survival and growth rates of coral transplants are higher in the windward side of El-Fanadir Reef than in the leeward side of the same site.
- (iv) The coral species *P. damicornis* grows well in clear water and on hard rocky substrate but it does not grow at all in turbid water and sandy substrate while the species *A. humilis* grows well in both sites but with higher rates in the clearer water.
- (v) At the Marine Biological Station, the massive coral *F. stelligera* recorded the highest survival rate of all coral species after one year while the branching coral *A. humilis* recorded the highest value among branching corals, however, at El-Fanadir, the branching coral *P. damicornis* recorded the highest survival rate after one year. At the present stage we can only speculate about the reasons for the differential survival of the transplants at the selected sites. It appears reasonable to assume that the strong differences of the sedimentation rates between the sites are one major factor.

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