

## ***The application of sexual coral recruits for the sustainable management of ex situ populations in public aquariums to promote coral reef conservation — SCORE Project***

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### ABSTRACT

1. SCORE (SEXual CORal REproduction) Project is an initiative of public aquariums and research institutions to produce and exchange sexual coral recruits for the sustainable management of *ex situ* populations. Here we present the results of the initial three years (2002–2004).

2. Primary polyps ( $n = 501$ ) of corals (*Acropora tenuis*, *Agaricia humilis*, *Favia fragum*) were transported from Rotterdam Zoo to Cologne, Burgers', Hagenbeck and London Zoos, where development of juveniles was monitored for 10 months. All polyps were produced at Rotterdam Zoo from laboratory colonies (*A. humilis*, *F. fragum*), and from larvae generated from field collected gametes at Akajima, Okinawa, Japan (*A. tenuis*). Additionally, planulae of *A. tenuis* ( $n = 1440$ ) were transported from Rotterdam Zoo to Burgers' Zoo and to London Zoo to obtain primary polyps.

3. Larval settlement (*A. tenuis*) was observed to be  $3.00 \pm 2.57\%$  (mean  $\pm$  SD;  $n = 1480$ ) in 2002 and  $17.36 \pm 6.01\%$  (mean  $\pm$  SD;  $n = 1480$ ) in 2003, significantly lower compared to settlement at Rotterdam Zoo ( $57.84 \pm 11.01\%$  in 2003; mean  $\pm$  SD,  $n = 1480$ ). High post-transport survival rates of  $95.18 \pm 4.86\%$  (mean  $\pm$  SD;  $n = 501$ ) were observed in primary polyps of all species.

4. Juvenile survival ( $t = 10$  months; *A. tenuis*: 18.4–86.2%; *A. humilis*: 0–19.7%; *F. fragum*: 13.3–72.7%) differed significantly between institutions. Mean colony sizes (measured 10 months after transportation) were, in all cases, similar or higher to those reported from literature.

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5. The results demonstrate the potential of this method to serve as an economical and sustainable alternative to existing mostly exploitative techniques for aquarium stocking. The use of sexual recruits provides an effective and low cost alternative, which is, in principle, applicable to all coral species.

6. The project was extended from 9 to 28 institutions across Europe, the USA and Japan in 2004. Copyright © 2006 John Wiley & Sons, Ltd.

KEY WORDS: coral; *ex situ* population; sustainability; conservation; aquarium; sexual reproduction; SCORE Project

## INTRODUCTION

Scleractinian corals (Anthozoa, Cnidaria), the main builders of the most diverse marine ecosystem of the world — the coral reef — are highly threatened by human activities (Grigg and Dollar, 1990; Brown, 1997). Anthropogenic disturbances include, sedimentation (Chansang *et al.*, 1982), sewage and eutrophication (Bell, 1992; Grigg, 1994), dive tourism (Price and Firaq, 1996; Rouphael and Inglis, 1997), and the collection of coral specimens for the ornamental trade (Green and Shirley, 1999; Best, 2002).

Zoos and public aquariums play an increasing role in the conservation of endangered species and ecosystems (Hutchins and Conway, 1995; Wetzel and O'Brien, 1995; Kelly, 1997). Today's aquariums are successful in husbandry and asexual propagation of corals (Carlson, 1987; Yates and Carlson, 1992; Atkinson *et al.*, 1995; Delbeek, 2001); exhibition tanks may be purely stocked with captive (asexually) propagated corals (e.g. Lisboa Aquarium, Falcato pers. comm.; Waikiki Aquarium, Delbeek, pers. comm.). However, the majority of exhibited animals are still derived from the field, from the trade or from customs' confiscations (Atkinson *et al.*, 1995; Green and Shirley, 1999).

Recent studies show a high potential for sexual reproduction techniques to contribute to coral reef restoration on a regional scale (Epstein *et al.*, 2001; Heyward *et al.*, 2002; Hatta and Iwao, 2003) including public aquariums (Nonaka *et al.*, 2003; see also Petersen and Tollrian (2001)). In addition, these techniques have been applied to produce colonies for display in public aquariums (Fan *et al.*, 2000). The application of sexual coral recruits on a large scale offers new possibilities in coral reef conservation to manage *ex situ* populations sustainably (Yates and Carlson, 1992; Delbeek, 2001; Petersen and Tollrian, 2001).

SCORE Project (SEXual CORal REproduction) is the first attempt to apply sexual coral recruits on a large scale to stock public aquariums sustainably. Several public aquariums and research institutions initiated the project in 2001, and it has been coordinated by Rotterdam Zoo, The Netherlands, and the University of Duisburg-Essen, Germany. The aims of this initial period were (1) to estimate the feasibility of the project, and (2) to show the potential for today's public aquariums to promote coral reef conservation.

## METHODS

### Species, origin and acquisition of larvae

Larvae of the Caribbean scleractinians *Favia fragum* and *Agaricia humilis* (for details on reproductive behaviour see Van Moorsel (1983) and Szmant-Froelich *et al.* (1985)) were constantly harvested from aquarium colonies at Rotterdam Zoo. Both species are mainly hermaphrodites (some gonochoric specimens were found in field populations of *A. humilis*; see Delvoye (1988)), which release settlement competent larvae following internal fertilization (= 'brooder'; for definition see Harrison and Wallace (1990)). *A. humilis* continuously releases larvae (= planulation) mainly at night (Van Moorsel, 1983) whilst field populations of *F. fragum* release larvae in a lunar-related cycle, with a peak 9–11 days after the new

moon (Szmant-Froelich *et al.*, 1985). In the laboratory, *F. fragum* showed maximum planulation 10–13 days after the new moon, mainly within 1–2 hr after the artificial lighting was switched off (Petersen, pers. obs.). *A. humilis* started planulating approximately 30 min after the artificial sunset occurred (Petersen, pers. obs.). The parental colonies used in this study to obtain larvae were collected in Curaçao, Netherlands Antilles, in October 2001, and transported to Rotterdam Zoo using a protocol adapted from Petersen *et al.* (2004). Colonies were transplanted onto Portland cement blocks (5 × 5 cm; H × W) for transportation and handling purposes.

