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Ein Beitrag zur Nachhaltigkeit in ex situ Populationen

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Breeding Techniques for Reefbuilding Corals

Towards Sustainability in Ex Situ Populations

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Für meine Eltern –
to my parents.

Contents

CHAPTER

1

General Introduction and Thesis Outline

CORAL REEFS AND CONSERVATION

Coral reefs, the most diverse ecosystems of the sea, are increasingly threatened by natural and anthropogenic disturbances (Grigg and Dollar, 1990; Veron, 1992). 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 trade and for supplying public aquariums (Best, 2002; Green and Shirley, 1999) play a major role in the decline of today's reefs.

Scleractinian corals, the key organisms of coral reefs, build a complex physical structure, which offers diverse ecological niches of multiple trophic interactions. This makes a coral reef to an oasis in the nutrient-desert of oligotrophic waters usually found in the tropics (Schuhmacher, 1976). An endosymbiosis with dinoflagellates (*Symbiodinium* spp.; = zooxanthellae) may lead to 10x higher calcification rates compared to azooxanthellate scleractinians (see Schuhmacher and Zibrowius, 1985). The net growth rate of a healthy coral reef is about 1 cm per year. Damages, caused by storms and by other natural factors, can be rapidly repaired (Schuhmacher, 1976). However, an increase of global and local threats such as rising sea temperatures and urban activities substantially disturb this fragile endosymbiosis and the whole balance of the ecosystem. This results in a gradually decrease of scleractinian coral cover and an overall decline of biodiversity (Veron, 1992, Glynn, 1993; Brown, 1997a, 1997b).

Therefore, conservation is an important goal of today's coral reef research. New restoration methods are developed such as the installation of artificial reefs and the transplantation of nubbins (Van Treeck and Schuhmacher, 1997; Schuhmacher et al., 2000; Schuhmacher, 2002). The application of sexual reproduction offers new possibilities for coral reef restoration, and to sustainably supply public aquariums and the commercial trade (Rinkevich and Shafir, 2000; Petersen and Tollrian, 2001; Heyward et al., 2002; Hatta and Iwao, 2003).

REPRODUCTION IN CORALS

Corals have evolved a tremendous diversity of asexual and sexual reproductive modes, (Fadlallah, 1983; Harrison and Wallace, 1990). The importance of asexual and sexual reproduction may differ species-specifically (Wallace, 1985). Therefore it is necessary to carefully evaluate for each species which mode is appropriate for ex situ propagation.

Asexual reproduction

Most reefbuilding corals are colonial animals, which undergo extra- or intrapolypal fission to develop new clones within a colony (Schuhmacher, 1976). Asexual propagules can be developed via budding (Zibrowius, 1985), polyp bail-out (Sammarco, 1982), polyp balls (Rosen and Taylor, 1969), anthocauli (Krupp et al., 1993) and skeleton fragmentation (Highsmith, 1982). New asexual modes such as polyp vesicles and polyp extrusion have been recently discovered (Borneman, personal communication). Due to the high plasticity of cnidarian tissue, the spectrum of asexual modes might be much wider (Harrison and Wallace, 1990).

Sexual reproduction

Three major steps characterize sexual reproduction in corals: (1) larval development including gametogenesis and fertilization, (2) larval planktonic stage and settlement, and (3) early development of post-settlement stages.

(1) Two principle modes can be distinguished: (A) brooding (internal fertilization, release of settlement competent planulae) and (B) broadcast spawning (external fertilization, planktonic embryogenesis). Most studied corals are simultaneous hermaphrodites (female and male gonads within a polyp); some species are gonochoric (one sex per colony). Exceptions are given: e.g. *Astroides calycularis* colonies are hermaphroditic with male and female polyps (Fadlallah, 1983), intracolonial polyps of *Porites astreoides* can be gonochoric and hermaphroditic (Chornesky and Peters, 1987). Brooding and spawning have been observed within one species located at different geographic regions (Fadlallah, 1983) and, in some regions, even within populations of the same locality such as *Pocillopora damicornis* (Ward, 1992), *Goniastrea aspera* (Nishikawa, personal communication) and *Oulastrea crispata* (Lin, personal communication). Depending on the species-specific properties and colony size, scleractinian corals may reach maturity after 3-5 years (Kojis and Quinn, 1985; Harrison and Wallace, 1990).

Most brooders have multiple gametogenic cycles while most spawners only have one annual gametogenic cycle. Cycles usually show lunar periodicity (Szmant-Froehlich et al, 1985; Szmant, 1986). Gametes of broadcast spawning species are synchronically released within a few nights soon after full moon. Multiple species spawning events have been observed in various geographic regions such as Eastern Australia (Babcock et al., 1986), Okinawa (Hayashibara et al. 1993) and Curaçao (Van Veghel, 1993). It is assumed that annual temperature cycles determine the month of spawning, the moon cycle defines the day, and the sunset determines the precise hour of spawning in a time frame of minutes (Jokiel et al., 1985; Babcock et al., 1986; Fukami et al., 2003). Egg-sperm bundles formed by each polyp or single gamete cells drift to the water surface where fertilization takes place. Sperm release in brooders can be lunar related (Szmant-Froehlich et al., 1985; Delvoye, 1988). Spermatozoa enter the polyp via the mouth and pharynx; fertilization takes place in the gastrovascular cavity where oocytes are located (Harrison and Wallace, 1990, Richmond, 1997). Although most brooders show a clear lunar rhythm of planulation (Fadlallah, 1983; Szmant-Froehlich et al., 1985), in some species a cycle could not be found (Van Moorsel, 1983).

Larvae of brooders are mostly bigger and already contain zooxanthellae; those of spawners are relatively small and usually obtain their symbiotic algae after settlement (Richmond, 1997). Although brooded larvae may settle within a few hours after release, they can stay in the water column for 100-200 days, if conditions are not appropriate for settlement (Fadlallah, 1983). Larvae of broadcast spawners are at least 2.5 days planktonic till they reach settlement competency (Miller and Mundy, 2003), a maximum planktonic period of 70 days was estimated from laboratory experiments (Wilson and Harrison, 1998).

(2) A crucial step in the life history of corals represents the settlement process: the change from a mobile planktonic stage into a sessile polyp. Therefore, choosing the ideal settlement location is essential for the further survival of the genotype. Within limits, the settlement spot can be corrected through reversible metamorphosis of the primary polyp (Richmond, 1985) and moving over the substrate, which is usually within the scale of a few centimeters (Vermeij and Bak, 2002a). Nevertheless, environmental conditions, which influence the recruitment success, may highly differ within such a spatial scale (see Vermeij and Bak, 2002b). Settlement depends generally on specific biological inducers related to

crustose coralline algae (Morse et al., 1996; Negri et al., 2001), light (Mundy and Babcock, 1998), sedimentation (Te, 1992), eutrophication (Tomascik, 1991), competition with algae (Tanner, 1995) and other cnidarians (Maida et al., 1995), grazing (Sammarco, 1980), and substrate orientation (Harriott and Fisk, 1987). It remains unclear, whether the orientation of the substrate plays an ultimate role in species-specific settlement patterns (Harriott and Fisk, 1987; Tomascik, 1991; Mundy and Babcock, 1998).

(3) The early life stages until the settler becomes visible to the naked eye of a diver (= recruitment; Harrison and Wallace, 1990) plays an important role in the reproductive success. Survival and growth are influenced by various factors such as light (Babcock and Mundy, 1996), sedimentation and eutrophication (Hunte and Wittenberg, 1992), competition (McCook et al., 2001) and grazing (Sammarco, 1980; Sato, 1985; Sorokin, 1995).

Although scleractinian corals may have a high fecundity of 240-2880 oocytes cm^{-2} in broadcast spawners and 48-528 oocytes cm^{-2} in brooders (calculated after Szmant, 1986), only a low percentage of propagules will settle and survive a period of more than 12 months. In general, 60-90% of settlers do not survive the initial 3-12 months (Sorokin, 1995). Field studies have shown that in *Litophyton arboreum*, of an estimated $1\text{-}3 \cdot 10^6$ larvae released only one established as a young colony (Gateno et al., 1998).

EX SITU CULTURE AND PROPAGATION

Corals can be maintained in open and closed systems for extended periods of time attaining similar or even higher growth rates compared to those in the field (Carlson, 1987; Atkinson et al, 1995; Adey and Loveland, 1998). Asexual propagation, mainly by fragmentation, is a common tool applied by professionals and hobbyists to produce high amounts of asexual recruits (Borneman and Lowrie, 2001). Although sexual reproduction has been randomly observed in aquariums (Delbeek and Sprung, 1994; Adey and Loveland, 1998), only very few institutions have attempted to apply sexual reproduction as a tool to manage ex situ populations sustainably (Fan et al, 2000). Techniques have been developed to rear and culture larvae of broadcast spawners for reef restoration (Epstein et al., 2001; Hatta and Iwao, 2003; Heyward et al, 2002), which can serve as a starting point to implement sexual coral reproduction in public aquariums and in mariculture. It is out of question that the aim of future coral farming and captive population management will be the large-scale application of sexual reproduction (Delbeek, 2001; Petersen and Tollrian, 2001).

THESIS OUTLINE

In order to establish mariculture techniques, I focused on the three essential steps of sexual coral reproduction (described above). Model species were chosen to study ex situ planulation, settlement and recruitment of reefbuilding corals. This dissertation contributes to fundamental knowledge of different fields of basic science (coral ecology) and applied science (mariculture and aquaculture). Therefore, I decided to divide my work in chapters dealing with specific subjects of these different fields. To ensure clearness, each chapter contains a brief introduction as well as the specific methods. Further on, the results are discussed in each chapter in detail with reference to relevant chapters; a general discussion at the end of the thesis gives a final conclusion on all chapters with special emphasize on

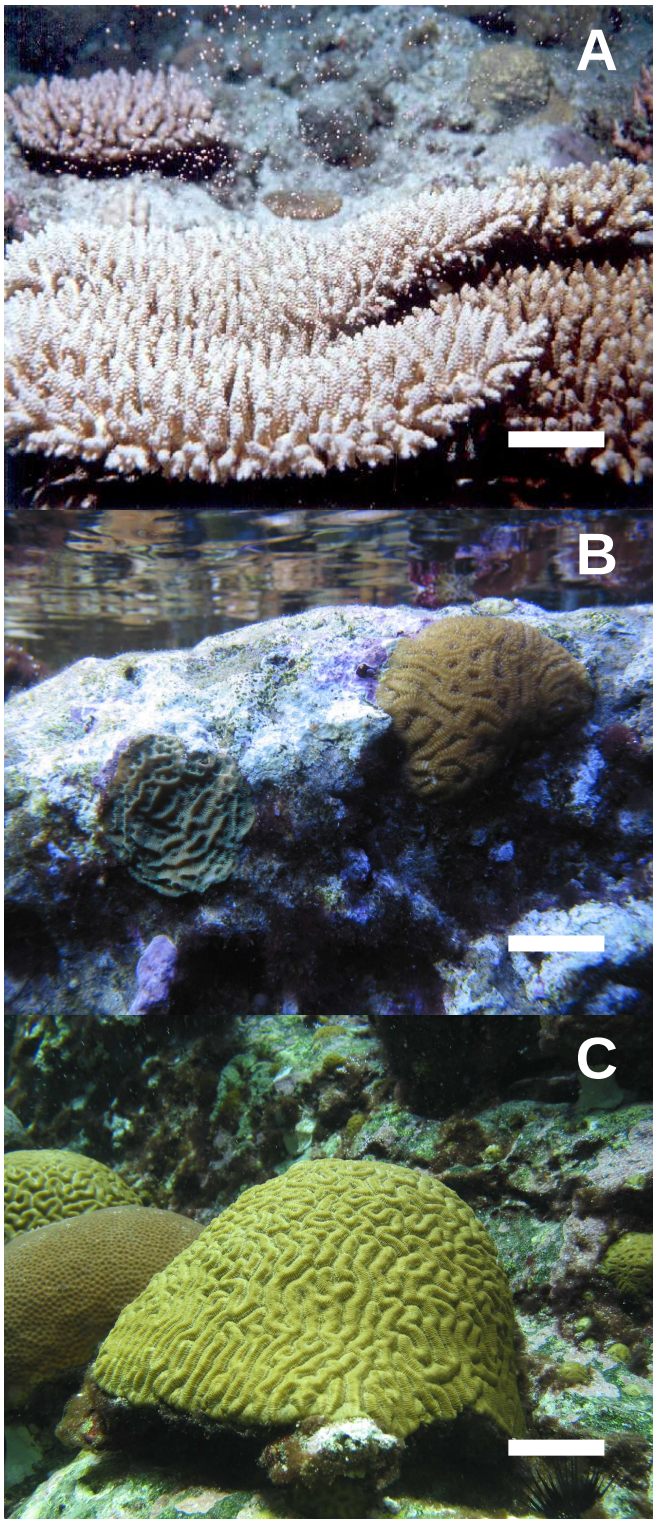
Fig. 1 Experimental investigations were mainly conducted with four coral species of the Caribbean and the Indo-Pacific. Data after Van Moorsel (1983), Veron (2000), Fukami et al. (2003) and Szmant-Froehlich et al. (1985).

(A) ***Acropora tenuis***, corymbose clumped colonies, inhabit upper reef slope; common in western Pacific and Red Sea; hermaphroditic broadcast spawner, annual spawning in June, 2-6 days after full moon at 19.30 hours. Size: several decimeters. Fig. 1A shows a spawning colony at Akajima, Okinawa, Japan in June 2002 (courtesy of M. Hatta). Scale bar: 5 cm.

(B, left) ***Agaricia humilis***, encrusting to submassive colonies, <10 cm, inhabits shallow reef flats, common in the Caribbean; hermaphroditic brooder with lunar gametogenesis, but no lunar planulation, reproduction year round.
(B, right) ***Favia fragum***, hemispherical to encrusting, usually <5 cm, inhabits shallow reef flats, common in the Caribbean; hermaphroditic brooder with 12 lunar-related cycles per year.
Scale bar: 2 cm.

(C) ***Diploria strigosa***, massive or encrusting, >1 m, inhabits especially shallow slopes and lagoons, common only in Caribbean; hermaphroditic broadcast spawner, annual spawning in September and/or October, 6-7 days after full moon, 22.30-23.00 hours (personal observation; 2001-2003). Scale bar: 5 cm.

Fig. 1 B-C show aquarium colonies.



possibilities and limitations in coral mariculture and on the establishment of breeding programs for endangered species.

Following the World Zoo Conservation Strategy [IUDZG/CBSG (IUCN/SSC), 1993], public aquariums have an important role in nature conservation. The new Oceanium at Rotterdam Zoo was officially opened in 2001. Apart from raising public awareness through the display of the typical fauna and flora of different regions of the world's oceans and coasts, conservation research and captive breeding of a maximum of the exhibited animals has been the philosophy from the beginning. Therefore, a marine laboratory co-ordinated by Michaël Laterveer was established, which in the present study served as the basis for experimental investigations. Additionally, the possibility to work in exhibits at the Rotterdam Zoo and in the field mainly in Curaçao, Netherlands Antilles, and partly in Akajima, Okinawa, created an ideal atmosphere to explore coral reproduction with regard to a potential application for ex situ population management. Due to the co-operation between Rotterdam Zoo and the Curaçao Sea Aquarium (CSA), the logistical background was given through David van Bergen, former park manager of the CSA, to carry out field-related experiments at Curaçao. Masayuki Hatta, who supported me already since my master thesis (see Petersen and Tollrian, 2001), ensured, together with Makoto Omori, an appropriate supply of planulae from the second field basis: Akajima Marine Science Laboratory (AMSL), Akajima, Okinawa, Japan.
The international aquarium community supported my attempts to evaluate the current status of sexual coral reproduction in public aquariums. In co-operation with the EUAC Coral ASP (Coral Animal Sustainability Program of the European Union of Aquarium Curators) a questionnaire was distributed via the common list servers (***chapter 2***). For the first time, an overview is given of species reproducing in aquariums. Using available literature and the results of this dissertation, principal trends are highlighted and techniques are outlined, which can also be applied by non-experienced staff to enhance the success in captive breeding.

In the current project, several techniques were developed to create the basis for large scale ex situ breeding: (1) depending on the species, the acquisition of relatively big colonies may be necessary to initiate captive reproductive events. A new method was developed to transport heavy coral colonies for >30 hr attaining post-transport survival rates of 100% (***chapter 3***). Although the transported colonies of *Montastraea annularis* and *Diploria strigosa* (see Fig. 1C) did not spawn during the present study, the described method may serve for future activities in various fields. During the incubation period for inducing spawning, *M. annularis* interestingly exhibited a syndrome called Dark Spots Disease, possibly due to temperature stimulations. The current observations describing the characteristics of the disease under ex situ conditions were published in Gil-Agudelo et al. (2004). (2) The present thesis further establishes transportation techniques for coral larvae - an important tool to exchange propagules between institutions and to supply mariculture centers with larvae reared from field-collected gametes. I studied the transportation of planulae of *D. strigosa* from Curaçao, and of *Acropora tenuis* (Fig. 1A) and *A. digitifera* from Akajima to Rotterdam (***chapter 4***). (3) Pre-studies, which I carried out during my master thesis (Petersen and Tollrian, 2001) indicated the necessity to create settlement substrates, which meet the specific needs of mariculture. Special designed ceramic tiles enabled me to control larval settlement of *Favia fragum* (Fig. 1B) and of *Acropora tenuis* species-specifically in a spatial scale of millimeters. The tiles can be used for research as well as for the mass production of recruit-units (***chapter 5***).

For the first time, a full life cycle of a reefbuilding scleractinian is documented in captivity. Captive bred specimens of *Favia fragum* spent the time till maturity isolated from natural triggers such as moonlight or the presence of adults. The colonies showed the same planulation cycle as those in the field, which might indicate that, contrary to previous

assumptions, timing of planulation is endogenously controlled (***chapter 6***). A new mode of reproduction was observed in *Agaricia humilis* (Fig. 1B) during the field research in Curaçao. Specimens under stress released pre-mature planulae. Contrary to previous assumptions, these embryos were able to develop into settlement competent planulae (***chapter 7***). It demonstrates the opportunistic life history strategy of this species, which favours its use as a model organism to develop mariculture techniques.

The biofilm of the substrate may highly influence species-specific settlement rates (***chapter 8***), which is important in mariculture. I studied the influence of the biofilm, which is mostly defined by benthic algae under aquarium conditions, on the settlement rate of *Favia fragum* and *Agaricia humilis.* Prior to the experiment, substrates were incubated under 2 different light spectra with and without grazers. The settlement rate of both species was mainly influenced by the factors 'grazing' and 'substrate orientation', and partly by the light spectrum. Especially algal management needs more attention in mariculture.

Light plays a major role in the development of zooxanthellate organisms. I studied the influence of different artificial light sources on the survival and growth of the F1 generation of *Agaricia humilis* and *Favia fragum* and additionally of the F2 generation of *F. fragum*. The early development of juveniles was dependent on the species, generation and light, and partly on the substrate orientation. I observed an overall higher survival and growth of the F2 generation of *F. fragum*, which may result from the selection of those genotypes of the F1 generation, which have a higher fitness under mariculture conditions (***chapter 9***).

Favia fragum and *Agaricia humilis* reproduced in a 30-m3 aquarium exhibit at Rotterdam Zoo. This gave us the unique chance to study the recruitment process of two brooders, commonly found in the same habitat, under semi-defined conditions. I found major differences in the recruitment rate. *Favia fragum* could even establish a F2 generation, while *A. humilis* only managed to establish a few specimens of the F1 generation (***chapter 10***).

Long before the project was started at Rotterdam Zoo, several German aquariums emphasized their interest to participate in a comparative study. Besides the Cologne Zoo, the Tiergarten Hagenbeck, and the Aquazoo Düsseldorf the aquariums of London Zoo, U.K., and Burgers' Zoo Arnhem, The Netherlands participated in the Sexual Coral Reproduction (SECORE) Project. The project, initiated as part of the present study, is aimed at applying the developed techniques by distributing captive reared primary polyps together with standardized protocols. It further aims at establishing a network of public aquariums and research institutions to promote the sustainable management of ex situ coral populations. The results show the high success of the presented techniques even when applied by non-experienced staff of public aquariums (***chapter 11***).

REFERENCES

Adey WH, Loveland K. 1998. Dynamic aquaria: building living ecosystems. London: Academic Press. 498 p.

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.

Babcock RC, Bull GD, Harrison PL, Heyward AJ, Oliver JK, Wallace CC, Willis BL. 1986. Synchronous spawning of 105 scleractinian coral species on the Great Barrier Reef. Mar Biol 90:379-394.

Babcock RC, Mundy C. 1996. Coral recruitment: consequences of settlement choice for early growth and survivorship in two scleractinians. J Exp Mar Biol Ecol 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.

Borneman EH, Lowrie J. 2001. Advances in captive husbandry and propagation: an easily utilized reef replenishment means from the private sector. Bull Mar Sci 69(2):897-913.

Brown BE. 1997a. Disturbances to reefs in recent times. In: Birkeland C (ed) Life and death of coral reefs. Chapman & Hall: New York, pp 354-379.

Brown BE. 1997b. Coral bleaching: causes and consequences. Coral Reefs 16, Suppl.:S129-S138.

Carlson BA. 1987. Aquarium systems for living corals. Int ZooYb 26:1-9.

Chansang H, Boonyanate P, Charuchinda M. 1982. Effect of sedimentation from coastal mining on coral reefs on the northwestern coast of Phuket Island, Thailand. Proc 4th Int Coral Reef Symp 1:129-136.

Chornesky EA, Peters EC. 1987. Sexual reproduction and colony growth in the scleractinian coral *Porites atreoides*. Biol Bull 172:161-177.

Delbeek JC, Sprung J. 1996. Das Riffaquarium. Vol. 1. Coconut Grove: Ricordea Publishing. pp. 544.

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 gamatogenesis 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

Fadlallah YH. 1983. Sexual reproduction, development and larval biology in scleractinian corals. A review. Coral Reefs 2:129-150.

Fan TY, Li JJ, Liu MC, Fang LS. 2000. The application of sexual reproduction to enrich a live coral tank. 5th Int Aquarium Congr, Monaco. Abstracts:41.

Fukami H, Omori M, Shimoike K, Hayashibara T, Hatta M. 2003. Ecological and genetic aspects of reproductive isolation by different spawning times in *Acropora* corals. Mar Biol 142:679-684.

Gil-Agudelo DL, Smith GW, Garzón-Ferreira J, Weil E, Petersen D. 2004. Dark Spots Disease and Yellow Band Disease, two poorly known coral diseases with high incidence in Caribbean reefs. In: Rosenberg E, Loya Y (eds). Coral Health and Diseases. Springer, Heidelberg. pp 337-348.

Gateno D, Barki Y, Rinkevich B. 1998. Aquarium maintenance of reef octocorals raised from field collected larvae. Aqua Sci Cons 2:227-236.

Glynn PW. 1993. Coral reef bleaching: ecological perspectives. Coral Reefs 12:1-17.

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. Mar Ecol Prog Ser 103:25 – 34.

Grigg RW, Dollar SJ. 1990. Natural and anthropogenic disturbance on coral reefs. In: Dubinsky Z (ed) Ecosystems of the world, vol 25, coral reefs. Elvesier, Amsterdam, pp 439-452.

Harriott V J, Fisk DA. 1987. A comparison of settlement plate types for experiments on the recruitment of scleractinian corals. Mar Ecol Prog Ser 37:201-108.

Harrison PL, Wallace CC. 1990. Reproduction, dispersal and recruitment of scleractinian corals. In: Dubinsky Z. (ed.) Ecosystems of the world. 25. Coral reefs. Elvesier, Amsterdam, pp 133-207.

Hatta M, Iwao K. 2003. Metamorphosis induction and its possible application to coral seedlings production. In: Saxena N (ed) Recent Advances in Marine Science and Technology, 2002. Japan International Marine Science and Technology Federation Akasaka, Minato-ku, Tokyo, pp 465-470.

Hayashibara T, Shimoike K, Kimura T, Hosaka S, Heyward A, Kudo K, Omori M. 1993. Pattern of coral spawning at Akajima Island, Okinawa, Japan. Mar Ecol Prog Ser 101:253-262.

Heyward AJ, Smith LD, Rees M, Field SN. 2002. Enhancement of coral recruitment by in situ mass culture of coral larvae. Mar Ecol Prog Ser 230:113-118.

Highsmith RC. 1982. Reproduction by fragmentation in corals. Mar Ecol Prog Ser 7:207-226.

Hunte W, Wittenberg M. 1992. Effects of eutrophication and sedimentation on juvenile corals. II. Settlement. Mar Biol 114:625-631.

IUDZG/CBSG (IUCN/SSC). 1993. The world zoo conservation strategy; the role of the zoos and aquaria of the world in global conservation. Chicago: Chicago Zoological Society, Brookfield, pp 76.

Jokiel P.L., Ito R.Y., Liu P.M. 1985. Night irradiance and synchronization of lunar release of planula larvae in the reef coral *Pocillopora damicornis*. Mar Biol 88:167-174.

Kojis BL, Quinn NJ. 1985. Puberty in *Goniastrea favulus*. Age or size limited? Proc 5th Int Coral Reef Symp 4:289-293.

Krupp DA, Jokiel PL, Chartrand TS. 1993. Asexual reproduction by *Fungia scutaria* on dead coralla. Proc. 7th Inter. Coral Reef Symp., Guam, 1:527-534.

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.

Maida M, Sammarco PW, Coll JC. 1995. Effects of soft corals on scleractinian coral recruitment. I: Directional allelopathy and inhibition of settlement. Mar Ecol Prog Ser 121:191-202.

Miller K, Mundy C. 2003. Rapid settlement in broadcast spawning corals: implications for larval dispersal. Coral Reefs 22:99-106.

Morse ANC, Iwao K, Baba M, Shimoike K, Hayashibara T, Omori M. 1996. An ancient chemosensory mechanism brings new life to coral reefs. Biol Bull 191:149-154.

Mundy CN, Babcock RC. 1998. Role of light intensity and spectral quality in coral settlement: Implications for depth-dependent settlement. J Exp Mar Biol Ecol 223:235-255.

Negri AP, Webster NS, Hill RT, Heyward AJ. 2001. Metamorphosis of broadcast spawning corals in response to bacteria isolated from crustose algae. Mar Ecol Prog Ser 223:121-131.

Petersen D, Tollrian R. 2001. Methods to enhance sexual recruitment for restoration of damaged reefs. Bull Mar Sci 69(2):989-1000.

Price ARG, Firaq I. 1996. The environmental status of reefs on Maldivian resort islands: a preliminary assessment for tourism planning. Aquatic Conservation: Marine Freshwater Ecosystems 6:93-106.

Richmond RH. 1985. Reversible metamorphosis in coral planulae larvae. Mar Ecol Prog Ser 22:181-185.

Richmond RH. 1997. Reproduction and recruitment in corals: critical links in the persistence of reefs. In: Birkeland C (ed) Life and death of coral reefs. Chapman and Hall, New York, pp 175-197.

Rinkevich B, Shafir S. 2000. Ex situ culture of colonial marine ornamental invertebrates: concepts for domestication. Aquarium Sciences and Conservation 2:237-250.

Rouphael AB, Inglis GJ. 1997. Impacts of recreational scuba diving at sites with different reef topographies. Biological Conservation 82(3):329-336.

Rosen BR, Taylor JD. 1969. Reef coral from Aldabra: new mode of reproduction. Science 166:119-121.

Sammarco PW. 1980. *Diadema* and its relationship to coral spat mortality: grazing, competition and biological disturbance. J Exp Mar Biol Ecol 45:245-272.

Sammarco PW. 1982. Polyp bail-out: An escape response to environmental stress and a new means of reproduction in corals. Mar Ecol Prog Ser 10:57-65.

Sato M. 1985. Mortality and growth of juvenile coral *Pocillopora damicornis* (Linaeus). Coral Reefs 4:27-33.

Schuhmacher H. 1976. Korallenriffe: Verbreitung, Tierwelt, Ökologie. BLV Verlagsgesellschaft, München.

Schumacher H, Zibrowius H. 1985. What is hermatypic? A redefinition of ecological groups in corals and other organisms. Coral Reefs 4:1-9.

Schuhmacher H. 2002. Use of artificial reefs with special reference to the rehabilitation of coral reefs. Bonner Zool Monogr 50:81-108.

Schuhmacher H, Van Treeck P, Eisinger M, Paster M. 2000. Transplantation of coral fragments from ship groundings on electrochemically formed reef structures. Proc 9th Int Coral Reef Symp, Bali, Indonesia, Vol. 2:983-990.

Sorokin YI. 1995. Coral reef ecology. 102. Ecological studies. 2nd printing. Berlin: Springer, 465 p.

Szmant-Froehlich A, Reutter M, Riggs L. 1985. Sexual reproduction of *Favia fragum* (Esper): lunar patterns of gametogenesis, embryogenesis and planulation in Puerto Rico. Bull Mar Sci 37(3):880-892.

Szmant AM. 1986. Reproductive ecology of Caribbean reef corals. Coral Reefs 5:43-54.

Tanner JE. 1995. Competition between scleractinian corals and macroalgae: An experimental investigation of coral growth, survival and reproduction. J Exp Mar Biol Ecol 190:151-168.

Te FT. 1992. Response to higher sediment loads by *Pocillopora damicornis* larvae. Coral Reefs 11:131-134.

Tomascik T. 1991. Settlement patterns of Caribbean scleractinian corals on artificial substrata along a eutrophication gradient, Barbados, West Indies. Mar Ecol Prog Ser 77:261-269.

Van Moorsel GWNM. 1983. Reproductive strategies in two closely related stony corals (*Agaricia*, Scleractinia). Mar Ecol Prog Ser 13:273-283.

Van Treeck P, Schuhmacher H. 1997. Initial survival of coral nubbins transplanted by a new coral transplantation technology - options for reef rehabilitation. Mar Ecol Prog Ser 150:287-292.

Van Veghel MLJ. 1993. Multiple species spawning on Curacao. Bull Mar Sci 51:1017-1021.

Vermeij MJA, Bak RPM. 2002a. Corals on the move: rambling of *Madracis pharensis* polyps early after settlement. Coral Reefs 21:262-263.

Vermeij MJA, Bak RPM. 2002b. How are coral populations structured by light? Marine light regimes and the distribution of *Madracis*. Mar Ecol Prog Ser 233:105-116.

Veron JEN. 1992. Conservation of biodiversity: a critical time for the hermatypic corals of Japan. Coral Reefs 11:13-21.

Veron JEN. 2000. Corals of the world. Vol. 1-3. Townsville: Australian Institute of Marine Science.

Wallace CC. 1985. Reproduction, recruitment and fragmentation in nine sympatric species of the coral genus *Acropora.* Mar Biol 88:217-233.

Ward S. 1992. Evidence for broadcast spawning as well as brooding in the scleractinian coral *Pocillopora damicornis*. Mar Biol 112:641-646.

Wilson JR, Harrison PL. 1998. Settlement-competency periods of larvae of three species of scleractinian corals. Mar Biol 131:339-345.

Zibrowius H. 1985. Asexual reproduction by bud-shedding in shallow-water *Balanophyllia* of the tropical Indo-Pacific (Cnidaria: Scleractinia: Dendrophylliidae). Proc 5th Int Coral Reef Symp 5:233-238.

CHAPTER

2

Sexual Reproduction of Scleractinian Corals in Public Aquariums

Current Status and Future Perspectives

INTRODUCTION

This chapter gives an overview on scleractinian species that have reproduced in public aquariums. It illustrates their reproductive biology and explores tendencies regarding modes of coral reproduction. In addition, methods are suggested that can be easily utilized to optimize ex situ reproduction and potentially broaden the spectrum of species reproduced *ex-situ*. I further aim at assessing the current status of today's public aquariums with regard to captive coral reproduction to estimate the potential for achieving self-sustaining *ex-situ* populations in future. By listing species and institutions, this chapter contributes to the exchange of knowledge and the co-operation within the aquarium community and with other conservation related institutions.

METHODS

Since little literature is available on captive coral reproduction in public aquariums, I designed a questionnaire, which was distributed by the EUAC Coral ASP (Coral Animal Sustainability Program of the European Union of Aquarium Curators) in March and April 2004 via the EUAC Coral ASP list server (49 aquarium professionals who specialize in corals, mainly from Europe) and via the AquaticInfo list server (682 institutional members, mainly aquarium professionals worldwide). The questionnaire was also sent to some institutions that are not members of these lists, but are known for their success in captive coral breeding. This allowed the questionnaire to be distributed to as many public aquariums as possible in order to obtain a maximum number of observations. The questionnaires was developed efficiently such that aquarium professionals required little time to answer the questions, but were able to provide the maximum amount of information possible. The following subjects were covered by multiple-choice questions:

1. Parental population: origin, population size, timing of first reproduction observed
2. Reproductive event: description, possible influence by the factors moonlight and temperature
3. Juveniles: population size
4. Aquarium system: size, life support system, environmental conditions

All observations were compared with field data based on available literature. In this chapter, sexual coral reproduction is defined as the process that involves either the release of gametes or planulae, or the appearance of juveniles, excluding an origin from asexual reproduction such as polyp bail-out (Sammarco, 1982) or from propagules produced in the field and introduced in the aquarium. The term 'spawning' is exclusively used for gamete release in broadcast spawning species. The release of sperm in brooders is difficult to observe and has only been reported from field observations, therefore this process was not considered in the questionnaire.

RESULTS

In total, 29 institutions completed and returned the questionnaire, of which 16 had observed reproduction in scleractinians. Table 1 gives an overview of all observations. Twenty-four species from 9 families, including one temperate coral (*Astroides calycularis*), were recorded as having reproduced in public aquariums. Among these, 16 species successfully established an F1 generation of a few to more than 100 individuals in at least one of the listed institutions. With regard to reproductive biology, all aquarium observations confirmed former field studies (see Table 2), with the exception of most brooders, which reproduced in captivity independent of the presence of moonlight. Parental colonies were mainly derived from the field or were captive propagated specimens (Fig. 1). The latter were mainly asexually propagated via fragmentation, however, in the case of *Favia fragum* at Rotterdam Zoo, recruits (F2 generation) were obtained from larvae released by captive bred specimens.

The release of gametes by broadcast spawning species was observed in all cases except for *Galaxea fascicularis* and *Echinopora lamellosa* in a 750,000 L exhibit at Burgers' Zoo, whereas the release of planulae was noticed in one third of all observations (see Fig. 2). In 41.6 % of all observations, spawning occurred regularly. In the remaining cases spawning was observed only once or more than once with a relative frequency of 25% each. Whenever larvae release was observed in brooders, it occurred regularly. Natural moonlight, in some cases with additional artificial moonlight, was present in all spawning observations. However, in the case of larvae release in brooders, the great majority of the systems were not exposed to moonlight at all (Fig. 3). Seasonal temperature fluctuations occurred in all systems where reproduction of broadcast spawners was observed, except for *Galaxea fascicularis* and *Echinopora lamellosa* at Burgers' Zoo (see Table 1), whereas brooders reproduced in systems with and without fluctuating temperature (Fig. 3). In broadcast spawners, almost 60% of all spawning events did not result in recruitment of juveniles (Fig. 4). Recruitment was only achieved by manipulating larval development and the settlement process, except for *G. fascicularis* and *E. lamellosa* at Burgers' Zoo, where less than 10 juveniles of each recruited in a 750,000 l system. In contrast to broadcast spawners all the reproductive activity observed in brooders resulted in recruitment of juveniles. However, the number of recruits was in around two-third of the cases less than 100 if settlement was not manipulated (Table 1 and Fig. 4).

Reproduction of broadcast spawners was exclusively observed in open systems, with two exceptions (*G. fascicularis* and *E. lamellosa* at Burgers' Zoo). The observed reproduction in brooders was almost equal in open and closed systems (Fig. 5). Systems in which broadcast spawners reproduced were mainly maintained under natural light, whereas those, in which brooders reproduced, were mostly equipped with artificial light. Except for skimming (in all closed systems), filtration was generally kept low (Fig. 5). Reproduction occurred in both brooding and broadcast spawning species in systems of all sizes. The smallest tank (all open systems) in which reproduction occurred was recorded at Waikiki Aquarium (56 L; *Tubastrea coccinea*), followed by 300 L systems at Kushimoto Marine Park (*Caulastrea tumida*) and at Musée océanographique de Monaco (*Astroides calycularis*) (Fig. 6).

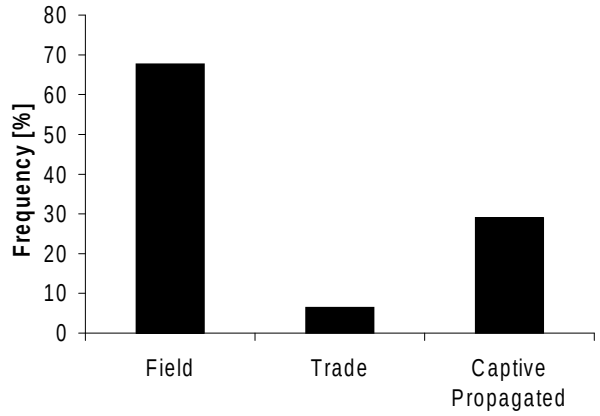


Fig. 1 Origin of parental colonies of all observations. Captive propagated parents derived either from asexual (fragmentation) or from sexual reproduction.

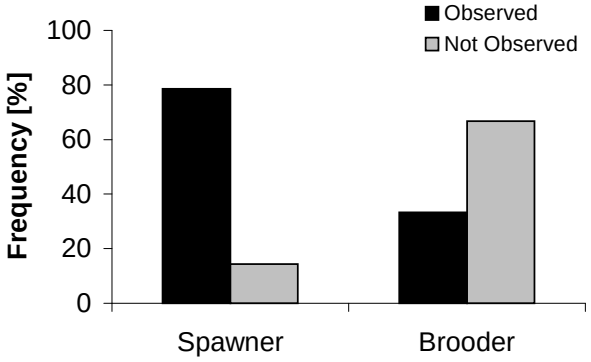


Fig. 2 Relative frequency of gamete or larvae release. The latter one was only observed when certain devices for larvae collection were applied.

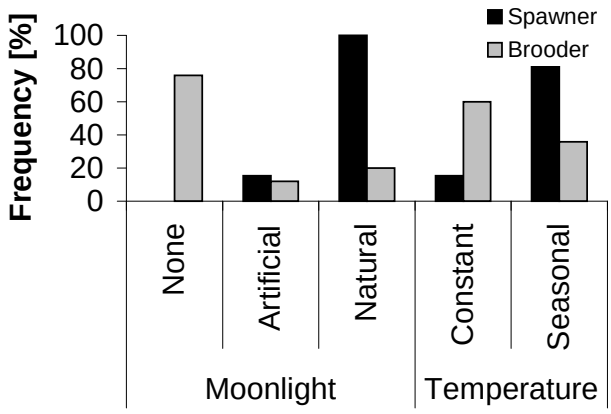


Fig. 3 Observation of reproduction in relation to the environmental factors moonlight and water temperature. Note that in some cases artificial and natural moonlight was applied at the same time.

