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REEFGUARD: A SCIENTIFIC APPROACH TO ACTIVE REEF REHABILITATION

ABSTRACT

In 2010, Dutch dredging and marine contractor, Van Oord, launched a Coral Rehabilitation Initiative as part of its Sustainability and Marine Ingenuity agenda. The key challenge was to demonstrate that already proven small-scale coral breeding techniques can be scaled-up and applied in practice to promote environmental gain around marine infrastructure projects. The Initiative's ultimate goal is to integrate the breeding and out-planting of tens of thousands to hundreds of thousands 'lab-cultured' juvenile corals in marine and coastal infrastructure development projects as a nature-based component. These corals are to be obtained from natural coral spawning events as well as from fragments of opportunity.

A key element of the Initiative has been the development of an innovative mobile laboratory – ReefGuard. This laboratory helps to ensure the availability of a highly controlled environment for the fertilisation, larval settlement and initial outgrowing of sexual recruits (as well as fragments) before outplacement. After its design in 2012 and construction in 2013, ReefGuard has been applied in four coral breeding experiments. The first two experiments were executed near Ningaloo Reef in Coral Bay, Australia (2014 and 2015) and the other two in Coral Harbour on New Providence, Commonwealth of the Bahamas (2015 and 2016).

The experiments involved three coral species of the genus *Acropora*, in-situ as well as ex-situ gamete collection, and employed 10,000, 36,000, 20,000 and 30,000 settlement tiles respectively. The tiles with settled larvae were used in various survival experiments under different laboratory and field conditions. This was to develop a scientific-base for the design of active reef rehabilitation campaigns. From these experiments it can be concluded that active reef rehabilitation is indeed a viable option that can be integrated in marine and coastal infrastructure development projects.

INTRODUCTION

The way we view the development of engineering infrastructure has been influenced by various societal trends – the progressing urbanisation of delta areas, growing global trade, energy demand and increasing stakeholder participation. Trends in the environment such as reducing bio-diversity, climate change and accelerated relative sea level rise are also influencers. Consequently, mono-functional (engineering) solutions without due consideration of the environment are less accepted nowadays. Instead, multi-functional, sustainable solutions and stakeholder involvement are becoming the new norm.

The evaluation of environmental impacts is an important aspect of the realisation of hydraulic infrastructure. Environmental impact assessment (EIA) procedures have a tendency to emphasise minimising the negative impacts of envisaged infrastructure projects and compensating for any residual negative effects. New design philosophies, like the 'Building with Nature' approach, aim to be proactive, utilising natural processes and providing opportunities for nature as part of the infrastructure development process (De Vriend and Van Koningsveld, 2012; De Vriend et al., 2015). Other similar philosophies have emerged, such as 'Working with Nature' promoted by PIANC (PIANC, 2011) and 'Engineering with Nature' promoted by the US Army Corps of Engineers (Bridges et al., 2014).

Above: Top view of a 15-month old ReefGuard coral, obtained from Coral Bay, Western Australia in a 2015 spawning. The coral is 3-4 cm in diameter at this point.

THE CORAL REHABILITATION INITITATIVE

In line with this wider trend, Dutch dredging and marine contractor, Van Oord, launched a Coral Rehabilitation Initiative in 2010 as part of its Marine Ingenuity and Corporate Social Responsibility program. The main idea of this Initiative was to demonstrate that already proven, small-scale, coral breeding techniques can be scaled-up. Thus, creating tens of thousands to hundreds of thousands 'lab-cultured' juvenile corals through sexual reproduction and from fragmentation to be outplaced in the field to promote true environmental gain around coastal and marine infrastructure projects.