During the annual mass spawning event at Aka Island, Okinawa, Japan (Hayashibara *et al.*, 1993), gametes of *Acropora tenuis* were collected *in situ* using non-invasive plankton nets covering, but not touching, the sampled colony (Hatta *et al.*, 2004). Eggs were *ex situ* fertilized, reared to larval stage following the protocol of Iwao *et al.* (2002), and transported to Rotterdam Zoo (Petersen *et al.*, in press b).

The species used in this study were not primarily chosen because of their importance for display. Instead, they served as model species representing both reproductive mechanisms. *A. humilis* and *F. fragum* are commonly found in the Caribbean (Van Moorsel, 1983; Szmant, 1986), and show maturity at a relatively small size (<2 cm; see Szmant (1986)) helping to reduce transportation costs for parental colonies. *Acropora tenuis* is commonly found at Okinawa (Hatta, pers. obs.). In this species, viable larvae were available owing to well established rearing protocols.

### Settlement

Larvae were settled on ceramic tiles in plastic aquariums at a constant temperature of 26°C using a water volume of approximately 1500 mL (natural sea water) and room light. We utilized two types of tiles, which were especially designed for mariculture (Petersen *et al.*, 2005; see also Figure 1). Pyramid tiles (truncated pyramid shaped, 17.0 × 17.0 mm, L × W) consisted of four vertical surfaces whilst flat tiles (22.0 × 22.0 mm, L × W) offered one horizontal surface for larval settlement. Twenty tiles of each type were temporarily

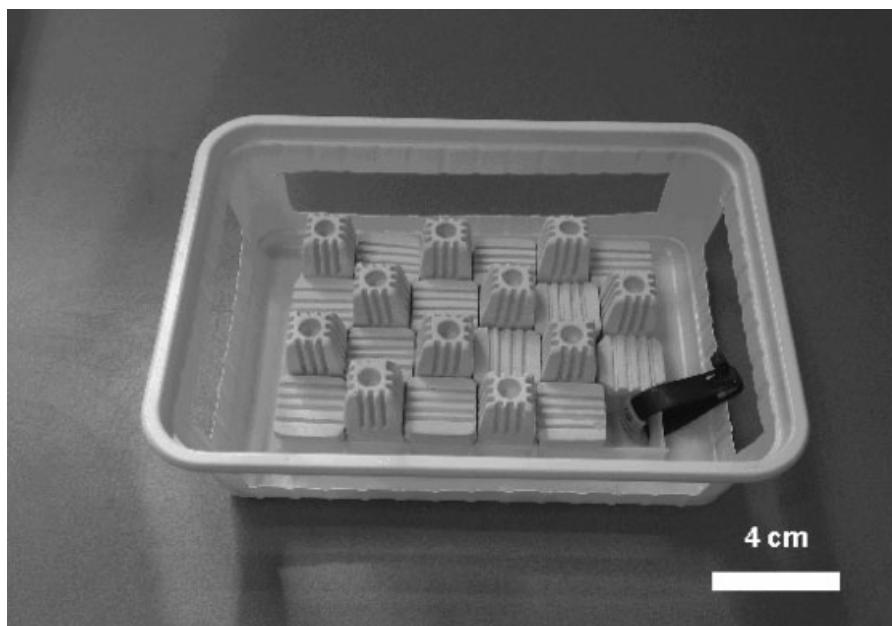


Figure 1. Arrangement of tiles in a polystyrene grid, which is placed in a polyethylene box for transportation of primary polyps.

arranged in a chessboard pattern in a polystyrene grid. All surfaces of the tiles exposed to the larvae had several parallel grooves that were highly attractive for larval settlement (Petersen *et al.*, 2005). Since the orientation of settlement substrates is crucial for the development of juveniles in the field (Sato, 1985; Maida *et al.*, 1994; Babcock and Mundy, 1996), it is an important consideration for successful mariculture. Tiles were checked for primary polyps after most larvae had settled, which occurred for the brooding species (*A. humilis* and *F. fragum*) within 24 hr and for the broadcast spawner *A. tenuis* over a period of 1–6 days (maximum settlement 2–4 days) after the start of the settlement experiments. In order to avoid reversible metamorphosis, which may occur because of stress related to sudden changes of the environment (see Richmond, 1985), primary polyps of *A. humilis* and of *F. fragum* were transferred 48 hr after the start of the experiment into a closed-system aquarium; those of *A. tenuis* were transferred in the same system after 6 days. Polyps remained in this system for approximately 1 month and were then transported to other European aquariums. During the entire period tiles were kept in polystyrene grids for stabilization and handling reasons (Petersen *et al.*, 2005). In addition to larval settlement experiments at Rotterdam Zoo, larvae of *A. tenuis* were shipped from Rotterdam Zoo to London Zoo once in 2002, and to Burgers' Zoo twice in 2002 and 2003, to settle under similar conditions following the protocol described above. However, in 2002, 12 tiles of each type were incubated in 250 mL sea water whilst in 2003 larger plastic aquariums (40 tiles in 1500 mL sea water) were used. A previous study using similar tiles showed that the total number of settlers was not dependent on available settlement area, but the number of settlers per tile is determined by the number of tiles (Petersen *et al.*, 2005). Therefore, and for economic reasons, we applied a higher number of tiles per plastic aquarium in 2003. Settlement data from Rotterdam Zoo in 2002 will be published elsewhere and are not included in this study.