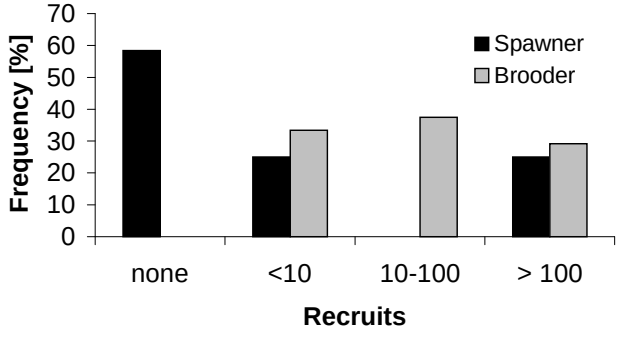


Fig. 4 Relative frequency of recruits in aquariums (data include cases of manipulation, which enhanced larval settlement).

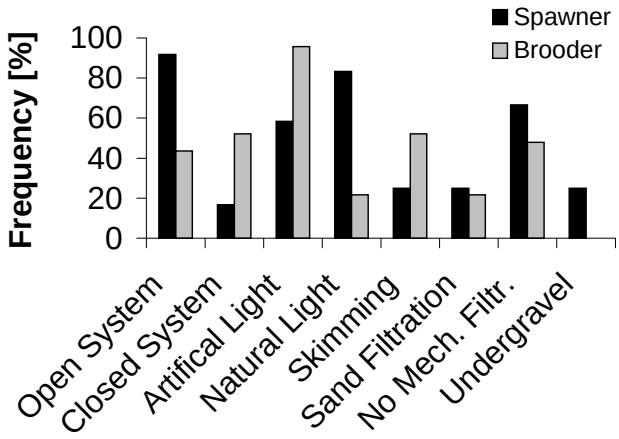


Fig. 5 Relative frequency of reproduction related to certain aquarium system properties, which might influence gamete development, gamete or larvae release and planktonic stage.

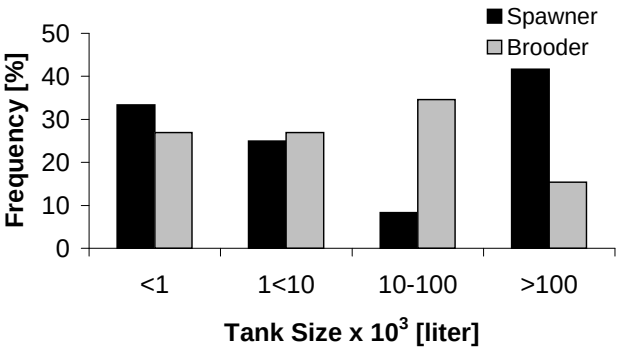


Fig 6 Relative frequency of reproduction related to tank size.

DISCUSSION

Factors Determining Captive Reproduction

At least 24 species of scleractinian corals have reproduced in public aquariums resulting in highly varied recruitment rates from none to more than 100 recruits. Apart from specific circumstances (e.g. low number of mature specimens; only gametes of one sex released at a time), several factors may determine the reproductive success in captivity: (1) environmental factors like moonlight and temperature, (2) tank size and life support system (LSS), (3) manipulation of reproduction and general experience of aquarium staff, and (4) other factors.

(1) The presence of natural moonlight has been shown in various field observations to trigger the precise spawning date (Babcock et al., 1994; Babcock et al., 1986; Harrison and Wallace, 1990). It can, therefore, be assumed to be essential for reproduction in the broadcast spawning species described in this study. Seasonal temperature fluctuations, which are supposed to determine the month of spawning in field populations (Richmond and Hunter, 1990), seem to be another factor that may stimulate reproduction within a species. Nevertheless all aquarium systems, which recorded reproductive behaviour in broadcast species, except the tank at Burgers' Zoo, were at the same time open systems, therefore other stimuli, such as hormones released during spawning events (Atkinson and Atkinson, 1992)

Family	Species	Mode[a]	Temp.	Moon[b]	Gamete/Larval Release	Generation		Manipulation[c]	Institution
Acroporidae	*Acropora formosa*	S	↑↓	N	regularly	>100	-	fertilization, settlement	Okinawa Churaumi Aquarium, Japan
	A. microphthalma	S	↑↓	N	regularly	>100	-	fertilization, settlement	Okinawa Churaumi Aquarium, Japan
	Acropora nobilis	S	↑↓	N	regularly	>100	–	fertilization, settlement	Okinawa Churaumi Aquarium, Japan
	Acropora valida	S	↑↓	N/A	>1 time	-	-	-	Birch Aquarium at Scribbs, USA
	Acropora yongei	S	↑↓	N	1 time[d]	-	-	-	Waikiki Aquarium, Hawaii
	Montipora capitata	S	↑↓	N	regularly	-	-	-	Waikiki Aquarium, Hawaii
Agariciidae	*Agaricia humilis*	B	→	-	>1 time	<10	-	-	Rotterdam Zoo, The Netherlands
		B	↑↓	A/-	regularly	>100	-	settlement	Rotterdam Zoo, The Netherlands
Caryophylliidae	*Euphyllia ancora*	S	↑↓	N	>1 time	-	-	-	Waikiki Aquarium, Hawaii
	Euphyllia glabrescens	B	↑↓	-	regularly	10-100	-	settlement	National Museum of Marine Biology and Aquarium, Taiwan
Dendrophyllidae	*Astroides calycularis*	B?[f]	→	-	not observed	>100	-	-	Musée océanographique de Monaco, Monaco
	Tubastrea coccinea	B	↑↓	-	not observed	10-100	-	-	Waikiki Aquarium, Hawaii
	Tubastrea sp.	B?	→	-	not observed	<10	-	-	London Zoo, U.K.
	Turbinaria reniformis	S?	↑↓	N/A	not observed	-	-	-	Birch Aquarium at Scribbs, USA
Faviidae	*Caulastrea tumida*	S?	↑↓	N	not observed	<10	-	fertilization, settlement	Kushimoto Marine Park, Japan
	Echinopora lamellosa	S?	→	N	not observed	<10	-	-	Burgers' Zoo, Netherlands
	Favia fragum	B?	↑↓	-	not observed	<10	-	-	Columbus Zoo and Aquarium, USA
		B	↑↓	A/-	regularly	>100	>100	settlement	Rotterdam Zoo, The Netherlands
		B	→	-	regularly	>100	>100 (+)[g]	-	Rotterdam Zoo, The Netherlands
Fungiidae	*Sandalolitha robusta*	S	↑↓	N	regularly	-	-	-	Waikiki Aquarium, Hawaii
Pocilloporidae	*Pocillopora damicornis*	B?	↑↓	-	not observed	<10	-	-	Cologne Zoo, Germany
		B?	→	-	not observed	<10[e]	-	-	London Zoo, U.K.
		B	↑↓	-	regularly	>100	0[h]	settlement	National Museum of Marine Biology and Aquarium, Taiwan
		B?	→	-	not observed	10-100	-	-	New England Aquarium, USA
		B?	→	N/A	not observed	>100[e]	-	-	Oceanopolis, France
		B?	→	A/-	not observed	10-100	-	-	Tokyo Sea Life Park, Japan

		B?	→	-	not observed	10-100	-	-	Vancouver Aquarium Marine Science Centre, USA
		B?	↑↓	N	not observed	10-100	-	-	Waikiki Aquarium, Hawaii
	Pocillopora sp.	B?	→	N	not observed	-	-	-	Oceanario de Lisoboa, Portugal
	Seriatopora hystrix	B	↑↓	-	regularly	>100	0[h]	settlement	National Museum of Marine Biology and Aquarium, Taiwan
		B?	→	N	not observed	<10[e]	-	-	Oceanopolis, France
	Stylophora pistillata	B	↑↓	-	regularly	>100	0[h]	settlement	National Museum of Marine Biology and Aquarium, Taiwan
		B?	→	-	not observed	<10	-	-	Musée océanographique de Monaco, Monaco
		B?	→	N	not observed	<10[e]	-	-	Oceanopolis, France
Poritidae	*Goniopora gigas*	S	↑↓	N	1 time	-	-	-	Waikiki Aquarium, Hawaii
Oculinidae	*Galaxea fascicularis*	S?	→	-	not observed	<10	-	-	Burgers' Zoo, Netherlands
	Galaxea sp.	B?	→	N	not observed	<10[e]	-	-	Skansen-Akvariet, Sweden

Table 1. Sexual reproduction of scleractinians in public aquariums including population sizes of F1 and F2 generations.

[a] S = broadcast spawner, B = brooder.
[b] Moonlight: N = natural, A = artificial.
[c] Experimental manipulation of reproduction process.
[d] One colony released egg/sperm bundles. Colony derived from asexual propagation (fragmentation).
[e] Juveniles originated at least partly from parents, which have been asexually propagated via fragmentation.
[f] '?' indicates that mode is suggested based on literature, but spawning / larval release was not observed in particular case.
[g] due to colony sizes of F2 generation and constant appearance of new primary polyps in exhibit, the existence of an F3 generation is suggested, although experimental evidence is missing.
[h] mature eggs and sperm, and early embryos present in coelenterons of specimen of F1 generation.

cannot be completely excluded. However, chemical stimuli can be neglected at least in the case of Waikiki Aquarium, where water is pumped up from deeper water depths to supply open systems (Delbeek, personal communication).

Photoperiod (= day length) suggested by Babcock et al. (1994) as a trigger for seasonal reproduction in high latitude coral reefs was not considered in the questionnaire. Brooding species show more plasticity in their reproductive behaviour. Although species like *Pocillopora damicornis, Seriatopora hystrix,* or *Favia fragum* show lunar cycles in the field, moonlight is not an ultimate trigger to induce reproduction in captivity. The aquarium observations listed in this study basically confirm the work of Jokiel et al. (1985), who showed the loss of synchronization, but a continual release of larvae in *P. damicornis* when night irradiance was kept constant. However, specimens of the F1 generation of *F. fragum* at Rotterdam Zoo showed lunar periodicity without the presence of moonlight (chapter 6). Seasonal temperature changes are generally not essential for reproduction in those brooding species, which reproduce all year round. Other species such as *Seriatopora hystrix* will continue to reproduce in aquariums, even when maintained at constant temperatures. Nevertheless, seasonal temperature variation may enhance sexual output of brooders in the field (Van Moorsel, 1983; Jokiel, 1985), therefore it might be beneficial to simulate seasonal temperature regimes in captivity to enhance reproduction. Regarding the enormous intra- and interspecific, regional and geographic variability in coral reproduction (see Fadlallah, 1983; Harrison and Wallace, 1990) the influence of temperature in the reproduction of brooders can currently be only underestimated, hence more investigation is necessary.

(2) The tank volume and LSS might influence the fertilization rate, the survivorship of planktonic embryos and larvae and the overall amount of settlers. Fertilization of broadcast spawners occurs externally at the water surface for a maximum of 2 hr after spawning (Harrison and Wallace, 1990). The embryos are then positively buoyant for at least 24 hr (Harrison and Wallace, 1990) before they develop benthic searching behaviour and reach settlement competency 2.5 d after spawning, at the earliest (Miller and Mundy, 2003). With regard to brooders in the field, a distance of 2 m between parents has been determined as the maximum in *Agaricia humilis* to ensure sperm transfer and internal fertilization (Morse et al., 1996). Successful fertilization and embryogenesis in aquariums is therefore dependent on the population density of brooders (see also chapter 10). Survival of coral plankton is generally limited due to the destructive impact of the LSS (especially mechanical filtration and skimming). In open systems, there is a high risk that coral plankton may be removed from the tank before settlement competency is reached. If the population density, especially that of brooding species, is comparably low in a large sized aquarium, fertilization might be impossible or insufficient to produce enough propagules for successful recruitment. Field populations of *Favia fragum* and *Porites astreoides* have shown high rates of self-fertilization (Brazeau et al., 1998). *Pocillopora damicornis* can produce planulae asexually, although sexual reproduction is assumed to be present in the field as well (Stoddart, 1983). Whether a coral will choose to cross- or self-fertilize or to produce planulae asexually might be highly dependent on environmental conditions and the population structure (e.g. density, sex ratio). Nothing is known yet about the influence of aquarium conditions on population genetics in corals. I cannot determine if the cases presented in this study might also include asexual reproduction. Genetic studies of *ex-situ* populations are necessary in order to better understand factors determining captive coral reproduction.

In conclusion, brooders have a higher chance of recruiting colonies from sexual reproduction under aquarium conditions than broadcast spawning species (see chapter 10). Recruitment was exclusively observed for broadcast spawners whenever the reproductive process was manipulated (see below). The only exceptions were found in *Galaxea fascicularis* and *Echinopora lamellosa*, which produced a few recruits in a 750,000 L closed

system at Burgers' Zoo. I assume that at least some embryos might have passively escaped from being removed from the system e.g. by sticking to the corners of the tank at the water surface or by 'hiding' at surface areas where no water was removed by the surface overflow. As soon as larvae have become benthic, the chance that they will remain in the system increases to the level expected from other benthos related plankton under aquarium conditions. However, the reported observations in this study indicate this is most likely only in very large systems. Local flow regimes of such systems might create conditions, which prevent coral plankton being flushed away by the LSS.

(3) The experience of the aquarium staff and the ability to manipulate the reproduction process is of fundamental importance in order to control and to promote captive reproduction. In total, four institutions – the National Museum of Marine Biology and Aquarium, Kushimoto Marine Park, Okinawa Churaumi Aquarium and Rotterdam Zoo – manipulated the reproductive process. In each of these cases, the amount of coral recruits (except for *Caulastrea tumida* at Kushimoto Marine Park and *Euphyllia glabrescens* at National Museum of Marine Biology and Aquarium of Taiwan) increased >100 by controlled settlement, and in the case of broadcast spawners, by *ex-situ* fertilization and larval rearing. Exceptions are found in the brooders *Pocillopora damicornis* at Oceanopolis, and *Astroides calycularis* at Musée océanographique de Monaco. In addition colonies of *Favia fragum* in an exhibit tank at Rotterdam Zoo (where reproduction was not manipulated) produced >100 juveniles, which managed to establish in the system (see chapter 10). In all other cases, there were mostly between 0-10 juveniles recorded. The ultimate evidence that captive sexual reproduction in brooders has occurred can only be given when larvae are collected (excluding genetic investigations). In most cases, reproduction of brooders was indirectly noticed by the sudden appearance of juveniles. However, recent studies in two brooding species, *Favia fragum* and *Agaricia humilis*, at Rotterdam Zoo have shown that when settlement is not manipulated larval behaviour between species can differ significantly and may therefore lead to very low recruitment rates or none at all (chapter 10). I assume that more species, especially brooders, reproduce in public aquariums without ever being noticed by the aquarium staff. Without any supportive manipulation, only the most opportunistic species such as *P. damicornis* or *F. fragum*, will establish recruits. However, the number of juveniles will be relatively low compared to the number of released larvae (chapter 10). An exception may be *Astroides calycularis* at the Musée océanographique de Monaco, which produced a few hundred recruits in a 300 L tank. The high success of this species might be the result of highly benthic planulae, which have been observed in the same family (*Tubastrea coccinea*; Stettler, personal communication) and in other scleractinians (e.g. in *Balanophyllia elegans*; Gerrodette, 1981). However, Cirino et al. (1993) observed also swimming larvae in *A. calycularis* in an open system at Naples Aquarium, Italy. The manipulation of captive reproduction in mass spawning species might be even necessary to avoid a negative impact of released gametes on water quality especially in smaller systems (e.g. rapid decrease in oxygen levels, toxic by-products of decaying gametes), which can cause mortalities in fish populations (Delbeek, personal communication). In order to avoid this risk, (1) it might be necessary to remove gametes within 2 hr after release before sperm dies off and starts decomposing or (2) the system should be designed in a way to avoid such spawning events. The simulation of natural temperature cycles to stimulate mass spawning, especially temperature regimes with maximum annual levels of nearly 30 ˚C might limit success in captive reproduction. As part of an experiment at Rotterdam Zoo to induce mass spawning in *Diploria strigosa* and *Montasraea annularis*, temperature was gradually increased above 28 ˚C resulting in a sudden outbreak of Dark Spots Disease, possibly in addition with other stressors (Gil-Agudelo et al., 2004). More investigation is necessary to better understand the impact of temperature fluctuations on coral physiology under aquarium conditions.

Family	Species	Sex[a]	Mode[b]	Lunar[c]	Timing	Region		Reference
Acroporidae	*Acropora formosa*	H	*S*	yes	seasonal	Pacific	GBR & Okinawa	Babcock et al. (1986), Fukami et al. (2003)
	Acropora microphthalma	H	S	yes	seasonal	Pacific	GBR	Babcock et al. (1986)
	Acropora nobilis	H	S	yes	seasonal	Pacific	GBR & Okinawa	Wallace (1985), Fukami et al. (2003)
	Acropora valida	H	S	yes	seasonal	Pacific	GBR	Babcock et al. (1986)
	Acropora yongei	H	S	yes	seasonal	Pacific	GBR	Babcock et al. (1986)
	Montipora capitata	H	S	yes	seasonal	Pacific	GBR	Babcock et al. (1986)
Agariciidae	*Agaricia humilis*	H	B	no	year round	Caribbean		Van Moorsel (1983)
Caryophylliidae	*Euphyllia ancora*	G	S	yes	seasonal	Pacific	GBR	Willis et al. (1985)
	Euphyllia glabrescens	x[d]	B	x	seasonal	Pacific	Central	Richmond and Hunter (1990)
Dendrophyllidae	*Astroides calycularis*	M	B	x	seasonal	Mediterranean		Cirino et al. (1993), Fadlallah (1983)
	Tubastrea coccinea	x	B	x	seasonal	Pacific	Hawaii	Richmond and Hunter (1990)
	Turbinaria reniformis	G	S	yes	seasonal	Pacific	GBR	Willis et al. (1985)
Faviidae	*Caulastrea furcata*	H	S	yes	seasonal	Pacific	GBR	Babcock et al. (1986)
	Echinopora lamellosa	H	S	yes	seasonal	Pacific	GBR	Babcock et al. (1986)
	Favia fragum	H	B	yes	year round	Caribbean		Szmant-Froehlich et al. (1985)
Fungiidae	*Sandalolitha robusta*	G	S	yes	seasonal	Pacific	GBR	Babcock et al. (1986)
Pocilloporidae	*Pocillopora damicornis*	H	B	yes	year round	Pacific	Eniwetok	Richmond and Jokiel (1984)
	Pocillopora damicornis	H	B	yes	seasonal	Pacific	GBR	Richmond and Hunter (1990)
	Pocillopora verrucosa	x	B	yes	seasonal	Pacific	Central	Richmond and Hunter (1990)
	Pocillopora verrucosa	H	S	yes	seasonal	Red Sea		Shlesinger and Loya (1985)
	Seriatopora caliendrum	H	B	x	seasonal	Red Sea		Shlesinger et al. (1998)
	Seriatopora hystrix	x	B	yes	seasonal	Pacific	Eniwetok	Fadlallah (1985)
	Stylophora pistillata	H	B	no	seasonal	Red Sea		Shlesinger and Loya (1985)
	Stylophora pistillata	PH	B	no	seasonal	Red Sea		Fadlallah (1985)
	Stylophora pistillata	x	B	yes	year round	Pacific	Palau	Fadlallah (1985)

Tab. 2. Field observations of sexual reproduction of all species related to those observed to reproduce in public aquariums. Whenever information on a particular species was not available, data of the genus respectively of other species of the same genus were added. Note different reproductive behaviour of certain species between geographic regions.

[a] H = hermaphroditic, G = gonochoric, PH = protandrous hermaphroditic, M = monoic female and male polyps in one colony
[b] S = broadcast spawner, B = brooder
[c] spawning / larval release shows lunar periodicity?
[d] 'x' indicates: no data available
[e] the same population showed hermaphroditic and gonochoric colonies
[f] probably based on wrong identification of species

(4) Species-specific properties have a major influence on the reproductive success in aquariums. Although larval development and settlement was manipulated in *Caulastrea tumida* at Kushimoto Marine Aquarium, <10 juveniles recruited following larvae rearing and settlement induction compared to >100 recruits in *Acropora* spp. treated similarly at Okinawa Churaumi Aquarium (see also Nonaka et al., 2003). Similar observations have been reported for brooding species as mentioned above. Furthermore, other factors such as colony size, asexual propagation by fragmentation, competition and predation may greatly influence reproductive success in captive corals (Chapter 10).

Enhancement of Reproductive Success

The observations reported here clearly show the advantage of manipulating reproduction in captive corals in order to enhance reproductive success. Although some methods such as *ex situ* fertilization and larval rearing in broadcast spawning species need specific knowledge and experience, other techniques can be easily applied, especially for brooders, even by non-experienced aquarium staff. To determine if certain brooding species release larvae, specimens can be held overnight in buckets (maintained at appropriate temperature) to check for released larvae at the following morning. When specimens cannot be removed from the system, plankton nets can either be put over the overflow of the system or above specific specimens to catch released larvae. Colonies can be sampled in the aquarium environment using certain devices using plankton netting, which allow water circulation and at the same time prevent released larvae from being removed by the current (Chapter 6). When the release of gametes is observed (eggs and sperm) in a system, eggs might be caught and maintained at the water surface of the aquarium system using a floating device that prevents propagules from being flushed into the overflow. It is possible that embryos might be able to develop to viable larvae. In this case, the concentration of embryos should be at low levels (water surface covered by less than 50% by a single layer of embryos) to enhance survival (personal observation). Finally, yet importantly, it would be beneficial to carry out histological examinations of potential parents to determine sexual maturity.

Future perspectives

This study shows a high potential in today's public aquariums to reproduce corals in captivity. Adey and Loveland (1998) recorded another 4 brooders (*Porites astreoides, P. porites, P. attenuata, Dichocoenia stokesi*), that had successfully reproduced in aquariums. Aquarium hobbyists have observed the release of gametes respectively larvae in at least 25 scleractinians (*Acropora* spp., *A. pulchra, A. microphthalma, A. pulchra, A. formosa, Balanophyllia* sp., *Catalaphyllia jardinei, Cynarina lacrymalis, Euphyllia* spp., *E. glabrescens, E. ancora, E. parancora, E. divisa, Favia* spp., *F. fragum, Fungia fungites, Galaxea fascicularis, Heliofungia actiniformis, Montipora* sp., *M. digitata, Pocillopora damicornis, P. verrucosa, Plerogyra sinuosa, Polyphyllia talpina, Seriatopora caliendrum, Tubastrea* sp., *T. aurea, T. coccinea, Trachyphyllia geoffroyi, Turbinaria patula*), in which mostly no recruitment resulted (Borneman, unpublished data). Maturity in the F1 generation of 3 species at the National Museum of Marine Biology and Aquarium of Taiwan (Fan et al., unpublished), and the generation of a full life cycle in captivity reported from Rotterdam Zoo in *Favia fragum* (chapter 6 and 10) emphasizes the future opportunity to initiate breeding programs. However, more research is necessary to estimate the benefits and costs of such programs for *ex-situ* and *in-situ* population management and for coral reef conservation in general (see chapter 12; see also Nonaka et al., 2003). The use of sexual coral recruits to stock public aquariums sustainably is effective and can be applied even when institutions are not at all experienced in handling primary polyps (Chapter 11). In order to apply breeding

techniques on a larger scale involving more public aquariums might, however, require special staff training, which could be done at regional and international workshops, for example.

REFERENCES

Adey WH, Loveland K. 1998. Dynamic aquaria: building living ecosystems. London: Academic Press. 498 p.

Atkinson S, Atkinson MJ. 1992. Detection of estradiol-17ß during a mass coral spawn. Coral Reefs 11:33-35.

Babcock RC, Willis BL, Simpson CJ. 1994. Mass spawning of corals on a high latitude coral reef. Coral Reefs 13:161-169.

Babcock RC, Bull GD, Harrison PL, Heyward AJ, Oliver JK, Wallace CC, Willis BL. 1986. Synchronous spawning of 105 scleractinian coral species on the Great Barrier Reef. Mar Biol 90:379-394.

Brazeau DA, Gleason DF, Morgan ME. 1998. Self-fertilization in brooding hermaphroditic Caribbean corals: evidence from molecular markers. J Exp Mar Biol Ecol 231:225-238.

Cirino P, Toscano A, Bentivegna F. 1993. Reproduction of *Astroides calycularis* in the Naples Aquarium, Italy. Proc 3rd Int Aquarium Congress, Boston, USA. p 183-187.

Fadlallah YH. 1983. Sexual reproduction, development and larval biology in scleractinian corals. A review. Coral Reefs 2:129-150.

Fukami H, Omori M, Hayashibara T, Hatta M. 2003. Ecological and genetic aspects of reproductive isolation by different spawning times in *Acropora* corals. Mar Biol 142:679-684.

Gil-Agudelo DL, Smith GW, Garzón-Ferreira J, Weil E, Petersen D. 2004. Dark Spots Disease and Yellow Band Disease, two poorly known coral diseases with high incidence in Caribbean reefs. In E. Rosenberg and Y. Loya, editors. Coral Health and Diseases. Heidelberg: Springer. p 337-348.

Gerodette T. 1981. Dispersal of the solitary coral *Balanophyllia elegans* by demersal planular larvae. Ecology 62:611-619.

Harrison PL, Wallace CC. 1990. Reproduction, dispersal and recruitment of scleractinian corals. In Z. Dubinsky, editor. Ecosystems of the world. 25. Coral reefs. Amsterdam: Elvesier. p 133-207.

Jokiel PL. 1985. Lunar periodicity of planula release in the reef coral *Pocillopora damicornis* in relation to various environmental factors. Proceedings 5th Int Coral Reef Congress, Tahiti 4:307-312.

Miller K, Mundy C. 2003. Rapid settlement in broadcast spawning corals: implications for larval dispersal. Coral Reefs 22:99-106.

Morse NC, Iwao K, Baba M, Shimoike K, Hayashibara T, Omori M. 1996. An ancient chemosensory mechanism brings new life to coral reefs. Biol Bull 191:149-154.

Nonaka M, Baird AH, Kamiki T, Yamamoto HH. 2003. Reseeding the reefs of Okinawa with larvae of captive-bred corals. Coral Reefs 22:34.

Richmond RH, Hunter CL. 1990. Reproduction and recruitment of corals: comparisons among the Caribbean, the Tropical Pacific, and the Red Sea. Mar Ecol Prog Ser 60:185-203.

Richmond RH, Jokiel PL. 1984. Lunar periodicity in larval release in the reef coral *Pocillopora damicornis* at Enewetak and Hawaii. Bull Mar Sci 34:280-287.

Sammarco PW. 1982. Polyp bail-out: An escape response to environmental stress and a new means of reproduction in corals. Mar Ecol Prog Ser 10:57-65.

Stoddart JA. 1983. Asexual production of planulae in the coral *Pocillopora damicornis*. Mar Biol 76:279-284.

Szmants-Froelich A, Reutter M, Riggs L. 1985. Sexual reproduction of *Favia fragum* (Esper): lunar patterns of gametogenesis, embryogenesis and planulation in Puerto Rico. Bull Mar Sci 37(3):880-892.

Shlesinger Y, Goulet TL, Loya Y. 1998. Reproductive patterns of scleractinian corals on the northern Red Sea. Mar Biol 132:691-701.

Shlesinger Y, Loya Y. 1985. Coral community reproductive patterns: Red Sea versus the Great Barrier Reef. Science 228:1333-1335.

Van Moorsel GWNM. 1983. Reproductive strategies in two closely related stony corals (*Agaricia*, Scleractinia). Mar Ecol Prog Ser 13:273-283.

Wallace CC. 1985. Reproduction, recruitment and fragmentation in nine sympatric species of the genus Acropora. Mar Biol 88:217-233.

Willis BL, Babcock RC, Harrison PL, Oliver TK. 1985. Patterns in the mass spawning of corals on the Great Barrier Reef from 1981 to 1984. Proc 5th International Coral Reef Congress, Tahiti 4:343-348.

CHAPTER

3

Transportation Techniques for Massive Scleractinian Corals

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INTRODUCTION

Ex situ research and mariculture requires an adequate supply of healthy, undamaged corals. This necessitates not only a careful selection of colonies regarding their general condition, but also the use of an appropriate technique to transport colonies from the field to the laboratory. One reason for the lack of information regarding the transportation of heavy scleractinians might be that most transports involve relatively small colonies (Green and Shirley, 1999). The collection of larger colonies from the field for commercial purposes and to supply public aquariums is generally not favoured in the context of coral reef conservation. However, for research purposes, it is sometimes necessary to collect larger colonies from the field or to transport colonies between institutions. The transport of heavy colonies presents several problems. The tissue of the colonies can be damaged by the weight of the skeleton when it is placed on live parts of the colony. The sharp edges of the skeleton and substrate can damage transport bags and cause leakage. The use of floating bodies to prevent the coral from touching the bag (especially the bottom of the bag) becomes ineffective when colonies weigh of more than 1,000 g (personal observation).

We planned the shipment of 100 adult colonies of the massive Caribbean species *Diploria strigosa* and *Montastraea annularis* from Curaçao, Netherlands Antilles, to Rotterdam, The Netherlands. To be able to study reproductive behavior, we had to ensure that the collected corals already had surpassed a species-specific minimum colony size indicating that they had reached maturity (see Szmant, 1986 and Soong, 1992). Therefore, a colony size of 20 cm was estimated as the minimum diameter per colony. With the goal of avoiding any damages and minimizing stress to the colonies, I developed a new technique to transport massive stony corals.

METHODS

Prestudy

To determine whether the dry method (transportation without water in a moisture environment; see Bronikowski, 1982 and Carlson, 1999) would be useful for the transport of massive stony corals, a transport simulation was conducted 4 months prior to the real transport.

Two colonies each of *Diploria strigosa* and of *Montastraea annularis* were collected in the shallow fringing reefs in front of the Curaçao Sea Aquarium in August 2001. The colonies were packed immediately after they were brought ashore, using the method described by Delbeek and Sprung (1996), with some modifications. A double plastic bag was put in a styrofoam box. Wet paper was placed on the bottom of the bag to make a soft bed, and then a layer of wet plastic strips was added. The colony was placed on top. All remaining space around the colony was filled with wet plastic strips. Before each bag was closed, 100 ml saltwater and 30 % oxygen (Nitrox) were added. The boxes were stored in an air-conditioned room (25 ºC) for 21 hr. The colonies were then unpacked and transferred to an open system aquarium to monitor behavior and survival.

Species

Diploria strigosa is described in chapter 1, page 13. *Montastraea annularis* is a common Caribbean species that is frequently found in shallow reef areas. It forms massive, lobed, dome-shaped colonies of several meters in diameter. Both species were identified following Humann (1996) and Veron (2000). Special regard was given to the reclassification of the species complex of *Montastraea annularis* by Szmant et al. (1997).

The colonies were carefully chosen according to their general condition and colony size. Only colonies in excellent condition, which showed no mechanical damages, bleached areas,

or ectoparasites, were collected. Only one lobe per colony of *Montastraea annularis* was collected.

Preparation for Transport

All of the colonies were collected from a depth of 5 - 10 m at the exposed fringing reefs in front of the Curaçao Sea Aquarium several weeks prior to transport. The colonies were broken off the substrate with a hammer and chisel. To avoiding causing damage to live tissue by direct contact, rubber gloves were worn during all handling activities. Divers then transferred the corals to a calm spot in the shallow reef near shore at a depth of 6 meters, where the colonies were stored until the day of transport. On the day of collection, they were prepared for stabilization during storage and transport in the following way: One colony after the other was brought ashore and placed in a plastic tub that contained about 50 litres of fresh saltwater. To stabilize the colonies for transport, plastic screws, to which a cross could be fixed, were cemented onto their basal plates. In the following paragraph I describe this procedure (see also Fig. 1 and 2).

During the entire procedure, one person holds the coral upside-down, using two flat sponges. Each sponge is put in a plastic bag to create a smooth surface and thus avoid any irritation of the coral by direct contact with the sponge. The colony is held upside-down in such a way that the basal plate is just emerging from the water. The weight of the colony must be equally distributed on the relatively high surface area of the sponges to prevent any tissue damage and reduce stress reactions. A second person prepares a pure, viscous mix of fresh water and Portland cement at a ratio of approximately 1 : 5. In general, Portland cement dries quickly in saltwater and can develop a permanent saltwater-resistant connection between all sorts of material. Depending on the surrounding temperature, it can dry in < 10 sec. The adhesive property increases gradually, and after 1 week the chemical reaction is complete. The cement is used to fix plastic U-shaped screws onto the dead skeleton of the colony.
Depending on the size of the colony, two to four screws are used, facing each other (this type of screw is normally used to fix electric cables in buildings). When the screws are fixed, a slight opening is left between the basal plate of the colony and the upper part of the screw. A tie wrap is subsequently inserted through this opening so that the colony can be fastened to an object. After the screws are fixed, the colony is put back into the sea for at least 24 hr to let the cement harden. If this is not done, both the cement and the screws will break off the colony. Each colony is then fixed to a PVC cross that is surrounded by a piece of polypropylene hose, thus forming a ring similar to a steering wheel. This construction is referred to as the “cross”. The diameter of the cross should be larger than the diameter of the colony. Each colony is fastened to a cross, using the cemented screws and tie wraps, by two divers. The corals were fixed to the centre of the cross in such a way that the edges of the colonies do not overlap the cross. Finally, the colonies are transferred again to the reef until the day of transport.

Transport and Acclimation Procedure

The colonies were packed separately in double plastic bags. They were completely submerged by fresh saltwater (approx. 5 - 10 L), and pure oxygen (100 %) was added in a ratio (oxygen : water) between 1 : 3 - 1 : 4. The colonies were shipped in styrofoam boxes by air cargo from Curaçao to The Netherlands. They were unpacked immediately after arrival at the Rotterdam

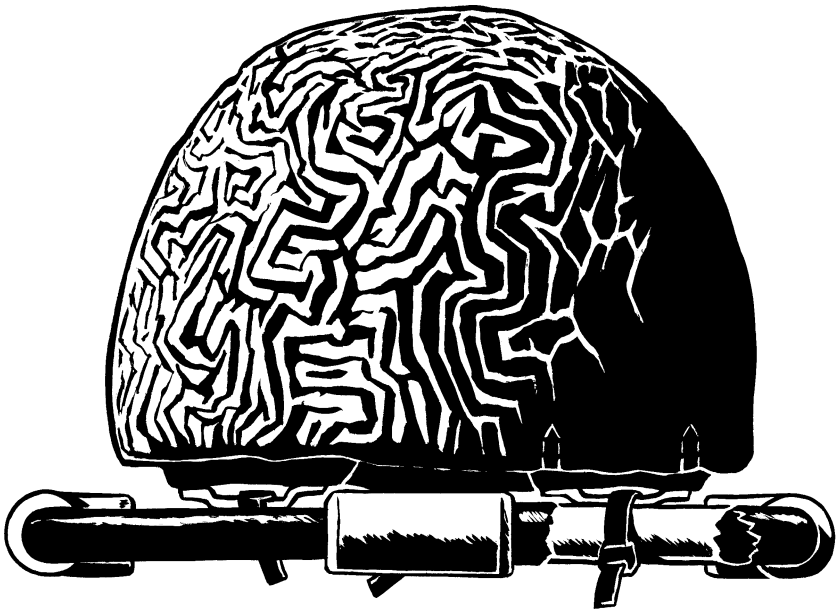

Fig. 1. Side view of a brain coral (*Diploria strigosa*) fixed on a PVC cross. The coral is positioned so that no live tissue touches the cross or the plastic bag. The drawing is semitransparent on the right side to emphasize the connection between the cross and the coral (Leen Zuydgeest, Rotterdam Zoo).

Zoo and slowly acclimatized to the aquarium water. During the first hour, 200 ml of water (2 - 4 % of the transport volume) were added every 10 min. After 1 hr, 400 ml of water were added every 10 min. After 2.5 hr, the corals were transferred to the experimental tanks.

Two transports were carried out (November 2001 and February 2002). I estimated the size of each coral colony by measuring the length and width of the basal plate, and the height of each colony. The weight of each colony was also recorded.

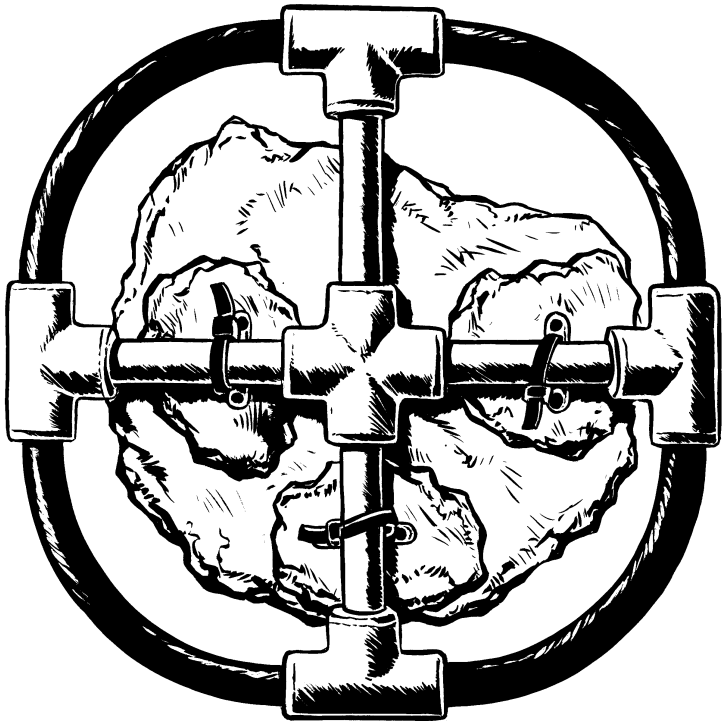

Fig. 2. View from the underside of the coral. Depending on the surface structure of the coral skeleton, a minimum of two (usually three) connections to the cross was necessary to fix the colony for transportation (Leen Zuydgeest, Rotterdam Zoo).

RESULTS

Pre Study

The first four colonies that were collected and used for the transportion simulation (dry method) showed a pale area after they were transferred to the aquarium in Curaçao, probably where the tissue had touched the bottom of the bag. Although this pale area did not necessarily lead to death, we could observe tissue necrosis.