Through the Coral Rehabilitation Initiative, Van Oord aimed to gain a deeper understanding of coral reefs in general and how they may best be protected on marine and coastal engineering projects around the world. The Initiative consisted of three phases:

- *Phase I: Feasibility*
  Literature review, expert consultation and training in coral breeding techniques (2010-2011)
- *Phase II: Construction of mobile lab facilities "ReefGuard"*
  Development of operating protocols and detailed design and construction of mobile laboratory, ReefGuard (2012-2013)
- *Phase III: Application in the field*
  Deployment of the ReefGuard in four field experiments (2014-2016)

Phase III focused on the ReefGuard's practical application in four field experiments to optimise large scale rehabilitation techniques, in the period between 2014 and 2016. Each field experiment had its own specific objectives:

- Coral Bay, Western Australia, 2014: delivering proof-of-concept ReefGuard functionality
- Coral Bay, Western Australia, 2015: test influence of environmental conditions on survival rates of sexual coral recruits
- Coral Harbour, Bahamas, 2015: expand capability of in-situ spawn collection with Caribbean coral species and test different settlement conditions
- Coral Harbour, Bahamas, 2016: test influence of aquaculture treatments on survival rates of sexual coral recruits

As of the first quarter of 2017, all four field experiments have been completed successfully. They have delivered crucial knowledge and practical know-how that is critical for the successful design of future projects.

The aim of this paper is to provide a more detailed background of the philosophy behind the Coral Rehabilitation Initiative and the mobile coral breeding facility, ReefGuard; the lessons learned from the four pilot applications; and what these results mean for future applications in hydraulic infrastructure development projects.

REEFGUARD PHILOSOPHY

Young (2000) advises to adopt a realistic perspective on restoration and rehabilitation. Though restoration/rehabilitation can enhance conservation efforts, it is important to be aware that it is always a poor second to the preservation of original habitats. Furthermore, rehabilitation efforts should take an evidence-based approach in order to be truly effective or formulated negatively in order to avoid being counterproductive. Young (2000) also states that this should not blind one to the tremendous potential of rehabilitation efforts when implemented appropriately. This section outlines some of the wider considerations that influenced the choices made in the ReefGuard approach.

Active vs. passive management measures

Coral reefs worldwide are under decline. While degrading, coral reefs lose original biological and ecological properties. This makes it harder for reefs to recover and makes them more vulnerable to regime shifts. This prospect may be accelerated in the face of climate change (Hughes et al., 2003).

In the past decades, management of coral reefs has gained momentum. Passive management tools have been applied worldwide, often in the form of defining Marine Protected Areas (MPAs) where use is highly regulated. With anthropogenic stresses under control, establishing MPAs is assumed to allow reefs to recover naturally, or at least will stop further degradation.
More recently, active management approaches have gained popularity as passive

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REMMENT TER HOFSTEDE
received an MSc in marine ecology at the University of Groningen, the Netherlands in 1999. He has since worked in applied sciences to facilitate integrated management of the marine and coastal environment. He joined Van Oord in 2014, bringing in science-based solutions to reduce the negative impact of infrastructure development projects on our marine ecosystems.

JESPER ELZINGA
holds an MSc with a specialisation in Aquatic Ecology and Water Quality Management (2013) at Wageningen University and Research Centre. He started working at Van Oord in 2013 after an internship. He has since been actively involved on site and in preparation works for all ReefGuard campaigns within Van Oord's Environmental Engineering Department.

TIJMEN SMOLDERS
graduated in 2010 as an MSc in Environmental Fluid Mechanics. from Delft University of Technology, the Netherlands. After three years of working for Royal Haskoning/DHV he joined Van Oord's Environmental Engineering Department working on a wide variety of environmental aspects of their dredging and maritime construction projects. This includes coral relocation projects, coral breeding, environmental monitoring and turbidity management both in the Netherlands and abroad.

MIRIAM SCHUTTER

studied animal biology at Wageningen University, The Netherlands, and received a PhD degree in 2010 on the importance of abiotic factors for optimizing coral growth in aquaculture. Afterwards, she spent almost 6 years abroad as a postdoctoral researcher in Taiwan and Mexico studying the sexual reproduction of corals, impacts of climate change, coral bleaching and coral reef rehabilitation. In 2016, she was hired as a coral expert by Van Oord for the large ReefGuard experiment in The Bahamas.

RONALD OSINGA

did an MSc in marine biology at the University of Groningen. After receiving his degree in 1991, he did, a PhD in marine microbiology at the Netherlands Institute for Sea Research, Texel, which he completed in 1996 at the University of Groningen. In the same year, he started as a post-doc at Wageningen University with the task to develop methods for culturing marine invertebrates such as sponges and corals. At present, Ronald Osinga is an assistant professor in marine animal ecology at Wageningen University. One of his current research concerns coral reef restoration, which encompasses a collaboration with the ReefGuard team of Van Oord.

management alone has proven ineffective in halting reef decline in many cases (McClanahan, 1999; Risk, 1999; Jameson et al., 2002; Epstein et al., 2005; Rinkevich, 2005; Coelho and Manfrino, 2007).