### Transportation of primary polyps

In 2003, 12 tiles of each type were arranged in a chessboard pattern in a polystyrene grid and put in a polyethylene box; one pyramid tile was not placed to fix a marker (Figure 1). The box had been previously prepared by cutting slits (width approximately 1 cm) along the whole length in each sidewall. After placing the grid with tiles in the box, insulation foil was put on top of the tiles and the box was closed. The insulation foil was aimed at preventing the tiles from tumbling over, the slits allowed water to circulate between the box and the plastic bag. Two boxes were placed in one double plastic bag; approximately 2 litres of water from the culture tank and pure oxygen (100%) were added before the bags were finally closed. They were put in Styrofoam boxes and transported by express service to: (1) Cologne Zoo, Germany, (2) Hagenbeck Zoo, Germany, (3) London Zoo, UK. After arrival, polyps were adapted to local water conditions by slowly adding fresh sea water for at least 2 hr and then transferred to culture tanks. In 2002, the fourth aquarium, Burgers' Zoo, acquired polyps of *A. tenuis* for this study from settlement experiments.

### Husbandry of juveniles

Specimens were maintained under the following conditions: (1) 250-watt metal halide lamps (HQI), 'daylight' colour (6000 kelvin), distance of tiles to water surface approximately 50 cm, (2) turbulent water to minimize accumulation of sediment, (3) algal control by grazing organisms such as herbivorous snails, hermit crabs or surgeonfish. Owing to the use of existing culture tanks at the different locations, (2) and (3) were uncontrolled, qualitative approaches (no standard between institutions). Culture tanks were either independent systems or connected to exhibits, but in all cases closed systems (i.e. no continuous water exchange using sea water from the field), (4) water chemistry was kept as close as possible to common field values, reported by Schuhmacher (1976), Sorokin (1995), and Adey and Loveland (1998) (see Table 4). During the whole experiment tiles were kept arranged in the grids as described before and were only removed temporarily to be checked one by one under a microscope.

## Monitoring and statistics

Protocols based on the results of previous experiments at Rotterdam Zoo were distributed to all aquariums to guarantee high conformity in the techniques for handling larvae and primary polyps (Petersen *et al.*, 2005, in press b). The study was mainly conducted by the regular zoo staff, so the experiments were designed in order to fit in with their daily operations. For logistical reasons (available time and space to conduct experiments) the number of species shipped differed between institutions. The available amount of specimens located on V-type and H-type tiles was influenced by species-specific settlement preferences (Petersen *et al.*, 2005).

Juveniles were counted separately for flat and pyramid tiles before and after transport, and then every second month for a period of 10 months. Settlement and survival rates were analysed with a chi-squared test. At the final count, the colony size of all specimens (overall age: 11 months) was measured by taking the maximum diameter of the colony (accuracy  $\pm 0.5$  mm). Colony sizes were transformed into size classes. Data of colonies situated on pyramid tiles (vertical) and on flat tiles (horizontal) were pooled when sub-sample size was  $\leq 2$ . If  $n_{\text{sub}} > 2$ , class frequencies were analysed for significance with a Mann–Whitney U-test. If no significant differences were found data were pooled and significance between institutions was tested using the same test. All statistical tests were carried out separately for each species using SPSS 12.0.

## RESULTS

### Larval settlement

Settlement data are shown in Table 1. In 2002, settlement of *A. tenuis* larvae was similar at London Zoo and at Burgers' Zoo ( $\chi^2 = 0.124$ ,  $p < 0.423$ ;  $n = 1480$ ). In 2003, settlement was overall higher; however, rates at Burgers' Zoo were significantly lower compared to those at Rotterdam Zoo ( $\chi^2 = 572.506$ ,  $p < 0.001$ ;  $n = 2960$ ). Water values showed elevated levels of nitrogen at London Zoo and at Rotterdam Zoo (Table 1) compared to common field values (Table 4). Additionally, phosphorus levels at Rotterdam Zoo were above those found in the field. All other values were within the range reported from the field.

Table 1. Larval settlement of *Acropora tenuis* at different zoo aquariums including water parameters. Accuracy may differ owing to different analytic methodology. For comparison of water quality with common field values see Table 4

	Burgers' Zoo	London Zoo	Burgers' Zoo	Rotterdam Zoo
Year	2002	2002	2003	2003
<i>n</i> (larvae)	690	790	1480	1480
Parallels	5	5	4	4
Water volume, mL	250	250	1500	1500
Total number of tiles	24	24	40	40
Total settlement, mean $\pm$ SD [%]	2.93 $\pm$ 2.52	3.30 $\pm$ 2.91	17.36 $\pm$ 6.01	57.84 $\pm$ 11.01
Salinity, ‰	34.0	34.0	34.0	34.0
Temperature, °C	26.0–26.2	26.0	26.0–26.2	26.0
pH	8.00–8.15	8.1–8.3	8.00–8.15	8.00–8.20
NH <sub>4</sub> <sup>+</sup> , mg L <sup>-1</sup>	0.00	0.0	0.00	0.00
NO <sub>2</sub> <sup>-</sup> , mg L <sup>-1</sup>	<0.004	0.0	<0.004	0.013
NO <sub>3</sub> <sup>-</sup> , mg L <sup>-1</sup>	<0.04	2.00	<0.04	3.1
PO <sub>4</sub> <sup>3-</sup> , mg L <sup>-1</sup>	0.01	0.0	0.01	0.15
Oxygen saturation, %	100.0	100.0	100.0	100.0