Collection, Preparation and Transport

A total of 50 colonies per species were collected. *Diploria strigosa* easily breaks off the substratum at the colony edge without any tissue damages. The lobes of *Montastraea annularis* can be cut off the substratum a few centimeters below the live tissue of the colony. If the colonies were not fastened to the PVC crosses, the corals were relatively unstable and tended to tumble over when current or surf motion increased. Unavoidably, some colonies tumbled over between the time they were collected and fastened to the crosses. They were carefully examined for any tissue damage. In most cases, the damages healed before transportation started. Not a single colony fastened to crosses tumbled over - even when the surf motion was medium to strong. Portland cement is very effective and easily manufactured. It provides a permanent, stable connection between the colony and the screws after 24 hr (in tropical waters of about 25 ºC). By using the cross as a handle, even the heaviest colonies (> 7,000 g) could be handled above the water surface without the screw connection breaking off. The cement must be mixed carefully, however; otherwise it can disintegrate after a few months.

The collection of each colony, its preparation for transport (fixing screws and crosses), and packaging (by 2 - 3 experienced persons) took approximately 45 - 60 min. In November 2001 and February 2002, 20 and 30 colonies of each species, respectively, were transported.

The November 2001 transport took 35 hr, and the February 2002 transport took 37 hr (from packing until unpacking). In November 2001 the transport water had a salinity of 36.0 ‰ and a temperature of 26.2 ^{o}C; in February 2002 the salinity was 36.3 ‰ and a temperature of 25.3 ^{o}C.

Colony Size

The colonies were measured and weighed after 3 months of acclimatization in the aquarium tanks. For *Diploria strigosa*, the average length was 18.3 cm (min. 12.4 cm, max. 22.8 cm), the width 16.5 cm (min. 11.2 cm, max. 21.0 cm) and height of 13.3 cm (min. 9.1 cm, max. 22.0 cm). The average weight per colony was 3,622 g (min. 2,112 g, max. 9,200 g). The lobes of *Montastraea annularis* had an average length of 14.0 cm (min. 9.1 cm, max. 19.1 cm), the width 11.1 cm (min. 6.2 cm, max. 15.0 cm) and height of 14.4 cm (min. 10.2 cm, max. 20.7 cm). The average weight per colony was 2,843 g (min. 1,810 g, max. 4,395 g).

Post Transport Survival Rates

A post-transport survival rate of 100% was measured 2 weeks after transportation. No damages were visible. During the first transport in November 2001, several colonies of *Diploria strigosa* were only partly submerged during the transport. This drop of the water level resulted from the use of inappropriate filling material in between the bags. The non-submerged tissue areas were pale and covered by a thick layer of mucus. During the following day the pale areas disappeared and the tissue recovered completely. The survival rate after 8 and 4 months, respectively, for the transports in November 2001 and in February 2002 was 96 % for *Diploria strigosa* and 100 % for *Montastraea annularis*, for a total survival rate of 98 %.

Further Development

Some colonies of both species (usually those with a ball-like shape) showed in the longer term a slow necrosis of those tissue areas that were not directly exposed to the light source (1 x 400 Watt 10 K HQI m^{-2}, distance of lamps to water surface = 40 cm, mean distance of colonies to water surface = 20cm, 450 ± 100 µmol m^{-2} s^{-1} light exposure at the top of the colonies). This necrosis stopped after the shaded parts disappeared, leading to a stable tissue cover. The colonies with a flatter and more pyramid-like shape did not show any tissue necrosis. To avoid disturbing the colonies, growth (as determined by the weight of the colonies) was not monitored. In both species, slow growth was indicated by the edges emerging from the surrounding, dead skeleton (estimated at 3 - 5 mm per year under optimal conditions). I observed a slow, but constant decrease of alkalinity from 3.3 to 1.8 meq l^{-1} until November 2002. After the calcium carbonate supply was optimized by the use of larger calcium reactors and the addition of calcium chloride/sodium hydrogen carbonate, I calculated a current consumption rate of 4,000 g per month per experimental tank (water volume = 2,000 L) to keep the calcium above 400 mg l^{-1} and the alkalinity between 3.2 and 3.5 meq l^{-1}. These tanks contained 30 massive coral colonies including other species (e.g. *Siderastrea siderea* and *Porites astreoides*), and approximately the same amount of smaller species (mostly *Favia fragum* and *Agaricia humilis*).

In November 2002, I noticed an outbreak of a syndrome termed Dark Spots disease (DSD) (Gil-Agudelo and Garzón-Ferreira, 2001, Gil-Agudelo et al., 2004). Within 1 month, two-third of the *Montastraea annularis* colonies were infected with DSD and showed dark pigmented spots that grew rapidly, leading to tissue necrosis. To prevent a further increase of DSD, I interrupted the temperature simulation and decreased the water temperature gradually from 28 ˚C to 25 ˚C. The simulation was aimed at inducing captive spawning events. After the disease was eliminated, I started the simulation again in February 2003. I did not observe reproduction in *M. annularis* and *D. strigosa* in 2002, but a regular release of planulae in *Favia fragum* and *Agaricia humilis* during the whole period from November 2001 up to the present date.

DISCUSSION

The results show that the presented technique is an appropriate method to transport heavy coral colonies over a large distance, for a period > 30 hr. All of the colonies survived and there was no visible damage. The deaths of two colonies of *Diploria strigosa* that occurred several months after transport cannot be directly correlated with the transport itself. Both colonies suddenly bleached for no obvious reason and died within a few days. However, the outbreak of DSD may have been resulted from transferring the pathogen with the transported corals in the aquariums (see below).

In comparsion with the conventional submerged and dry methods, the current method requires a careful and relatively time-intensive (15 - 20 min to fix 3 screws and one cross to a colony) preparation of the colonies prior to transportation. In addition, at least one person is needed to assist during the fixing of the screws and crosses. Portland cement increases the pH of saltwater after it is manufactured; therefore, the freshly fixed corals have to be kept in a relatively large volume of water to prevent pH changes. We fixed the last cement plugs to the corals two days before the transport and did not observe any negative reaction of these corals after they had been in a relatively small water volume for > 30 hr. However, potential pH fluctuations should be considered when Portland cement is applied in closed and semi-open systems with a limited water exchange. In this context, I emphasize the importance of mixing

the cement carefully with water to achieve a medium viscosity. Only a homogenous mixture of water and cement can guarantee a proper and long-lasting attachement of the screws to the colony. If they are not properly prepared, the cement plugs may decompose several months later, as I observed in some of the colonies.

To keep the colonies submerged, 3-5 liters of saltwater had to be added to each plastic bag, which increased the transportation costs. To reduce the weight, and therefore shipping costs, it is possible that the corals could be transported using the dry method, in addition to be fixed to the crosses to insure no direct contact between the colonies (in the transport box) and the bottom of the bag. This idea was not tested in the present study, although the results of the pre study indicated it was feasible if direct contact of the tissue with the bottom of the bag could be avoided. Another observation in favor of the dry method is that unsubmerged parts of colonies showed no visible damages after acclimation. Bronikowski (1982) and Carlson (1999) suggested that the total transport time for the dry method should be < 20 hr, and no large temperature changes should occur during transport. With the dry method, there is no water (which functions as a temperature buffer) surrounding the coral. However, the experience of the Monaco Aquarium (Ounaïs, personal communication) shows that it is possible to transport large colonies using the dry method, even for transportation times of 39 hr.

Whether the corals should be transported by the dry or the submerged method may also depend on the species. Borneman (personal communication) suggests that the dry method should be used for species that produce a lot of mucus in order to avoid increased bacteria growth during transport and thus prevent large oxygen consumption and high concentrations of metabolic toxins in the water. He further recommends that trials should be conducted with tetracycline or similar antibiotics, which reduce bacteria growth, to find out more about the influence of microbes on transport conditions and survival rates.

The use of antibiotics may be important in eliminating and avoiding the transfer of potential pathogens that cannot be easily detected, as was the case with DSD in the present study. DSD has been increasingly observed in the Caribbean (Gil-Agudelo et al., 2004). Although DSD was present in some colonies of *Montastraea annularis* at the collection site, none of the collected colonies showed any abnormal pigmentation. We noticed a small dark spot in one colony of *M. annularis* in August 2002, 5 months after its collection, and did not observe any major increase till October 2002. In October/November the spots increased rapidly and new colonies were infected daily. Finally, we had to move all infected colonies (two-third of the stock) to a separate tank, and conduct a major water exchange (80 %) to stop the disease in the experimental setup. In addition to the relative high temperature (28 ºC), we had to assume that other factors, such as insufficient water movement and low alkalinity, might have contributed to the outbreak. Therefore we optimized these factors. The pathogen causing DSD has not yet been clearly identified. For further details on our observations regarding DSD, see Gil-Agudelo et al. (2004). In general, more research on coral diseases, adequate quarantaine procedures and medical treatments are necessary to prevent outbreaks such as those in the present study.

In a previous study, Bronikowski (1982) drilled holes into the colony skeletons to fix them with screws to the rock framework in the exhibit tanks. Thinking this simple method might also be useful in preparing massive colonies for transport, we attempted to use it in the current study; however, it resulted in heavy mucus production, mesentery filament ejection, and even tissue damage and broken colonies. Species with massive, dense skeletons were very difficult to drill, even when we used diamond drills. Moreover, screws stuck in these holes often broke off the colony during handling.

Special attention should be given to temperature changes during transport and acclimation. Jones (personal communication) uses ice or heat blocks, depending on the

shipping route used, to avoid an increase or drop of temperature. He also recommends giving the receiver of the shipment as much information as possible about the environmental conditions the corals have been exposed to prior to transport. We adjusted the aquarium water before the arrival of the corals to the temperature and salinity of the collection site. Nevertheless we observed a temperature decrease of 1 – 2 ºC in the transport bags during the acclimation procedure due to a relatively low room temperature. This temperature drop could only be stopped by adding more aquarium water to the corals per time unit. In addition to the corals, we shipped other aquatic animals (mainly grazing organisms for the experimental tanks), resulting in a total of 100 boxes per shipment. Depending on the total quantity of a transport, special attention should be given to formulating a detailed plan - especially for packing and unpacking to minimize handling time. In the present case more than 15 people helped with each, packing and unpacking to minimize handling time.

We still keep the colonies fixed to the crosses in the experimental tanks. This allows us to handle the colonies easily without touching the coral itself. The PVC crosses provide a stable basis for a permanent placement on horizontal surfaces in aquariums.

In conclusion, deciding which transportation method to apply depends mainly on species-specific properties (e.g. mucus production), and the colony size, and partly on the maximum expected transportation. In future transports of live corals for commercial, educational, or research purposes, there will probably be a shift away from field-collected specimens towards captive propagated corals for coral farms, public aquariums, research facilities and commercial uses (Rinkevich and Shafir, 1998; Delbeek, 2001; Petersen and Tollrian, 2001). This may result in an increase of transports of cultured fragments using the dry method. Recent studies have shown that it is possible to transport coral larvae over large distances, which can be used to supply species, which are difficult to fragment (Petersen and Tollrian, 2001; chapter 4). Furthermore, newly settled primary polyps have shown high survival rates during intercontinental transports (unpublished data). In the future, the transfer of fragments, planulae, and primary polyps will not only reduce transportation costs and ensure a supply for public aquariums and commercial trade, it will also enable us to establish an international frame work of breeding centres for in and ex situ conservation (see also Petersen and Tollrian 2001; Petersen et al., 2002; and chapter 11).

The setup and maintenance of stable inland breeding stocks will further require the safe and gentle transfer of mature colonies from the field to breeding facilities, as well as between facilities. For massive species in particular, relatively heavy and large colonies are necessary to initiate reproductive events in captivity (Szmant, 1986). Appropriate transportation and handling techniques are therefore essential to minimize stress and damage to parental colonies, and prevent a lack of fecundity.

REFERENCES

Bronikowski EJ, Jr. 1982. The collection, transportation, and maintenance of living corals. AAZPA Conference Proceedings 1982: 65-70.

Carlson BA. 1999. Organism responses to rapid changes: what aquaria tell us about nature. American Zoologist 39: 44-55.

Delbeek JC. 2001. Coral farming: past, present and future trends. Aquarium Sciences and Conservation 3: 171-181.

Delbeek JC, Sprung J. 1996. Das Riffaquarium: Ein umfangreiches Handbuch zur Bestimmung und Aquarienhaltung tropischer wirbelloser Meerestiere. Coconut Grove: Ricordea Publishing. 544 p.

Gil-Agudelo DL, Garzón-Ferreira J. 2001. Spatial and seasonal variation of the Dark Spots

Disease in coral communities of the Santa Marta area (Colombian Caribbean). Bulletin of Marine Science 69(2):619-629

Gil-Agudelo DL, Smith GW, Garzón-Ferreira J, Weil E, Petersen D. 2004. Dark spots disease and yellow band disease, two poorly known coral diseases with high incidence in Caribbean reefs. In Rosenberg E, editor. Coral Health and Diseases. Springer, Heidelberg. pp. 337-348.

Green EP, Shirley F. 1999. The global trade in coral. World Conservation Monitoring Centre. Cambridge: World Conservation Press. 70 p.

Humann P. 1996. Reef coral identification: Florida, Caribbean, Bahamas. 3rd edn. Jacksonville: Paramount Miller Graphics. 239 p.

Petersen D, Laterveer M, van Bergen D. 2002. SECORE - Steinkorallen-Projekt: Internationales Projekt zur Steinkorallen-Vermehrung. Deutsche Aquarien- und Terrarienzeitschrift (DATZ) 55(10): 36-39.

Petersen D, Tollrian R. 2001. Methods to enhance sexual recruitment for restoration of damaged reefs. Bulletin of Marine Science 69(2): 989-1000.

Rinkevich B, Shafir S. 1998. Ex situ culture of colonial marine ornamental invertebrates: concepts for domestication. Aquarium Sciences and Conservation 2: 237-250.

Soong K. 1992. Colony size as a species character in massive reef corals. Coral Reefs 12: 77-83.

Szmant AM, Weil E, Miller MW, Colon DE. 1997. Hybridization within the species complex of the scleractinian coral *Montastraea annularis*. Marine Biology 129: 561-572.

Szmant AM. 1986. Reproductive ecology of Caribbean reef corals. Coral Reefs 5: 43-54.

Veron JEN. 2000. Corals of the world. Vol. 3. Townsville: Australian Institute of Marine Science. 490 p.

CHAPTER

4

Ex situ Transportation of Coral Larvae for Research, Conservation, and Aquaculture

INTRODUCTION

Coral larvae of broadcast spawning species can be obtained in large quantities due to recent advances in ex situ fertilisation and rearing methods (Willis et al. 1997, Hatta et al. 1999, Heyward and Negri 1999). Larvae may serve for laboratory and field experiments as well as for restoration and aquaculture purposes. However, the use of cultured larvae was so far mostly limited to their place of origin. Aiming reef restoration by enhancing recruitment, Heyward et al. (2002) shipped several million larvae obtained from coral slick using floating culture ponds that were carried by boats over a distance of several kilometers for field experiments. Omori (pers. communication) cultured millions of larvae in a floating culture pond, packed the larvae in containers, and transported them by ferry boat to the destination over 1 hr away. For research purposes, small numbers of larvae of acroporids have been successfully delivered by regular postal mail within Japan (Hatta, unpublished). The transportation of coral larvae in high amounts over large distances might give new opportunities for research, conservation and aquaculture.

We transported coral larvae of three different broadcast spawning species in several transports from Okinawa, Japan, and from Curaçao, Netherlands Antilles, respectively, to Rotterdam. Here, I summarise our experiences with transportation methodology and exemplify possible applications of the presented technique in research, conservation and aquaculture.

METHODS

Gametes of *Acropora tenuis* and *A. digitifera*, and of *Diploria strigosa* were collected in the field during the annual spawning events at Aka Island, Okinawa in May/June and at Curaçao, Netherlands Antilles in September, respectively. Fertilisation and rearing of larvae was carried out following Iwao et al. (2002). Larvae were shipped 4 - 6 days after fertilisation in 10 µm-filtered seawater in 5 different container volumes: (1) 15 ml and (2) 50 ml centrifuge tubes (polyethylene), (3) 200 ml tuberware boxes (polypropylene), (4) 500 ml and (5) 1,000 ml bottles (polyethylene). No oxygen or germicidal chemicals were added. Larvae in different densities (3.3-13.3 larvae ml^{-1}; see Table 1) were transported by regular airmail, express airmail, express service or as hand luggage.

Larvae in ≤ 200 ml units were accurately counted while transferring them with a pipette from a petridish to centrifuge tubes and tuberware boxes. If necessary, water was added to attain the precise volume for transportation. Centrifugal tubes were either transported in thermal-isolated envelopes (airmail, express airmail) or in carton boxes filled with styrofoam eggs (express service, hand luggage). Regarding the preparation of the 500 ml and 1,000 ml bottles, larval density was determined by well mixing the culture and then counting planulae in replicate 10 ml aliquots. The density was then adjusted to 4 larvae ml^{-1} by adding or removing water. Bottles were filled with the well-mixed culture and finally put in a carton box filled with styrofoam eggs.

After arrival, larvae were transferred with transport water to petridishes (≤ 200 ml transport volume) and into plastic containers (500 and 1,000 ml transport volume), respectively, to calculate survival rates using the same counting methods as before transportation. The number of larvae settled on container walls during transportation was additionally determined. Larvae were gradually acclimated to 26 ºC in 2 hr. Seawater used for the experiments was adapted to the local salinity (34 ‰) prior to the settlement experiment.

Immediately after arrival, water analyses were conducted for particular samples by taking 100 ml transport water from 200 and 500 ml bottles. First the samples were filtered

(0.45 µm), then nitrogen bound as NH_4^+, NO_2^-, NO_3^{2-}, and phosphorous bound as PO_4^{3-} was measured using photo spectrometry (DR/4000U Photospectrometer, HACH Company, U.S.A.). The pH was determined by potentiometric titration (Titro Line easy, Schott GmbH, Germany).

Metamorphosis competency of *Acropora tenuis* ("hand luggage" transport, see table 1) was determined by inducing metamorphosis with the peptide Hym-248, following a modified protocol of Iwao et al. (2002). We used ceramic tiles presented in chapter 5, which were incubated in sterilised seawater prior to the experiment for 2 months to exclude any toxins, which may be released in the water by ceramics (see chapter 5). The tiles were placed in 250 ml, and then 100 larvae and Hym-248 in a final concentration of 3×10^{-6} M were added (room light: 40 µmol m^{-2} s^{-1}; 26 ºC). The number of metamorphosed animals (attached and non-attached disc-shaped planulae; see Harrison and Wallace 1990) was counted 12 hr after the start of the experiment.

RESULTS AND DISCUSSION

High survival rates of > 90% were determined at ≤ 4 larvae ml^{-1} for *Acropora tenuis* even when transportation took 10 d while no larvae survived at densities >6.6 larvae ml^{-1} during the 10 d transport (Table 1). However, shorter transport duration (≤ 4 d) may lead to high survival rates even at densities of 10 larvae ml^{-1}. High post-transport survival rates were verified for *Diploria strigosa* by shipping more than 48,000 larvae from Curaçao to The Netherlands (4 larvae ml^{-1}). Post-transport metamorphosis of *Acropora tenuis* was 67.6 ± 6.03 % (mean ± SD; n = 3) compared to 93.8 ± 2.20 % (mean ± SD; n = 6) prior to transportation when using Hym-248.

Natural rates of larval survival and settlement can be hardly compared with those of the present study since various environmental factors may highly affect these life history stages in the field (Westneat and Resing 1988, Maida et al. 1995, Fearon and Cameron 1996, Fabricius and Metzner 2004; see also McCook et al. 2001). However, survival rates of 5-10 % (7-10 d after fertilization) and max. metamorphosis rates of 30-80 % were determined for broadcast spawners from larval cultures and settlement experiments in situ and in flow-through aquariums (Heyward et al. 2002, Miller and Mundy 2003). Rates obtained in the present study are within this range. Nevertheless, lower metamorphosis rates after transportation when applying Hym-248 may indicate transport stress.

Analyses of the transport water indicate reduced temperatures and pH as well as higher nitrate levels compared to the field (Table 2). A temperature decrease in the observed range probably does not affect survival and metamorphosis (Edmunds et al. 2001), however, temperatures > 30 ºC would influence larvae negatively if occurring during transportation (Bassim and Sammarco 2003). Changes in pH and nitrate concentration are presumably the result of deteriorating organic material. The influence of pH and nitrate on the viability of planulae has been hardly investigated. However, a pH slightly below 7.7 did not affect the growth of adult scleractinians cultured in aquariums (Atkinson et al. 1995). More research is necessary to determine the impact of nitrate concentration on larval physiology. In this context, antibiotics, which have been successfully applied in larval culture (Heyward, personal communication), could help to reduce bacteria growth and the accumulation of toxic metabolic products.

Regarding these changes of water chemistry and physics, I assume (1) larval density and (2) transport duration to be crucial for the successful ex situ transportation of coral larvae. (3) Packing (thermal isolation) and (4) transport logistics (reliability, CITES formalities, monetary costs, see Table 1) may play an additional role. We obtained best results when

Species	Density	Volume	Replicates	n	Duration	Logistics	Monetary costs[a]	S±SD
	larvae ml^{-1}	ml			d		€	%
Acropora tenuis	3.3	15	8	400	10	airmail	25.00	98.3±2.5
	6.6	15	4	400	10	airmail	25.00	0.0
	13.3	15	4	800	10	airmail	25.00	0.0
	3.3	15	2	100	6	Express airmail	20.00	74.0±14.1
	6.6	15	2	200	6	Express airmail	20.00	60.5±2.1
	4	200	1	800	6	Express airmail	20.00	78.6
	3	15	9	405	4	airmail	25.00	99.2±1.5
	5	15	6	450	4	airmail	25.00	99.5±0.6
	10	15	6	900	4	airmail	25.00	98.4±3.5
	4	500	5	10,000	3	express service	20.00	103.5±16.4
	4	50	4	800	2	hand luggage	30.00	100.0
	8	50	2	800	2	hand luggage	30.00	98.4±2.3
	4	200	4	3,200	2	hand luggage	30.00	99.2±0.9
Acropora digitifera	3	15	3	135	4	airmail	25.00	85.2±10.5
	5	15	3	225	4	airmail	25.00	69.3±5.0
	10	15	3	450	4	airmail	25.00	98.0±2.9
	4	500	5	10,000	3	express service	20.00	78.7±23.8
Diploria strigosa	4	50	4	800	2	hand luggage	30.00	95.0±5.0
	4	1,000	12	48,000	2	hand luggage	30.00	93.8±3.1

Table 1 Data of coral larval transports from Akajima (Japan) and Curaçao (Netherlands Antilles) to Rotterdam Zoo (The Netherlands). n = total number of larvae per transport, S = mean post-transport survival. [a] per 1,000 ml volume (= 1 kg). [b] calculated for an overweight charge of 30.00 Euro kg^{-1}.

Species	Density	DT	Temp.	pH	NH4-N	NO2-N	NO3-N	PO4-P
	larvae ml^{-1}	d	°C		µM	µM	µM	µM
A. tenuis	4	6	22.1	7.34	0.56	0.15	20.98	-
	4	3	23.9	7.63	0.56	0.07	22.91	0.19
A. digitifera	4	3	24.0	7.44	0.00	0.13	28.72	0.64
Field			25.0-27.0[a]	8.00-8.40[b]	0.05-11.00[c]	n.a.	0.05-5.00[c]	0.02-1.36[c]

Table 2 Water analysis of 3 different larval transports of *Acropora* spp. compared to common field values [[a] at Akajima in May/June, [b] after Sorokin (1995), [c] after Adey and Loveland (1998)]. DT = duration of transport, n.a. = not available.

shipping larvae by express services or transporting them personally as hand luggage. (5) The shape of the transport container may be important regarding thermal effects and potential settlement during transportation. 11.5 % of larvae settled in edges and corners of the tuberware boxes compared to 0.06 % in the bottles. The phenomenon that edges may initiate settlement has been generally observed in laboratory experiments (Harrison and Wallace 1990).

In conclusion, our results demonstrate that it is possible to transport coral larvae over large distances without any life support with an optimum density of 4 larvae ml^{-1} and a transportation time of $\leq$ 4 days. To ensure appropriate experimental results, survival and metamorphosis competency should be verified prior to and after transportation, especially when larvae are shipped for research. For this purpose standardised testing methods can be applied such as Hym-248, which exclusively induces metamorphosis in the genus *Acropora* (Iwao et al. 2002).

The presented method has been successfully applied to supply more than 10 European public aquariums with primary polyps cultured at Rotterdam Zoo (unpublished data; see also chapter 11). Today's public aquariums have an important role to promote nature conservation by raising public awareness, self-sustaining ex situ populations and co-ordinating breeding programs for endangered species [IUDZG/CBSG (IUCN/SSC), 1993]. The effective transfer of specimens will be an important step to reach these aims. When assuming settlement rates of > 50 % and recruitment rates of > 20 %, which are common values in mariculture (see chapter 8 and 9), intercontinental larval transports are at least 200 times more economical compared to the common method to ship adults (1 kg transport weight per coral; see Green and Shirely 2000, and chapter 3). In addition, utilization of larvae would replace collection of coral colonies thereby reduce impacts on coral populations (chapter 12).

Therefore the transportation of planulae may be an attractive alternative in aquaculture to supply the trade by inland mariculture facilities, which may be directly located in those countries that are of major importance for the trade in ornamentals (e.g. USA, Japan, Germany; after Green and Shirely 2000).

The ex situ transfer of planulae can be useful in research, if local logistics at the collection site are not appropriate to carry out experiments under laboratory conditions. Further on, it may be attractive for research institutions, which are not directly located at coral reefs, to safe the costs for a field trip to obtain planulae, e.g. for bio assays, if these institutions can receive high quality larvae delivered by aquaculture facilities located at these reefs. Larval supply from different regions, e.g. Japan and Australia, serves multiple chances of research using larvae that are obtained only once a year at each region.

REFERENCES

Adey WH, Loveland K. 1998. Dynamic aquaria: building living ecosystems. Academic Press, London. p 498.

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.

Bassim KM, Sammarco PW. 2003. Effects of temperature and ammonium on larval development and survivorship in a scleractinian coral (*Diploria strigosa*). Mar Biol 142:241-252.

Edmunds PJ, Gates RD, Gleason DF. 2001. The biology of larvae from the reef coral *Porites astreoides*, and their response to temperature disturbance. Mar Biol 139:981-989.

Fearon RJ, Cameron AM. 1996. Larvotoxic extracts of the hard coral *Goniopora tenuidens*: allelochemicals that limit settlement of potential competitors? Toxicon 34(3):361-367.

Fabricius KE, Metzner J. 2004. Scleractinian walls of mouths: predation on coral larvae by corals. Coral Reefs 23:245-248.

Green EP, Shirley F. 1999. The global trade in coral. World Conservation Monitoring Centre. Cambridge: World Conservation Press. 70 p.

Harrison PL, Wallace CC. 1990. Reproduction, dispersal and recruitment of scleractinian corals. In: Dubinsky Z. (ed.) Ecosystems of the world. 25. Coral reefs. Elvesier, Amsterdam, pp 133-207.

Hatta M, Fukami H, Wang W, Omori M, Shimoike K, Hayashibara T, Ina Y, Sugiyama T. 1999. Reproductive and genetic evidence for a reticulate evolutionary history of mass spawning corals. Mol Biol Evol 16:1607-1613.

Heyward AJ, Negri AP. 1999. Natural inducers for coral larval metamorphosis. Coral Reefs 18:273-279.

Heyward AJ, Smith LD, Rees M, Field SN. 2002. Enhancement of coral recruitment by *in situ* mass culture of coral larvae. Mar Ecol Prog Ser 230:113-118.

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.

IUDZG/CBSG (IUCN/SSC). 1993. The world zoo conservation strategy; the role of the zoos and aquaria of the world in global conservation. Chicago: Chicago Zoological Society, Brookfield, pp 76.

Maida M, Sammarco PW, Coll JC. 1995. Effects of soft corals on scleractinian coral recruitment. I: Directional allelopathy and inhibition of settlement. Mar Ecol Prog Ser 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.

Miller K, Mundy C. 2003. Rapid settlement in broadcast spawning corals: implications for larval dispersal. Coral Reefs 22:99-106.

Sorokin YI. 1995. Coral reef ecology. 102. Ecological studies. Springer, Berlin Heidelberg New York. p 465.

Westneat MW, Resing J. 1998. Predation on coral spawn by planktivorous fish. Coral Reefs 7:89-92.

Willis BL, Babcock RC, Harrison PL, Wallace CC. 1997. Experimental hybridization and breeding incompatibilities within the mating systems of mass spawning reef corals. CoralReefs 16:S35-S65

CHAPTER

5

Innovative Substrate Tiles to Spatially Control Larval Settlement in Coral Culture

INTRODUCTION

Various substrata such as coral skeleton (Babcock and Davies 1991; Babcock and Mundy 1996), ceramic or terracotta tiles (Tomascik 1991; Hunte and Wittenberg 1992; Maida et al. 1995; Mundy and Babcock 1998; Wilson and Harrison 1998), cement blocks (Hunte and Wittenberg 1992), glas jars or petridishes (Hodgson 1990; Oren and Benayahu 1997; Epstein et al. 2001), PVC plates (Soong et al. 2003), crustose coralline algae fragments (Morse et al. 1996; Heyward and Negri 1999), and more complex set ups like polystyrene grates (Van Moorsel 1988) have been used for settlement experiments to study coral biology. Settlement and metamorphosis are generally favored in crevices and on ridges of biologically conditioned surfaces (= biofilm) (Harrison and Wallace 1990). Rough surfaces such as on ceramic tiles and on coral skeletons are more attractive than substrata like petridishes (Harriott and Fisk 1987). A comparative study of Harriott and Fisk (1987) emphasized the use of ceramic tiles as an economical and attractive substratum for larval settlement. Nevertheless, commercially available terracotta tiles often used in settlement studies usually are not a favorable substratum for further use in mariculture or restoration, simply due to the fact that a few hundred settlers located on one plate are difficult to separate from each other. Besides such terracotta tiles are not principally designed to avoid damaging young settlers e.g. by handling and by grazing organisms. Smaller sized tiles made from clay may reduce the number of settlers per unit, but need specific attachment to avoid tumbling over in the water current (Petersen and Tollrian, 2001).

Currently there are no standardized substrata available, which allow to control coral settlement and to handle recruits for specific purposes in a reproducible way. Designing such substrata, which can be manufactured in large numbers will be increasingly important in coral mariculture to serve the needs of field restoration projects, public aquaria, the trade in marine ornamentals or other fields.

Identifying the special needs for settlement tiles to serve in mariculture based on the studies of Petersen and Tollrian (2001), and of Harriott and Fisk (1987), I designed a new type of settlement substratum suitable to gain primary polyps and juvenile colonies, and to exchange coral stocks in large quantities among public aquaria. The aim was to: (1) attract and (2) spatially control larvae settlement to a maximum, (3) further more produce substrate units for appropriate handling in coral mariculture.

MATERIALS AND METHODS

Principal design and methodology

I designed 2 different types of substrate tiles representing horizontal (= ‘flat tiles’) and vertical surfaces (= ‘pyramid tiles’) for settlement (see Figure 1). The tiles, 22.0 x 22.0 ± 1.0 mm (L x W) for the flat tiles and 17.0 x 17.0 ± 1.0 mm for the pyramid tiles are relatively small to serve as basic units; they can be arranged in a chessboard pattern (Fig. 2). Depending on specific demands and available space, these units can temporarily be put together in any number to create settlement surfaces of any size. The tiles are placed into a polystyrene grid next to each other. Whereas the flat tiles show only 1 flat horizontal surface, the pyramid tiles display 4 vertical surfaces. All exposed surfaces have several parallel grooves (width and depth 2.0 ± 0.1 mm). The lower non-exposed bases of both types have the same conically tapered shape (17.0 x 1.70 x 11.0 ± 1.0 mm, L x W x H) to fit exactly into the grid (see Fig. 1).

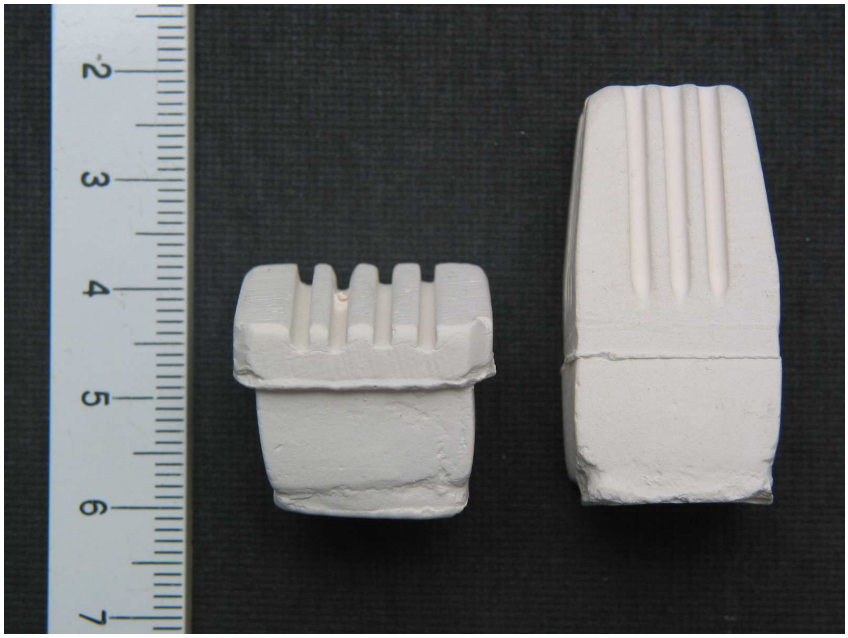

Fig.1 Side view of both tile-types. The flat tile provides 1 horizontally, while the pyramid tile provides 4 vertically oriented settlement surfaces. The grooves give shelter for initial life stages of settlers.

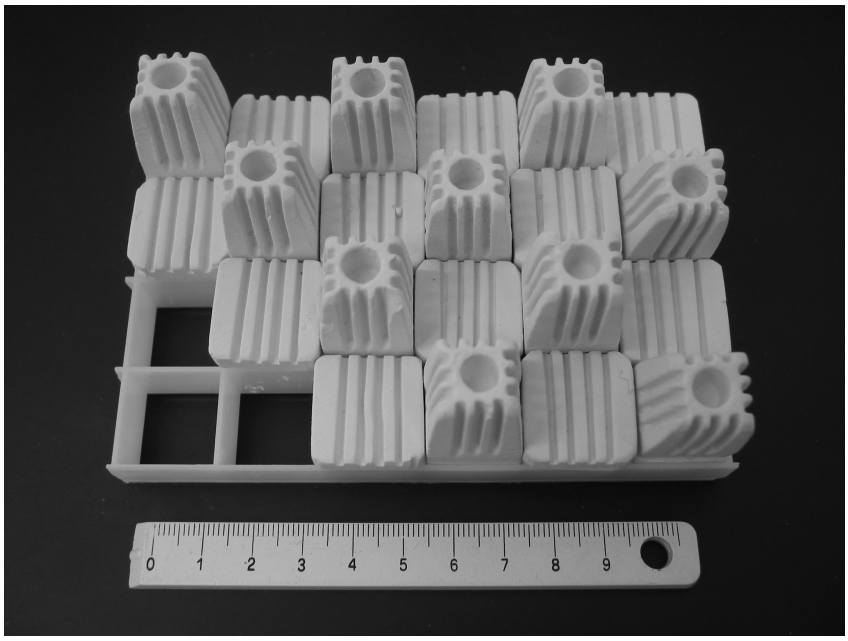

Fig. 2 Chessboard-like arrangement of flat and pyramid tiles. Three tiles are not placed to emphasize the polystyrene grid. Due to the arrangement of the tiles in the grid and due to the low water surface not covering the top of the pyramid tiles, larvae could settle only surfaces with grooves. The majority of settlers were located in these grooves. Four of such tile units were used to replicate each treatment.

Manufacture of tiles

The tiles were produced from commercially available liquid clay (product: GK100, producer: VeKa BV, The Netherlands), which was pressed under vacuum in specially designed molds made from plaster. After a drying period of 1-2 days the tiles were baked at 1040 ºC. We started with an oven that had been used for baking and glazing all sorts of pottery (Oven 1), but this one was soon replaced by a brand-new oven (Oven 2). All tiles used in settlement experiments were manufactured in Oven 2.

Chemical analysis

White-baking clay consists mainly of kaolinite $Al_2(OH)_4[Si_2O_5]$ and limespar $CaCO_3$ (Holleman and Wiberg 1985). We analyzed tiles freshly baked in Oven 1 and 2 whether they released chemical elements into seawater, which could disturb larvae settlement. 250 ml UV-sterilized seawater was incubated for 1 week at 26 ºC with and without tiles. Water samples were prepared for ICP spectrometry by adding 1.6 ml of HNO_3 (65%) per 100 ml. All further measurements (salinity, pH, Ca^{2+} and alkalinity) and settlement experiments were only carried out with tiles from Oven 2, which were incubated in seawater for at least 2 months. Because of logistical and financial reasons, no parallel analyses were conducted.

Salinity, pH and alkalinity were measured before and after settlement experiments. Nitrogen bond as NH_4^+, NO_2^-, NO_3^- and phosphorous (PO_4^{3-}) were analyzed using photo spectrometry (DR/4000U Photospectrometer, HACH Company U.S.A.).

Studied species

Experiments were carried out with the Caribbean brooder *Favia fragum* and with the Indo Pacific broadcast spawner *Acropora tenuis*. Colonies of *F. fragum* were collected in Curaçao, Netherlands Antilles and transported to Rotterdam Zoo, where they regularly released larvae. Larvae of *A. tenuis* were reared from field-collected gametes at Aka Island, Okinawa, Japan following the protocol of Iwao et al. (2002) and shipped to Rotterdam Zoo following the protocol of chapter 4.

Settlement experiments

In order to develop a biofilm, some of the tiles (flat- and pyramid-types) were placed in a grid and incubated for at least 2 months in a laboratory aquarium (5,000 l water volume; closed system with a monthly water exchange rate of approx. 10%), which contained marine algae and other benthic organisms (= conditioned tiles). Other tiles were incubated for 2 months under sterile conditions (external UV-sterilizers; 500 L tank with a water exchange rate of 100 % weekly) without any light exposure (= non-conditioned tiles). Twelve tiles of each type were placed into a non-incubated grid in a flat polyethylene box (normally used to pack fast-food), 250 ml seawater was added to partially submerge the tiles in such a way that in the end the top of the pyramid tiles was just above the water surface (see also Fig. 1). Three treatments were tested: (1) only non-conditioned tiles, (2) only biologically conditioned tiles, (3) alternating non-conditioned with biologically conditioned tiles. Temperature was constantly at 26 ºC. The amount of light was kept constant (room light: 40 µmol m^{-2} s^{-1}; measured with a spherical light sensor, LI-193SA, LICOR). No water was exchanged during the settlement period. Forty larvae of *Favia fragum* (2 replicates per case) respectively 100 larvae of *Acropora tenuis* (4 replicates per case) were added per treatment. The number of larvae per case and the number of replicates was determined by the amount of larvae available. Settled *F. fragum* larvae were counted after 24 hr, *A. tenuis* larvae after 6 days by checking each settlement tile separately under a microscope. Settlers were defined as attached larvae showing minimum initial metamorphosis (flat disk shape).