Rinkevich (2008), recognises that active management measures are still under development. However, he makes a compelling case for their increased future importance comparing to the history of terrestrial forestation approaches. The key successful element in this analogy was the drastic upscaling of already working smaller scale techniques.

Efforts undertaken by Van Oord as part of its Coral Rehabilitation Initiative was to promote the feasibility of active management approaches. This was aimed to demonstrate that already proven, small-scale coral breeding techniques can be scaled-up and applied in the field to promote environmental gain around marine infrastructure projects.

Scientific knowledge on factors influencing reef rehabilitation

Adverse environmental conditions for the survival of coral recruits (juvenile corals less than one year of age) in combination with diminished quantity and quality of brood stocks are major factors affecting the ability of degraded reefs to recover naturally. To enhance the feasibility of active management measures we need:

- a better our understanding of the early stages of coral survival;
- to improve our understanding of causal linkages between environmental parameters (e.g. small scale temperature fluctuations, algal/coral interactions, grazer abundance) and coral recruitment.

Many coral reefs around the world have suffered major degradation in the face of natural as well as anthropogenic environmental impacts (Depczynski et al., 2013, Moore et al., 2012, Speed et al., 2013). Under a natural disturbance regime, coral reefs are capable of recovering rapidly from acute disturbances, provided that there is sufficient supply of new larvae and there are stable populations of herbivorous grazers keeping algal growth under control (Gilmour et al., 2013). Under severely changed environmental conditions (e.g. high rates of sedimentation, eutrophication, low rates of herbivory, climate change), however, recovery from acute disturbances can be much slower. Furthermore, reefs have a greater chance of shifting from a coral-dominated state to one dominated by algae (regime shift). This effect may be exacerbated when the frequency of stressors, such as related to storms or high seawater temperature events increases. This effectively leaves the reefs with less time to recover.

Many factors influence recovery rates of reefs. In some cases recovery may be substrate-limited, but in all cases coral reproduction followed by successful settlement and recruitment are key processes contributing to the persistence and resilience of coral assemblages. Consequently, a better understanding of these processes is critical to fully understand how and why recovery rates vary among reefs and what would be a sensible rehabilitation strategy given this variability.

To achieve its objectives, the Coral Rehabilitation Initiative aims to adhere and contribute to the state-of-the-art in scientific understanding of early stage coral survival. It also aims to strengthen the understanding of causal linkages between environmental parameters and coral recruitment. The ReefGuard enables the production of tens of thousands of settlement tiles with coral spat. This enhances the ability to conduct scientific experiments on the survival rates of coral recruits at a scale that was not previously possible (both in size and numbers). This added knowledge is crucial for the design of effective active rehabilitation strategies.

Promoting active reef rehabilitation with 'lab-cultured' juvenile corals

In order to achieve the Coral Rehabilitation Initiative's objectives it was essential to enhance the understanding of the positive or and/or negative effects of in- and ex-situ culturing intervals, potentially in combination with various treatment regimes. In addition, it was crucial to learn about the most effective outplacement approaches.

Transplanted corals that were cultured ex-situ for a period of time were found to have a higher survival rate than corals transplanted to the reef earlier (Guest et al., 2014). Thus, the selection of an optimal aquaculture period is an important design parameter for rehabilitation efforts. However, costs of maintaining corals in nurseries are high (Edwards, 2010). As such, part of the Coral Rehabilitation Initiative was designed to:

- increase practical understanding of what optimal aquaculture lengths are;
- discover what possible management approaches could be utilised when the optimal aquaculture length is not feasible due to practical reasons.

It is important to realise that aquaculture duration affects the size of the corals. The size the corals should be before outplacement is an important design criterion. When they are large enough they pass through the stage when they are vulnerable to being destroyed by a single bite from a predator.