### Juvenile survival

The primary polyps of the studied species arrived within 24 hr at their destination attaining post-transport survival rates of 90.83–100.00% ( $n = 501$ ; Table 2). Figure 2 gives an overview of the survival rates for the

Table 2. Post-transport survival of primary polyps shipped from Rotterdam Zoo to European aquariums approximately 1 month after larval settlement

Species	Destination	$n$	Duration (hr)	Survival (%)
<i>Acropora tenuis</i>	London Zoo	104	~23	94.23
<i>Acropora tenuis</i>	Burgers' Zoo	29	~23	93.43
<i>Agaricia humilis</i>	Hagenbeck Zoo	94	~24	90.83
<i>Agaricia humilis</i>	London Zoo	71	~23	98.51
<i>Favia fragum</i>	Cologne Zoo	150	~23	100.00
<i>Favia fragum</i>	London Zoo	66	~23	98.51

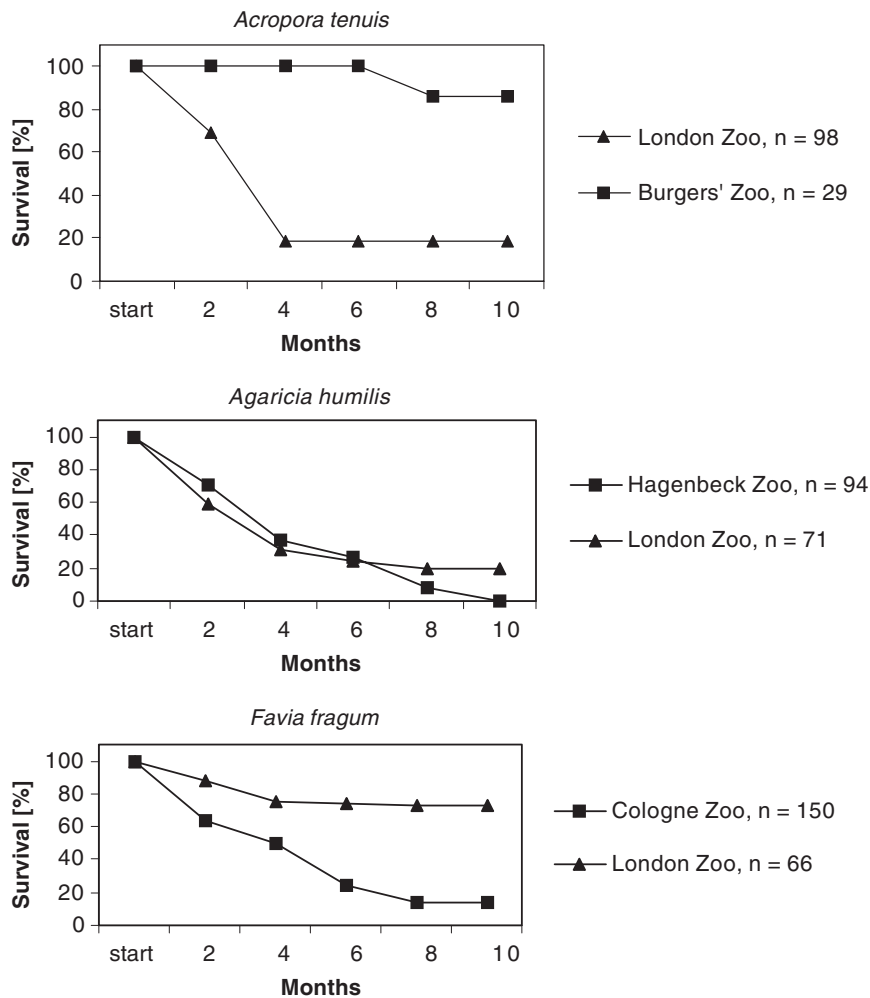


Figure 2. Survival of juveniles maintained in culture tanks at different zoo aquariums for 10 months.

Table 3. Survival and juvenile sizes of corals located on flat tiles (= horizontal) and on pyramid tiles (= vertical), and maintained for 10 months in culture tanks at different zoo aquariums

Species	Institution	<i>n</i>		Survival		Diameter <sup>a</sup>		
		vertical	horizontal	vertical (%)	horizontal (%)	mean $\pm$ SD (all in mm $\pm$ 0.5 mm)	min.	max.
<i>Acropora tenuis</i>	London Zoo	82	16	19.5	12.5	13 $\pm$ 11	4	43
<i>Acropora tenuis</i>	Burgers' Zoo	26	3	84.6	100.0	5 $\pm$ 3	1	15
<i>Agaricia humilis</i>	London Zoo	47	24	25.5	8.3	6 $\pm$ 4	1	15
<i>Agaricia humilis</i>	Hagenbeck Zoo	76	18	0.0	0.0	—	—	—
<i>Favia fragum</i>	London Zoo	32	34	75.0	70.6	11 $\pm$ 5	3	20
<i>Favia fragum</i>	Cologne Zoo	80	70	21.3	4.3	13 $\pm$ 4	3	17

<sup>a</sup>Age of juveniles approximately 11 months.