Data analysis

Settlement preferences were tested using a 2-factor ANOVA with the factors ‘substrate condition’, ‘surface’, and ‘surface nested within substrate condition’. Substrate condition was categorized as mentioned before. Four different surface categories were defined to describe potential settlement spots on the substrata: (1) within the grooves (2.70 ± 0.33 cm^2; mean ± SD) or (2) outside the grooves (4.27 ± 0.13 cm^2; mean ± SD) of horizontal tiles, and (3) within the grooves (6.18 ± 0.18 cm^2; mean ± SD) or (4) outside the grooves (11.13 ± 0.34 cm^2; mean ± SD) of vertical tiles. Settlers located between 2 tiles were categorized as

'outside the grooves' of the tile on which they were attached after separating the tiles. The number of settlers per surface category was transformed into density cm^{-2}. The surface area of each category was determined by measuring 10 tiles of each type with a computer supported digital microscope camera (AxioCam MRc, AxioVision 3.1, Carl Zeiss Vision GmbH Germany). Statistical tests were performed for both species separately. When significance was shown, multiple comparisons of means were carried out to identify differences between groups (Tukey's test). All statistical analyses were carried using SPSS 12.0.

RESULTS

Influence of settlement tiles on water chemistry

Seawater from Oven 1 baked tile incubations showed high concentrations of lithium (10^4 times higher) and certain heavy metals (10 to more than 3 x 10^3 times higher) compared to water with tiles baked in Oven 2 (Table 1). Tiles from Oven 2 still released a considerable amount of chromium into the water compared to the control, whereas elements such as copper, zinc and lead showed lower concentrations. No lithium was detected in the control, whereas the amount of heavy metals was slightly higher compared to common field data (after Adey and Loveland 1998). New tiles baked in Oven 2 caused a quantitative increase of calcium and a decrease of pH and alkalinity (Table 2). After 6 days of incubation water samples, which were taken after a settlement experiment with *Acropora tenuis* including tiles that had been incubated for at least 2 months in seawater, showed a slight decrease in pH and alkalinity, whereas an increase of PO_4^{3-} and NO_3^- was observed (Table 3).

Conc in ppb	Li	Cr	Mn	Co	Ni	Cu	Zn	Cd	Pb
Tiles of oven 1	5203.4	547.8	560.8	13.7	14.7	120.4	517.8	35.3	98.7
Tiles of oven 2	<0.1	20.9	1.4	1.3	8.3	7.3	0.1	0.5	0.2
Control[a]	<0.1	<0.1	1.2	0.9	8.9	16.6	70.1	0.1	0.5
Field[b]	180	0.3	0.2	0.05	1.7	0.5	4.9	0.1	0.03

Table 1. Selection of metal contents in salt water incubated for one week with tiles baked in Oven 1 or 2 (ICP spectrometry). [a] Natural seawater supplied by tank ships. [b] Common values after Adey & Loveland (1998).

	pH	Salinity (in ‰)	Ca (in mg l^{-1})	Alkalinity (in meq l^{-1})
Tiles (52.8g)	6.92	36.1	535.02	0.40
Tiles (26.5g)	7.66	36.2	517.00	1.60
Control (start)	8.15	36.0	469.20	2.74
Control (end)	8.03	36.1	464.90	2.72

Table 2. Water chemistry after one-week incubation of new tiles in 250 ml seawater per treatment.

Settlement behaviour of *Favia fragum* and *Acropora tenuis*

Tiles incubated in the aquarium showed mainly initial growth of green turf algae (Chlorophyta; approx. 30% total surface cover) and crustose coralline algae (Rhodophyta; approx. 10% total surface cover), those maintained under sterile conditions did not show any visible biofilm after incubation.

Table 4 gives an overview of the settlement experiments. Settlement differed between the conditions, and between the surface categories, and showed significant interaction between both factors for *Favia fragum* (2 factor ANOVA, $p \leq 0.001$) and for *Acropora tenuis*

(2 factor ANOVA, $p < 0.001$). The significant interaction between tile condition and surface shows that both factors had a different effect on larval settlement (Table 4).

Overall settlement rates of *Favia fragum* (60.7 ± 13.3 %; mean ± SD) using biologically conditioned tiles were similar to those of alternating non-biologically with biologically conditioned tiles (Tukey's test, $p = 1.0$). In all treatments settlement occurred only on biologically conditioned tiles. Larvae highly preferred to settle in the grooves of pyramid tiles compared to all other surfaces (Tukey's test, $p < 0.001$), whereas no significant differences were detected among the latter ones (Tukey's test, $p > 0.5$). The majority of larvae was negatively buoyant during the experiment.

Acropora tenuis showed with 16.3 ± 3.1 % (mean ± SD) lower total settlement compared to *Favia fragum* (see Table 4). Nevertheless, overall settlement rates were again similar when only using conditioned tiles or when alternating non-conditioned with conditioned tiles (Tukey's test, $p = 0.395$). Settlement occurred only on biologically conditioned tiles, except for 2 settlers attaching to non-conditioned tiles, which showed initial algae growth at the end of the experiment (treatment: alternating non- with conditioned tiles; see Table 4). Larvae highly preferred to settle in the grooves of vertical tiles (Tukey's test, $p < 0.001$) compared to all other surfaces, which showed no settlement differences between each other (Tukeys test, $p > 0.1$). Already from the start of the experiment larvae were swimming near the water surface.

	pH	Salinity (in ‰)	Alkalinity (in meq l^{-1})	NH_4-N (all in mg l^{-1})	NO_2-N	NO_3-N	PO_4-P
BC	8.01	35.8	1.76	0.00	0.006	1.4	0.08
NC	7.92	35.7	2.72	0.00	0.003	1.3	0.15
Start	8.11	35.8	2.80	0.00	0.004	0.7	0.06

Table 3. Water chemistry of settlement experiments with *Acropora tenuis* after 6 days of incubation using biologically conditioned (BC) and non-conditioned tiles (NC) incubated for at least 2 months in seawater prior to the experiment.

DISCUSSION

Surface relief, substrate orientation and the presence of a biofilm were used to control larvae settlement in the highest possible spatial scale. Larvae of both species clearly preferred to settle in grooves of biologically conditioned surfaces. Species-specific larval behaviour resulting in attachment on flat or pyramid tiles further restricted potential settlement surface. Due to the grooved surface structure of the tiles, settlement at edges or between tiles, which might not be beneficial for further handling in aquaculture or certain research purposes, was highly reduced. In conclusion, although settlement rates were comparable (Lewis 1974), or in the case of *A. tenuis* even lower compared with studies, in which normal terracotta tiles or other substrate types were used (Mundy and Babcock 1998, Petersen and Tollrian 2001), the presented design shows the high advantage of the arrangement of tiles and the grooved surface structure. It gives the possibility to spatially control larval settlement within square centimeters. Due to the use of single units (tiles), which can be arranged to create surfaces of any size, this high spatial differentiation of settlement behaviour might principally still function even in large scale applications using thousands of tiles. Settlement in the grooves provided shelter and enabled us to handle tiles without damaging primary polyps. Using temporarily arranged basic units (flat and pyramid tiles) we could separate settlers for further

		Larval settlement on tiles								
		Total	Biologically conditioned				Non-conditioned			
			Flat tiles		Pyramid tiles		Flat tiles		Pyramid tiles	
	Variable		In grooves	Elsewhere	In grooves	Elsewhere	In grooves	Elsewhere	In grooves	Elsewhere
F. fragum	NC	0.0	-	-	-	-	0.00	0.00	0.00	0.00
	BC	61.3 ± 12.4	0.48 ± 0.22	0.02 ± 0.00	0.10 ± 0.07	0.004 ± 0.005	-	-	-	-
	NC/BC	60.0 ± 14.1	1.05 ± 0.44	0.0	0.13 ± 0.04	0.008 ± 0.011	0.0	0.0	0.0	0.0
A. tenuis	NC	0.0	-	-	-	-	0.0	0.0	0.0	0.0
	BC	14.75 ± 1.7	0.02 ± 0.03	0.0	0.19 ± 0.03	0.0	-	-	-	-
	NC/BC	17.8 ± 3.6	0.06 ± 0.04	0.0	0.44 ± 0.06	0.0	0.0	0.0	0.01 ± 0.05	0.0

Table 4 Total larval settlement (mean ± SD %), and density of settlers (mean ± SD cm^{-2}) per surface category (in grooves or elsewhere on flat and on vertical tiles) and tile condition (biologically conditioned, non-conditioned) for *Favia fragum* and *Acropora tenuis* in 3 different treatments: only non-conditioned tiles (NC), only biologically conditioned tiles (BC), and alternating non- with biologically conditioned tiles (NC/BC).

use in research (see chapter 9) and mariculture (chapter 11), which shows another advantage compared to commonly used terracotta tiles. The mean density of settlers per unit, which might be important to control in coral culture, was determined by the amount of biologically conditioned tiles per set up. Light intensity, kept constant at a relatively low level in the present study could be varied (Maida et al. 1995; Mundy and Babcock 1998) or tiles could be prepared with metamorphosis inducers (Morse et al. 1996; Heyward and Negri 1999; Negri et al. 2001; Iwao et al. 2002) to maximize and to spatially control settlement, presumably up to the species level. The design and dimensions of the tiles could be adapted to specific needs in settlement and recruitment studies, or in restoration and mariculture, which might highly differ between each other. Small settlement units are generally more favorable for transferring recruits from mariculture centers to public aquaria, whereas for field applications far more robust tiles might be necessary. The presented tiles are successfully used to supply public aquaria with maricultured coral settlers attaining high post-transport survival rates and recruitment rates (chapter 11 of this thesis). Contrary to simply attaching single tiles to the aquarium decoration using epoxy, the proper attachment of tiles in the field might be more complex (Mundy 2000).

Apart from the selection of appropriate material, the production process of substrata has to be carefully performed to avoid toxic effects. Although clay does not contain potential toxins, we measured high concentrations of heavy metals reaching lethal levels of copper (Esquivel 1983) when tiles were burned in an oven, which had been regularly used to glaze commercial pottery. Regarding the high settlement of *F. fragum*, the incubation of the tiles in the presented re-circulation systems was sufficient to neutralize negative effects of chemicals released by the tiles. Low settlement rates of *Acropora tenuis* could be caused either by sub-optimum settlement conditions or by low fitness of larvae. Sub-optimum conditions could be e.g. the low light intensity used in the present study. Mundy and Babcock (1998) showed significant lower settlement of *A. tenuis* at low light intensities. This could also explain the preference for the vertical tiles as larvae spent more time at the water surface due to positive photo-taxis (see also Mundy and Babcock 1998). Furthermore, larvae fitness may differ between spawning seasons (Hatta personal communication). We achieved settlement rates up to 72 % in similar experiments with *A. tenuis* carried out 1 year later, which might indicate fitness differences between both seasons (unpublished data). It is difficult to estimate whether the shown increase of nitrogen and phosphorous concentrations and the decrease of alkalinity during the relatively long incubation period (6 days) in a water volume of 250 ml reduced settlement, since influences of these factors on larval settlement are hardly known yet.

Regarding mariculture and restoration purposes the design of settlement tiles will be increasingly important. Besides aspects such as high survival and growth rates of juveniles, the control of settlement, handling and the possibility to automatize processes will be crucial to produce large numbers of coral recruits in an economic way.

REFERENCES

Adey WH, Loveland K (1998) Dynamic aquaria: building living ecosystems. Academic Press, London

Babcock RC, Davies P (1991) Effects of sedimentation on settlement of *Acropora millepora*. Coral Reefs 9: 205-208

Babcock RC, Mundy C (1996) Coral recruitment: consequences of settlement choice for early growth and survivorship in two scleractinians. J Exp Mar Biol Ecol 206: 179-201

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. Res Ecol 9(4): 1-11

Esquivel I (1983) Short term copper bioessay on planula of the reef coral *Pocillopora damicornis*. In: Jokiel PL, Richmond RH, Rogers RA (eds) Coral reef population biology. Hawaii Insitute of Marine Biology Technical Report No. 37, pp 465-472

Harrison PL, Wallace CC (1990) Reproduction, dispersal and recruitment of scleractinian corals. In: Dubinsky Z (ed) Ecosystems of the world vol 25, coral reefs. Elvesier, Amsterdam, pp 133-207

Harriott VJ, Fisk DA (1987) A comparison of settlement plate types for experiments on the recruitment of scleractinian corals. Mar Ecol Prog Ser 37: 201-108

Heyward AJ, Negri AP (1999) Natural inducers for coral larval metamorphosis. Coral Reefs 18: 273-279

Hodgson G (1990) Sediment and the settlement of larvae of the reef coral *Pocillopora damicornis*. Coral Reefs 9: 41-43

Holleman AF, Wiberg N (1985) Lehrbuch der anorganischen Chemie, 91st - 100th edn. de Gruyter, Berlin, New York, p 1451

Hunte W, Wittenberg M (1992) Effects of eutrophication and sediment on juvenile corals. Mar Biol 114: 625-631

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

Lewis BL. 1974. The settlement behaviour of planulae larvae of the hermatypic coral *Favia fragum* (Esper). J Exp Mar Biol Ecol 15:165-172

Maida M, Sammarco PW, Coll JC (1995) Effects of soft corals on scleractinian coral recruitment. I: Directional allelopathy and inhibition of settlement. Mar Ecol Prog Ser 121: 191-202

Morse ANC, Iwao K, Baba M, Shimoike K, Hayashibara T, Omori M. 1996. An ancient chemosensory mechanism brings new life to coral reefs. Biol Bul 191:149-154

Moorsel Van GWNM (1988) Early maximum growth of stony corals (Scleractinia) after settlement on artificial substrata on a Caribbean reef. Mar Ecol Prog Ser 50: 127-135

Mundy CN (2000) An appraisal of methods used in coral recruitment studies. Coral Reefs 19: 124-131

Mundy CN, Babcock RC (1998) Role of light intensity and spectral quality in coral settlement: Implications for depth-dependent settlement. J Exp Mar Biol Ecol 223: 235-255

Negri AP, Webster NS, Hill RT, Heyward AJ (2001) Metamorphosis of broadcast spawning corals in response to bacteria isolated from crustose algae. Mar Ecol Prog Ser 223: 121-131

Oren U, Benayahu Y (1997) Transplantation of juvenile corals: a new approach for enhancing colonization of artificial reefs. Mar Biol 127: 499-505

Petersen D, Tollrian R (2001) Methods to enhance sexual recruitment for restoration of damaged reefs. Bull Mar Sci 69(2): 989-1000

Soong K, Chen M, Dai CCC, Fan T, Fan JLH (2003) Spatial and temporal variation of coral recruitment in Taiwan. Coral Reefs 22: 224-228

Tomascik T (1991) Settlement patterns of Caribbean scleractinian corals on artificial substrata along a eutrophication gradient, Barbados, West Indies. Mar Ecol Prog Ser 77: 261-269

Wilson JR, Harrison PL (1998) Settlement-competency periods of larvae of three species of scleractinian corals. Mar Biol 131: 339-345

CHAPTER

6

Planulation in a Captive Raised Population of *Favia fragum*

INTRODUCTION

From November 2001 until January 2002, thirty adult colonies of *Favia fragum* previously collected in Curaçao, Netherlands Antilles, were maintained in a 30 m^3 closed system exhibit aquarium at Rotterdam Zoo, The Netherlands. In February 2002, I observed > 100 sexual recruits situated on the artificial rockwork of the exhibit. With a mean growth rate of 0.16 cm $month^{-1}$, recruits rapidly established an F1 population of approx. 25 colonies m^{-2} (see chapter 10). Approx. 12 months after recruitment was firstly observed, specimens reached maturity and started developing an F2 generation.

From February until May 2004, I daily collected planulae from 20 colonies of the F1 generation to study the planulation behaviour of captive raised specimens.

METHODS

Two months prior to the experiment, 20 colonies of *Favia fragum* (diameter 3.85 ± 0.42 cm; mean ± SD) were transplanted on the end of a PVC pipe (d = 50 mm, h = 50 mm) using epoxy cement. Molds made from polyurethane casting resin (UR 5859, Axson North America) were placed on a polyethylene grid in the same 30-m^3 aquarium. The shape of the molds allowed placing the PVC pipes in the center and temporarily fixing a cylinder (d = 100 mm, h = 200 mm) made from plankton mesh (60 μm) around the colony. The shape of the mesh was supported by PVC rings, which guaranteed a stable connection between the mold and the cylinder to avoid any larvae from escaping. The top of the cylinders was located above the water surface; therefore larvae could easily be collected from the surface. Mesh cylinders were placed on the molds from the late afternoon till the next morning when larvae were collected using a plastic pipette. Following Van Moorsel (1983) planulation was measured in released larvae per square unit living tissue area. In order to estimate the influence of the sampling method on the general condition of the colonies, I measured their growth as a proxy for fitness by monthly taking a digital picture and then calculating the surface area of live tissue with a computer supported microscopy camera (AxioCam MRc, AxioVision 3.1, Carl Zeiss Vision GmbH Germany).

RESULTS AND DISCUSSION

All colonies planulated during the sampling period. Larvae were mostly released around 2 hr after switching off the lights, which I could observe using a torch with red light of low intensity (quantum flux < 10 μmol m^{-2} s^{-1}). However, I assume that larval release probably occurred all night long since larvae were still released 3 hr after artificial sunset when I stopped monitoring. Larvae [0.171 ± 0.06 mm^3; mean ± SD; calculated after Van Moorsel (1983)] were significantly bigger than those of colonies previously collected in the field (0.117± 0.071 mm^3; mean ± SD) (t-test, $t = 7.969$, $n = 341$, $p < 0.001$).

Planula release showed periodicity and peaked between Day 10 and Day 13 after new moon (= ANM) (Fig. 1). Similar tendencies were observed within the same population at Curaçao [March-May 2004: peak between Day 8 and Day 12 ANM; n = 20 (unpublished data)]. Contrary to field specimens, a few larvae were released by the aquarium colonies in between the cycles. Our results confirm Szmant-Froehlich et al. (1985), who observed *F. fragum* planulating in a similar lunar cycle in Puerto Rico (peak 9-11 days ANM).

In the present study, larval production decreased over time from a maximum of 6.9 cm^{-2} $week^{-2}$ in March to a max. of 2.8 cm^{-2} $week^{-2}$ in May (Fig. 2) possibly due to several

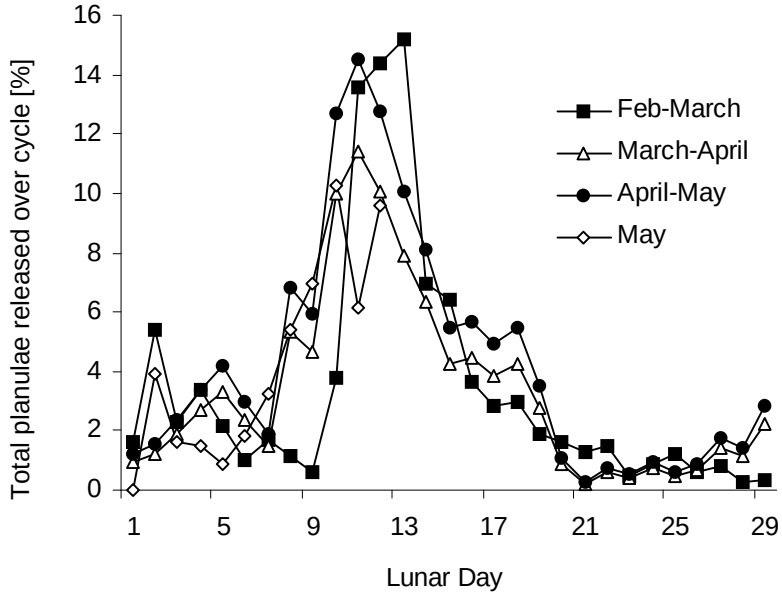

Fig. 1 Planulation of a captive F1 generation of *Favia fragum* without natural triggers such as moonlight (n = 20).

possible reasons: (1) stress and/or (2) reduction of sperm transfer caused by sampling method, (3) influence by environmental conditions. I cannot exclude that the applied method caused some stress, however, sampled colonies (growth: 0.47 ± 0.12 cm^2 $month^{-1}$; mean ± SD; n = 20) did not show significantly reduced growth compared to 2 control groups, which were fixed on PVC pipes and molds, and incubated (1) with plankton nets and not sampled (0.37 ± 0.20 cm^2 $month^{-1}$; mean ± SD; n = 10), (2) without nets (0.68 ± 0.36 cm^2 $month^{-1}$; mean ± SD; n = 10) (Kruskal Wallis ANOVA, H = 5.443, p = 0.066). Little is known about spawning in brooding species (Harrison and Wallace 1990). I did not observe any sperm release.

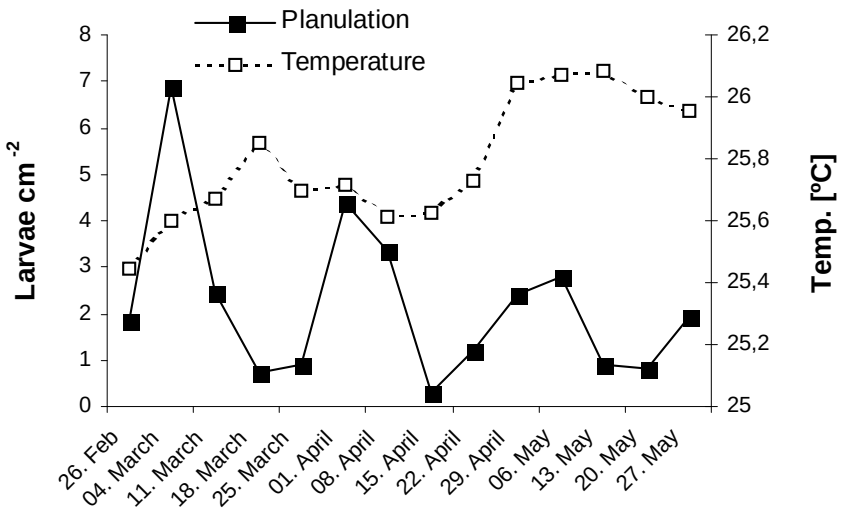

Fig. 2 *Favia fragum*. Mean planulation cm^{-2} living tissue area wk^{-1} (n = 20) in relation to mean temperature wk^{-1}.

Since field populations of *F. fragum* may show high rates of self-fertilization (Brazeau et al. 1998), genetic investigation would be necessary to estimate the importance of successful sperm transfer in the present population, which might have been reduced due to the application of mesh cylinders during the night, when spawning probably occurs (Harrison and Wallace 1990). Increasing temperatures, which can increase planulation success (Van Moorsel 1983), obviously did not stimulate planulation (Fig. 2); I observed an overall temperature increase from 25.4 ºC in February to 26.1 ºC in May. However, regarding short-term temperature changes as a possible stimulus for planulation (Harrison and Wallace 1990),

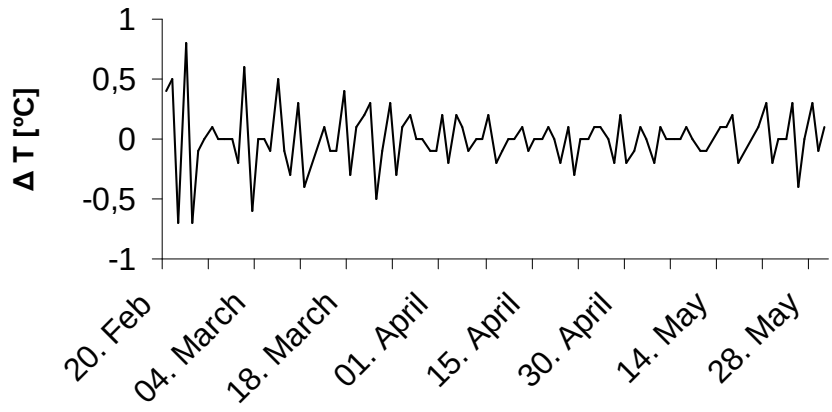

Fig. 3 Temperature fluctuation (between consecutive days) during the sampling period.

fluctuations on a daily basis decreased over time in the present study (Fig. 3). Longer sampling periods under different temperature regimes would be necessary to better understand possible controls.

It remains a miracle how captive raised specimens of *Favia fragum* could time planulation similar to field colonies when never being exposed to natural triggers such as moonlight. Due to the removal of field-collected adults at a time when juveniles were still in an early pre-recruitment stage, parental influence (e.g. by sperm transfer) can be excluded. No additional specimens were present in the tank at any time. My observations contradict with previous observations such as those of Jokiel et al. (1985), who showed the loss of lunar periodicity in *Pocillopora damicornis*, when field collected specimens were maintained under constant new moon and full moon, respectively. In the present study, constant nocturnal lighting from surrounding building safety installations partly illuminated the tank (quantum flux: 0.217 ± 0.106 μmol m^{-2} s^{-1}), which was 20 times more intensive than natural full moon (Jokiel et al. 1985). I conclude that reproduction in *F. fragum* might be at least partly endogenously controlled, which so far has only been shown in the temperate scleractinian *Balanophyllia elegans* (Beauchamp 1993).

REFERENCES

Beauchamp KA (1993) Gametogenesis, brooding and planulation in laboratory populations of a temperate scleractinian coral *Balanophyllia elegans* maintained under contrasting photoperiod regimes. Invert Reprod Develop 23(2-3):171-182.

Brazeau DA, Gleason DF, Morgan ME (1998) Self-fertilization in brooding hermaphroditic Caribbean corals: evidence from molecular markers. J Exp Mar Biol Ecol 231:225-238

Jokiel PL, Ito RY, Liu PM (1985) Night irradiance and synchronization of lunar release of planula larvae in the reef coral *Pocillopora damicornis*. Mar Biol 88:167-174.

Szmant-Froehlich A, Reutter M, Riggs L (1985) Sexual reproduction of *Favia fragum* (Esper): lunar patterns of gametogenesis, embryogenesis and planulation in Puerto Rico. Bull Mar Sci 37(3):880-892.

CHAPTER

7

Pre-planular External Development in the Brooding Coral *Agaricia humilis*

INTRODUCTION

In the diverse reproductive ecology of corals (see chapter 1) unusual patterns are known like asexual production of planulae (Stoddart 1983), intra-polypal self-fertilization (Brazeau et al. 1998), bud formation (Boschma 1936, Hoeksema 1989), reversible metamorphosis (Richmond 1985) or polyp bail-out (Sammarco 1982). At least the latter three have been shown to be a direct response to environmental stress. The reproductive group of the brooders has been connected so far generally to the release of propagules in the planula stage (Harrison & Wallace 1990; Richmond 1997). In this chapter, I show for the first time the extra-polypal development of premature released propagules (embryos) in the brooding species *Agaricia humilis*. The term embryo has been defined by Harrison and Wallace (1990) for life stages between "… the early development of fertilized eggs up until the stage where the epidermis begins to differentiate and cilia form, at which stage the developing propagule is termed a planula." Fadlallah (1983) proposed to include all free-living stages of coral larval ontogeny up to settlement in the term planula. In accordance to Harrison & Wallace (1990), I define a planula as a ciliated propagule, which is capable of active movement (swimming or crawling).

The Caribbean coral *Agaricia humilis* is common in shallow reef areas (Van Moorsel 1983, Szmant 1986). The life history of *A. humilis* has adapted to persist in this high-disturbance environment by evolving reproductive characteristics such as early maturity, year-round reproduction and an overall high reproductive output (Van Moorsel 1983).

MATERIAL AND METHODS

In order to test an *in situ* method to collect larvae of *Agaricia humilis* for research and aquaculture purposes (for ex situ collection method see chapter 8), 19 colonies (max. diameter 45 ± 0.7 mm, mean ± SD) were collected using hammer and chisel at the fringing reef of Curaçao. Colonies were brought to shore in separate plastic bags in a transport container and transferred into a 50-liter container with seawater. PVC-pipes (length 50 mm) were filled with Portland cement (Hydraulic Water Stop Cement, Quikrete Companies USA) and colonies were mounted at the end. The colonies were transferred back to the reef within 30 min, and fixed to a grid located at the collection site at a depth of 7 m. Two days later cone-shaped nets (mesh size 60µm) with a sampling tube at the top were deployed above each colony to collect larvae every morning for the next eight days. To ensure quantitative sampling, the bottom of each unit was closed with a disc-shaped plexi glass-plate (Fig. 1). The number of released propagules per colony was monitored for 8 days. Data were normalized per cm^2 colony surface. Surface area was determined from a digital image of each colony, followed by a computer-supported measurement of the surface covered by live tissue (AxioVision 3.1, Carl Zeiss Vision GmbH Germany). Propagule sizes are estimates based on photography. Propagules were kept in 100 ml plastic cups in the laboratory; settlement competence was tested with ceramic tiles that were incubated in aquaria for at least two months prior to the experiments to develop a biofilm including crustose coralline algae.

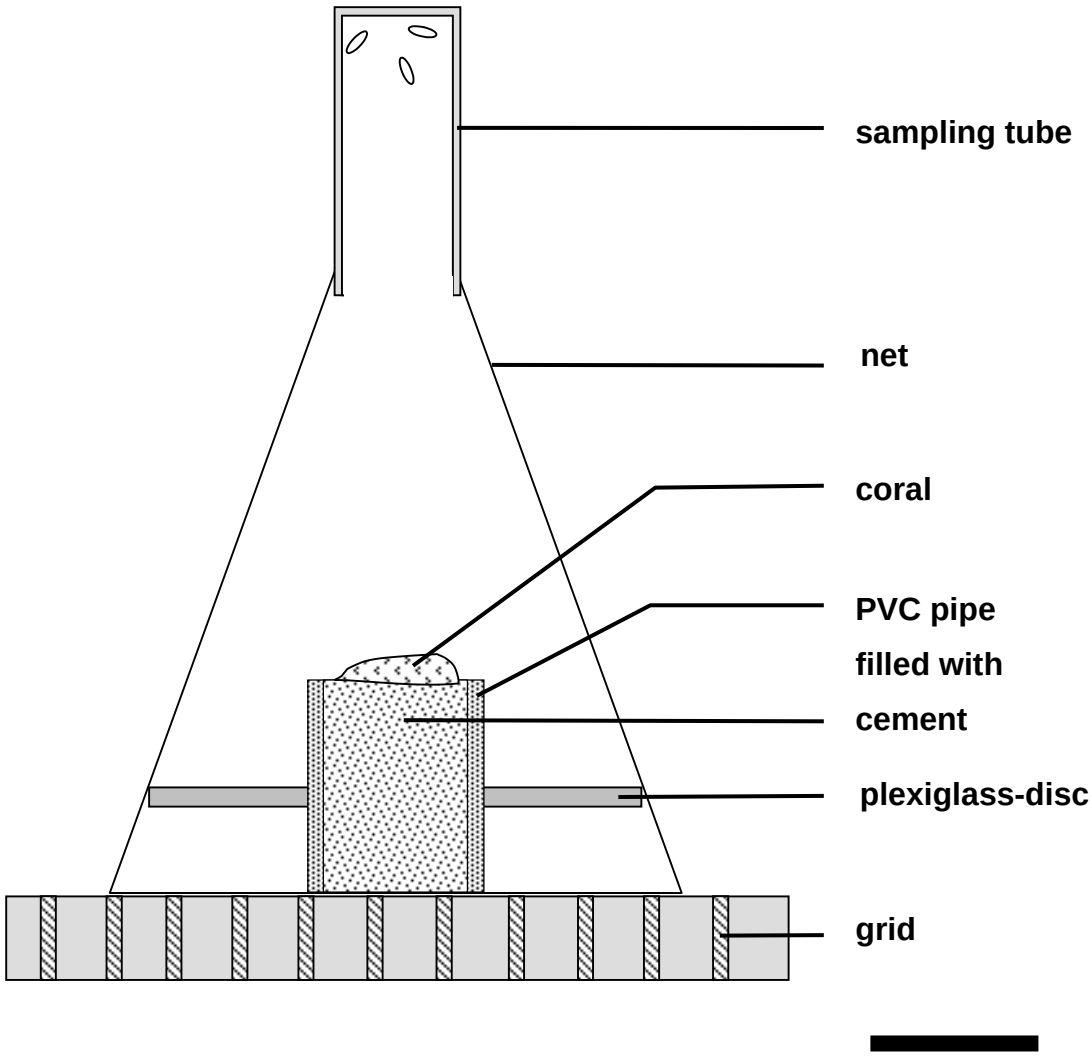

Fig. 1 In situ collection of propagules using a cone-shaped 60 µm net with a sampling tube (cross section). *Agaricia humilis* colonies were mounted on PVC pipes using Portland cement. Scale bar: 5 cm.

RESULTS

In the first three days of the sampling period, all colonies released spheroid propagules (diameter approx. 0.35 mm, volume approx. 0.02 mm^3; for calculation see Van Moorsel 1983). These pre-planulae did not show an outer epidermis yet, lacked ciliate activity and drifted passively at the water surface (Fig. 2A). In agreement with most brooding corals, the propagules had acquired zooxanthellae from the parent colonies. Release peaked at the second day of the sampling period with a mean of 8.02 ± 7.60 (mean ± SD) of propagules per cm^2 colony surface. A total of 9.29 ± 7.86 propagules cm^{-2} (mean ± SD; n = 19; see Fig. 3) was released in eight days.

Between 36 to 60 hr after release, the embryos developed into small planulae with a length of ~ 0.5 mm and a similar volume as the stage at release (Fig. 2B). Most of these small planulae settled in the following 24 hr near the water surface on the walls of the plastic cups whether or not tiles with a biofilm were present. No settlement was observed on the tiles, but due to the small size of primary polyps our ability to detect them on the tiles was limited.

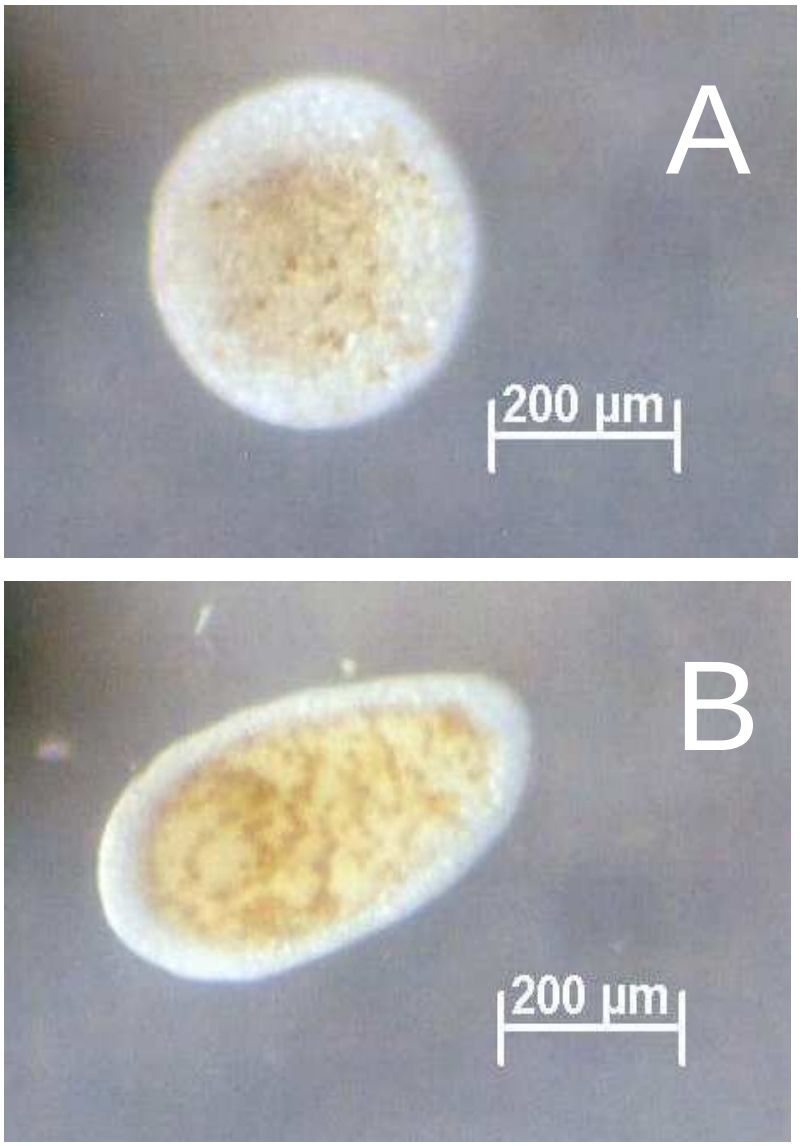

Fig. 2. Morphology of *Agaricia humilis* propagules. (A) Pre-planular stage showing no outer epidermis at time of release. (B) Actively swimming planula after approx. 36 hr of external development.

During the eight-day monitoring period, 65 % of the 19 colonies also released some fully developed planulae (length ~ 1 mm, total 0.27 ± 0.39 larvae cm^{-2} $(8\ days)^{-1}$; mean ± SD; n = 19, see Fig. 3). More than 90 % of these planulae settled within the first 36 hr after release, exclusively on biologically conditioned tiles (for comparison see chapter 8).

DISCUSSION

I assume that the pre-planulae were in a late embryogenetic stage (Harrison & Wallace 1990). A comparison of the volume of these propagules with the size range of planulae from 35 *Agaricia humilis* colonies collected at the reef of Curaçao in 1980 and 1981 (Fig. 4) demonstrates the small size: the average volume of all larvae was 0.167 mm^3, and the smallest average volume for a colony was 0.041 mm^3, almost twice the volume of the premature larvae in this study. The volumes of the embryos and resulting planulae were comparable to planula volumes of *Madracis* species (*M. decactis* & *M. pharensis*: down to 0.021 mm^3), which are the smallest known for scleractinian corals (Vermeij et al. 2003).