For the sexually propagated corals, recommended sizes in a study by Edwards (2010) could mean that aquaculture lengths of up to two years are needed. In practice, one might mitigate the risk of post outplacement mortality due to earlier outplacement with the production of more juveniles or by outplacing juveniles in a combination with larger fragments.

Once a large number of sexually reproduced juveniles has been cultured for a sufficiently long period of time, the next step is to design an appropriate outplacement strategy. Various strategies may be considered, ranging from covering as wide an area as possible for reduced competition between corals to creating concentrated patches in order to promote the new reef's ability to engineer its own environment (Griffin et al., 2015).

REEFGUARD HARDWARE: DESIGN OF A MOBILE CORAL BREEDING FACILITY

Coral rehabilitation efforts, passive or active, rely to a great extent on nature's capability to help itself. The previous section highlighted that while in some cases this recovery may be substrate-limited, in all cases coral reproduction followed by successful settlement and recruitment are key processes that contribute to the natural persistence and resilience of reefs. Consequently, a decision was made to focus on a better understanding of these processes for the Coral Rehabilitation Initiative.

A critical first step in coral rehabilitation is to understand how the coral reproductive cycle works. Corals can be distinguished by their reproductive mode. Brooding corals have larvae that develop fully inside the colony and are released in small numbers on a regular basis to find a spot to settle. Broadcast-spawning corals release their gametes (usually in packages of eggs and sperm) in a massive spawning event that occurs over the course of just a couple of days only once a year.

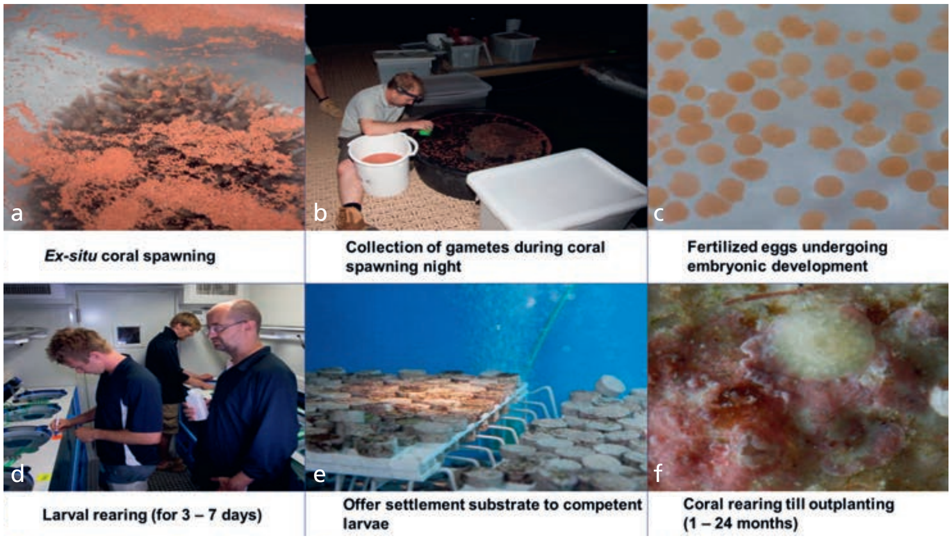

Figure 1. The coral reproductive cycle ex-situ: colonies of the same coral species spawn simultaneously in-situ on the reef or ex-situ in aquaria. (a) Their buoyant gamete bundles rise to the water surface after spawning after which they are carefully collected using plastic cups and/or pipettes (b). (c) Gamete bundles from different colonies are gently mixed for fertilisation after which the embryos go through several developmental stages to become coral larvae. (d) These larvae are reared for a couple of days until they become settlement-competent and are offered suitable substrates to settle on (e). (f) Once attached and metamorphosed into small coral recruits, their goal is to grow into adult colonies that can contribute to the yearly mass spawning, while facing numerous threats in the process.

Depending on the size of the reef and the number of colonies that spawn at the same time, millions of gametes enter the water column during mass spawning. After 10-15 minutes the buoyant gametes reach the top of the water column and break open to release the eggs and sperm. The current and waves causes sperm and eggs of different colonies to meet after which, the eggs may fertilise. The resulting embryos take several days to pass through a number of development stages after which they develop into settlement-competent larvae. The larvae then swim down in the water column to look for suitable substrate to settle.