first 10 months after transportation of propagules. Total survival rates ranged between 0 and 86% (see also Table 3). In all cases species-specific survival differed highly between the institutions over the entire monitoring period of 10 months (*A. tenuis*:  $\chi^2 = 41.919$ ,  $p < 0.001$ ,  $n = 127$ ; *A. humilis*:  $\chi^2 = 20.254$ ,  $p < 0.001$ ,  $n = 165$ ; *F. fragum*:  $\chi^2 = 74.955$ ,  $p < 0.001$ ;  $n = 216$ ). At London Zoo, the number of juveniles of *A. tenuis* decreased in the first 2 months significantly compared to those at Burgers' Zoo ( $\chi^2 = 11.623$ ,  $p < 0.001$ ;  $n = 127$ ). The same effect was observed for *F. fragum* at Cologne Zoo compared to specimens at London Zoo ( $\chi^2 = 12.770$ ,  $p < 0.001$ ;  $n = 216$ ). However, *A. humilis* showed a similar decrease at London Zoo and Hagenbeck Zoo for the first 6 months (start–2 months:  $n = 165$ ,  $\chi^2 = 2.187$ ,  $p = 0.095$ ; 2–4 months:  $n = 108$ ,  $\chi^2 = 0.004$ ,  $p = 0.552$ ; 4–6 months:  $n = 57$ ,  $\chi^2 = 0.238$ ,  $p = 0.434$ ). Six to eight months after the start of the experiment, colonies at London Zoo stabilized whereas those at Hagenbeck Zoo further decreased ( $\chi^2 = 10.286$ ,  $p = 0.002$ ;  $n = 42$ ) and finally completely died in the last period (8–10 months after start). The overall survival rate of *A. humilis* was relatively low compared to all other species (Figure 2, Table 3). Survival rates of juveniles located on pyramid tiles were significantly higher compared to those on flat tiles for *F. fragum* at Cologne Zoo ( $n = 150$ ,  $\chi^2 = 12.321$ ,  $p = 0.001$ ). In all other cases, no significant influence of the surface orientation on colony survival was found; however, relative survival was lower on flat tiles than on pyramid tiles (Table 3). *A. tenuis* at Burgers' Zoo was not tested because of the low number of specimens ( $n = 3$ ) located on flat tiles. At Cologne Zoo, without any obvious reason a bloom of cyanobacteria was observed approximately 4 months after the start of the experiment. At Hagenbeck Zoo, grazing of turf algae was insufficient over the entire period, leading to a relatively high cover (approximately 50%) of filamentous green algae (Chlorophyta).

Water parameters of all experimental systems are listed in Table 4. Nitrogen levels found at Cologne Zoo and at London Zoo were above those reported from the field. Furthermore the concentration of phosphorus and the pH at Cologne Zoo were slightly out of the range of natural values. All other values were within common field levels.

### Juvenile sizes

Table 3 gives an overview of the colony sizes at the end of the monitoring period. The surface orientation did not have any significant influence on the final juvenile sizes ( $U < 26.0$ ,  $p > 0.483$ ). Colony sizes of *F. fragum* were similar at London Zoo and at Cologne Zoo ( $n = 54$ ,  $U = 268.5$ ,  $p = 0.485$ ), whereas colonies of *A. tenuis* were larger at London Zoo compared to those at Burgers' Zoo ( $U = 71.5$ ,  $p = 0.012$ ;  $n = 38$ ). The highest colony diameters were recorded for all species at London Zoo (see Table 3 and Figure 2).

Table 4. Water parameters of culture tanks used to maintain juvenile corals for a duration of 10 months. Accuracy may differ owing to different analytic methodology

	Salinity (‰)	Temperature (°C)	pH	NH <sub>4</sub> <sup>+</sup> (all in mg L <sup>-1</sup> )	NO <sub>2</sub> <sup>-</sup>	NO <sub>3</sub> <sup>-</sup>	PO <sub>4</sub> <sup>3-</sup>	Oxygen (%)
Burgers' Zoo	34.0	26.0–26.2	8.00–8.15	0.00	<0.004	<0.04	0.01	100.0
Cologne Zoo	32.0	26.0–28.0	7.8–8.1	n.a.	n.a.	15.0	0.092–0.14	n.a.
Hagenbeck Zoo	32.0	24.0	8.1–8.3	0.018	0.002	0.021	0.004	95.0
London Zoo	34.0	26.0	8.1–8.3	0.0	0.0	2.00	0.0	100.0
Field	34.0–37.0 <sup>a</sup>	20.0–29.0 <sup>b</sup>	8.00–8.40 <sup>c</sup>	0.0009–0.198 <sup>c</sup>	n.a.	0.0136–0.310 <sup>c</sup>	0.0019–0.129 <sup>c</sup>	n.a.

Field values after

<sup>a</sup>Adey and Loveland (1998),

<sup>b</sup>Schuhmacher (1976) and

<sup>c</sup>Sorokin (1995); n.a. = not available.

## DISCUSSION

### Survival and growth of juveniles

Survival rates of all species differed highly between institutions, whereas colony sizes partly differed between institutions and varied highly between individual colonies.

Coral recruitment was defined by Harrison and Wallace (1990) as the life history stage when propagules reach a size to become visible to the naked eye. Since this definition may lead to significant inter-observer variation, we define coral recruitment as the life history stage when the mortality rate of settlers drops to below 10%, leading to a semi-stable sub-population size. We propose this range since it leads to a clear distinction between pre-recruitment and recruitment stages. Mortality rates in the pre-recruitment stage are far higher: 60–90% mortality (Sorokin, 1995) are commonly observed in field populations within the first 3–12 months after settlement (here defined as 'pre-recruitment' stage) as a result of the effects of sedimentation (Wittenberg and Hunte, 1992), and by benthic competitors such as algae (McCook *et al.*, 2001) and cnidarians (Maida *et al.*, 1995; Atrigenio and Aliño, 1996).