The amount of released pre-planulae is an order of magnitude higher compared to the average of 0.86 planulae cm^{-2} wk^{-1} found in *Agaricia humilis* by Van Moorsel (1983). My

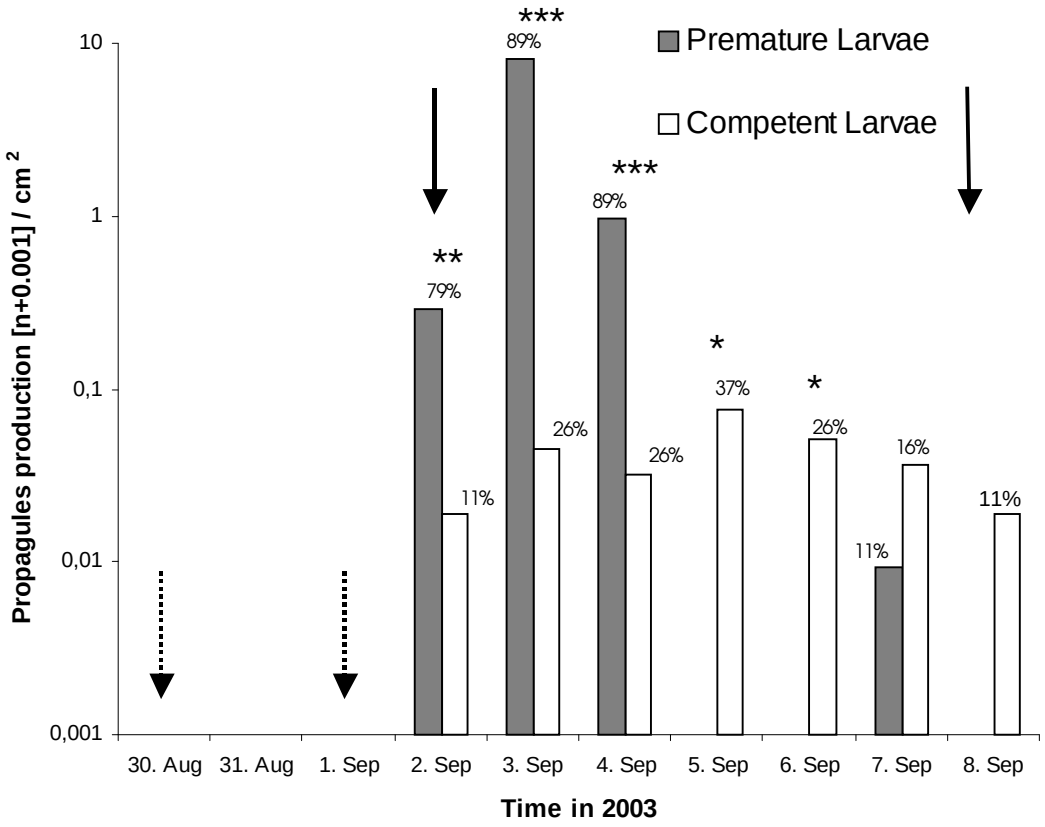

Fig. 3. Release of premature and settlement-competent planulae in *Agaricia humilis* per cm^2 colony surface after deployment. Note logarithmic scale. Above bars: percentage of colonies releasing propagules (n = 19). Arrows at 30 Aug and 1 Sep 2003 mark day of colony preparation and start of net deployment, other arrows indicate sampling period. Significant differences in the fraction of corals releasing pre-mature and settlement-competent planuale are indicated with * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$ (Wilcoxon test).

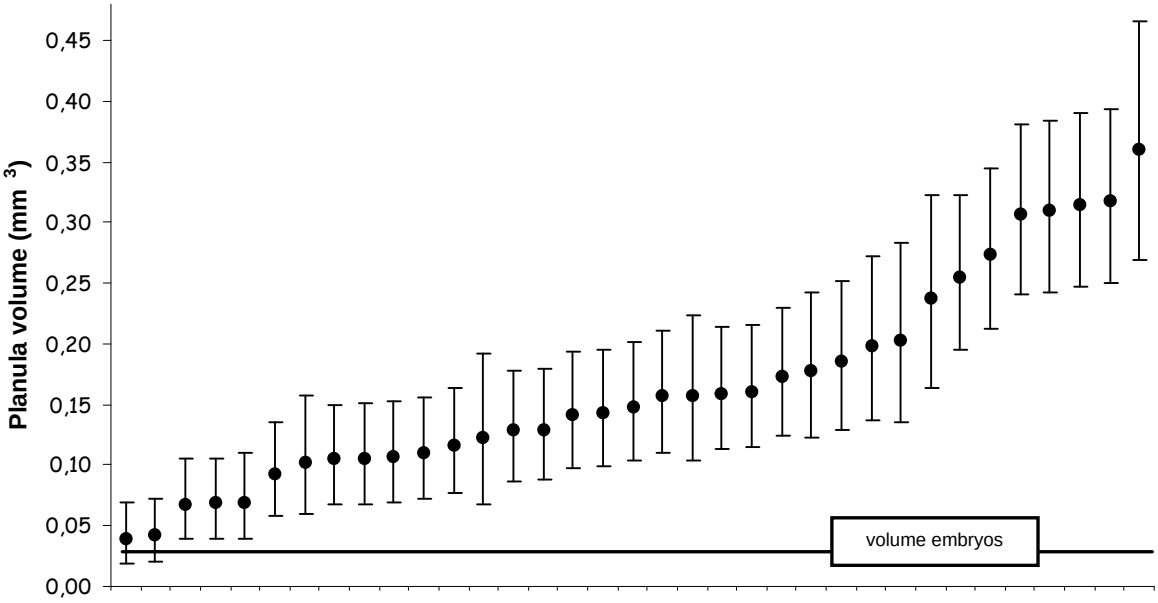

Fig. 4. *Agaricia humilis*. Volume of embryos found during the present study (0,022 mm^3) compared to the volume of 319 planulae from 35 *A. humilis* colonies (each with 95 % conf. interval) collected in Curaçao from 1980-1981. The 95 % conf. intervals were calculated after root-transformation of planula volumes.

observations further show that coralline algae on the tiles were not ultimately necessary for settlement (some of these algal species are known to induce settlement in *A. humilis*; Morse et al. 1994, Raimondi and Morse 2000). The catholic settlement of the small planulae may indicate a choice for a less optimal substrate due to a low survival chance. Isomura and Nishihira (2001) demonstrated a positive relation between planular size and survival in several brooding species.

I assume that during hydration process, lime (dissolved as Ca^{2+} and CO_3^{2-}) released by Portland cement caused stress in the colonies by increasing the pH of the surrounding seawater. A reduced water flow around the colony due to collection nets may have enhanced the effect. Nevertheless, the technique to transplant corals using Portland cement has been applied previously without any visible negative effects on coral colonies even when freshly transplanted colonies were kept in a low water volume for more than 30 hr (chapter 3). All 19 colonies did not show signs of deterioration during the 8-day monitoring period. Six months later, the same specimens were sampled again for a period of two months. By then they only released normal-developed planulae (unpublished data).

Planula release as a reaction to stress has been documented before in scleractinians (Edmondson 1929, Fadlallah 1983, Rinkevich & Loya 1987) and other corals (Henry et al. 2003), but details on larval development are usually not provided. Only Harii et al. (2001) mention the copious release of premature larvae in one colony of *Alveopora japonica*, which was cut in half. These larvae reached settlement competency within 120 days. For the parent colony it may be a reaction to dispose of resource-costly propagules.

In addition, my observations demonstrate that in the brooder *Agaricia humilis* the aborted embryos are able to develop externally into viable planula larvae. Due to external development of these passively floating propagules in the water column, settlement is delayed for at least 2-5 days compared to normal planulae known to be settlement competent immediately after release. This has consequences for dispersal. External development of propagules increases the risk of predation during a longer planktonic stage, but it may be an adaptation to escape from the stress location. As such it may represent an ultimate attempt to maintain instead of taking a risk of colony mortality together with undeveloped planulae. It corroborates the opportunistic life history strategy of *A. humilis*, which enables this species to persist in an unpredictable environment. The reported phenomenon, successful external development of embryos in a species known as brooder, adds another mode to the broad spectrum of coral reproduction already known.

REFERENCES

Boschma H (1936) Koralen. Voordracht Diligentia ‘s-Gravenhage, p 22-33

Brazeau DA, Gleason DF, Morgan ME (1998) Self-fertilization in brooding hermaphroditic Caribbean corals: evidence from molecular markers. J Exp Mar Biol Ecol 231:225-238

Edmondson CH (1929) Growth of Hawaiian corals. Bernice P. Bishop Mus Bull 58:1-38

Fadlallah YH (1983) Sexual reproduction, development and larval biology in scleractinian corals. A review. Coral Reefs 2:129-150

Harii S, Omori M, Yamakawa H, Koike Y (2001) Sexual reproduction and larval settlement of the zooxanthellate coral *Alveopora japonica* Eguchi at high latitudes. Coral Reefs 20:19-23

Harrison PL, Wallace CC (1990) Reproduction, dispersal and recruitment of scleractinian corals. In: Dubinsky Z (ed) Ecosystems of the world vol 25, coral reefs. Elvesier, Amsterdam, p 133-207

Henry LA Kenchington ELR, Silvaggio A (2003) Effects of mechanical experimental disturbance on aspects of colony responses, reproduction, and regeneration in the cold-water octocoral *Gersemia rubiformis.* Canadian Journal of Zoology 81:1691-1701

Hoeksema BW (1989) Taxonomy, phylogeny and biogeography of mushroom corals (Scleractinia: Fungiidae). Zool Verh Leiden 254:1-295

Isomura N, Nishihira M (2001) Size variation and its effect on the lifetime of planulae in three pocilloporid corals. Coral Reefs 20:309-315

Morse DE, Morse ANC, Raimondi T, Hooker N (1994) Morphogen-based chemical flypaper for *Agaricia humilis* coral larvae. Biol Bull 186:172-181

Raimondi PT, Morse ANC (2000) The consequences of complex larval behaviour in a coral. Ecology 81(11):3193-3211

Richmond RH (1985) Reversible metamorphosis in coral planulae larvae. Mar Ecol Prog Ser 22:181-185

Richmond RH (1997) Reproduction and recruitment in corals: critical link in the persistence of reefs. In: Birkeland (ed) Life and death of coral reefs, Chapman & Hall, N.Y, p 175-197

Sammarco PW (1982) Polyp bail-out: An escape response to environmental stress and a new means of reproduction in corals. Mar Ecol Prog Ser 10:57-65

Stoddart JA (1983) Asexual production of planulae in the coral *Pocillopora damicornis*. Mar Biol 76:279-284

Szmant AM (1986) Reproductive ecology of Caribbean reef corals. Coral Reefs 5:43-54.

Van Moorsel GWNM (1983) Reproductive strategies in two closely related stony corals (*Agaricia*, Scleractinia). Mar Ecol Prog Ser 13:273-283

Vermeij MJA, Sampayo E, Bröker K, Bak RPM (2003) Variation in planulae release of closely related coral species. Mar Ecol Prog Ser 247:75-84

CHAPTER

8

Spatial and temporal variation in larval settlement of reefbuilding corals in mariculture

INTRODUCTION

The production of large amounts of sexual recruits requires appropriate techniques to achieve high settlement rates. In mariculture, factors negatively influencing settlement such as competition by sessile animals and predation by corallivors can be effectively excluded, whereas other crucial factors such as competition by algae and biological settlement inducers might play a major role. However, there is currently no literature available on the influence of the biological composition of substrates on coral settlement in mariculture and aquaculture.

I investigated the influence of substrate condition on the settlement rate of two brooding coral species. In a pre-study I noticed temporal variation in settlement. For a better understanding of the importance of this phenomenon in coral mariculture, I included it in this study.

METHODS

Collection of planulae

Larvae of 20 colonies of the Caribbean reefbuilding corals *Agaricia humilis* (diameter 4.14 ± 0.58 cm; mean ± SD) and *Favia fragum* (diameter 3.85 ± 0.42 cm; mean ± SD) were daily collected for 3 months in the morning following the protocol of chapter 6. Each colony was fixed to a device using plankton mesh cylinder to allow water exchange and to collect planulae colony-specifically. The basis of each collection device consisted of a mold (polyurethane casting resin), which developed a biofilm. To exclude the growth of filamentous algae, this biofilm was daily maintained (directly after larvae collection) by cleaning the mold with a brush. Due to the arrangement of the collection devices near the water surface (open top of mesh cylinder was not submerged), larvae could be easily collected using a plastic pipette without disturbing the colonies. Larvae of both species were generally released within 2 hr after sunset (chapter 6).

Incubation of settlement tiles

I used two different types of substrate tiles, which were arranged chessboard-like on polystyrene grids (see chapter 5) and incubated for 3 months under different light conditions with and without grazers to develop a biofilm.

Tiles were located 10 cm below the water surface under (1) 250 W HQI 6,000 K (= 6 K; daylight spectrum) with a quantum flux of 420.3 ± 32.4 µmol m^{-2} s^{-1} (mean ± SD) and under (2) 250 W HQI 20,000 K (= 20 K; blue light spectrum) with a quantum flux of 303.8 ± 25.7 µmol m^{-2} s^{-1} (mean ± SD), respectively. Half of the tiles under each light condition were maintained (3) without any grazers, the other half of the tiles were (4) grazed by herbivorous hermit crabs (*Paguristes* spp.) in a density of 100 specimens m^{-2}. Pre studies using sea urchins (*Echinometra lucunter*) to control algae growth during tile conditioning showed patchiness of highly grazed spots and non-grazed spots within one treatment.

All tiles were incubated in a 10 m^3 closed re-circulation system designed similarly to the system (5,000 L volume) described in chapter 9. The system was started 12 months prior to the experiment by introducing approx. 200 kg of Live Rock (coral reef substrate containing all its associated organisms; here: aquacultured in Florida, USA, and field-collected at Curaçao, Netherlands Antilles).

The water quality of the system was weekly checked using potentiometric titration (Titro Line easy, Schott GmbH, Germany), galvanomerty (Oxi 330i, WTW GmbH, Germany) and photo spectrometry (DR/4000U Photospectrometer, HACH Company U.S.A.): Temp. 25,5 ºC, dissolved oxygen 103.0 %, pH 8.26, salinity 36.0 ‰, alkalinity 3.37 meq l^{-1}, NH3-N

0,004 mg l^{-1}, NO2-N 0.005 mg l^{-1}, NO3-N 4.1 mg l^{-1}, Ca^{2+} 431.7 mg l^{-1}, Mg^{2+} 1307.5 mg l^{-1}, PO4-P 0.013 mg l^{-1}. These values are within the range of those in the field, however, nitrate showed slightly higher concentrations (see Sorokin, 1995; Adey and Loveland, 1998).

Temporal settlement

In pre studies, I observed that a reasonable number of larvae of *Favia fragum* already settled on the molds of the larval collection device already during the night of planulation. Since this phenomenon may significantly reduce the number of available propagules for the production of sexual recruits, I quantified the number of settlers 12 hr after release. Each colony of both species was monitored over the entire collection period. Settlers located on the biologically conditioned molds were immediately removed from the system after being recorded. Remaining larvae were pooled and used for spatial settlement experiments.

Spatial settlement

Twenty tiles of each type (flat and pyramid) were placed chessboard-like in a polystyrene grid, which prior to the experiment was fixed on the bottom of a 1.5 L polystyrene container with silicon. One grid giving space for 40 tiles fits exactly in the plastic container with margins of <5 mm around filled with silicon. Prepared containers were incubated in seawater with daily 100 % water exchange at room temperature until the pH was not reduced any longer by acid released by freshly applied silicon (~ 7 days). After placing the tiles, containers were carefully filled with 1.2 L seawater (36 ‰) to avoid any changing of the algal distribution among the tiles. Finally 100 larvae were added per treatment (4 replicates) and incubated at 26 ˚C for 24 h at room light (60 μmol m^{-2} s^{-1}).

Data acquisition and analysis

Each tile was checked separately under the microscope for settled larvae. I define settlers as attached larvae in a flattened disc shape showing initial metamorphosis (see Harrison and Wallace, 1990). Regarding potential settlement locations on the tiles, 4 surface categories were defined following chapter 5: (1) within the grooves (2.70 ± 0.33 cm^2; mean ± SD) or (2) outside the grooves (4.27 ± 0.13 cm^2; mean ± SD) of horizontal tiles, and (3) within the grooves (6.18 ± 0.18 cm^2; mean ± SD) or (4) outside the grooves (11.13 ± 0.34 cm^2; mean ± SD) of vertical tiles. Settlement data were root-transformed and tested with a 2-factor ANOVA with the factors “surface category” (see above) and “ tile incubation”: (1) 6 K light with grazing, (2) 6 K light without grazing, (3) 20 K light with grazing, and (4) 20 K without grazing.

From 4 tiles per treatment (randomly chosen), digital microscopic pictures were taken (AxioCam MRc, Carl Zeiss Vision GmbH Germany), which were used to identify algal groups and to measure their surface cover for each surface category (AxioVision 3.1, Carl Zeiss Vision GmbH Germany). In order to identify the influence of the 3-month incubation period of the tiles on the developing biofilm, data were root-transformed, and tested with a 2-factor ANOVA using the factors defined above. Analyses were conducted separately and only for the 4 most dominant algal groups.

Whenever analysis of variance indicated significant differences, a multiple comparison of means was conducted using Tukey’s test. Each coral species was separately analyzed (SPSS 12.0).

RESULTS

Temporal settlement

A total of 5,801 larvae were released in *Agaricia humilis* (18 colonies planulated) of which 14.5 % (from 5 colonies) settled within 12 hr after release before larval collection occurred. The relative number of settled larvae differed significantly between the colonies (Kruskal-Wallis ANOVA, $H = 373.62$, $p < 0.001$) with one colony showing a settlement rate of 52.6 % under optimum conditions within the first 12 hr. Over the entire period, the same colony released almost one third of the total number of larvae whilst others released < 10 (214.8 ± 336.9 larvae colony^{-1}; mean ± SD). Under optimum settlement conditions, ~ 40 % of the overall collected larvae settled 12-36 hr after being released. 36 hr and later, < 10 % of the remaining larvae settled.

Favia fragum released a total of 4,020 larvae (all colonies planulated; 134.0 ± 86.3 larvae colony^{-1}; mean ± SD) of which 42.1 % settled under optimum conditions within 12 hr after planulation. All colonies released larvae that settled in the night of planulation, however, the relative number of settlers per colony differed significantly (Kruskal-Wallis ANOVA, $H = 244.34$, $p < 0.001$). Approximately 55 % of the collected larvae settled 12-36 hr after planulation. Less than 10 % of the remaining larvae settled later than 36 hr after being released.

		Larval settlement on tiles				
			Flat tiles		Pyramid tiles	
		Total	In grooves	On ridges	In grooves	On ridges
	Variable	Mean±SD %	Mean±SD cm^{-2}			
A. humilis	DL/+G	17.5±8.2	0.060±0.046	0.003±0.006	0.113±0.055	0.000
	DL/-G	0.5±1.0	0.000	0.000	0.004±0.008	0.000
	BL/+G	40.5±13.2	0.268±0.058	0.000	0.211±0.085	0.000
	BL/-G	7.8±3.8	0.000	0.000	0.063±0.031	0.000
F. fragum	DL/+G	57.0±4.1	0.504±0.070	0.021±0.011	0.223±0.025	0.002±0.004
	DL/-G	5.5±2.6	0.014±0.018	0.006±0.007	0.032±0.007	0.001±0.002
	BL/+G	43.0±6.1	0.435±0.076	0.006±0.007	0.148±0.049	0.003±0.004
	BL/-G	14.0±4.9	0.079±0.071	0.011±0.010	0.068±0.027	0.002±0.004

Table 1 Larval settlement in *Agaricia humilis* and *Favia fragum* on differently incubated tiles. DL = "daylight", BL = blue light, +G = grazed, -G = non-grazed.

Spatial settlement

Agaricia humilis showed lower total settlement (mean 16.6 %; n = 1600) compared to *Favia fragum* (mean 29.9 %; n = 1600) (Table 1). Settlement highly differed between surface categories and differently incubated tiles, and showed significant interaction between both factors for *Agaricia humilis* and for *Favia fragum* (Table 2), which indicate that the non-biotic surface structure of the tiles had a different influence on settlement success than their biotic structure (Fig. 1 and 2). Multiple comparisons showed significant differences in settlement in *Agaricia humilis* between all tile incubations (Tukey's test, $p \leq 0.004$) with overall highest settlement on tiles previously incubated with grazers under blue light (87.5 % of all settlers; see Table 1). Lowest settlement was recorded on non-grazed tiles under daylight (Fig. 1). 99,6% of settlers

	df	F	p
A. humilis			
Incubation of tiles	3	77.385	<0.001***
Surface	3	134.001	<0.001***
Surface*Incubation of Tiles	9	31.996	<0.001***
F. fragum			
Incubation of tiles	3	55.117	<0.001***
Surface	3	150.747	<0.001***
Surface*Incubation of Tiles	9	19.858	<0.001***

Table 2 Influence of differently incubated tiles (light spectrum, grazing) and tile surfaces (horizontal/vertical, grooves/ridges) on larval settlement in *Agaricia humilis* and *Favia fragum* (2-factor ANOVA).

		Surface cover				
			Flat tiles		Pyramid tiles	
		Total	In grooves	On ridges	In grooves	On ridges
	Variable	Mean±SD %	Mean±SD %			
Empty	DL/+G	25.07±22.36	15.89±12.60	9.79±4.18	38.57±26.07	36.03±28.60
	DL/-G	3.68±6.49	0.00	8.60±6.73	0.81±1.63	5.31±5.29
	BL/+G	46.50±31.50	44.46±24.79	68.57±37.88	28.81±19.43	44.15±38.14
	BL/-G	2.30±5.26	0.00	0.67±0.71	7.90±4.01	0.62±0.49
Turf algae	DL/+G	53.08±39.16	80.53±12.01	90.17±4.21	17.85±11.71	23.78±15.14
	DL/-G	0.00	0.00	0.00	0.00	0.00
	BL/+G	40.64±28.55	54.18±24.77	31.33±27.95	43.94±20.55	33.12±23.92
	BL/-G	0.00	0.00	0.00	0.00	0.00
Filament. alg.	DL/+G	0.00	0.00	0.00	0.00	0.00
	DL/-G	95.05±7.20	100.00±0.00	91.40±10.72	95.97±8.06	92.85±7.20
	BL/+G	0.00	0.00	0.00	0.00	0.00
	BL/-G	79.62±31.49	97.53±4.94	99.05±0.57	48.92±5.46	73.00±10.94
Coralline algae	DL/+G	21.52±33.02	2.27±1.58	0.04±0.05	43.58±41.00	40.19±39.30
	DL/-G	2.16±4.62	0.00	0.01±0.02	6.77±5.84	1.84±2.04
	BL/+G	9.21±19.69	1.09±1.05	0.10±0.20	13.43±9.81	22.23±21.33
	BL/-G	7.23±11.80	2.47±2.94	0.29±0.58	21.73±16.13	4.41±3.13

Table 3 Surface cover of tiles by different algal groups depending on incubation conditions. DL = "daylight", BL = blue light, +G = grazed, -G = non-grazed.

was located in grooves (Tukey's test, $p < 0.001$) with significant differences between flat and pyramid tile (Tukey's test, $p < 0.001$). The latter preferences varied depending on tile incubation.

83.7 % of all settlers in *Favia fragum* were located on grazed surfaces (Tukey's test, $p < 0.001$; see Table 1). On these surfaces, settlement rate was independent of the light source used for incubating the tiles (Tukey's test, $p = 0{,}056$). Regarding non-grazed surfaces, reasonably more larvae settled on those tiles incubated under blue light (Tukey's test, $p = 0.006$). Similar to *A. humilis*, the majority of all settlers were located in the grooves (95.2 % of all settlers; Tukey's test, $p < 0.001$) showing a high preference for horizontal surfaces (= flat tiles; Tukey's test, $p < 0.001$). Overall highest settlement rates were obtained in the grooves of flat tiles independently of the light used for tile-incubation (see Fig. 2).

	df	F	p
EMPTY			
Incubation	3	19.273	<0.001***
Surface	3	0.436	0.728
Surface*Incubation	9	1.725	0.109
TURF ALGAE			
Incubation	3	34.298	<0.001***
Surface	3	4.552	0.007**
Surface*Incubation	9	4.224	<0.001***
FILAMENT. ALGAE			
Incubation	3	226,926	<0.001***
Surface	3	3.178	0.032*
Surface*Incubation	9	3.159	0.005**
CORALLINE ALGAE			
Incubation	3	3.390	0.025*
Surface	3	5.886	0.002**
Surface*Incubation	9	1.278	0.273

Table 4 Influence of 3 month-incubation (light spectrum, grazing) and surface category (horizontal/vertical, grooves/ridges) on biological composition of tiles (2-factor ANOVA). The 4 dominant groups were analyzed.

Biofilm

Six ecologically relevant categories were sufficient to describe more than 99% of the biological surface structure of tiles incubated in the aquarium system (Fig. 3 and Table 3). We focused on the 4 most abundant groups: (1) "empty" (no visible biofilm), (2) "turf algae" (thin layer of green algae turf, Chlorophyta), (3) "filamentous algae" (thick layer of filamentous mats, Chlorophyta), and (4) "coralline algae" (encrusting, Rhodophyta). Cyanobacteria (fifth group) were only associated with tiles incubated under blue light; low quantities of

"sediments" (sixth group) (feces of hermit crabs) were identified on almost all tiles. Organisms such as microscopic sponges and worm-snails (*Petaloconchus* spp.) are listed under "others" (see Fig. 3).

The 4 dominant groups above were highly influenced by the factor "light/grazing" and mostly by the surface category (Fig. 3 and Table 4). Filamentous algae were massively and exclusively present on non-grazed tiles (Tukey's test, $p < 0.001$) with a slightly higher occurrence on flat tiles (Tukey's test, in grooves: $p = 0.04$, on ridges: $p = 0.574$). Overall frequency of these algal mats was lower on tiles exposed to blue light (Tukey's test, $p = 0.011$). Grazed tiles were mainly characterized by short algal turfs, empty space and coralline algae (Fig. 3). The latter was more abundant on pyramid tiles (Tukey's test, $p \leq 0.045$) with highest abundance on grazed surfaces incubated under daylight spectrum. Grazed tiles under blue light had the overall lowest algal cover (= "empty") compared to all other treatments (Tukey's test, $p \leq 0.013$), whereas the surface category did not have any influence (see Table 4). Short algal turf was only present on grazed surfaces (Fig. 3). These turfs preferred to grow on flat tiles (Tukey's test, $p \leq 0.041$) independently of the light spectrum (Tukey's test, $p = 0.254$).

Filamentous algae inhibited settlement in both species, whereas empty space and short turf algae showed a positive influence on larval settlement. The presence of coralline algae was not correlated to the larval settlement rate (Table 5). Contrary to *Favia fragum*, *Agaricia humilis* consequently settled on "clean" surfaces and never near filamentous algae or sediments.

	Empty		Turf algae		Filament. algae		Coralline algae	
	R^2	p	R^2	p	R^2	p	R^2	p
Agaricia humilis								
Horizontal	0.741	<0.001***	0.192	0.090	0.501	0.002**	0.002	0.863
Vertical	0.091	0.257	0.434	0.005**	0.534	0.001**	0.027	0.541
Favia fragum								
Horizontal	0.271	0.023*	0.829	<0.001***	0.901	<0.001***	0.05	0.406
Vertical	0.412	0.007**	0.268	0.04*	0.617	<0.001***	0.191	0.090

Table 5 Influence of different "algal groups" on settlement success in grooves of flat (= horizontal) and pyramid (= vertical) tiles. Due to low settlement rates, the remaining surfaces were not analyzed.

DISCUSSION

In the present study, the settlement of a reasonable number of larvae within the night of planulation represents an important loss of available propagules for mariculture. Individual and species-specific differences in larval settlement competency are significant. Although differences in settlement behaviour between larval released by individual colonies were at least mentioned elsewhere (Richmond, 1985; Babcock and Heyward, 1986), there is no specific literature available on this phenomenon. However, besides its ecological relevance, such differences may be of major importance in coral mariculture. In case of *Favia fragum*, almost half of all larvae settled before they could be collected. Assuming that larvae may delay settlement if the environment is not appropriate (see Harrison and Wallace, 1990), the genotypes of these early settlers might be better adapted to

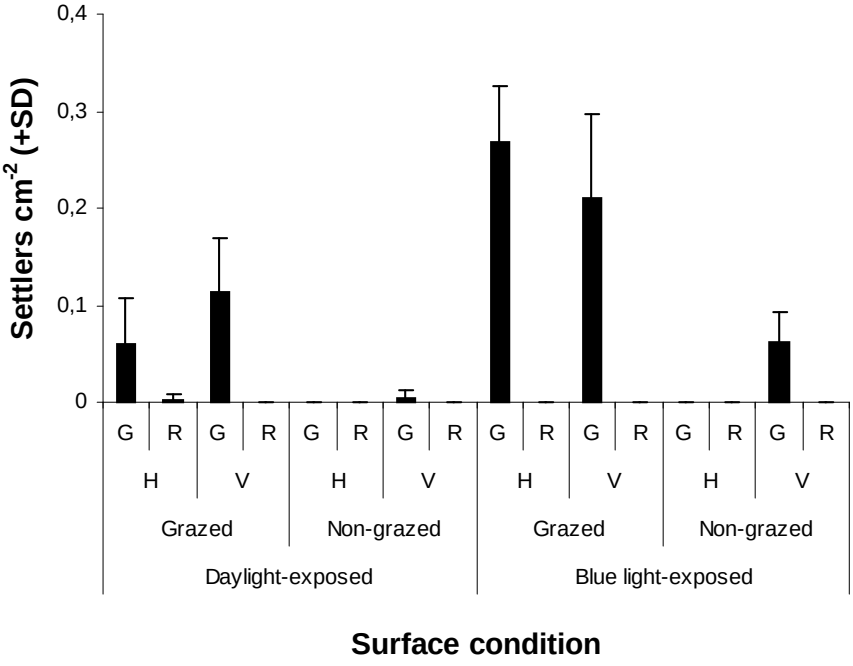

Fig. 1 *Agaricia humilis.* Larval settlement after 24 hr depending on tile shape and tile incubation. H = horizontal (flat tile), V = vertical (pyramid tile), G = in grooves, R = on ridges.

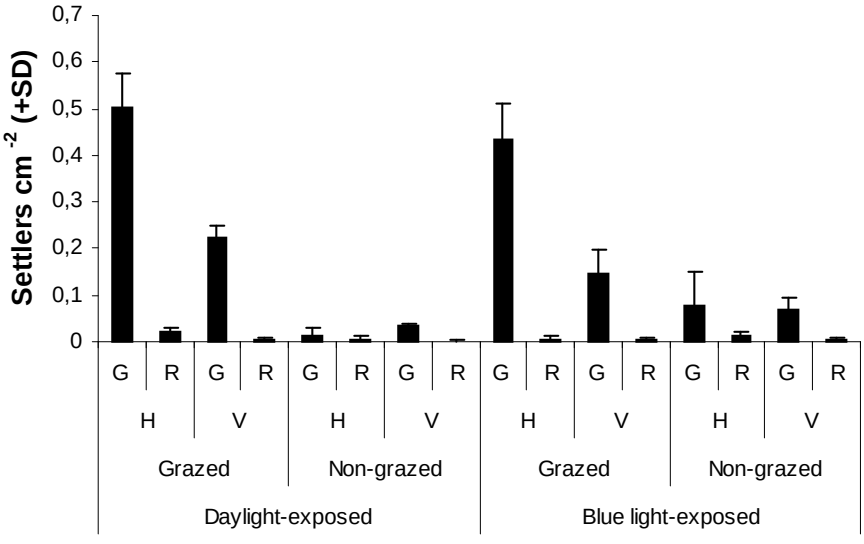

Fig. 2 *Favia fragum*. Larval settlement after 24 hr depending on tile shape and tile incubation. H = horizontal (flat tile), V = vertical (pyramid tile), G = in grooves, R = on ridges.

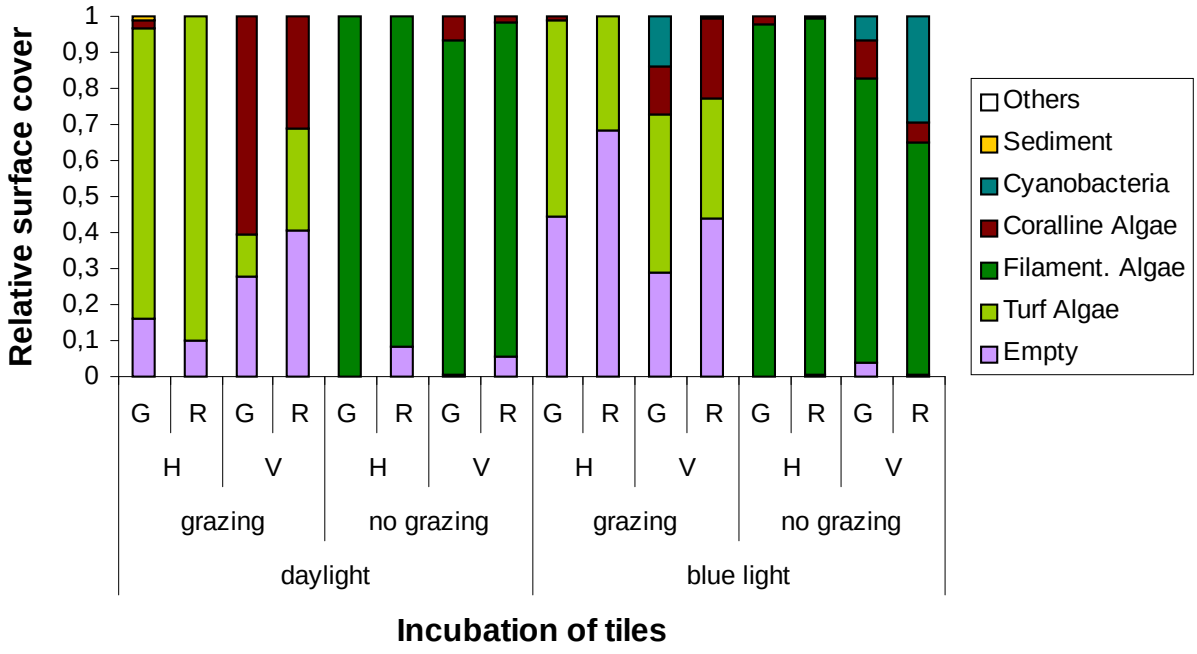

Fig. 3. Biofilm composition on settlement tiles incubated for 3 months under different environmental conditions. Relative cover is shown for each surface category: H = horizontal (flat tile), V = vertical (pyramid tile), G = in grooves, R = on ridges.

mariculture conditions, and therefore more valuable for an aquarium culture, e.g. to supply the ornamental trade. However, it is well known that larvae may be released in different developmental stages (Richmond, 1997; Harii et al. 2001), which could explain temporal variation in settlement. Especially stress, e.g. as a result of the collection method, can trigger the release of pre-mature larvae, which may lead to a significant delay of settlement (chapter 7). I did not carry out histological or genetic analyses to determine whether one or both factors influenced timing of settlement in the present study. If the first 36 hr after planulation could be used for inducing settlement on tiles, more than 60 % of the total amount of released larvae in *Favia fragum* could be used for recruit production (under the conditions of the present study). Thus and furthermore to automate breeding methods, tiles could be placed prior to planulation together with parental colonies in flow-through aquariums, which should be designed to increase the contact time of released larvae and tiles. Genetic investigation is necessary to better understand individual differences in settlement and to estimate relative fitness variation of aquarium-derived progenies under mariculture conditions. Indeed, settlement (unpublished data) and recruitment studies (chapter 9) using *Favia fragum* indicated rapid domestication of aquarium-derived propagules.

I could confirm high preferences of both species for the grooves in tiles, shown in chapter 5. However, preferences for the tile-type changed in *Agaricia humilis* depending on the treatment of the tiles (daylight vs. blue light). The incubation of tiles had a great influence on overall settlement rate and specific settlement preferences, which so far has not been recognized in previous studies. Certain algae have been identified to influence settlement of scleractinians in the field: coralline algae may trigger (Morse et al., 1996; Negri et al., 2001) whilst cyanobacteria may reduce settlement (Kuffner and Paul, 2004). Nevertheless, regarding previous settlement experiments, substrates were mostly incubated in the field or in open-

systems for basic research (Hunte and Wittenberg, 1992; Mundy and Babcock, 1998) and for aquaculture (Gateno et al., 1998; Epstein et al., 2001) without considering possible influences of the developing biofilm on the settlement rate. As shown in the present study, the incubation of substrates can be essential for the settlement success, especially if tiles are incubated ex situ without the common herbivorous fauna found in coral reefs (see Lirmann, 2001). In the present study, filamentous algae, which were negatively correlated to the presence of grazers, highly reduced settlement in the studied species. Besides grazing, the light spectrum applied for tile incubation additionally affected settlement, at least in *Agaricia humilis*. The combination of both conditions resulted in the overall lowest algal cover on horizontal surfaces compared to all other treatments. Due to the extremely flat shape of the primary polyps and the encrusting colony morphology in *A. humilis*, already a relatively thin algal layer (< 0.5 mm) might be a reasonable threat, which could explain lower settlement on horizontal surfaces, previously incubated under 'daylight' spectrum. However, this does not explain, why settlement in *A. humilis* was significantly higher on vertical surfaces incubated under blue light compared to those incubated under daylight spectrum, since the particular surface cover with algal turf and coralline algae should have clearly favored the opposite. Indeed, our observations do not necessarily confirm those of Morse et al. (1996) and Negri et al. (2001), who found coralline algae triggering larval settlement. Settlement in both species was not correlated to the presence of coralline algae; however, conditions (light spectrum, grazing) that favored growth of coralline algae did also have a positive effect on larval settlement. In this study, we excluded interaction (e.g. competition) between algal groups, however, it seems obvious that observed turf and filamentous algae might be different morphs of the same algal species exhibited under different grazing pressure. This was indicated by the gradual change of morphotypes into each other when transferred between conditions (unpublished data). My observations emphasize the importance of algal control in coral mariculture, which clearly includes the process of substrate pre-conditioning. Further research is necessary to estimate optimum incubation periods for settlement tiles, which could influence settlement success species-specifically and the importance of grazing generally. However, if incubation periods are too short, specific algal succession commonly found on newly introduced substrates might highly reduce survival of settlers (personal observation). Primary polyps of *Acropora* spp., which were settled on non-incubated tiles using a neuropeptide (Iwao et al., 2002; see also chapter 5), showed low post-settlement survival due to rapid algal succession on tiles when located in flow-through aquariums (Hatta, personal communication).

Algal growth also depends on nutrients (nitrogen and phosphorous), which are of major importance in closed systems with relatively limited water volume. In the present study, nitrate levels were slightly higher than commonly found in the field. I showed that, at least, relatively low elevations of nitrate might be neutralized by appropriate grazing when we assume that such nitrate concentrations do not directly affect settlement. The appropriate supply of grazing organisms in high amounts will be crucial for future coral mariculture. The hermit crabs, used in the present study were collected in the field. In order to enhance sustainability, it should be envisaged to additionally breed grazing organisms like commonly done to supply plankton food in fish breeding (Artemia spp., Brachionus spp.). We recently succeeded in breeding *Paguristes* spp. (unpublished data), which will help to sustainably manage the coral culture at Rotterdam Zoo.

Contrary to the field, mariculture conditions usually exclude potential competitors such as cnidarians, sponges, or mollusks (Maida et al., 1995; Sorokin, 1995), however, specific organisms may be favored in monocultures such as the anemone *Aiptasia* spp. and worm snails of the genus *Petaloconchus*. We managed to keep the number of these competitors low by not adding any organic food to the system.