For rehabilitation efforts, each of these steps can be performed in a laboratory under controlled circumstances that increase survival rates (Figure 1) of the larvae. Broadcast-spawning coral species are especially attractive for rehabilitation efforts due to their concentrated massive release of reproductive material combined with the potential to increase survival rates under controlled conditions.

In substrate-limited systems, reef recovery is slowed because the settlement-competent larvae have difficulties finding a suitable spot to settle. In recruitment-limited systems, reef recovery is slow because the larvae may find a place to settle but do not survive the first year in the field as a consequence of all kinds of natural and anthropogenic pressures. In supply-limited systems, reef recovery is slow because the supply of larvae is the inhibiting factor (low connectivity). Whichever the situation, they suffer great casualties along the journey from the mass spawning event to post-settlement survivorship after two years (Figure 2).

Though the annual spawning event may release billions of eggs and sperm bundles into the water column, the fertilisation process may only yield millions of fertilised eggs. These embryos may develop into hundreds of thousands of settlement competent larvae. Of these only tens of thousands may make it through the actual settlement process and perhaps only several thousands of recruits survive the first year on the reef. After which, perhaps only hundreds of juvenile corals may grow to a size of approximately 5cm – a size where they themselves can participate in the reproductive cycle – effectively escaping the size

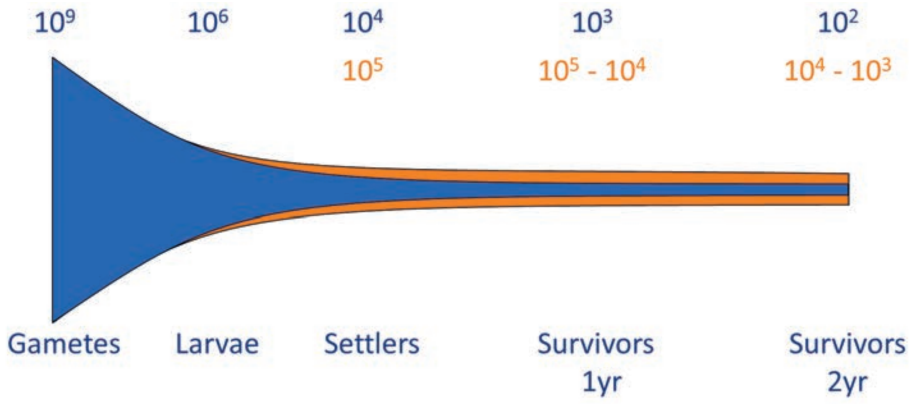

Figure 2. The funnel of progressive mortality (blue). The ReefGuard facility provides controlled circumstances with the aim to provide as much ‘light at the end of the tunnel’ as possible (orange). If scaling up currently proven small-scale breeding techniques is feasible, it should be possible to increase survival rates ultimately enabling rehabilitation at an ecologically relevant scale.

Figure 3. ReefGuard facility in the Bahamas (August 2015). The ReefGuard is a mobile facility which can be shipped anywhere in the world to perform coral research.