In the present study, recruitment occurred mainly after 4 to 8 months. Especially during the first 4 months, algal turf and sedimentation were threatening factors for propagules in all cases (except for *A. tenuis* at Burgers' Zoo), mainly on a microhabitat level, invisible to the naked eye. Both factors are considered as limiting recruitment, especially on horizontal surfaces in the field (Sato, 1985; Maida *et al.*, 1994; Babcock and Mundy, 1996). In the present study, algal growth and sedimentation were highly reduced, mostly invisible on a macro scale and there was little surface orientation effect (flat tiles versus pyramid tiles) on survival or growth. However, the constant presence of filamentous green algae at Hagenbeck Zoo and the cyanobacteria bloom at Cologne Zoo affected survival, mainly on light-exposed surfaces (flat tiles). Except for nitrogen at London Zoo and at Cologne Zoo, and for phosphorus at the latter, water parameters were within the range reported from the field. Elevated nitrogen and phosphorus may have contributed to the sudden cyanobacteria bloom in the culture tank at Cologne Zoo. Since the experiments of the present study were not primarily aimed at studying influences of water chemistry on juvenile development, it is difficult to estimate its influence on the growth and survival of coral propagules. In order to evaluate the quantitative and qualitative influence of particular parameters, it might be more appropriate to carry out a comparative study under laboratory conditions at one institution. We did not measure carbon, available either as particulate organic material (POM) or as dissolved organic carbon (DOC). In the systems at London and Burgers' Zoos, live plankton was either regularly added or produced by the system itself, whereas no additional food was supplied in the systems at Cologne and Hagenbeck Zoos. Culture tanks at Cologne, Hagenbeck and London Zoos were connected to exhibition tanks. POM and DOC levels probably were indirectly affected in these culture tanks owing to a regular supply of the

exhibits with organic food. The tank at Burgers' Zoo represented an independent recirculation system. In order to reduce differences between institutions and to increase overall survival and growth rates in future, we plan to further investigate the role of planktonic food, POM and DOC as potential carbon sources in coral mariculture.

Nevertheless, maximum survival rates above 70–80% achieved in *F. fragum* and in *A. tenuis* in the present study greatly exceeded those reported from the field, which generally reach a maximum of 10–40% depending on the species and the location (Sorokin, 1995; see also: Sato, 1985; Maida *et al.*, 1994; Babcock and Mundy, 1996). The relatively low survival rate observed in *A. humilis* is in accordance to similar observations in culture tanks at Rotterdam Zoo (Petersen *et al.*, unpublished). This might indicate sub-optimum conditions for this species in common closed systems and needs further investigation, preferably involving comparative field studies.

Maximum colony diameters of the studied species mostly exceeded diameters reported for reefbuilding corals in the field, which reach generally less than 1 cm in their first year (Harrison and Wallace, 1990); for the genus *Acropora* at London Zoo the growth rate was 3–4 times higher (mean diameter 1.3 cm; maximum 4.3 cm; see Figure 3) than that observed in the field (mean 0.5 cm; maximum 1.2 cm; Harrison and Wallace, 1990).

### Distribution of larvae versus primary polyps

Except for Rotterdam Zoo, none of the participating zoos had previous experience of working with coral planulae or primary polyps. This lack of experience at the participating institutions was important (1) to establish whether there would be interest of such institutions in expanding SCORE Project after this initial period and develop it into a permanent institution, and (2) to compare the efficiency of various distribution techniques for larvae and primary polyps to inexperienced institutions.

Two different methods for supplying public aquariums with sexual recruits were tested: (1) 'inexperienced' aquariums received larvae and carried out larval settlement by themselves or (2) larvae were first settled at an 'experienced' aquarium, which regularly works with coral planulae (i.e. Rotterdam Zoo) and then primary polyps were distributed. In 2001 the settlement rates of *A. tenuis* achieved at London Zoo and Burgers' Zoo were much lower than at Rotterdam Zoo ( $16.3 \pm 3.1\%$ ; Petersen *et al.*, in press b). Although in the following year overall settlement was higher — possibly due to other factors such as seasonal and species-specific fluctuations in larval fitness observed in the field (Hatta, unpublished) — settlement was significantly lower at Burgers' Zoo compared to Rotterdam Zoo. In comparison, intra-European transportation of primary polyps as an alternative method to supply coral recruits showed high survival for all species. Propagules that had already passed the planktonic stage and had undergone settlement and metamorphosis were much easier to handle by inexperienced institutions than the larvae and the associated procedures required to initiate settlement. Up to 4000 larvae (Petersen *et al.*, in press b) versus 24 tiles with settlers (resulting in a maximum 24 corals) can be transported in one litre of water. Although the shipment of larvae even at a settlement rate of 1% would be still more economical when looking purely at transportation costs, we demand an effective and sustainable use of natural resources, which favours the distribution of primary polyps to inexperienced institutions. 'Dry transportation' of juveniles, which was not tested in this study, could further help reduce transportation costs. In the past, the dry-method has been successfully applied, mainly for coral fragments (Carlson, 1999). Besides the methods applied in this study, the distribution of coral fragments may further serve as an alternative to sustainably supply public aquariums and the trade. Carlson (1999) and Yates and Carlson (1992) reported survival rates of nearly 100% when fragments were transported dry or submerged within 24–30 hr. Transportation costs using this method (transport volume per specimen: approximately 250 mL; estimated after Carlson (1999)) might prove to fall between those of the tile method presented in the present study and commercial transportation of whole colonies (mean weight per colony = 166.4 g + > 200 mL transport water, estimated



Figure 3. Colonies at an age of 11 months at London Zoo. (A) *Acropora tenuis*; (B) *Favia fragum*; (C) *Agaricia humilis*. Note the arrangement of tiles in the polystyrene grid. Scale bar: 1 cm (Courtesy: Rachel Jones, Zoological Society of London, UK).

after Green and Shirley (1999)). Asexual techniques, such as fragmentation, may be useful whenever the particular species allows such an approach (e.g. massive species are difficult to fragment); however, it is likely that sexual propagation techniques will become more important in the future (see also Delbeek, 2001).