In conclusion, temporal and spatial variation in larval settlement may differ inter- and partly intraspecifically with major importance in coral mariculture. Temporal variation could be partly compensated by a proper design of breeding facilities, however, more investigation is needed to evaluate the importance of genetics in parental colonies. Spatial variation in settlement is determined by the shape and the biological condition of settlement substrates. Certain algae, which can be highly influenced by culture conditions, may inhibit settlement. More research is necessary to estimate optimum incubation times for substrates, species-specific preferences and the influence of isolated algal species on settlement in order to maximize the breeding success.

REFERENCES

Adey, W.H., Loveland, K. 1998. Dynamic aquaria: building living ecosystems. London: Academic Press, 498 p.

Babcock, R.C., Heyward, A.J. 1986. Larval development of certain gamete-spawning scleractinian corals. Coral Reefs 5, 111-116.

Epstein, N., Bak, R.P.M., 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.

Gateno, D., Barki, Y., Rinkevich, B. 2000. Aquarium maintenance of reef octocorals raised from field collected larvae. Aquarium Sciences and Conservation 2, 227-236.

Harii, S., Omori, M., Yamakawa, H., Koike, Y. 2001. Sexual reproduction and larval settlement of the zooxanthellate coral *Alveopora japonica* Eguchi at high latitudes. Coral Reefs 20:19-23.

Harrison, P.L., Wallace, C.C. 1990. Reproduction, dispersal and recruitment of scleractinian corals. In: Dubinsky Z. (ed.) Ecosystems of the world. 25. Coral reefs. Elvesier, Amsterdam, pp 133-207.

Hunte, W., Wittenberg, M. 1992. Effects of eutrophication and sedimentation on juvenile corals. II. Settlement. Mar Biol 114, 625-631.

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.

Kuffner, I.B., Paul, V.J. 2004. Effects of the benthic cyanobacterium *Lyngbya majuscula* on larval recruitment of the reef corals *Acropora surculosa* and *Pocillopora damicornis*. Coral Reefs 23(3), 455-458.

Lirmann, D. 2001. Competition between macroalgae and corals: effects of herbivore exclusion and increased algal biomass on coral survivorship and growth. Coral Reefs 19, 392-399.

Maida, M., Sammarco, P.W., Coll, J.C. 1995. Effects of soft corals on scleractinian coral recruitment. I: Directional allelopathy and inhibition of settlement. Mar Ecol Prog Ser 121, 191-202.

Morse, A.N.C., Iwao, K., Baba, M., Shimoike, K., Hayashibara, T., Omori, M. 1996. An ancient chemosensory mechanism brings new life to coral reefs. Biol Bull 191, 149-154.

Mundy, C.N., Babcock, R.C. 1998. Role of light intensity and spectral quality in coral settlement: Implications for depth-dependent settlement. J Exp Mar Biol Ecol 223, 235-255

Negri, A.P., Webster, N.S., Hill, R.T., Heyward, A.J. 2001. Metamorphosis of broadcast spawning corals in response to bacteria isolated from crustose algae. Mar Ecol Prog Ser 223, 121-131.

Richmond, R.H. 1985. Reversible metamorphosis in coral planulae larvae. Mar Ecol Prog Ser 22, 181-185.
Richmond, R.H. 1997. Reproduction and recruitment in corals: critical links in the persistence of reefs. In: Birkeland C (ed) Life and death of coral reefs. Chapman and Hall, New York, pp 175-197.
Sorokin, Y.I. 1995. Coral reef ecology. 102. Ecological studies. 2nd printing. Berlin: Springer, 465 p.

CHAPTER

9

The Influence of Light on the Early Growth and Survival of Reefbuilding Corals in Mariculture

INTRODUCTION

Light represents an important factor in the development of zooxanthellate corals (Falkowski et al, 1990) and showed significant influence on the metabolism of asexual propagules and adults under laboratory conditions (Marubini et al., 2001; Titlyanov et al., 2001; Anthony and Hoegh-Guldberg, 2003). However, no data are currently available on the application of artificial light sources in the mariculture of sexual propagules. Light might be the most cost-intensive factor to run a land-based culture, especially if aimed at being independent of natural sunlight. Carlson (1999) gives an overview of available light sources and their intensity levels; Jones (2002) examined the spectral quality of different commercially available metal halide lamps (HQI) widely used in coral husbandry.

I studied the influence of 2 different light spectra of HQI lamps, which are commonly used by professionals and hobbyists (see Adey and Loveland, 1998; Carlson, 1999; Delbeek and Sprung, 1996), on the early survival and growth of primary polyps of 2 coral species under laboratory conditions. Additionally, I had the possibility to compare the development of the F1 and F2 generations of one species to evaluate domestication effects. The results are discussed in context with other factors such as nutrition, algal control, genetics and economical aspects.

METHODS

System Description

All experiments were carried out in a closed re-circulation system, consisting of 2 culture tanks (400 x 100 x 60 cm; L x W x H) and 1 sump tank (300 x 100 x 50 cm; L x W x H). The total gross water volume of the 3 tanks is 5,000 L. Parts of the life support system (LSS) are either situated in the sump tank itself or are connected to this tank via separate bypasses. Two surface overflows situated on the right and left hand side of the tanks lead the water directly to the LSS. Twelve commercial aquarium heaters (500 W) situated directly in the sump tank and 2 external coolers (1300 W, SK5 AquaMedic GmbH, Germany) are controlled digitally (EY3600 novaPro32, Sauter Ltd. Switzerland) to keep the temperature at 26.5 ± 0.5 ˚C. A calcium reactor (Jetstream 2, Schuran Seawater Equipment, Germany) is installed and commercial water additives are supplied to avoid the lack of essential elements, which are continuously consumed by corals. Water from the sump tank enters the culture tanks via several inflows situated lengthwise on both sides of each tank in a distance of 50 cm. As a result 7 inflows per side of each tank are exactly positioned opposite each other to create a turbulent flow regime along the entire length of the tank (total flow: 20,000 l h^{-1} $tank^{-1}$).

No organic food (frozen or live food) was added during the entire period. One culture tank was used for the experiments; the other tank was used to maintain reefbuilding corals. Five herbivorous fish (*Scartella cristata*) were kept in each culture tank. Algae were mainly controlled by herbivorous hermit crabs (*Paguristes* spp.) cultured in the tank in a high density of approx. 700 specimens m^{-2} (calculated from taking the mean density 100 cm^{-2}; n = 10). Approx. 100 kg of Live Rock (coral reef substrate containing all its associated organisms; here: aquacultured in Florida and field-collected at Curaçao, Netherlands Antilles) were introduced to start up the system 12 months prior to the experimental period. The system was dominated by crustose coralline algae (Rhodophyta, ~ 50 % surface cover) and algae turfs (Chlorophyta, ~ 30 %), approx. 20 % of the substrate was not visibly covered by any algae. Due to the high grazer density, green algae turfs were kept short. The following water parameters were measured using potentiometric titration (Titro Line easy, Schott GmbH,

Germany), galvanomerty (Oxi 330i, WTW GmbH, Germany) and photo spectrometry (DR/4000U Photospectrometer, HACH Company U.S.A.): dissolved oxygen 102.9 %, pH 8.19, salinity 36.0 ‰, alkalinity 3.09 meq l^{-1}, NH3-N 0,00 mg l^{-1}, NO2-N 0.007 mg l^{-1}, NO3-N 3.0 mg l^{-1}, Ca^{2+} 454.5 mg l^{-1}, Mg^{2+} 1310.8 mg l^{-1}, PO4-P 0.014 mg l^{-1}. The values are within the range of the field (see Sorokin, 1995; Adey and Loveland, 1998)

Sediments, mainly located on the settlement tiles (see below) were eliminated by weekly conducting a water exchange of approx. 10 % of the total water volume.

Studied species

I used primary polyps of *Favia fragum* and *Agaricia humilis*. Planula larvae were constantly obtained from colonies previously collected at Curaçao, Netherlands Antilles (chapter 8).

Parallel to the experiments, specimens of an F1 generation of *F. fragum* surprisingly started to release larvae (chapter 6 and 10). The specimens were raised in an exhibit tank at Rotterdam Zoo and originated from the same population used for the present study. I decided to use this unique chance to involve F2 primary polyps in the experiments for comparison.

Experimental design

I used the tiles described in chapter 5 for the experiments. Settlement occurred in standing seawater using 1.5 L plastic containers at 26 ˚C, at a salinity of 36 ‰, and low light intensity of 60 µmol m^{-2} s^{-1}. Four to 6 days after settlement, primary polyps situated on tiles (density: ~ 5 settler $tile^{-1}$) were transferred to the culture tank. Primary polyps were slowly adapted to new light conditions by moving them in 3 steps (weekly in the first month) from the edge of the tank to the center, directly under the HQI lamps. Two different light regimes were tested: 400 W HQI 6,000 K (= 'daylight' spectrum) and 400 W HQI 20,000 K (= blue light spectrum) (illumination 12 h d^{-1}). Both light spectra are widely used in aquariology; an intensity of 400 W represents an upper light level to maintain aquariums with a water depth similar to the culture tank of the present study (50 cm) (see Adey and Loveland, 1998; Carlson, 1999, Delbeek and Sprung, 1996). Prior to their application, bulbs were illuminated for more than 100 hr to stabilize the halide gases (see Jones, 2002). The housing and reflector type of each lamp was identical. Irradiance was measured < 5 cm below the surface and in a depth of 50 cm where polyps were located during the experiment. To equalize refraction effects of the water surface, quantum flux was integrated over 30 sec (1 measurement sec^{-1}). Three measurements were taken per lamp: directly under, and at the left and right periphery of the reflector. Additionally, irradiance at 3 acclimation-spots from the edge of the tank to the center (= directly under the lamp), were measured by taking 3 spot checks across the whole length where the polyps were located (in 50 cm depth). Photosynthetic active radiance (PAR) was measured using a spherical light sensor (LI-193SA; LICOR, USA) connected to a LI-1400 data logger (LICOR, USA).

Juveniles of *Agaricia humilis* and *Favia fragum* (F1) were monitored for 12 months. Survival and size (max. diameter) were measured after 1 and 2 months, and then every 2 months. F2 juveniles of *F. fragum*, which were involved in a later stage of the experiment, were measured after 1, 2, 4 and 6 months. Contrary to the experiment using F1 polyps, one part of the juveniles was placed directly under the 'daylight' lamps while the other part was adapted like described above. Observations in captive recruitment of *Acropora tenuis* had indicated higher growth rates when newly settled primary polyps were not light-adapted after being transferred to culture tanks (R. Jones, London Zoo, personal communication). Microscopic pictures were taken whenever the position of the specimens on the tiles was appropriate for measurements (AxioCam MRc, Carl Zeiss Vision GmbH Germany). Max.

diameters of specimens were then measured digitally (AxioVision 3.1, Carl Zeiss Vision GmbH Germany).

Data analysis

Data were statistically analyzed for differences between horizontally (flat tiles) and vertically oriented (pyramid tiles) polyps per light treatment and between light treatments for each orientation. Species groups were analyzed separately. Since 1 aquarium system was available for the experiments, survival data were analyzed with a Chi-Square homogeneity test. Growth data were first tested for normality and then analyzed with a Student's t-test.

RESULTS

Light measurements

Table 1 gives an overview of all measurements. When measured just below the water surface, quantum flux of 400 W HQI 6,000 K lamps was 2 times higher than of 400 W HQI 20,000 K. However, both lamp types showed almost equal irradiance in a water depth of 50 cm. At the edge of the tank, where polyps were first located for acclimation, irradiance was comparable with irradiance during settlement, and 3 times lower compared with irradiance in the center of the tank, under the lamps.

Light type	**Water depth**	**Position**	**Irradiance**
	cm		**µmol m^{-2} s^{-1}**
400W HQI 6K	<5	center	570.2 ± 22.1
	50	edge	59.8 ± 8.3
	50	edge-center	125.8 ± 12.3
	50	center	176.7 ± 41.1
400W HQI 20K	<5	center	282.3 ± 43.2
	50	edge	52.9 ± 6.4
	50	edge-center	110.9 ± 9.8
	50	center	157.8 ± 41.05

Table 1 Irradiance of different light sources dependent on water depth and position in the culture tank.

Growth

In the first month, primary polyps of *Agaricia humilis* (initial diam. 1.03 ± 0.25 mm; mean ± SD) showed growth under all conditions, except for those located on vertical surfaces under blue light: polyps showed lower mean diameters compared to the start of the experiment (Fig. 1A). This phenomenon can be explained by shrinking polyps (= tissue-necrosis and -retraction towards the center of the polyp; in a more advanced stage tentacles are lost; see Fig. 2B). Shrinking was generally observed in individuals of both species under all conditions. However, the frequency differed between species groups and conditions (see Fig. 1A-B). Regarding vertical surfaces, polyps of *A. humilis* grew significantly faster under 6 K lamps only in the first month ($t = -6.19$, $p < 0{,}001$). In the first 6 months, mean diameters decreased for both light conditions below those measured at the start. Although not significantly different ($t = 0.64$, $p = 0.53$), polyps under blue light had a slightly higher growth, which was, however, with 45.8 ± 37.6 % (mean ± SD) still relatively low compared to the growth of *Favia fragum* (Table 2). In a period of 12 months, no specimen produced any daughter polyps (fission).

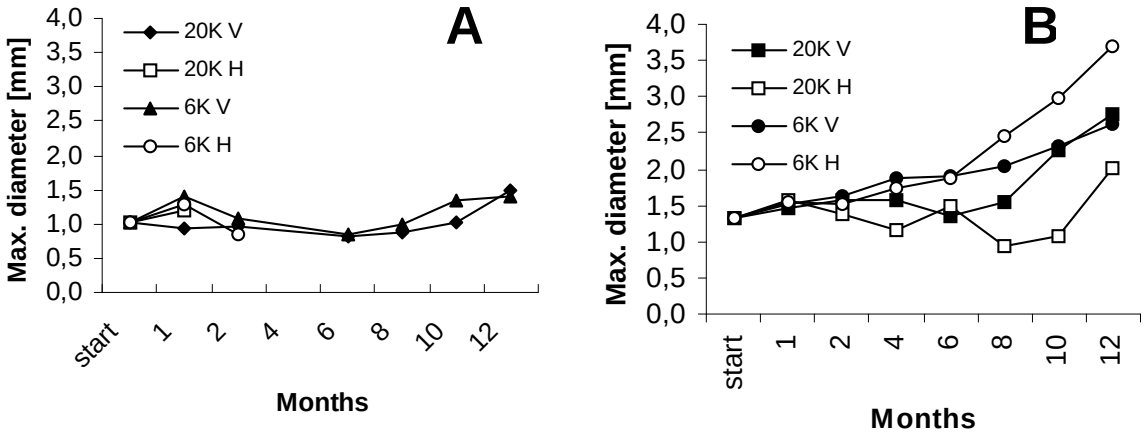

Fig. 1 A-B Growth of *Agaricia humilis* (A) and *Favia fragum* (B) on vertical (= V) and horizontal (= H) surfaces under different light conditions (6 K = 400W HQI 6 K; 20 K = 400W HQI 20 K).

In the first 2 months, primary polyps of the F1 generation of *Favia fragum* (initial diam. 1.33 ± 0.24 mm; mean ± SD) showed no significant differences regarding surface orientation and light spectra ($t \geq 1.25$, $p > 0.05$) (Fig. 1B). However, settlers located horizontally under blue light showed reduced growth and necrosis, which increased reasonably from month 4, compared to polyps located vertically under blue light ($t = -2.56$, $p = 0.012$) and to polyps located horizontally under daylight ($t = -2.21$, $p = 0.029$) (see Fig. 2A-B). Except for month 6, these settlers had the smallest mean sizes until the end of the experiment with 3 times less growth compared to horizontal polyps under daylight ($t \leq -2.92$, $p \leq 0.006$) with overall highest growth between 6 and 12 months (Table 2 and Fig. 1B). Final sizes of juveniles located vertically were intermediate and were not influenced by the light spectrum ($t = 0.57$, $p = 0.58$). Polyp fission was observed for the first time after 4 months. Comparing overall growth rates per light treatment and species, polyps of *F. fragum*, which were initially slightly larger than *Agaricia humilis*, had at all times higher mean diameters (Fig. 1A-B).

For both light conditions, primary polyps of the F2 generation of *Favia fragum* (initial diam. 1.40 ± 0.18 mm; mean ± SD) showed comparable growth to the F1 generation under daylight (Fig. 3). Significant differences of F2-polyps were only observed between blue light and daylight on horizontal surfaces, and only for the first 2 months ($t \leq -2.45$, $p \leq 0.024$), after

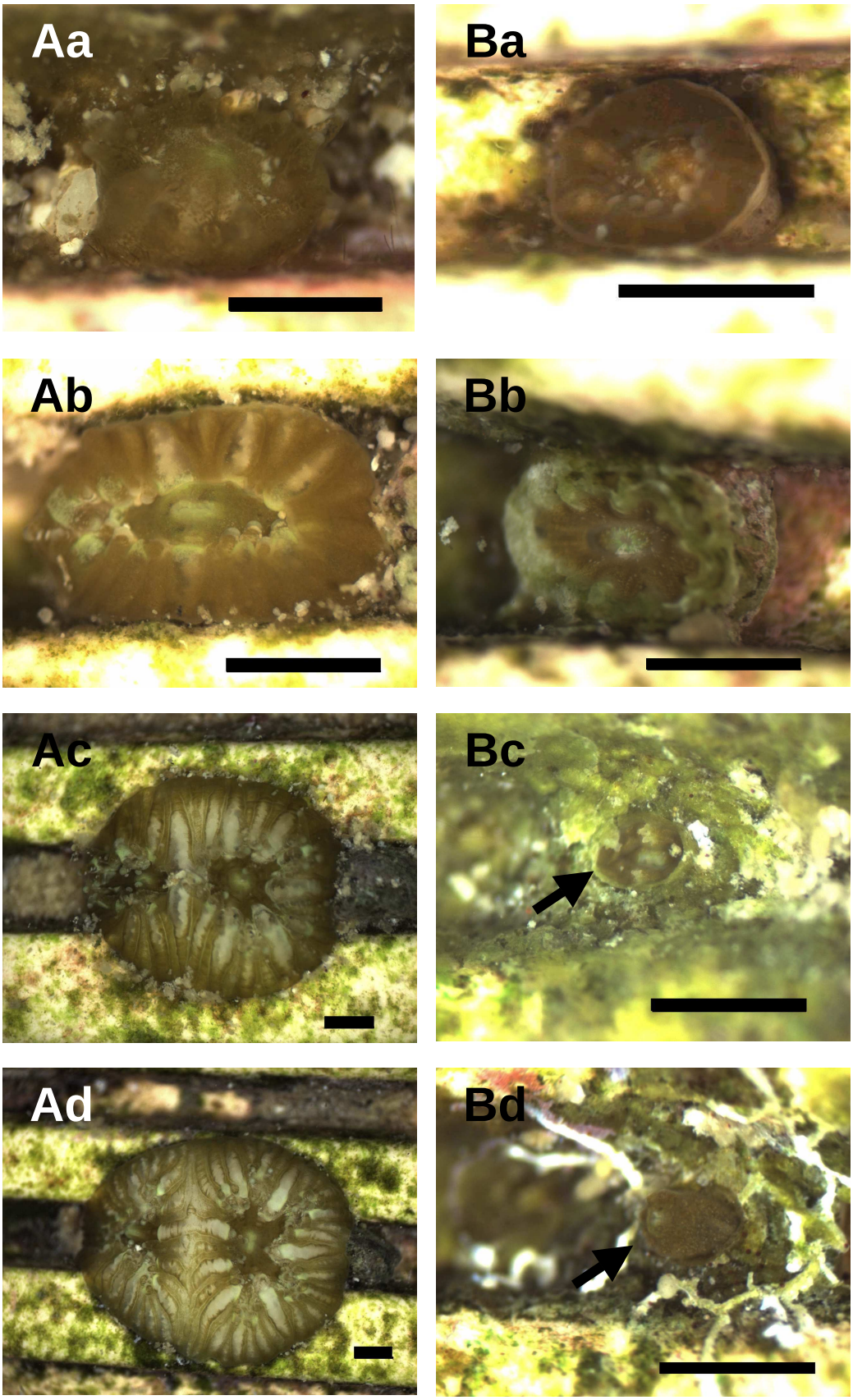

Fig. 2 A-B *Favia fragum*. Examples of a well-developing juvenile (A) under 400W HQI 6 K lighting and a shrinking juvenile (B) under 400W HQI 20 K lighting (a = 1, b = 6, c = 10, d = 12 months). Scale bar: 1 mm.

6 months mean diameters were almost identical for both light conditions (Fig. 3) and surface orientations (Table 3). Horizontally oriented primary polyps, which were directly exposed to 400 W daylight without any adaptation when transferred after settlement, did not show any growth in the first month compared to those polyps with adaptation ($t = 2.22$, $p = 0.04$) (Fig 4). However, although not significantly different ($t = -1.40$, $p = 0.17$), these non-adapted juveniles had larger diameters after 6 months compared to horizontally located polyps with adaptation (Table 3). Contrary, no significant differences were found between adapted and non-adapted polyps on vertical surfaces at any time ($t \geq -1.34$, $p \geq 0.196$).

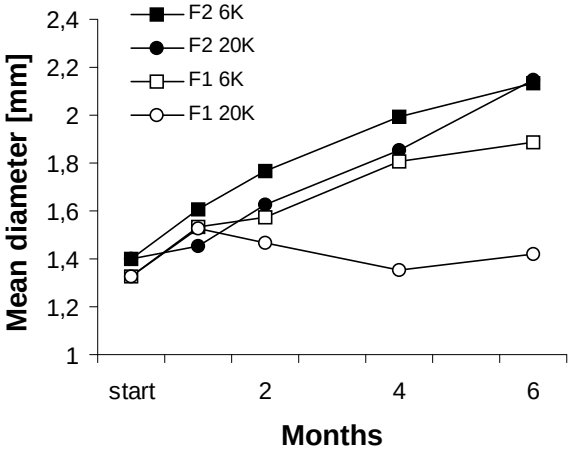

Fig. 3 Comparison of overall growth between the F1 and F2 generation of *Favia fragum* under different light conditions (6 K = 400W HQI 6 K; 20 K = 400W HQI 20 K). Note: d = 6 months.

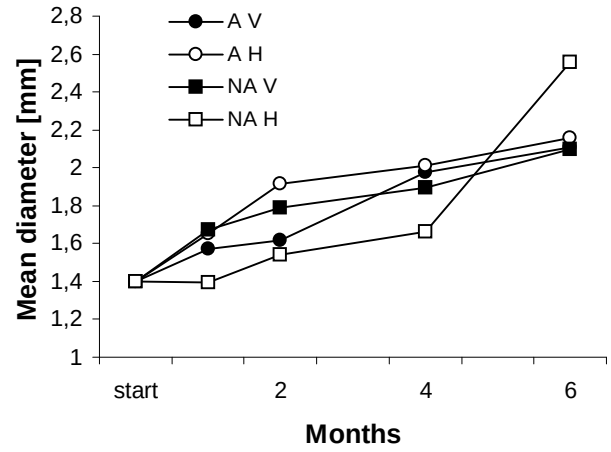

Fig. 4 Effects of light acclimation on the initial growth of F2 juveniles of *Favia fragum* on vertical (V) and horizontal (H) surfaces. A = juveniles adapted in 3 steps; NA = juveniles not adapted.

Survival

All juveniles of *Agaricia humilis* located on horizontal surfaces died within 4 months (Fig. 5A). A drastic decrease of vertically oriented polyps could be observed for both light treatments in the first 2 months. Except for month 8 and 10, survival rates of vertically oriented polyps were at all times significantly higher under daylight ($\chi^2 \geq 3.82$, $p \leq 0.037$). After 12 months, total survival on vertical surfaces was 19.4 ± 10.4 % (mean ± SD) (Table 2).

Highest mortality of primary polyps of the F1 generation of *Favia fragum* was observed under all conditions during the first 2 months (Fig. 5B). Over the entire period, polyps located vertically under 6 K lamps survived better than on horizontal surfaces within the same light treatment ($\chi^2 \geq 5.13$, $p \leq 0.017$) and on vertical surfaces under 20 K lamps ($\chi^2 \geq 5.12$, $p \leq 0.02$), respectively. A total of 54.0 % of vertically oriented juveniles survived under daylight compared to a total of 25.0 ± 9.6 % (mean ± SD) juveniles under the remaining conditions (see Table 2).

Over the entire period of 6 months, primary polyps of the F2 generation of *Favia fragum* showed high survival rates of more than 75 % (Table 3 and Fig. 6). From month 2 after settlement, significant higher survival was recorded for horizontally oriented polyps under blue light compared to polyps located vertically under the same light source ($\chi^2 \geq 4.18$, $p \leq 0.033$). During the whole monitoring period, these horizontally located polyps (blue light) survived better than under daylight ($\chi^2 \geq 4.26$, $p \leq 0.042$). Similar survival rates were found under the remaining conditions (Fig. 6). Regarding the first 2 months, horizontally located polyps showed higher survival rates (= 100 %) when directly placed under the light source ($\chi^2 \geq 8.04$, $p \leq 0.005$). For both light conditions, overall survival of the F2 generation was higher compared to the F1 generation (Fig. 7 and Table 3).

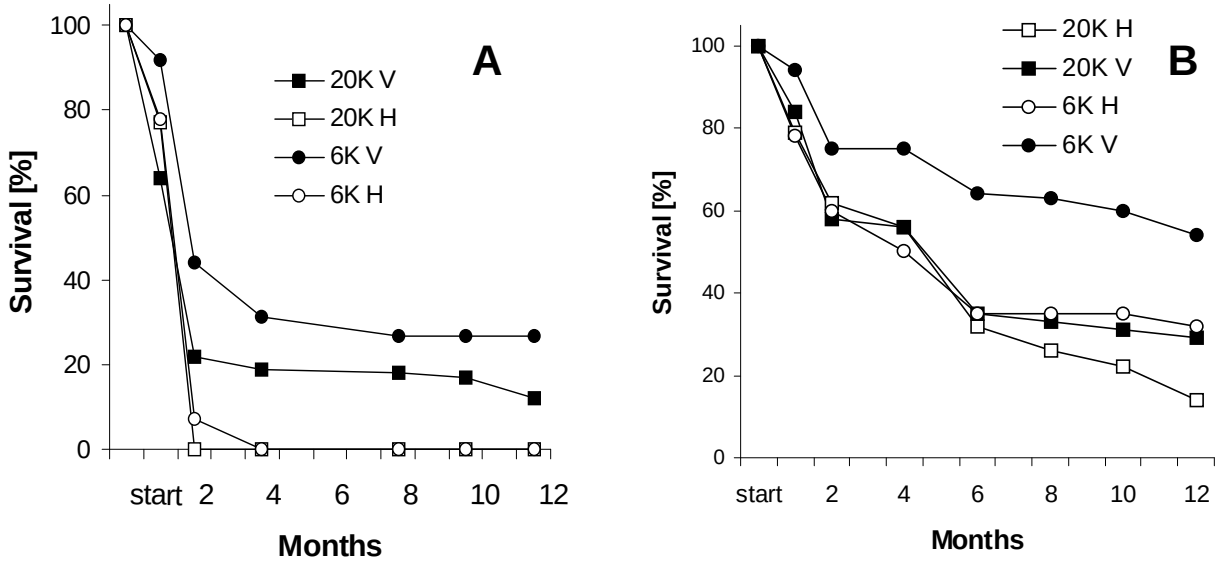

Fig. 5A-B Survival of *Agaricia humilis* (A) and *Favia fragum* (B) on vertical (= V) and horizontal (= H) surfaces under different light conditions (6 K = 400W HQI 6 K; 20 K = 400W HQI 20 K).

DISCUSSION

Although *Favia fragum* and *Agaricia humilis* show similar reproductive characteristics in the field and occur in the same habitat (Szmant, 1986), overall recruitment rates highly differed between both species under mariculture conditions in the present study. However, both species showed preferences for daylight conditions. While *Favia fragum* showed both, higher survival and growth, *A. humilis* only showed significantly higher survival under daylight spectrum. The overall low survival and growth in *A. humilis* may indicate a general limitation of this species in the present study (see below). Preferences for different light spectra observed for other scleractinians under natural light conditions (Babcock and Mundy, 1996) are only clearly shown for *F. fragum*. Irradiance of both light sources was below those measured in the field [0-10 m: >500 µmol m^{-2} s^{-1}; Vermeij and Bak (2002)]. However, quantum flux directly affecting early settlers may highly differ in the natural habitat due to the complex surface structure of coral reefs (Vermeij and Bak, 2002). Larvae generally prefer to settle protected in crevices and on grooves. Those locations have usually lower light levels compared to exposed surfaces at the same water depth. With increasing size, juveniles can persist to higher competition of other benthic organisms such as algae, and therefore then may

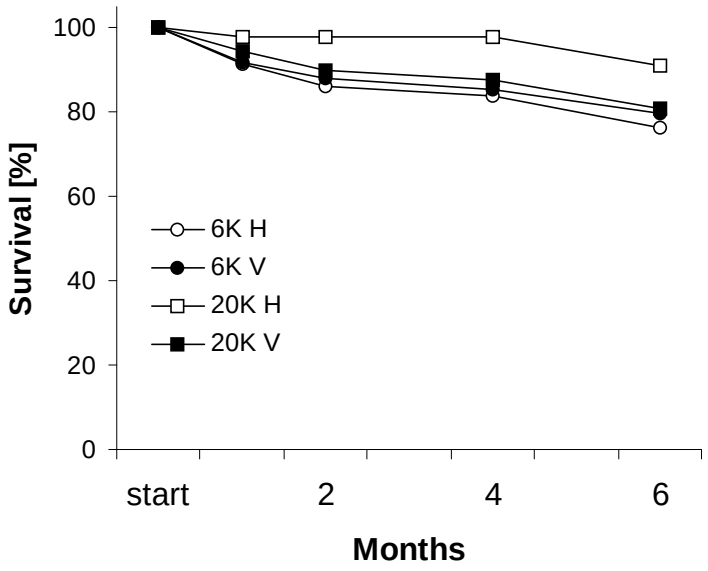

Fig. 6. Survival of F2 juveniles of *Favia fragum* on vertical (= V) and horizontal (= H) surfaces under different light conditions (6 K = 400W HQI 6 K; 20 K = 400W HQI 20 K). Note: d = 6 months.

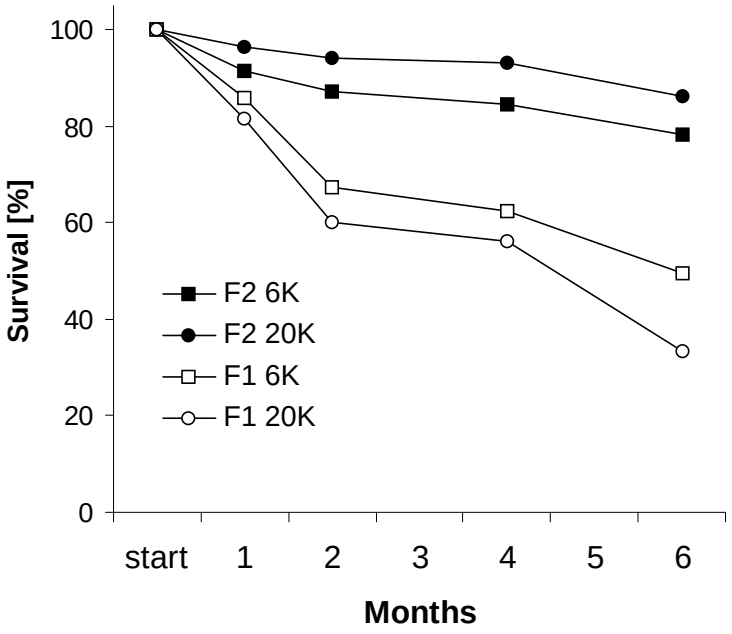

Fig. 7. Comparison of overall survival between the F1 and F2 generation of *Favia fragum* under different light conditions (6 K = 400W HQI 6 K; 20 K = 400W HQI 20 K). Note: d = 6 months.

invade from the protected settlement spot onto more light exposed surfaces (Harrison and Wallace, 1990). Natural recruitment rates are usually higher on vertical surfaces compared to horizontal surfaces due to higher sedimentation and algae growth on the latter ones (Sato 1985; Maida et al. 1994; Babcock and Mundy 1996). In the present study, irradiance in 50 cm depth directly under 400W HQI lamps is within the range of those measured on such vertical surfaces in the field (Vermeij and Bak, 2002). Limiting factors such as algal growth and sedimentation were minimized in the present study. Appropriate algal control was achieved by high densities of grazers. However, weekly removal of sediments could not avoid accumulation of feces excreted by these grazers, especially by hermit crabs. Feces mainly filling the grooves of the flat tiles probably affected survival of *F. fragum* and excluded recruitment of *A. humilis* on horizontal surfaces. Especially *A. humilis* might be affected due to its encrusting morphology, which is easily covered by sediments and algae. Algal growth plays a major role in coral mariculture (Bausoch et al., 2000; Gateno et al., 2000; Petersen and Tollrian, 2001) and may be closely related to irradiance. Effective control is an essential step

towards achieving high survival and growth rates. In the past, filamentous algae were mainly mechanically reduced using razor blades, brushes, and tweezers (Bausoch et al., 2000; Gateno et al., 2000). However, such techniques are labor intensive and bear the risk of damaging juveniles. Biological control of algal growth, successfully applied in the present study, has not yet been paid attention to in coral mariculture. However, my observations indicate the need for more research to evaluate optimum grazing densities (see chapter 8), which might depend on grazer-type (e.g. hermit crabs vs. sea urchins).

In both species, survival and growth rates varied over time, which may be explained by temporal changes of specific needs depending on the life history stage. These changes have to be considered when applying sexual reproduction in mariculture. Beside light, other energy sources such as live or frozen food were eliminated in the present study. Due to the preference of cryptic habitats in early life stages as described above, such energy sources could be of major importance in the first weeks after settlement to reduce initial mortality (see also Sebens et al., 1998). Indeed, survival and growth of the same species was higher under comparable light regimes, when culture tanks were connected to exhibits with frequent feeding of the fish stock. The latter is confirmed by chapter 10 and 11. Growth rates in the field are only available for recruits that were already visible to the naked eye (Van Moorsel, 1988), and therefore can hardly be used for comparison.

The use of light sources with higher irradiance (≥ 1,000 W) might increase growth rates, which may secondarily lead to higher survival rates. However, apart from high investment and running costs of such light regimes, higher light intensities do not necessarily lead to higher survival and growth. As chapter 10 shows, juvenile colonies of *Favia fragum* had similar sizes and were similar distributed under light intensities ranging between 280 and 1,500 µmol m^{-2} s^{-1} on exposed surfaces. It has to be mentioned that corals may partly neutralize different light intensities by photoadaptation, e.g. by varying zooxanthellae densities (Falkowski et al., 1990). However, an economical way to increase light intensity is achieved by reducing water depth. The light intensity of 400W HQI 6,000 K is 3 times higher just below the surface compared to a depth of 50 cm. In the present study, this alternative could not be tested. Pre-studies showed that tiles placed elevated from the bottom of the culture tank were not any longer accessible for hermit crabs and therefore showed high growth rates of turf algae leading to high mortality of corals. In this context, daylight spectrum (6,000 K) is more favorable than a blue light spectrum (20,000 K) as lamps of the same wattage may loose irradiance when shifted to the blue spectrum as shown in the present experiment. Irradiance differences in the 400W HQI lamps used in the present study were nearly neutralized in a depth of 50 cm due to the filtration effect of water for longer wavelengths (Falkowski et al., 1990). Observations of juveniles of *Acropora tenuis* at Burgers' Zoo Arnhem, The Netherlands, however, showed relatively high growth rates even under 70W HQI lamps. Again, this re-circulation system was regularly supplied with planktonic food (Janse, personal communication).

Acclimation processes might be of major importance when transferring propagules to different light conditions. In the present study, transferring primary polyp of the F2 generation of *F. fragum* to 3 times higher light intensities right after settlement without any acclimation, resulted in highest survival and juvenile sizes after 6 months. However, growth was possibly photo-inhibited in the first month (see also Falkowski et al., 1990). It should be emphasized that photoacclimation studied in adult corals requires energy, which can be supplied by organic food sources such as live plankton and deep-frozen food (Titlyanov et al., 2000; Anthony and Hoegh-Guldberg, 2003).

Besides having much higher survival and growth rates, juveniles of the F2 generation of *Favia fragum* showed higher similarities between light conditions with highest survival under blue light. This behaviour contradicts with the F1 generation, which clearly favored daylight

Species	Light	Orientation	n	Total survival	Diameter	Total growth	Mean growth	
				%	mm	%	mm month^{-1}	% month^{-1}
Agaricia humilis	daylight	horizontal	41	0	-	-	-	-
		vertical	86	18.1	1.4 ± 0.5	35.4 ± 45.6	0.03 ± 0.04	3.0 ± 3.8
	blue light	horizontal	22	0	-	-	-	-
		vertical	100	12.0	1.5 ± 0.4	45.8 ± 37.6	0.04 ± 0.03	3.8 ± 3.1
Favia fragum	daylight	horizontal	100	32.0	3.7 ± 2.1	177.2 ± 158.4	0.20 ± 0.18	14.8 ± 13.2
		vertical	100	54.0	2.6 ± 1.0	96.3 ± 73.9	0.11 ± 0.08	8.0 ± 6.2
	blue light	horizontal	100	14.0	2.0 ± 1.1	52.1 ± 81.7	0.06 ± 0.09	4.4 ± 6.8
		vertical	100	22.0	2.8 ± 1.4	108.0 ± 108.6	0.12 ± 0.12	9.0 ± 9.1

Table 2 Early development of *Agaricia humilis* and *Favia fragum* under different light sources (400W HQI) over 12 months (mean ± SD). [a] Diameter after 12 months.

