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## Ex situ transportation of coral larvae for research, conservation, and aquaculture

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**Abstract** Due to the lack of appropriate methods to transport high amounts of larvae ex situ over large distances, the availability of coral larvae was so far mainly limited to their place of origin. For a research project at Rotterdam Zoo, The Netherlands, we transported several thousand larvae of three broadcast spawners (*Acropora tenuis*, *A. digitifera*, *Diploria strigosa*) from the Indo Pacific and the Caribbean to Europe. Beside logistics and packing techniques, post-transport survival rates were mainly influenced by larvae density and transport duration. Our results indicate optimum survival rates of >90% at densities of 4 larvae ml<sup>-1</sup> when not exceeding a transportation time of 4 days. The ex situ transport of coral larvae over large distances might offer new possibilities for research, conservation, and aquaculture.

**Keywords** Coral larvae · Ex situ transportation · Conservation · 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, which were obtained from coral slick, in situ using floating culture ponds that were carried by boats over a limited distance of several kilometers for field experiments. Omori (personal communication) cultured millions of larvae in a floating culture pond, packed the larvae in containers, and transported them to the destination over 1 h by the ferry boat. For research purposes, larvae of acroporids have been successfully delivered by regular postal mail within Japan (M. Hatta, unpublished). The transportation of coral larvae in high amounts over large distances might give new opportunities for research, conservation, and aquaculture.

As part of a research project (SECORE; Petersen et al. in press a), we planned to transport coral larvae of three different broadcast spawning species in several transports from Okinawa, Japan respectively from Curaçao, Netherlands Antilles to the marine laboratory of Rotterdam Zoo, The Netherlands. In this paper, we summarise our experiences concerning 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 using 15 and 50 ml centrifuge

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tubes (polyethylene), 200 ml tuberware boxes (polypropylene) and 500 ml, respectively 1,000 ml bottles (polyethylene). No oxygen or germicidal chemicals were added. Larvae in different densities (3.3–13.3 larvae ml<sup>-1</sup>; see Table 1) were either 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 petri dish 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<sup>-1</sup> 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) and into plastic containers (500 and 1,000 ml), 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°C in 2 h. 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 the 200 and 500 ml bottles. First the samples were filtered (0.45 µm), then nitrogen bound as NH<sub>4</sub><sup>+</sup>, NO<sub>2</sub><sup>-</sup>, NO<sub>3</sub><sup>-</sup>, and phosphorous bound as PO<sub>4</sub><sup>3-</sup> was measured using photo spectrometry (DR/4000U Photospectrometer, HACH Company, USA). 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, 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 (Petersen et al. 2005). The tiles were placed in 250 ml, then 100 larvae and Hym-248 in a final concentration of 3×10<sup>-6</sup> M were added (room light: 40 µmol m<sup>-2</sup> s<sup>-1</sup>; 26°C). The number of metamorphosed animals (attached and non-attached disc-shaped planulae; see Harrison and Wallace 1990) was counted 12 h after the start of the experiment.

## Results and discussion

High survival rates of >90% were determined at ≤ 4 larvae ml<sup>-1</sup> for *Acropora tenuis* even when transportation took 10 days while no larvae survived at densities > 6.6 larvae ml<sup>-1</sup> during the 10-days transport (Table 1). However, shorter transport duration (≤ 4 days) may lead to high survival rates even at densities of 10 larvae ml<sup>-1</sup>. High post-transport survival rates were verified for *D. strigosa* by shipping more than 48,000 larvae from Curaçao to The Netherlands (4 lar-

**Table 1** Data of coral larval transports from Akajima (Japan) and Curaçao (Netherlands Antilles) to Rotterdam Zoo (The Netherlands)

Species	Density larvae (ml <sup>-1</sup> )	Volume (ml)	Replicates	n	Duration (days)	Logistics	Monetary costs <sup>a</sup> (€)	S ± SD (%)
<i>Acropora tenuis</i>	3.3	15	8	400	10	normal airmail	25.00	98.3 ± 2.5
	6.6	15	4	400	10	normal airmail	25.00	0.0
	13.3	15	4	800	10	normal 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	normal airmail	25.00	99.2 ± 1.5
	5	15	6	450	4	normal airmail	25.00	99.5 ± 0.6
	10	15	6	900	4	normal 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 <sup>b</sup>	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
<i>A. digitifera</i>	3	15	3	135	4	normal airmail	25.00	85.2 ± 10.5
	5	15	3	225	4	normal airmail	25.00	69.3 ± 5.0
	10	15	3	450	4	normal airmail	25.00	98.0 ± 2.9
<i>Diploria strigosa</i>	4	500	5	10,000	3	express service	20.00	78.7 ± 23.8
	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

n total number of larvae per transport.

S mean post-transport survival.

<sup>a</sup>Per 1,000 ml volume (= 1 kg).

<sup>b</sup>Calculated for an overweight charge of 30.00 Euro kg<sup>-1</sup>

vae ml<sup>-1</sup>). Post-transport metamorphosis of *A. 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 1998; 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 days after fertilization) and maximum 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 the transport 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°C 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 (A. J. Heyward, personal communication) could help to reduce bacteria growth and the accumulation of toxic metabolic products.

Regarding these changes of water chemistry and physics, we 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 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 tubeware 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 show the possibility to transport coral larvae over large distances without any life support with an optimum density of 4 larvae ml<sup>-1</sup> and a transportation time of ≤ 4 days. To ensure appropriate experimental results, survival and metamorphosis competency should be verified prior 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 (SCORE project; Petersen et al. in press a). 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 (Petersen et al. in press a; Petersen et al. in press b), 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 1999; Petersen et al. 2004). In addition, utilization of larvae would replace collection of coral colonies thereby reduce impacts on coral populations.

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 1999).

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

**Table 2** Water analysis of three different larval transports compared to common field values

Species	Density larvae (ml <sup>-1</sup> )	Duration of transport (days)	Temperature (°C)	pH	NH <sub>4</sub> -N (µM)	NO <sub>2</sub> -N (µM)	NO <sub>3</sub> -N (µM)	PO <sub>4</sub> -P (µM)
<i>A. tenuis</i>	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
<i>A. digitifera</i>	4	3	24.0	7.44	0.00	0.13	28.72	0.64
Field			25.0–27.0 <sup>a</sup>	8.00–8.40 <sup>b</sup>	0.05–11.00 <sup>c</sup>	Not available	0.05–5.00 <sup>c</sup>	0.02–1.36 <sup>c</sup>

<sup>a</sup>At Akajima in May/June

<sup>b</sup>After Sorokin (1995)

<sup>c</sup>After Adey and Loveland (1998)

conditions. Further on, it may be attractive for research institutions, which are not directly located at coral reefs, to save 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.

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