### Conclusions and future perspectives

Our results show there is great potential for today's public aquariums to use sexual reproduction to achieve self-sustaining *ex situ* coral populations. Institutions experienced in coral husbandry, but not in sexual propagation, were able to achieve relatively high survival and growth rates at the first attempt. Public aquariums not only have a great responsibility for nature conservation, but should also play an exemplary role in demonstrating how to use ornamental organisms sustainably. Since the beginning of SECORE Project, there has been a high level of interest from the international aquarium community which has resulted in an increase of membership in 2004. Currently 28 institutions in Europe, the USA and Japan are involved in the project. It is planned to establish breeding centres in Europe, the USA and Asia by organizing workshops for a limited number of public aquariums that have the capacity to specialize in breeding coral species from specific regions. During the workshops, these future breeding centres will learn how to collect propagules, and to culture and settle larvae. Cultured primary polyps may then be distributed from these breeding centres to other public aquariums. This will enable SECORE to apply the captive sexual reproduction techniques to far more coral species, from a wider range of geographical regions than only one centre could do. A well established network such as SECORE Project can also assist in managing legislative regulations such as CITES and other logistical challenges faced by individual members.

With regard to captive sexual propagation in general, the *ex situ* generation of a full life cycle, which has been observed in *F. fragum* (Petersen, unpublished data) represents the next step towards captive coral breeding and the initiation of breeding programmes for endangered coral species (see Petersen, in press a). It is an essential consideration for any *ex situ* related breeding activity, that it should not negatively impact the local gene pools of corals. Therefore, aquarium-derived progenies should be traced and not released to the field without considering the genetic aspects of locality and special regard should be given to the guidelines for re-introduction of the Species Survival Commission (SSC) of the IUCN from May 1995. Captive breeding will give future public aquariums an important role in coral reef conservation by promoting sustainability and by helping to preserve coral species from extinction.

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### REFERENCES

- Adey WH, Loveland K. 1998. *Dynamic Aquaria: Building Living Ecosystems*. London: Academic Press; 498 pp.  
Atkinson MJ, Carlson B, Crow GL. 1995. Coral growth in high-nutrient, low-pH seawater: a case study of corals cultured at the Waikiki Aquarium, Honolulu, Hawaii. *Coral Reefs* **14**: 215–223.

- Atrigenio MP, Aliño PM. 1996. Effects of the soft coral *Xenia puertogalerae* on the recruitment of scleractinian corals. *Journal of Experimental Marine Biology and Ecology* **203**: 179–189.
- Babcock RC, Mundy C. 1996. Coral recruitment: consequences of settlement choice for early growth and survivorship in two scleractinians. *Journal of Experimental Marine Biology and Ecology* **206**: 179–201.
- Bell PRF. 1992. Eutrophication and coral reefs – some examples in the Great Barrier Reef Lagoon. *Water Research* **26**: 553–568.
- Best BA. 2002. Coral reefs in crisis: trade in coral reef animals and products. *Tropical Coasts* **9**(2): 4–11.
- Brown BE. 1997. Disturbances to reefs in recent times. In Birkeland C (ed.) *Life and Death of Coral Reefs*. Chapman & Hall: New York; 354–379.
- Carlson BA. 1987. Aquarium systems for living corals. *International Zoo Yearbook* **26**: 1–9.
- Carlson BA. 1999. Organism responses to rapid changes: what aquaria tell us about nature. *American Zoologist* **39**: 44–55.
- Chansang H, Boonyanate P, Charuchinda M. 1982. Effect of sedimentation from coastal mining on coral reefs on the northwestern coast of Phuket Island, Thailand. In *Proceedings of the Fourth International Coral Reef Symposium*, Gomez ED, Birkeland CE, Buddemeier RW, Johannes RE, Marsh JA, Tsuda RT (eds). Marine Science Center, University of the Philippines, Manila, Philippines; Vol. 1, 129–136.
- Delbeek JC. 2001. Coral farming: past, present and future trends. *Aquarium Sciences and Conservation* **3**: 171–181.
- Delvoye L. 1988. Gametogenesis and gametogenetic cycles in *Agaricia agaricites* (L) and *Agaricia humilis* Verrill and notes on gametogenesis in *Madracis mirabilis* (Duchassaing & Michelotti) (Scleractinia). Studies in honour of Dr Pieter Wagenaar Hummelinck. Foundation for Scientific Research in Surinam and the Netherlands Antilles, Amsterdam **123**: 101–134.
- Epstein N, Bak RPM, Rinkevich B. 2001. Strategies for gardening denuded coral reef areas: the applicability of using different types of coral material for reef restoration. *Restoration Ecology* **9**(4): 1–11.
- Fan TY, Li JJ, Liu MC, Fang LS. 2000. The application of sexual reproduction to enrich a live coral tank. *Fifth International Aquarium Congress, Monaco*. Abstracts; 41.
- Green EP, Shirley F. 1999. *The Global Trade in Coral*. World Conservation Monitoring Centre. World Conservation Press: Cambridge, UK.
- Grigg RW. 1994. Effects of sewage discharge, fishing pressure and habitat complexity on coral ecosystems and reef. *Marine Ecology Progress Series* **103**: 25–34.
- Grigg RW, Dollar SJ. 1990. Natural and anthropogenic disturbance on coral reefs. In *Ecosystems of the World*, Vol. 25, *Coral Reefs*, Dubinsky Z (ed.). Elsevier: Amsterdam; 439–452.
- Harrison PL, Wallace CC. 1990. Reproduction, dispersal and recruitment of scleractinian corals. In *Ecosystems of the World*, Vol. 25, *Coral Reefs*, Dubinsky Z (ed.). Elsevier: Amsterdam; 133–207.
- Hatta M, Iwao K. 2003. Metamorphosis induction and its possible application to coral seedlings production. In *Recent Advances in Marine Science and Technology, 2002*, Saxena N (ed.). Japan International Marine Science and Technology Federation Akasaka: Minato-ku, Tokyo; 465–470.
- Hatta M, Iwao K, Taniguchi H, Omori M. 2004. Restoration technology using sexual reproduction. In *Manual for Restoration and Remediation of Coral Reefs*, Omori M, Fujiwara S (eds). Nature Conservation Bureau, Ministry of the Environment, Japan; 14–28.
- Hayashibara T, Shimoike K, Kimura T, Hosaka S, Heyward A, Harrison P, Kudo K, Omori M. 1993. Patterns of coral spawning at Akajima Island, Okinawa, Japan. *Marine Ecology Progress Series* **101**: 253–262.
- Heyward AJ, Smith LD, Rees M, Field SN. 2002. Enhancement of coral recruitment by in situ mass culture of coral larvae. *Marine Ecology Progress Series* **230**: 113–118.
- Hutchins M, Conway WG. 1995. Beyond Noah's Ark: the evolving role of modern zoological parks and aquariums in field conservation. *International Zoo Yearbook* **34**: 117–130.
- Iwao K, Fujisawa T, Hatta M. 2002. A cnidarian neuropeptide of the GLWamide family induces metamorphosis of reef-building corals in the genus *Acropora*. *Coral Reefs* **21**: 127–129.
- Kelly JD. 1997. Effective conservation in the twenty-first century: the need to be more than a zoo. One organization's approach. *International Zoo Yearbook* **35**: 1–14.
- Maida M, Coll JC, Sammarco PW. 1994. Shedding new light on scleractinian coral recruitment. *Journal of Experimental Marine Biology and Ecology* **180**: 189–202.
- Maida M, Sammarco PW, Coll JC. 1995. Effects of soft corals on scleractinian coral recruitment. I: Directional allelopathy and inhibition of settlement. *Marine Ecology Progress Series* **121**: 191–202.
- McCook LJ, Jompa J, Diaz-Pulido G. 2001. Competition between corals and algae on coral reefs: a review of evidence and mechanism. *Coral Reefs* **19**: 400–417.
- Nonaka M, Baird AH, Kamiki T, Yamamoto HH. 2003. Reseeding the reefs of Okinawa with larvae of captive-bred corals. *Coral Reefs* **22**: 34–47.