Generation	Light	Orientation	N	Total survival	Diameter	Total growth	Mean growth	
				%	mm	%	mm month^{-1}	% month^{-1}
F1	daylight	horizontal	100	35.0	1.9 ± 1.0	40.9 ± 78.5	0.09 ± 0.17	3.5 ± 6.6
		vertical	100	64.0	1.9 ± 0.8	55.7 ± 81.5	0.10 ± 0.14	4.7 ± 6.8
	blue light	horizontal	100	32.0	1.5 ± 1.0	10.6 ± 99.5	0.02 ± 0.17	0.9 ± 8.3
		vertical	100	35.0	1.4 ± 1.0	9.1 ± 94.2	0.02 ± 0.16	1.0 ± 8.1
F2	daylight	horizontal	80	76.3	2.2 ± 1.0	54.1 ± 70.5	0.13 ± 0.16	9.0 ± 11.7
		vertical	109	79.8	2.1 ± 0.9	50.4 ± 61.9	0.12 ± 0.14	8.5 ± 10.3
	blue light	horizontal	100	91.0	2.1 ± 0.9	52.0 ± 63.4	0.12 ± 0.15	8.7 ± 10.6
		vertical	88	80.7	2.2 ± 0.7	54.4 ± 50.7	0.13 ± 0.12	9.1 ± 8.5
	daylight, -A	horizontal	88	83.0	2.6 ± 0.9	82.7 ± 61.9	0.19 ± 0.14	13.8 ± 10.3
		vertical	82	82.9	2.1 ± 0.9	49.7 ± 60.8	0.12 ± 0.14	8.3 ± 10.1

Table 3. Early development of the F1 and F2 generation of *Favia fragum* under different light sources (400W HQI) including non-acclimation (-A) over 6 months (mean ± SD). [a] Diameter after 6 months.

conditions similar to field populations. The gene pool of the captive raised adult population (F1 generation) is the result of selection under the specific conditions of re-circulation systems. Therefore, it might be possible that genotypes of the F2 propagules are better adapted to mariculture conditions than those of F1 propagules originating from field-collected parents. Our observations imply rapid domestication in corals, which may be of major importance in mariculture and breeding to supply the ornamental trade and public aquariums (see Rinkevich and Shafir, 2000). However, this hypothesis has to be critically evaluated through genetic investigation. In this context, it should be emphasized that the development of individual primary polyps highly differed in a treatment, e.g. primary polyps sometimes exhibited high growth rates while others were shrinking and showed necrosis, even when located on the same tile just a few millimeters next to each other. Another explanation for the higher survival and growth in the F2 generation could be the slightly larger initial diameter of primary polyps. Larger initial sizes may lead to higher initial survival and growth. The observed primary polyp sizes may result from slightly larger larvae volumes found in the F2 generation compared to the F1 generation (chapter 6).

In conclusion, light spectrum can influence the survival and growth of early life stages of scleractinian corals in mariculture. Irradiance of the applied light sources was sufficient to achieve high survival and medium growth rates. My results further indicate that other factors such as surface orientation, additional energy sources (live and frozen food), densities of algal grazers, and population genetics may be at least as important as the factor light to achieve high survival and growth rates. Regarding the most economic method to mariculture corals using artificial light sources, more research is necessary for better understanding species-specific needs and temporal changes, depending on life history stage. Such temporal changes might require the application of different light regimes over time. In order to evaluate interaction between factors, multifactorial analysis of variance involving more species is needed. However, such an experimental design would require more than one culture system and higher amounts of primary polyps. Since corals have high requirements on culture conditions, the set up of a series of identical culture systems will be cost and labor intensive. Furthermore, genetic aspects will play a major role in future coral mariculture regarding potential fitness threats (e.g. inbreeding and genetic drift; see chapter 12). Depending on the specific application such as the sustainable management of ex situ populations in public aquariums, the supply of the trade in ornamentals, and the establishment of breeding programs for endangered species, either high genetic diversity or the isolation of certain genetic lines (domestication) might be favorable.

REFERENCES

Aden, W.H., Loveland, K. 1998. Dynamic aquaria: building living ecosystems. London: Academic Press. 498 p.

Anthony, K.R.N., Hoegh-Guldberg, O., 2003. Kinetics of photoacclimation in corals. Oegologia 134(1), 23-31.

Babcock, R.C., Mundy, C. 1996. Coral recruitment: consequences of settlement choice for early growth and survivorship in two scleractinians. J Exp Mar Biol Ecol 206, 179-201.

Bausoch, K. J., S. Leota, S. Victor, Richmond, R. 2000. Laboratory cultivation of coral juveniles. 9th Int. Coral Reef Symp., Bali. Abstracts: new posters.

Brown, B.E., 1997. Disturbances to reefs in recent times. In: Birkeland C (ed) Life and death of coral reefs. Chapman & Hall: New York, pp 354-379.

Carlson, B.A., 1987. Aquarium systems for living corals. Int. Zoo Yb. 26, 1-9.

Carlson, B.A. 1999. Organism responses to rapid change: what aquaria tell us about nature. Amer. Zool. 39, 44-55.

Delbeek, J.C., Sprung J. 1996. Das Riffaquarium. Vol. 1. Coconut Grove: Ricordea Publishing. p. 544.

Delbeek, J.C. 2001. Coral farming: past, present and future trends. Aquarium Sciences and Conservation 3: 171-181.

Epstein, N., Bak, R.P.M., 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.

Falkowski, P.G., Jokiel, P.L., Kinzie III, R.A. 1990. Irradiance and corals. In Dubinsky Z, editor. Ecosystems of the world vol 25, coral reefs. Amsterdam: Elvesier, p 89-107.

Fan, T.Y., Li, J.J., Liu, M.C., Fang, L.S., 2000. The application of sexual reproduction to enrich a live coral tank. 5th International Aquarium Congress, Monaco. Abstracts, p 41.

Gateno, D., Barki, Y., Rinkevich, B. 2000. Aquarium maintenance of reef octocorals raised from field collected larvae. Aquarium Sciences and Conservation 2, 227-236.

Green, E.P., Shirley F, 1999. The global trade in coral. World Conservation Monitoring Centre. World Conservation Press: Cambridge, UK.

Grigg, R.W., Dollar, S.J., 1990. Natural and anthropogenic disturbance on coral reefs. In: Dubinsky Z (ed) Ecosystems of the world, vol 25, coral reefs. Elvesier: Amsterdam, pp 439-452.

Harrison, P.L., Wallace, C.C. 1990. Reproduction, dispersal and recruitment of scleractinian corals. In: Dubinsky Z. (ed.) Ecosystems of the world. 25. Coral reefs. Elvesier, Amsterdam, pp 133-207

Hatta, M., Iwao, K., 2003. Metamorphosis induction and its possible application to coral seedlings production. In: Saxena N (ed) Recent Advances in Marine Science and Technology, 2002. Japan International Marine Science and Technology Federation Akasaka, Minato-ku, Tokyo, pp 465-470.

Heyward, A.J., Smith, L.D., Rees, M., Field, S.N., 2002. Enhancement of coral recruitment by in situ mass culture of coral larvae. Mar. Ecol. Prog. Ser. 230, 113-118.

Jones, S.H., 2002. Artificial lighting in the reef aquarium. Sea Scope 19, 3-4.

Maida, M., Coll, J.C., Sammarco, P.W. 1994. Shedding new light on scleractinian coral recruitment. Journal of Experimental Marine Biology and Ecology 180: 189-202.

Marubini, F., Barnett, H., Langdon, C., Atkinson, M.J., 2001. Dependence of calcification on light and carbonate ion concentration for the hermatypic coral *Porites compressa*. Mar. Ecol. Prog. Ser. 220, 153-162.

Petersen, D., Tollrian, R. 2001. Methods to enhance sexual recruitment for restoration of damaged reefs. Bull. Mar. Sci. 69(2): 989-1000.

Rinkevich, B., Shafir, S., 2000. Ex situ culture of colonial marine ornamental invertebrates: concepts for domestication. Aquarium Sciences and Conservation 2, 237-250.

Sato, M. 1985. Mortality and growth of juvenile coral *Pocillopora damicornis* (Linaeus). Coral Reefs 4: 27-33.

Sebens K.P., Grace S.P., Helmuth B., Maney Jr. E.J., Miles J.S. 1998. Water flow and prey capture by three scleractinian corals, *Madracis mirabilis*, *Montastraea cavernosa* and *Porites porites*, in a field enclosure. Mar. Biol. 131:347-360.

Szmant, A.M. 1986. Reproductive ecology of Caribbean reef corals. Coral Reefs 5, 43-54.

Sorokin, Y.I. 1995. Coral reef ecology. 102. Ecological studies. 2nd printing. Berlin: Springer, 465 p.

Titlyanov E., Titlyanova, T.V., Yamazato, K., 2000. Formation, growth and photo-acclimation of colonies of the hermatypic coral *Galaxea fascicularis* under different light conditions. Symbiosis 30(4), 257-274.

Van Moorsel, G.W.N.M., 1988. Early maximum growth of stony corals (Scleractinia) after settlement on artificial substrata on a Caribbean reef. Mar. Ecol. Prog. Ser. 50: 127-135.

Vermeij, M.J.A., Bak, R.P.M., 2002. How are coral populations structured by light? Marine light regimes and the distribution of *Madracis*. Mar. Ecol. Prog. Ser. 233:105-116.

CHAPTER

10

Sexual Recruitment of the Corals *Favia fragum* and *Agaricia humilis* in a 30 m^3 Exhibit Aquarium

Species-Specific Limitations and Implications on Reproductive Ecology

INTRODUCTION

Today's public aquariums show a continuous trend to build larger exhibits (i.e. see Alvarez and Garcia, 2001; Janse and Wensing, 2001) offering a great experience to the visitor and new opportunities for research. Multi-species exhibits like live coral aquariums allow us to study the ecology of displayed animals in a relatively well defined biological and non-biological environment (Carlson, 1999; see also Adey and Loveland, 1998). In the past, the sudden appearance of juvenile scleractinian corals in exhibit tanks, in particular cases the result of sexual reproduction, has been observed for some species (see chapter 2). Scientific investigation to gain better understanding of this phenomenon have hardly been carried out. The process described in this chapter has been defined in the field as 'coral recruitment' (see chapter 1). When recruitment occurs, a noticeable decrease of mortality can be observed (Sorokin, 1995) as a result of juveniles with increasing size being less affected by environmental factors such as sedimentation (Wittenberg and Hunte, 1992) and algal growth (McCook et al., 2001). Recruitment rate can be understood as a measure the reproductive success under local environmental conditions. These conditions can particularly influence gamete production, fertilization, planktonic stage and settlement of larvae, and early life stages of settlers.

In this chapter, I have investigated the process of coral recruitment in a closed-system exhibit aquarium. I observed recruits of *Favia fragum* and *Agaricia humilis* after field-collected specimens were temporarily maintained in a $30m^3$ closed-system mesocosm (for definition see Adey and Loveland, 1998). One and half years later, I observed recruitment of the F2 generation of *F. fragum*. I analyzed the population structure of both species and estimated the recruitment rate in relation to environmental factors and depending on species-specific reproductive behavior.

METHODS

Aquarium System

Technical properties

The exhibit was 19 months in operation (start April 2000) when parental colonies of the studied species were introduced. The tank (8.0 x 1.6 x 2.3 m; L x W x H) has a gross water volume of 29,440 l with additionally 11,300 L water volume of the life support system (= LSS; Intensive Aquaculture Technology LTD., U.K.). Artificial rockwork made from a sand and concrete mixture sealed by acid stain (Dave Manwarren Corp., USA) takes approx. 25 % of the total tank volume. At the start approx. 300 kg of 'Live Rock' (coral reef substrate containing all its associated organisms; here: aquacultured in Florida) was introduced. After the artificial rockwork had been covered with marine algae and other benthic organisms most of it was removed again.

A residence time of 1.8 hr was calculated before water passes again the LSS. First the water is collected in a sump tank (2.5 x 1.5 m; diameter x H) to which a protein skimmer (1.8 x 2.5 m; diameter x H; flow rate 20,000 l h^{-1}) and a heat exchanger are connected in separate bypasses. Before water is pumped into the exhibit tank it has to pass 2 sand pressure filters. No ozone or UV sterilization are applied.

In the exhibit water motion is generated by controlling water circulation by means of pneumatic valves connected to a digital timer (the valves were installed in the later phase of the study period) and an independent circulation system: three automatic siphon devices (volume 500 L) are placed approx. 2.5 m above the water surface of the exhibit. The devices

principally function according to the Carlson Surge Device designed by Bruce Carlson, Waikiki Aquarium, Hawaii (for further details see Delbeek & Sprung 1996). Approx. every 10 minutes the devices unload their complete volume in less than 30 s creating a strong and alternating surge. A total flow of approx. 50,000 l h^{-1} is generated in the tank.

Regarding lighting, we use five 2 KW HQI 6,000 K (daylight spectrum) and 1 KW HQI 6,000 K (replaced by 1 KW HQI 20,000 K = blue spectrum at the end of the study period) of each in the deeper zones, and seven 400 W HQI 10,000 K (intermediate between daylight and blue spectrum) for variable use in the shallow zones and behind the artificial rock wall of the tank (total: ± 1.39 KW m^{-2}). The 2 KW bulbs operate daily for 5 hr, all others for 12 hr. All lamps are switched on and off successively in pairs of two. The tank is neither exposed to artificial nor to natural moonlight. A constant nocturnal lighting from surrounding building safety installations partly illuminates the tank (0.217 ± 0.106 µmol m^{-2} s^{-1}; see chapter 6).

Approx. 25 % of the total water volume of the system (33,380 L) is monthly exchanged due to sand filter backwashing. We use natural seawater, regularly collected by tank ships offshore in the Atlantic Ocean. Two calcium reactors (Jetstream 2, Schuran Seawater Equipment, Germany) are used and commercial water additives are regularly added to supply certain elements, which are continuously consumed especially by corals. Temperature and salinity are constantly kept at 26 ºC and at 36 ‰.

Live Stock

The mesocosm aquarium exclusively exhibits organisms from the Caribbean. Here I only list organisms, which might be relevant for the present study.

The benthos is mainly dominated by algae (crustose coralline algae, green turf algae and cyanobacteria) and by cnidarians like scleractinians (*Agaricia humilis, Diploria strigosa, Eusmilia fastigiata, Favia fragum, Madracis* spp., *Meandrina meandrites, Montastraea annularis, Porites asteroides, Siderastraea siderea*), gorgonians (*Eunicea* spp., *Gorgonia vandalia, Muricea muricata, Pterogorgia citrina, Pseudoptergogorgia* spp., *Pleaxaurella* spp.), and anemones (*Condylactis gigantea*).

Fish can be divided into 3 relevant groups: (1) herbivorous, (2) planktivorous and (3) coralivorous. (1) The tank contains a group of 18 Acanthuridae (*Acanthurus bahianus, A. coeruleus*) and 15 *Scartella cristata*. (2) and (3) During the study period only three planktivorous *Gramma loreto* were living in the tank and a couple of the Indo Pacific butterfly fish *Chelmon rostratus* was temporarily introduced to remove *Aiptasia* sp. Consumption of coral plankton by other fish (e.g. Labridae) may not be completely excluded, due to possible changes in feeding habits in captivity.

For additional algal control 250 hermit crabs (*Paguristes* spp.), 585 snails (*Astraea* spp., *Cerithium litteratum, Nodolittorina tuberculata*) and 335 sea urchins (*Echinometra lucunter*) were successively introduced.

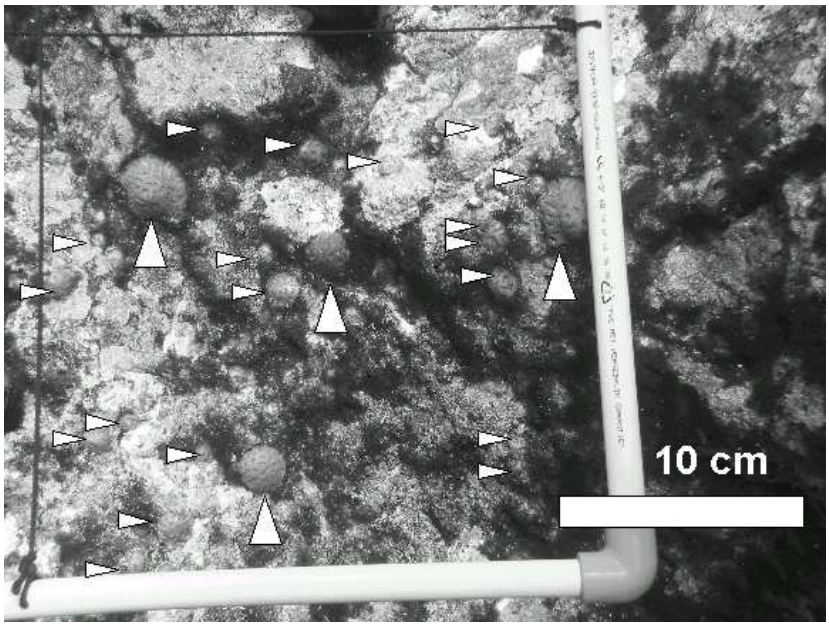

Fig. 1 High local densities of the F1 and F2 generations of *Favia fragum*, here in a sub-square of a large transect (F1 specimens: vertical large arrows; F2 specimens: horizontal small arrows).

Parental Corals

In November 2001, 30 adult colonies of each, *Agaricia humilis* and *Favia fragum* were collected at the fringing reef of Curaçao and transported to Rotterdam Zoo using a modified protocol of chapter 3. After arrival at Rotterdam Zoo, corals were acclimated to local water conditions (for protocol see chapter 3) and placed in the coral exhibit onto polystyrene grids at the back part of the tank behind the artificial rock wall, not visible to the visitor. The coral specimens that were aimed for the experiments of this dissertation stayed in the exhibit tank until the experimental tanks in the laboratory were fully operational. After 3 months all specimens were removed; no additional specimens of both species were present in the exhibit at any time.

Population Assessment of Juveniles

I aimed at assessing (1) the total number of recruits and (2) the mean density as standard values for possible comparison with other studies. I used 2 different methods: (1) the surface relief of the tank was divided into fields defined by landmarks and surface orientation. All visible specimens of the F1 and F2 generation of both species were counted 3 times for each field on subsequent days by one person positioned in the public area in front of the acrylic panel. In order to calculate the mean density per field, the surface of each field was measured 2-dimensionally (AxioVision 3.1, Carl Zeiss Vision GmbH Germany). (2) transects of 50 x 50 cm and 25 x 25 cm (subdivided into 4 squares, each 25 x 25 cm and 12.5 x 12.5 cm) were placed randomly on the artificial rockwork, a digital picture of each transect and of each square was taken (Canon Powershot G2; Ikelite Digital Underwater Housing) by a diver, and the amount of colonies per surface unit was then counted (Adobe Photoshop 7.0). The surface area and max. diameter of the transected colony was measured digitally whenever the position of the specimen on the photograph ensured an appropriate measurement (AxioVision 3.1, Carl Zeiss Vision GmbH Germany). The large transects were used to assess the F1, the small transects served for the F2 generations. Additionally, the max. diameter of 10 colonies was monthly measured for a duration of 5 months. These colonies were situated horizontally behind the artificial rock wall of the exhibit.

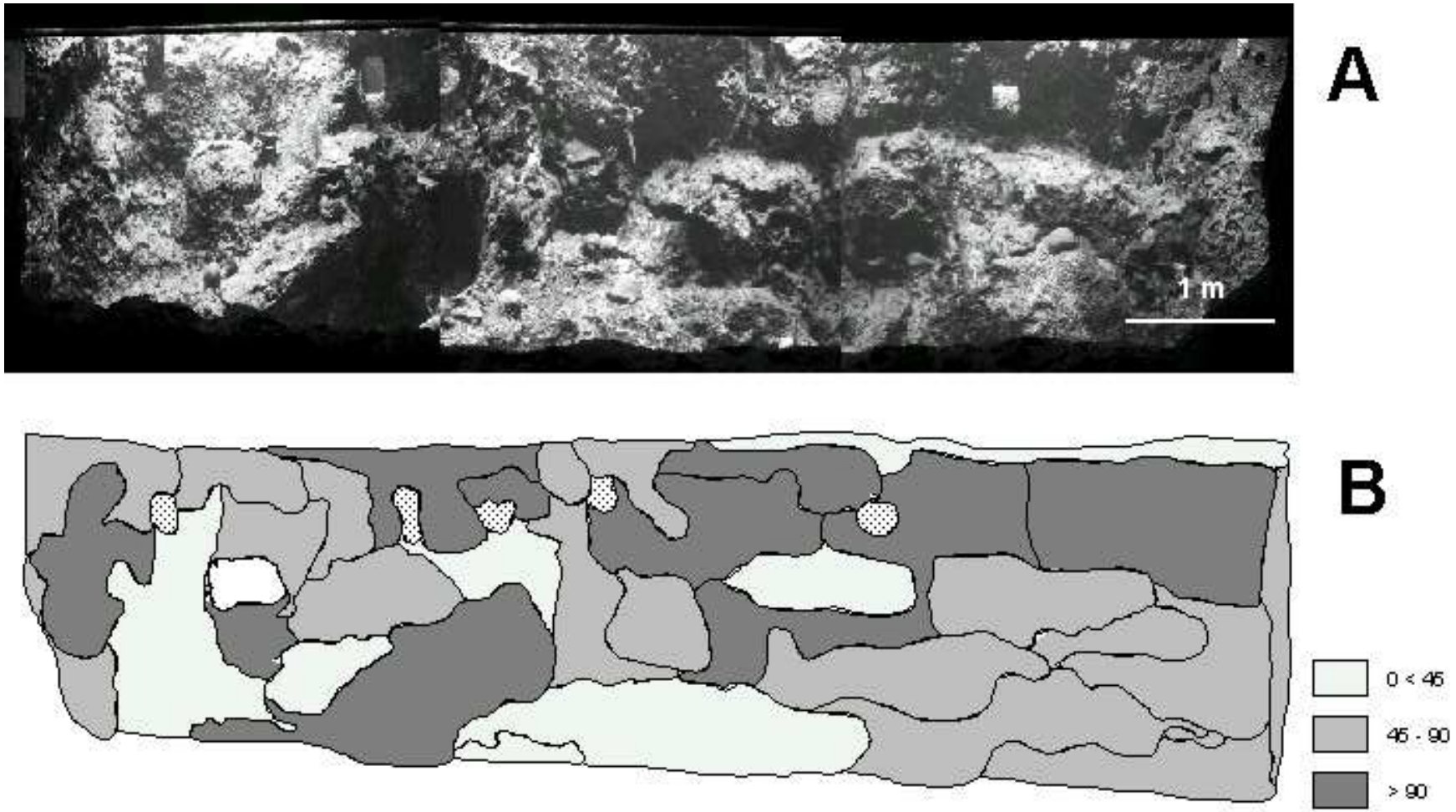

Fig. 2 A) Overview of the exhibit made from a series of 5 photographs. B) Map showing distribution of fields with different surface orientation (0º < 45º, 45º-90º, > 90º). Note: since fields were defined using photographs and the exhibit itself, Fig. 2A does not consequently reflect the distribution of the fields in Fig. 2B.


A
B

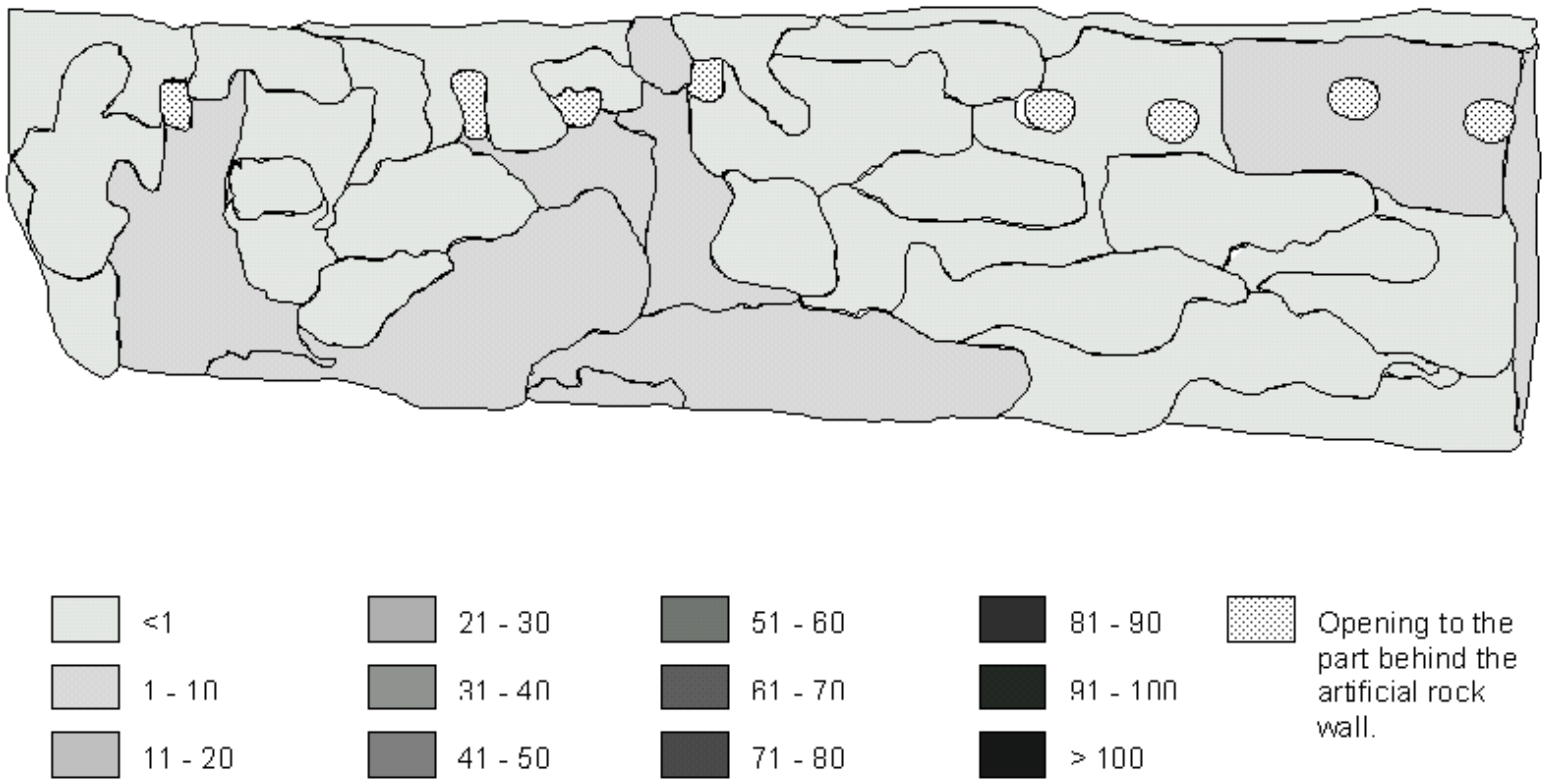

Fig. 3 Recruit densities of all populations per m^2. A) F1 generation of *Favia fragum*. B) F2 generation of *F. fragum*. C) F1 generation of *Agaricia humilis*.

Measurement of Environmental Factors

Substrate orientation was analyzed to estimate its influence on the recruitment of *Agaricia humilis* and *Favia fragum* in the exhibit tank. Substrate orientation was related to the surface relief fields and transects by categorizing them in (1) horizontal (0 < 45º), (2) vertical (45 – 90º), and (3) overhanging (> 90º). The category of each field and transect was determined by the general orientation of the surface area. Light intensity was measured in different water depths below the 1 KW and 2 KW HQI lamps using a spherical light sensor (LI-193SA; LICOR) connected to a LI-1400 datalogger (LICOR). The sensor measures the quantum flux between 400 and 700 nm (photosynthetically active radiance = PAR). The average amount of light was calculated by taking a measurement every second for duration of 30 s to minimize the influence of light refraction at the water surface.

To estimate the influence of established corals on the recruitment of the studied species, their mean density (surface area covered by corals m^{-2}) was measured using the large transects.

Water chemistry was routinely monitored during the entire period. Analyses for calcium, nitrogen, phosphorous and magnesium were carried out using photo spectrometry (DR/4000U Photospectrometer, HACH Company U.S.A.).

Data Analysis

Analysis of variance (ANOVA) Model Typ III for a non-balanced design was originally planned to be used for data evaluation. As samples did not fulfill assumption of homogeneity of variance, the influence of substrate orientation on recruit density and colony size was studied with a Kruskal-Wallis ANOVA (SPSS 12.0). Whenever significance was detected, a posteriori comparisons of mean ranks of all pairs of groups were carried out following Siegel and Castellan (1988). Due to the topography of the artificial rock wall with almost only vertical and overhanging surfaces especially in the upper part of the tank, it is difficult to statistically study a potential influence of water depth on the population structure. Therefore I used the field maps to discuss possible influences.

RESULTS

In 3 subsequent counts, the method to divide the surface relief into fields showed a total of 146.7 ± 31.9 (mean ± SD) recruits of the F1 generation, 427 ± 87.6 (mean ± SD) recruits of the F2 generation in *Favia fragum*, whereas *Agaricia humilis* showed a total of 5 ± 1.2 (mean ± SD) recruits of the F1 generation and no F2 recruits. Using the transecting method (28 transects for F1, 21 transects for F2 generation), a total of 101 recruits of the F1 and 261 recruits of the F2 generation were found in *F. fragum* (see Fig. 1), whereas only 7 colonies of the F1 generation in *A. humilis* were counted in total (F2 generation: no recruits). F1 colonies (n = 64) of *F. fragum* had a colony surface of 5.48 ± 3.85 cm^2 (mean ± SD) with a maximum diameter of 3.12 ± 0.98 cm (mean ± SD), F2 recruits (n = 217) had a surface of 0.54 ± 0.53 cm^2 (mean ± SD) with a max. diameter of 0.83 ± 0.41 (mean ± SD). *A. humilis* recruits (n = 7) showed a surface of 7.15 ± 3.89 cm^2 (mean ± SD) and a max. diameter of 3.80 ± 1.14 cm (mean ± SD). The F1 specimens of both species were approx. 2 years old, when they were measured. Because of the low number of recruits in *A. humilis*, this species was not statistically analyzed.

The tank (see Fig. 2A) divided in 35 field sections of different surface orientation (Fig. 2B) clearly shows a dominance of vertical and overhanging surfaces. Fig 3A-C gives an overview of the densities of each population m^{-2}. When comparing mean densities per orientation category, calculated after the field method (Fig. 4B) with those of the transecting

method (Fig. 4A) similar tendencies are shown. Recruit densities were highest on horizontal surfaces (0 < 45˚), followed by vertical surfaces (45 – 90˚), and lowest on overhanging surfaces (> 90˚). Nevertheless, in both generations of *F. fragum* the absolute values of the means differ between both methods, especially on the horizontal and vertical surfaces. Recruit densities of *F. fragum* differ between differently oriented fields (Kruskal-Wallis ANOVA, F1: n = 35, H = 8.020, p = 0.018; F2: n = 35, H = 16.140, p < 0.001) and between differently oriented transects (Kruskal-Wallis ANOVA, F1: n = 28, H = 7.652, p = 0.022; F2: n = 21, H = 7.684, p = 0.021). In both assessment methods densities of the F1 generation were only significantly higher on horizontal surfaces compared to those on overhanging surfaces (Table 1). The analysis of the F2 generation showed similar tendencies, except for the field method, where densities on horizontal surfaces were significantly higher compared to vertical and overhanging surfaces (Table 1 and Fig 3). It has to be noted that the field method showed higher significance compared to the transect method. Higher densities (> 10 recruits m^{-2}) of the F1 generation of *F. fragum* were found in all depth zones of the exhibit, highest densities of > 40 and > 100 recruits m^{-2} occurred at horizontal surfaces on top and near the bottom of the tank (Fig. 3A). Similar tendencies are shown in the F2 generation, however, highest densities of > 50 recruits m^{-2} can be observed more towards the center of the tank (Fig. 3B). It should be emphasized that the only horizontal surface area at the top of the rock wall represents a connection between the front and back part of the tank, where the parental colonies were maintained (Fig. 2B). More than half of the rock wall (to the left) reaches throughout the water surface. Additional connections to the back part are represented by several holes with a diameter of up to 30 cm in the upper part of the tank. Through these holes larvae could additionally reach the front part of the tank.

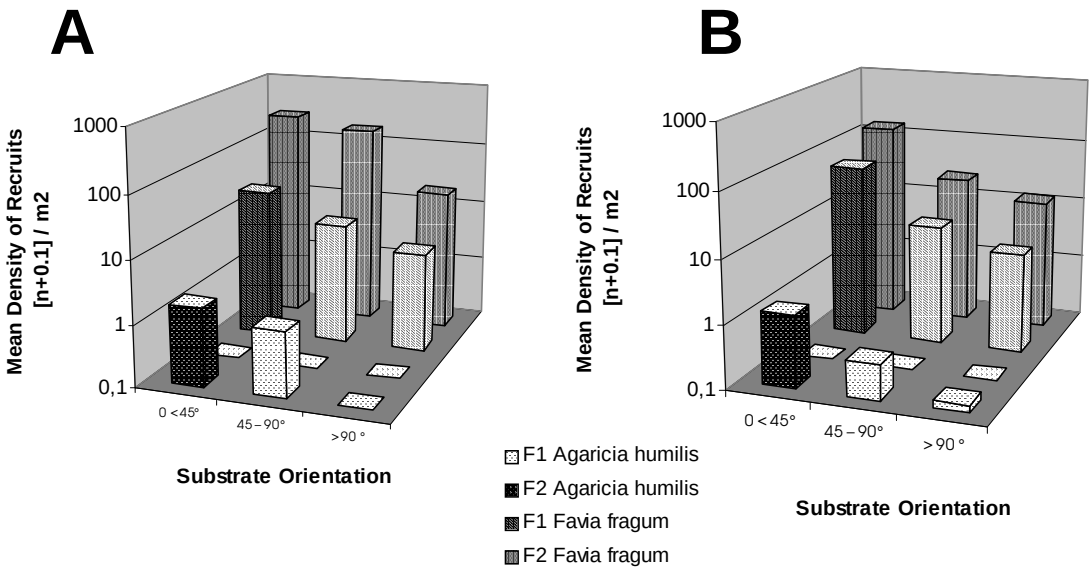

Fig. 4 Recruit densities for all populations depending on substrate orientation. Assessment using A) transects or B) field maps. Note logarithmic scale.

The colony size of the recruits was not dependent on the surface orientation (Kruskal-Wallis ANOVA, F1: n = 64, H = 2.424, p = 0.298; F2: n = 217, H = 0.798, p = 0.671) in both generations (see also Fig. 4). Maximum colony sizes occurred in all depths of the tank. Colony sizes highly varied between specimens of the F1 generation as indicated in Fig. 5. A mean growth rate of 0.16 ± 0.06 cm $month^{-1}$ (mean ± SD) was calculated for 10 colonies, which had a max. diameter of 2.94 ± 0.49 cm (mean ± SD) at the beginning of the monitoring period.

Light intensity was with 1537.33 ± 162.68 µmol m^{-2} s^{-1} (mean ± SD) highest below the 2KW lamps in a water depth of 0.1 m. Differences between different types of lamp decreased with increasing water depth (Fig. 6) and finally ranged between 561.37 ± 4.01 µmol m^{-2} s^{-1} (mean ± SD; 1KW HQI 6,000 K) and 283.57 ± 6.09 µmol m^{-2} s^{-1} (mean ± SD; 1KW HQI 20,000 K).

All water parameters are within natural limits except nitrate, which shows slightly higher levels (Table 2).

Using the large transects, I calculated a total coral cover of 4.69 % (including scleractinians, gorgonians and anemones).

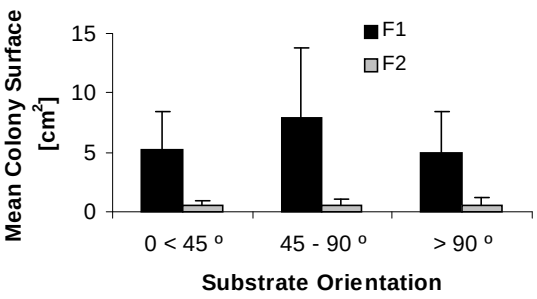

Fig. 5 Colony size of the F1 and F2 generations of *Favia fragum* on different oriented surfaces (n_{F1} = 64, n_{F2} = 217; mean + SD).

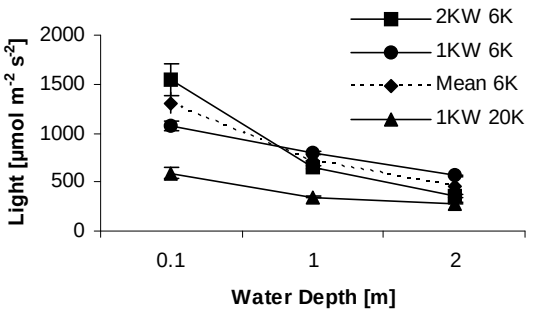

Fig. 6 Light distribution in the exhibit below different light units. 2KW 6 K = 2,000 W HQI 6,000 K; 1 KW 6K = 1,000 W HQI 6,000 K; 1 KW 20 K = 1,000 W HQI 20,000 K

DISCUSSION

The population structure of *Favia fragum* and *Agaricia humilis* reflects their life history under the specific circumstances of an aquarium exhibit. The distribution of the F2 generation of *F. fragum* is the result of the number of released larvae, larval survivorship, settlement rate and early recruitment rate of juveniles. The distribution of the F1 generations of both species involves the whole recruitment process until the specimens reached sexual maturity (proven in the case of *F. fragum*, see below). The water chemistry of the exhibit shows a high water quality similar to field conditions. The higher nitrate concentrations are at levels that did not show growth limitation in captive corals (see Atkinson et al., 1995).

The applied methods seem to be appropriate to assess the different populations and generally reflect the same tendencies in the present study. However, the transect method showed the tendency to overestimate densities, whereas the field method might lead to an underestimation of the real population densities. Additionally, the latter method showed a relatively high standard deviation of approx. 20 % in 3 subsequent counts by one person. Whether to spot-check or to count all specimens in a particular case might depend on the size of the system and on logistical reasons, such as available equipment, the possibility to dive in a system, and available assistance (aquarium staff or students).

Planulation and Coral Plankton

From controlled planulation studies of the same populations (chapter 8) I can estimate that 30 colonies of *Favia fragum* release approx. 6,000 larvae compared to approx. 8,700 larvae in *Agaricia humilis* in 3 months. Under laboratory conditions (chapter 8), approx. 40 % of released planulae in *F. fragum* settled within 12 hr after release compared to less than 14.5 % in *A. humilis*. Within 12-36 hr after release, 55 % of the remaining larvae of *F. fragum* and 40 % of *A. humilis* settled. Larvae of *F. fragum* are generally negatively buoyant right after release (Carlon, 2002; personal observation), whereas those of *A. humilis* usually stay at the water surface for at least 12 hr (personal observation). Although the number of released larvae is higher in *A. humilis*, far less propagules will get the chance to attach due to a delay in settlement. The majority of larvae, especially of *A. humilis* will either be lost when passing the filtration units of the LSS or be consumed by planktivorous fish (Westneat and Resing, 1988). Additionally, established corals may consume high amounts of planktonic planulae (Fabricius and Metzner, 2004; see also Sebens et al., 1998). However, predation by planktivorous organisms is probably of minor importance in the present study due to the extremely low density of planktivorous fish and adult corals.

			z	**p**
Fields	F1	horizontal*vertical	1.998	0.137
		horizontal*overhanging	2.802	0.015*
		vertical*overhanging	1.255	0.628
	F2	horizontal*vertical	3.059	0.007**
		horizontal*overhanging	3.925	<0.001***
		vertical*overhanging	1.487	0.411
Transects	F1	horizontal*vertical	2.133	0.100
		horizontal*overhanging	2.402	0.048*
		vertical*overhanging	0.358	1.000
	F2	horizontal*vertical	0.650	1.000
		horizontal*overhanging	2.617	0.027*
		vertical*overhanging	2.280	0.068

Table 1 *Favia fragum*, F1 and F2 generation. Recruit density differences depending on substrate orientation. A posteriori multiple comparison after Siegel and Castellan (1988). Stars indicate significance level.