- Petersen D, Tollrian R. 2001. Methods to enhance sexual recruitment for restoration of damaged reefs. *Bulletin of Marine Science* **69**(2): 989–1000.
- Petersen D, Laterveer M, Van Bergen D, Kuenen M. 2004. Transportation techniques for massive scleractinian corals. *Zoo Biology* **23**: 165–176.
- Petersen D, Laterveer M, Schuhmacher H. 2005. Innovative substrate tiles to spatially control larval settlement in coral culture. *Marine Biology* **146**(5): 937–942.
- Petersen D. In press a. How can future public aquariums contribute to coral reef conservation? Some thoughts on the feasibility of coral breeding programs to manage *in-situ* and *ex-situ* populations. *Proceedings of the Sixth International Aquarium Congress, Monterey, USA*.
- Petersen D, Hatta M, Laterveer M, Van Bergen D. In press b. *Ex situ* transportation of coral larvae for research, conservation and aquaculture. *Coral Reefs*.
- Price ARG, Firaq I. 1996. The environmental status of reefs on Maldivian resort islands: a preliminary assessment for tourism planning. *Aquatic Conservation: Marine and Freshwater Ecosystems* **6**: 93–106.
- Richmond RH. 1985. Reversible metamorphosis in coral planulae larvae. *Marine Ecology Progress Series* **22**: 181–185.
- Rouphael AB, Inglis GJ. 1997. Impacts of recreational scuba diving at sites with different reef topographies. *Biological Conservation* **82**(3): 329–336.
- Sato M. 1985. Mortality and growth of juvenile coral *Pocillopora damicornis* (Linnaeus). *Coral Reefs* **4**: 27–33.
- Schuhmacher H. 1976. *Korallenriffe: Verbreitung, Tierwelt, Ökologie*. BLV Verlagsgesellschaft: Munich.
- Sorokin YI. 1995. *Coral Reef Ecology. 102, Ecological studies*, 2nd edn. Springer: Berlin.
- Szmant AM. 1986. Reproductive ecology of Caribbean reef corals. *Coral Reefs* **5**: 43–54.
- Szmant-Froelich A, Reutter M, Riggs L. 1985. Sexual reproduction of *Favia fragum* (Esper): lunar patterns of gametogenesis, embryogenesis and planulation in Puerto Rico. *Bulletin of Marine Science* **37**(3): 880–892.
- Van Moorsel GWNM. 1983. Reproductive strategies in two closely related stony corals (*Agaricia*, Scleractinia). *Marine Ecology Progress Series* **13**: 273–283.
- Veron JEN. 2000. *Corals of the World*, Vols 1–3. Australian Institute of Marine Science: Townsville.
- Wetzel JA, O'Brien M. 1995. Aquariums: a look to the future. *International Zoo Yearbook* **34**: 1–6.
- Wittenberg M, Hunte W. 1992. Effects of eutrophication and sedimentation on juvenile corals. I. Abundance, mortality and community structure. *Marine Biology* **112**: 131–138.
- Yates KR, Carlson BA. 1992. Corals in aquariums: how to use selective collecting and innovative husbandry to promote reef conservation. *Proceedings of the Seventh International Coral Reef Symposium, Guam, 1992*, 2, 1091–1095.