Settlement and Recruitment

Inhibition of settlement by established corals observed in the field (Maida et al., 1995; Fearon and Cameron, 1996) might play a minor role in the present study due to the low coral cover (< 5 %) as mentioned above. Other factors, such as (1) light (Mundy and Babcock, 1998), (2) filamentous algae (Tomascik, 1991) and (3) sedimentation (Hunte and Wittenberg, 1992) may have mainly influenced the settlement rate in the aquarium exhibit. The same effects may further influence the early development of juveniles as shown by various field studies (McCook et al., 2001; Babcock and Mundy, 1996; Wittenberg and Hunte, 1992; see also Falkowski et al., 1990).

(1) The F1 and F2 generation in *Favia fragum* showed highest densities on horizontal followed by vertical surfaces. Although not statistically tested, colony densities seemed to be independently of water depth. Colonies of the F1 generation of *Agaricia humilis* showed the same tendency. The overall light regime of the exhibit from the surface to a depth of 2 m represents the light distribution found in the field on light exposed surfaces in shallow waters to a depth of 20-30 m (Vermeij and Bak, 2002). Laboratory studies with larvae of the same populations showed high settlement rates (> 50%) even under lower light regimes than recorded in the exhibit (< 100 µmol m^{-2} s^{-1}; see chapter 8), which confirms my hypothesis of depth independent settlement in the exhibit provided that other factors like

	Temp.	DO	pH	Salinity	Alkalinity	NH3-N	NO2-N	NO3-N	Ca	Mg	PO4-P
	℃	%		‰	meq/l	all in mg/l					
Exhibit (± SD)	26.0 ± 0.4	100.4 ± 1.3	8.0 ± 0.1	36.0 ± 0.4	3.1 ± 0.6	0.001 ± 0.005	0.004 ± 0.001	1.2 ± 0.4	457.4 ± 13.8	1338.7 ± 20.9	0.010 ± 0.005
Field	25.4 – 29.0[1]	-	8.0 – 8.4[2]	35.8 – 36.2[3]	-	0.0003 - 0,023[5]	0.0003 – 0.015[5]	0.0016 – 0.016[5]	416.0[4]	1300.0[4]	0.0007 – 0.023[5]

Table 2. Water chemistry of the exhibit tank. Parameters were measured weekly, approximately 2 hr after the start of illumination. [1] at Curaçao (Van Moorsel, 1983); [2] after Sorokin (1995); [3] at Curaçao (personal observation); [4] after Adey and Loveland (1998), [5] calculated after Delbeek and Sprung (1996)

dispersal are excluded. As a result, specimens are found in the exhibit under light conditions of a water depth, which does not correspond to the distribution of the species in the field (Szmant-Froehlich et al., 1985; Van Moorsel, 1983; Van den Hoek and Breeman, 1978). Depth-dependent settlement was shown for some scleractinians under laboratory conditions with light as a proxy for water depth (Mundy and Babcock, 1998). However, a recent study of Vermeij and Bak (2002) emphasizes the importance of the light regime of the microhabitat, which represents an environment of a few square centimeters to even only a few square millimeters. In the present study, the distribution of both species emphasizes the role of the microhabitat on the settlement and recruitment, which may offer low light intensities even in shallow waters. Therefore, other factors such as a limited supply of larvae in greater depths of the reef, higher competition by other benthic organisms (sponges, etc.) or lower growth rates under low light regimes might be important factors affecting the distribution of *F. fragum* and *A. humilis* in the field. Light intensity can change drastically within a range of a few centimeters due to the surface structure (orientation) of the microhabitat (Vermeij and Bak, 2002). These small scale differences were not assessed in the present study. Nevertheless, corals can neutralize low light levels till a species-specific limit by photoadaptation (Falkowski et al., 1990), which can explain a depth and orientation-independent colony size in the F1 and F2 population of *F. fragum* in the present study. It should be emphasized that corals can use other energy sources than their symbiotic zooxanthellae. The feeding of the fish population in the tank could be an additional food source for the studied corals. The max. growth rate of *F. fragum* and *Agaricia humilis* in laboratory tanks, where no additional organic food was added was less (< 1.0 mm month^{-1}; chapter 9) compared to the growth in systems with additional food sources such as live and deep frozen plankton (> 1.0 mm month^{-1}) (see chapter 11). However, the importance of planktonic food on coral growth and reproduction needs further investigation. In the present study colony growth was only monitored under one condition for a limited time of 5 months, therefore we can not determine whether the specimens of the F1 generations reached maturity at different time e.g. depending on different growth rates related to surface orientation respectively to light intensity. In general, growth rates are influenced by substrate orientation; highest rates may be found on horizontal (= light exposed) surfaces (Babcock and Mundy, 1996). Using the colony sizes measured approx. 2 years after larval settlement, I can estimate a mean growth rate of 0.13 mm month^{-1} in *F. fragum*, which corresponds with extension rates under field conditions (Van Moorsel, 1988). Mean growth of *A. humilis* of 0.16 mm month^{-1} was less than those observed in the field (2.4 mm month^{-1}; Van Moorsel, 1988). Further, I cannot exclude that growth rates can change over time and between different life stages (see Van Moorsel, 1988). Furthermore, some individuals of the F1 generation in *F. fragum* might have shown lower growth rates than the average and were still below a diameter of 2 cm, thus were assessed as F2 specimens.

(2) and (3) Although algal cover and sedimentation was not monitored, we observed certain tendencies. Total cover of Chlorophyta, Rhodophyta and cyanobacteria varied over time, especially cyanobacteria temporarily bloomed in certain areas of the tank where water movement was less turbulent. Sediments accumulated in these regions of the tank as well. Sediments were removed successively once a month, whereas cyanobacteria were removed whenever necessary. Sedimentation occurred mainly on horizontal surfaces, whereas cyanobacteria appeared independent of orientation and light intensity on all surfaces of low flow regimes. The presence of herbivorous grazers kept green algal turfs short, which favored the growth of crustose coralline algae, which may induce settlement in scleractinians (Morse et al., 1996; Negri et al., 2001). Sedimentation and algal growth limit coral recruitment on horizontal surfaces in the field (Babcock and Mundy, 1996; Hunte and Wittenberg, 1992; Tomascik, 1991; Wittenberg and Hunte, 1992). These negative influences can be minimized in captivity as shown in the present study. However, the spatial distribution, especially of the

F2 generation of *F. fragum* shown in the field maps, shows higher recruit densities in the center of the tank, where highest turbulence was present before the pneumatic valves were installed. Research on the influence of turbidity on the settlement and recruitment of corals in the aquarium environment would help to better understand captive reproduction.

In conclusion, the extremely high annual recruitment rate of *F. fragum* (F2 generation: 137 recruits m^{-2} with field method, 272 recruits m^{-2} with transects) compared to a general annual recruitment of scleractinians of 5 – 10 recruits m^{-2} (Sorokin, 1995) can be explained by the favorable conditions of the studied exhibit: low predation, low competition by other benthic cnidarians, reduction of sedimentation and algal growth, appropriate light levels and high water quality. Nevertheless, the extremely low recruitment rate of *A. humilis* emphasizes species-specific limitation under captive conditions. Such differences may limit captive reproduction to a degree that although reproduction occurs, no recruitment is observed.

Sexual Maturity of F1 Generations

When looking at the massive appearance of F2 recruits in *Favia fragum* 18 months after the field colonies were introduced in the system, specimens of the F1 generation reached sexual maturity approx. 12 months after birth, some with higher growth rates probably even earlier. This calculation corresponds with field observations that showed initial reproduction at a colony size of 5 – 10 polyps (diameter of approx. 1 – 1.5 cm) (Szmant-Froehlich et al., 1985). Although not investigated in the present study, specimens of the F2 generation might have reached sexual maturity as well, due to a colony size of > 1.5 cm of at least some individuals. *Agaricia humilis* may release larvae at a size of 2.8 cm in diameter (Van Moorsel, 1983). In the present study the direct evidence of planulation in the F1 generation of *F. fragum* was given by collecting larvae after specimens were transplanted on a certain larval collection set up (chapter 6); obtained larvae settled successfully (unpublished data).

When looking at the colony sizes of the F1 recruits of *Agaricia humilis* in the present study (max. diameter recorded: 5.3 cm), at least some of the assessed specimens should have reached maturity as well. However, no recruits were found until the present date. If we assume that gametes were developed, a reason might be the low population density, which can lead to ineffective sperm transfer and therefore to a lack of fertilization (Morse et al., 1996). The few recruits in the exhibit were left undisturbed and were not used for further investigation to determine whether gametes were produced or whether larvae were released. Assuming the latter, the relatively low amount of propagules in combination with a species specific delay in settlement shown for *A. humilis* under captive conditions could be another explanation for the lack of a F2 generation. At the zoos of Hamburg, London and Rotterdam recruitment rates of *A. humilis* were relatively low (chapter 11). The overall sensitivity of *A. humilis* regarding reproduction in captivity contradicts with its high abundance in shallow and highly disturbed natural reef areas (Van Moorsel, 1983; see chapter 8), thus needs further investigation.

REFERENCES

Adey WH, Loveland K. 1998. Dynamic aquaria: building living ecosystems. London: Academic Press. 498 p.

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.

Alvarez J, Garcia M. 2001. The Oceanic Park of Valencia. In Ounais N, Theron D, editors. Proceedings Fifth International Aquarium Congress, Monaco. Bulletin de l'Institut Oceanographique, Monaco, No. special 20(2):175-180.

Babcock RC, Mundy C. 1996. Coral recruitment: consequences of settlement choice for early growth and survivorship in two scleractinians. J Exp Mar Biol Ecol 206:179-201.

Carlon DB. 2002. Production and supply of larvae as determinants of zonation in a brooding tropical coral. J Exp Mar Biol Ecol 268:33-46.

Carlson BA. 1999. Organism responses to rapid changes: what aquaria tell us about nature. Am Zool 39: 44-55.

Delbeek JC, Sprung J. 1996. Das Riffaquarium. Vol. 1. Coconut Grove: Ricordea Publishing. p. 544.

Fabricius KE, Metzner J. 2004. Scleractinian walls of mouths: predation on coral larvae by corals. Coral Reefs 23:245-248.

Falkowski PG, Jokiel PL, Kinzie III RA. 1990. Irradiance and corals. In Dubinsky Z, editor. Ecosystems of the world vol 25, coral reefs. Amsterdam: Elvesier, p 89-107.

Fearon RJ, Cameron AM. 1996. Larvotoxic extracts of the hard coral *Goniopora tenuidens*: allelochemicals that limit settlement of potential competitors? Toxicon 34(3):361-367.

Harrison PL, Wallace CC. 1990. Reproduction, dispersal and recruitment of scleractinian corals. In Dubinsky Z, editor. Ecosystems of the world vol 25, coral reefs. Amsterdam: Elvesier, p 133-207.

Harriott V J, Fisk DA. 1987. A comparison of settlement plate types for experiments on the recruitment of scleractinian corals. Mar Ecol Prog Ser 37:201-108.

Hunte W, Wittenberg M. 1992. Effects of eutrophication and sedimentation on juvenile corals. II. Settlement. Mar Biol 114:625-631.

Janse M, Wensing J. 2001. Burgers' Ocean, a new Indo-Pacific ecodisplay at Burgers' Zoo, Arnhem, The Netherlands. In Ounais N, Theron D, editors. Proceedings Fifth International Aquarium Congress, Monaco. Bulletin de l'Institut Oceanographique, Monaco, No. special 20(2): 331-334.

Maida M, Sammarco PW, Coll JC. 1995. Effects of soft corals on scleractinian coral recruitment. I: Directional allelopathy and inhibition of settlement. Mar Ecol Prog Ser 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.

Morse ANC, Iwao K, Baba M, Shimoike K, Hayashibara T, Omori M. 1996. An ancient chemosensory mechanism brings new life to coral reefs. Biol Bull 191:149-154.

Mundy CN, Babcock RC. 1998. Role of light intensity and spectral quality in coral settlement: Implications for depth-dependent settlement. J Exp Mar Biol Ecol 223:235-255.

Negri AP, Webster NS, Hill RT, Heyward AJ. 2001. Metamorphosis of broadcast spawning corals in response to bacteria isolated from crustose algae. Mar Ecol Prog Ser 223:121-131.

Sebens KP, Grace SP, Helmuth B, Maney Jr. EJ, Miles JS. 1998. Water flow and prey capture by three scleractinian corals, *Madracis mirabilis, Montastraea cavernosa* and *Porites porites,* in a field enclosure. Mar Biol 131:347-360.

Siegel S, Castellan NJ. 1988. Nonparametric statistics for the behavioral sciences. 2nd ed. New York: McGraw-Hill, 399 p.

Sorokin YI. 1995. Coral reef ecology. 102. Ecological studies. 2nd printing. Berlin: Springer, 465 p.

Szmant-Froehlich A, Reutter M, Riggs L. 1985. Sexual reproduction of *Favia fragum* (Esper): lunar patterns of gametogenesis, embryogenesis and planulation in Puerto Rico. Bull Mar Sci 37(3):880-892.

Tomascik T. 1991. Settlement patterns of Caribbean scleractinian corals on artificial substrata along a eutrophication gradient, Barbados, West Indies. Mar Ecol Prog Ser 77:261-269.

Van Moorsel GWNM. 1983. Reproductive strategies in two closely related stony corals (*Agaricia*, Scleractinia). Mar Ecol Prog Ser 13:273-283.

Van Moorsel GWNM. 1988. Early maximum growth of stony corals (Scleractinia) after settlement on artificial substrata on a Carribbean reef. Mar Ecol Prog Ser 50:127-135.

Van den Hoek C, Breeman AM. 1978. The distribution of algae, corals and gorgonians in relation to depth, light attenuation, water movement and grazing pressure in the fringing coral reef of Curaçao, Netherlands Antilles. Aquat Bot 5:1-46.

Vermeij MJA, Bak RPM. 2002. How are coral populations structured by light? Marine light regimes and the distribution of *Madracis*. Marine Ecology Progress Series 233:105-116.

Westneat MW, Resing J. 1998. Predation on coral spawn by planktivorous fish. Coral Reefs 7:89-92.

Wittenberg M, Hunte W. 1992. Effects of eutrophication and sedimentation on juvenile corals. I. Abundance, mortality and community structure. Mar Biol 112: 131-138.

CHAPTER

11

The Application of Sexual Coral Recruits for Sustainable Management of Ex Situ Populations in Public Aquariums to Promote Coral Reef Conservation - SECORE Project

INTRODUCTION

SECORE (SExual COral REproduction) Project is the first attempt to use sexual coral recruits on a larger scale to stock public aquariums sustainably. All methods applied in this project were developed as part of this dissertation. This initial stage of SECORE Project was aimed at estimating the feasibility of such a project, but also to show the potential of today's public aquariums to promote coral reef conservation. Several European aquariums were involved in monitoring the early development of primary polyps.

METHODS

Species

Larvae of *Favia fragum* and *Agaricia humilis* were constantly harvested from aquarium colonies at Rotterdam Zoo following the protocol of chapter 8. Larvae of *Acropora tenuis* were supplied by Masayuki Hatta from Akajima, Okinawa, Japan (see chapter 4).

Settlement

Larvae were settled on ceramic tiles (see chapter 5). Tiles were checked for primary polyps after most larvae had settled, which occurred in 1 - 6 days depending on the species, and then transferred to a closed system aquarium, which was the same system used for the experiments in chapter 9. Polyps remained in this system for approx. one month and were then transported to other European aquariums. During the entire period tiles were kept in polystyrene grids for stabilization and handling reasons (see chapter 9). Additionally to the larvae settlement at Rotterdam Zoo, larvae of *Acropora 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 of chapter 5. In 2002, twenty-four tiles in 250 ml seawater were used per set up. In 2003 the protocol had been adjusted to 40 tiles in 1,500 ml seawater per set up.

Transport of Primary Polyps

In 2003, twelve tiles of each type were arranged chessboard-like in a polystyrene grid and put in a polyethylene box (normally used for fast food). The box was previously prepared by cutting slits (width approx. 1 cm) over the whole length in each sidewall. After placing the grid with tiles in the box, isolation foil was put on top of the tiles and the box was closed. The isolation foil was aimed at preventing the tiles from tumbling over; the slits were aimed at allowing water circulation. Two boxes were placed in one double-sided plastic bag; approx. two liters of water from the culture tank and 100 % oxygen were added before the bags were finally closed. They were put in styrofoam boxes and transported by express service to the following institutions: (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 seawater for at least 2 hours and then transferred to culture tanks. In 2002, the forth aquarium, Burgers' Zoo, acquired polyps of *Acropora tenuis* for this study from settlement experiments conducted at Burgers' Zoo.

Husbandry of juveniles

Specimens were maintained under 250W HQI (distance of tiles to water surface approx. 50 cm). Culture tanks were either independent systems or connected to exhibits, but in all cases closed systems. Water chemistry was kept as close as possible to the optimum.

During the whole experiment, tiles were kept arranged in the grids as described above and were only removed temporarily to be checked one by one under a microscope.

Monitoring and statistics

Standard protocols based on the results of previous experiments at Rotterdam Zoo (chapter 5, and 9) were distributed to all aquariums to guarantee a high similarity of handling larvae and primary polyps, respectively, and of their husbandry. The regular zoo staff mainly conducted the study, thus I had to design the experiments in a way to fit in the daily operations. Sample size and amount of species transported to each institution was dependent on the availability of specimens at a particular time and available capacity (time, space, staff) at a particular institution. The available amount of specimens located on flat and pyramid tiles was influenced by species-specific settlement preferences, shown in chapter 5 and 8.

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 analyzed with a Chi-Square test. At the final count colony size of all specimens (overall age: 11 months) was measured by taking the maximum diameter of the colony (accuracy ± 1mm). 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 ≤ 2. If $n_{sub} > 2$, class frequencies were analyzed for significance with a Mann-Whitney U-Test. If no significant differences were found, data were pooled and significance between institutions tested using the same test. All statistical tests were carried out separately for each species using SPSS 12.0.

RESULTS

Larval settlement

In 2002 settlement of *Acropora tenuis* larvae was with 2.93 ± 2.52 % (mean ± SD) at London Zoo similar to 3.30 ± 2.91 % (mean ± SD) at Burgers' Zoo ($\chi^2 = 0.124$, $p < 0.423$). In 2003, settlement was higher with 17.36 ± 6.01 % (mean ± SD) at Burgers' Zoo, but still significantly lower compared to 57.84 ± 11.01 % (mean ± SD) settlement at Rotterdam Zoo ($\chi^2 = 572.506$, $p < 0.001$).

Juvenile Survival

Primary polyps arrived within 24 hr at their destination. They showed a mean post-transport survival rate of 95.18 ± 4.86 % (± SD; n = 501; Fig 1) for all three species. Figure 2 gives an overview of the survival rates for the first 10 months after transportation of propagules. Survival rates ranged between 0 and 86%. In all cases species-specific survival highly differed between the institutions over the entire monitoring period of 10 months (*Acropora tenuis*: $\chi^2 = 41.919$, $p < 0.001$; *Agaricia humilis*: $\chi^2 = 20.254$, $p < 0.001$; *Favia fragum*: $\chi^2 = 74.955$, $p < 0.001$). These differences developed at different time. At London Zoo the number of juveniles of *A. tenuis* decreased already in the first 2 months significantly compared with those at Burgers' Zoo ($\chi^2 = 11.623$, $p < 0.001$). The same effect was observed for *F. fragum* at Cologne Zoo compared to specimens at London Zoo ($\chi^2 = 12{,}770$, $p < 0.001$). Contrary, *A. humilis* showed a similar decrease at London Zoo and Hagenbeck Zoo for the first 6 months between the measurements ($\chi^2 < 2.187$, $p > 0.05$). Between 6 - 8 months after the start of the experiment, colonies at London Zoo stabilized whereas those at Hagenbeck Zoo further decreased ($\chi^2 = 10.286$, $p = 0.002$) and finally completely died in the last period (8 – 10 months after start). Fig. 2 shows that the overall survival rate of *Agaricia humilis* was relatively low compared to the other species. Survival rates of juveniles located on pyramid

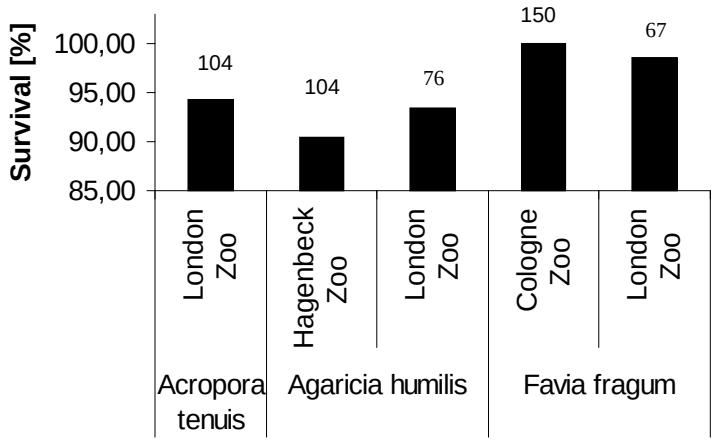

Fig. 1 Post-transport survival rate of primary polyps shipped from Rotterdam Zoo to other European zoos. N written above bars.

tiles were significantly higher compared to those on H-type tiles for *F. fragum* at Cologne Zoo (χ^2 = 12.321, p = 0.001). Except for *A. tenuis* at Burgers' Zoo in all other cases there was no significant influence of the surface orientation found, although relative survival was lower on flat tiles than on pyramid tiles (Fig. 3). *A. tenuis* at Burger's Zoo was not tested due to the low amount of specimen (n = 3) located on flat tiles.

Juvenile sizes

Figure 4 gives an overview of the mean colony sizes at all institutions. The colony sizes situated on flat tiles were not in any case significantly different from those situated on pyramid tiles. The overall comparison of colony sizes was similar for *Favia fragum* in London and Cologne (U = 268.5, p = 0.485), whereas colonies of *Acropora tenuis* were larger at London Zoo compared to those at Burgers' Zoo (U = 71.5, p = 0.012). The highest colony diameters were recorded for all species at London Zoo (min. and max. diameter observed: *Acropora tenuis*: min. 0.3 cm, max. 4.3 cm; *Agaricia humilis*: min. 0.1, max. 1.5 cm; *Favia fragum*: min. 0.2 cm, max. 2.0 cm; see also Figure 5).

DISCUSSION

Survival and growth of juveniles

Highly different survival rates between the institutions were found for all species. Colony sizes partly differed between institutions and highly varied between individual colonies. In the present study, recruitment occurred mainly after 4 - 8 months indicated by a noticeable decrease of mortality. Initially, algal turf and sedimentation were threatening factors for propagules in all cases (except for *Acorpora tenuis* at Burgers' Zoo), mainly on a microhabitat level, invisible to the naked eye. Therefore sediment removal and algal control was increased. Both factors were limiting recruitment mainly on horizontal surfaces in the field (Sato, 1985; Maida et al., 1994; Babcock and Mundy, 1996). We were able to reduce the influence of these factors over time; therefore, we did not observe different survival or growth depending on surface orientation (flat vs. pyramid tiles) except for *Favia fragum* at Cologne Zoo and *Agaricia humilis* at Hagenbeck Zoo. At Cologne Zoo, a bloom of cyanobacteria occurred approx. 4 months after the start of the experiment, mainly affecting survival on light-exposed surfaces of flat tiles. At Hagenbeck Zoo, turf algae were difficult to control over the entire period. Differences in water chemistry and nutrition might have contributed to highly differing survival rates between the aquarium systems. In the systems at London and Burgers' Zoo live plankton was regularly added, whereas no additional food was supplied in the

systems at Cologne Zoo and Hagenbeck. Culture tanks at Cologne, Hagenbeck Zoo and London Zoo were connected to exhibit tanks, which might influence the factors mentioned above additionally. The tank at Burgers' Zoo represented an independent re-circulation

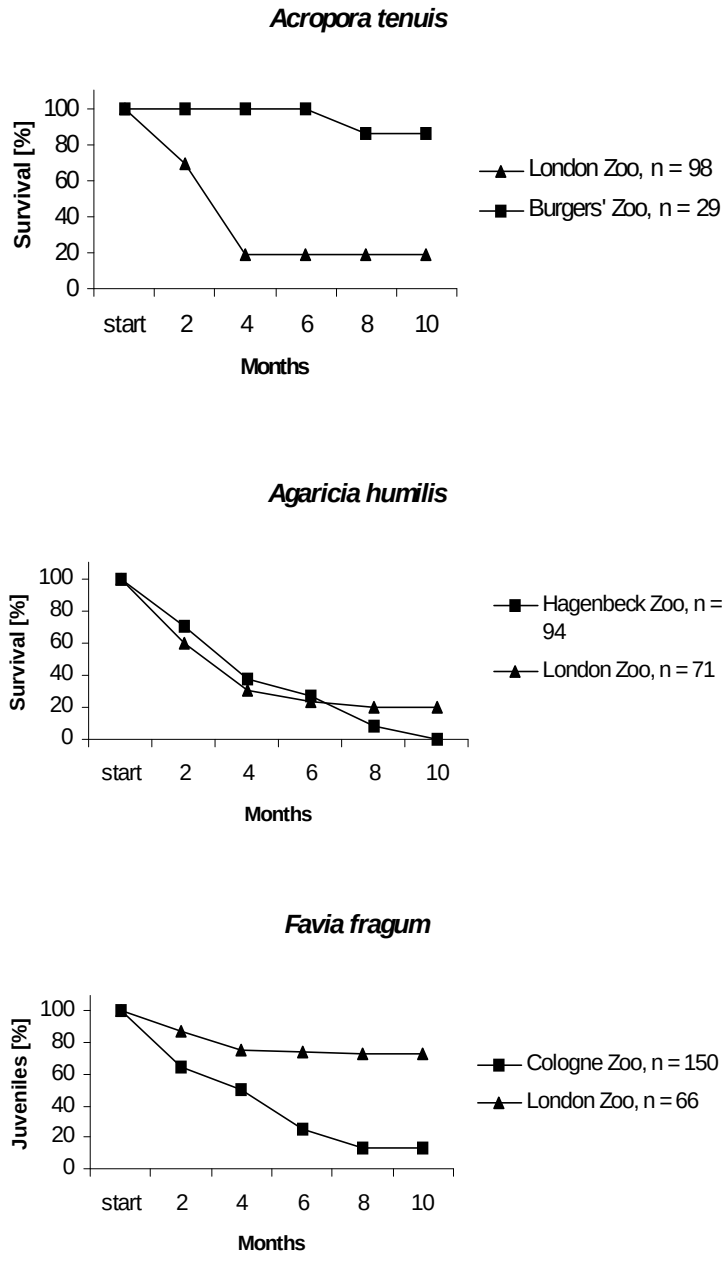

Fig. 2 Overall survival of juveniles at different zoo aquariums.

system. In accordance to chapter 9, further investigation is necessary to estimate the influence of water chemistry, nutrition, and other factors (i.e. temperature, water flow, etc.) in coral mariculture.

Maximum survival rates above 70 and 80 % of *Favia fragum* and *Acropora tenuis* in the present study exceeded those reported from the field, which generally reach max. 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 for *Agaricia humilis* in the study was in accordance with the results of chapter 9 and 10, which might indicate sub-optimum conditions for this species in common closed systems. This needs further investigation.

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 the initial year (Harrison and Wallace, 1990). Regarding the genus *Acropora* at London Zoo, we even achieved 3 – 4 times higher growth (mean diameter 1.3 cm; max. 4.3 cm) compared to reports from the field (mean 0.5 cm; max. 1.2 cm; Harrison and Wallace, 1990).

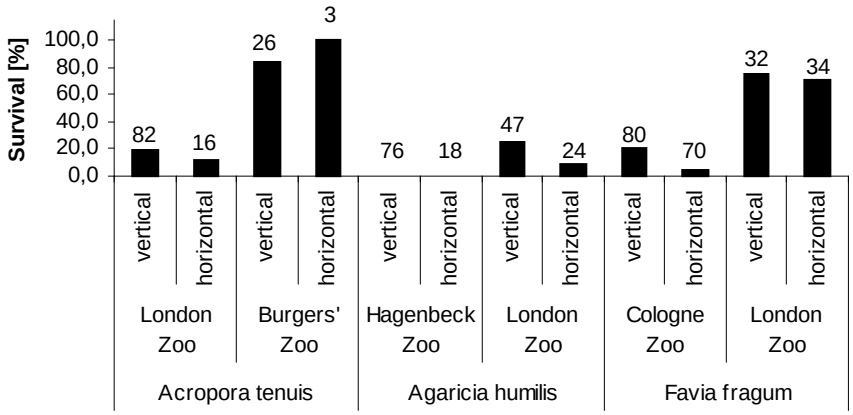

Fig. 3 Survival on pyramid and flat tiles after 10 months incubation. N written above bars.

Distribution of larvae versus primary polyps

Except for Rotterdam Zoo, none of the participating zoos had any practical experiences in handling coral larvae and primary polyps. By distributing standard protocols, which described procedures in detail, necessary basic tools were given to non-experienced institutions for a successful culture of juvenile corals.

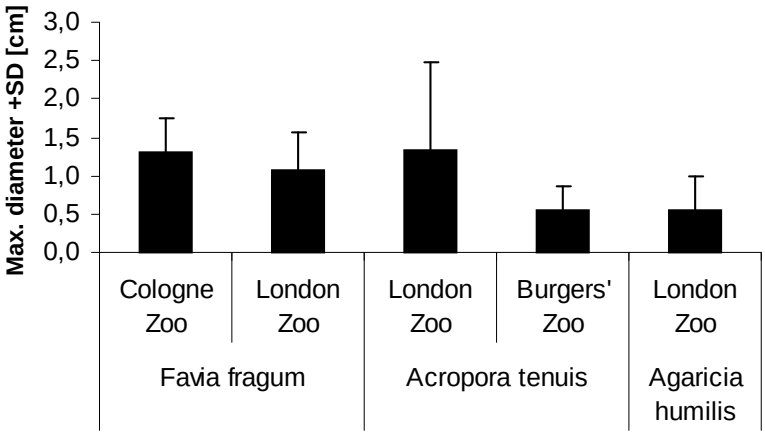

Fig. 4 Mean colony sizes, 10 months after distribution to European zoo aquariums (age of juveniles: 11 months).

I tested two different methods to supply public aquariums with sexual recruits: (1) larvae or (2) primary polyp. In 2001, achieved settlement rates of *Acropora tenuis* at London Zoo and at Burgers' Zoo were much lower than at Rotterdam Zoo (16.3 ± 3.1 %; unpublished data). 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, personal observation) - 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 in all species. In this study, propagules that had already passed the planktonic stage and that had undergone settlement and metamorphosis were much easier to handle by non-experienced institutions than larvae and the procedure to initiate settlement. Currently we can transport up to 4,000 larvae l^{-1} water volume (chapter 4) versus 24 tiles with settlers (resulting in max. 24 corals) in the same

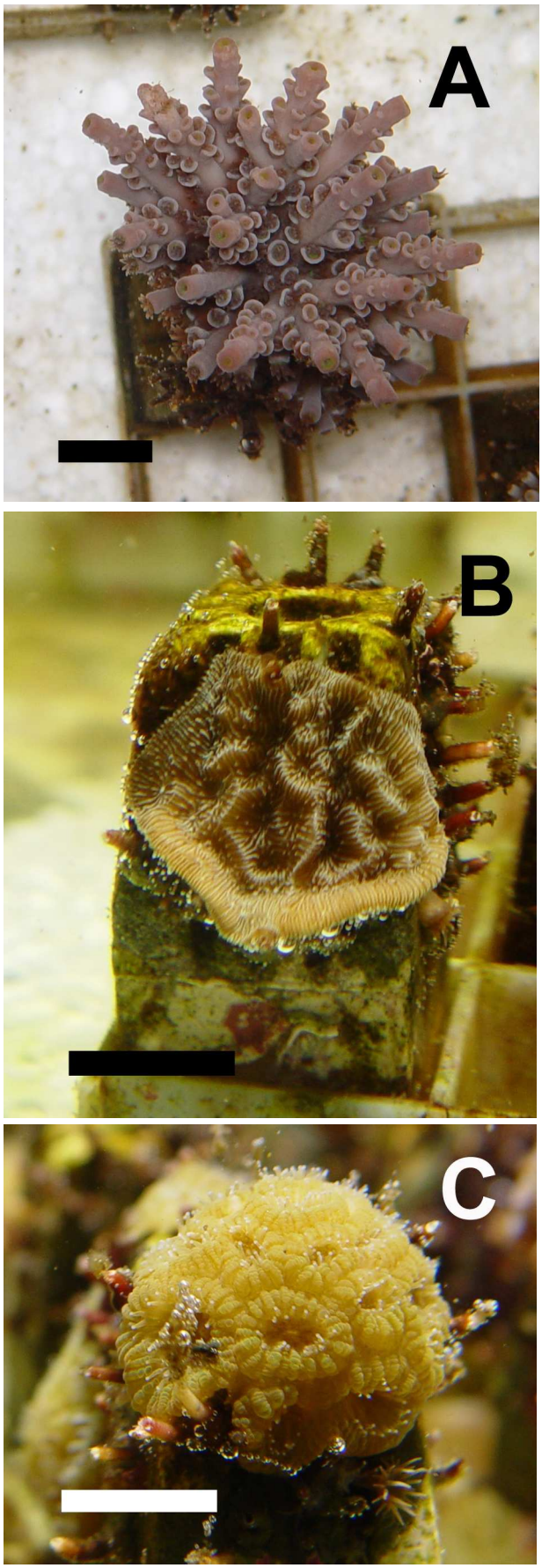

Fig. 5 Colonies (age: 11 months) at London Zoo: (A) *Acropora tenuis*, (B) *Agaricia humilis*, (C) *Favia fragum*. Note the arrangement of tiles in the polystyrene grid. Scale bar: 1 cm (Courtesy: Rachel Jones, Zoological Society of London, U.K.).

water volume. Although the shipment of larvae even at a settlement rate of 1 % would be still more economical when purely looking at transportation costs, we demand an effective and sustainable use of natural resources, which favors the distribution of primary polyps to non-experienced institutions. 'Dry transportation' of juveniles not tested in this study could further help reducing transportation costs. In the past, the dry-method has been successfully applied mainly for coral fragments (see chapter 3).

Conclusions and future perspectives

Our results show a great potential of today's public aquariums to use sexual reproduction for self-sustaining *ex situ* coral populations. Institutions, experienced in coral husbandry, but not in sexual propagation, were able to achieve high survival and growth rates at the first attempt. Public aquariums have not only a great responsibility in nature conservation, but also play an exemplary role in the sustainable use ornamental organisms. Since the beginning, there was a high interest of the international aquarium community in SECORE that resulted in a membership extension in 2004. Currently more than 25 institutions in Europe, the USA and Japan are involved in the project.

Regarding captive sexual propagation in general, the generation of a full life cycle *ex situ*, which is described in chapter 6 and 10, might be the next step towards captive coral breeding and the initiation of breeding programs for endangered coral species (see chapter 12).

REFERENCES

Babcock RC, Mundy C (1996) Coral recruitment: consequences of settlement choice for early growth and survivorship in two scleractinians. J Exp Mar Biol Ecol 206:179-201.

Harrison PL, Wallace CC (1990) Reproduction, dispersal and recruitment of scleractinian corals. In: Dubinsky Z. (ed.) Ecosystems of the world. 25. Coral reefs. Elvesier, Amsterdam, pp 133-207

Maida M, Coll JC, Sammarco PW (1994) Shedding new light on scleractinian coral recruitment. J Exp Mar Biol Ecol 180:189-202.

Sato M (1985) Mortality and growth of juvenile coral *Pocillopora damicornis* (Linaeus). Coral Reefs 4:27-33.

Sorokin YI (1995) Coral reef ecology. 102. Ecological studies. 2nd printing. Springer, Berlin.

LEBENSLAUF

Seit 01/02	Promotion an der Universität Duisburg-Essen, Institut für Ökologie, Abt. Hydrobiologie Projektstandort: Rotterdam Zoo, Rotterdam, Niederlande
01/02-12/04	Promotionsstipendium der Deutschen Bundesstiftung Umwelt (DBU)
01/00-05/01	Wissenschaftlicher Leiter am Meereszentrum Fehmarn, Burg auf Fehmarn
09/92-07/99	Studium der Biologie (Dipl.) an der Ludwig-Maximilians-Universität München Abschluß: Diplom
10/91-09/92	Studium der Chemie (Dipl.) an der Technischen Universität München
07/90-06/91	Grundwehrdienst
09/81-06/90	Holbein-Gymnasium Augsburg Abschluß: Abitur

Essen, 3. Dezember 2004

gez.
Dirk Petersen

Erklärung:

Hiermit erkläre ich, gem § 6 Abs. 2, Nr. 7 der Promotionsordnung der Fachbereiche 6 bis 9 zur Erlangung des Dr. rer. nat., dass ich das Arbeitsgebiet, dem das Thema “Zuchtmethoden für riffbildende Steinkorallen – ein Beitrag zur Nachhaltigkeit in ex situ Populationen” zuzuordnen ist, in Forschung und Lehre vertrete und den Antrag von Herrn Dirk Petersen befürworte.

Essen, 3. Dezember 2004

gez.
Prof. Dr. Helmut Schuhmacher

Erklärung:

Hiermit erkläre ich gem. § 6 Abs. 2, Nr. 6 der Promotionsordnung der Fachbereiche 6 bis 9 zur Erlangung des Dr. rer. nat., dass ich die vorliegende Dissertation selbstständig verfasst und mich keiner anderen als der angegebenen Hilfsmittel bedient habe.

Essen, 3. Dezember 2004

gez.
Dirk Petersen

Erklärung:

Hiermit erkläre ich gem. § 6 Abs. 2, Nr. 8 der Promotionsordnung der Fachbereiche 6 bis 9 zur Erlangung des Dr. rer. nat., dass ich keine anderen Promotionen bzw. Promotionsversuche in der Vergangenheit durchgeführt habe und dass die Arbeit von keiner anderen Fakultät abgelehnt wurde.

Essen, 3. Dezember 2004

gez.
Dirk Petersen