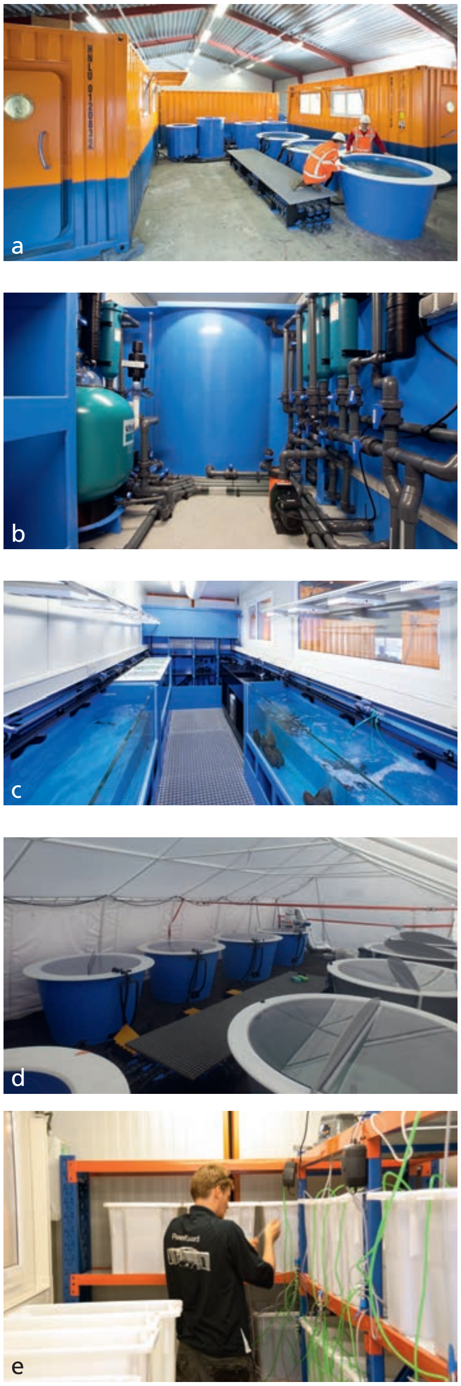

Figure 4. ReefGuard basic layout. (a) Basic layout with three containers positioned in a U-shape around the patio area. (b) Filter container aimed at maintaining proper water quality (container in the back in the top panel). (c) Aquarium laboratory designed to provide a high level of environmental control (container on the left in the top panel). (d) Patio area with a range of basins that can be used in various stages of the breeding process (central part in the top panel). (e) Wet laboratory designed to handle multiple smaller basins and perform microscope inspections (container on the right in the top panel).

bottleneck. This process of ever reducing numbers of survival can be seen as a ‘funnel of death’ as shown in Figure 2 (in blue). Rehabilitation approaches that rely on sexual coral reproduction should try to keep the end of this funnel as wide as possible as seen in Figure 2 (in orange) allowing as many corals to survive as possible.

Broadcast spawning corals make use of a strategy to spawn in massive numbers that is particularly designed to overcome the high mortality that exists throughout the entire process. Despite that, survival rates are still very low, causing only a few corals to make it through the first year. It is clear that the most gain to be made is in the first few days, from spawning (billions of gametes) until initial settlement (tens of thousands). The availability of controlled circumstances is an important condition to influence mortality rates in these very early stages of larvae development.

To address this issue, Van Oord decided to develop a coral breeding facility, ReefGuard, with high quality water filtration and climate control systems in combination with light control measures. The filtration systems consist of various sieve filters (down to 0.2 µm), UV filters and protein skimmers removing lipids and proteins from the water column. Climate control is provided by various

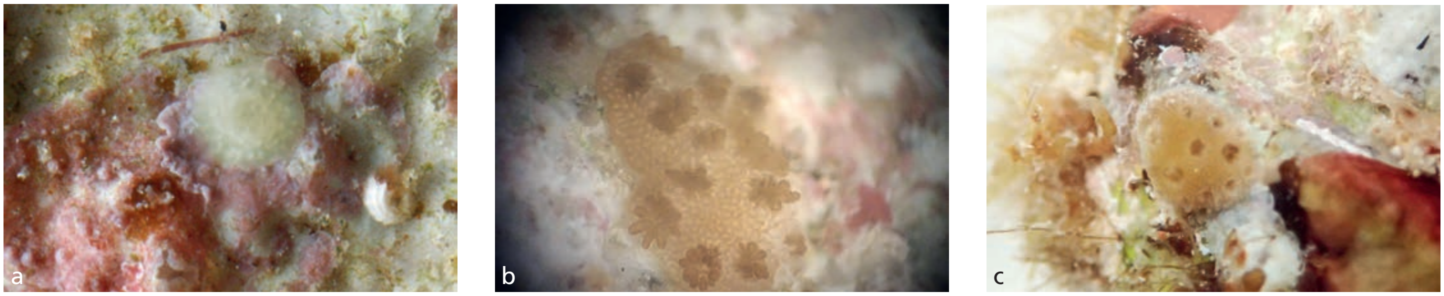

Figure 5. Various stages of development. (a) Coral spat just after settlement (2 weeks after spawning, diameter of 1 mm). (b) Coral juvenile (2 months after spawning, diameter of 1-2 mm). (c) Young coral colony (8 months after spawning, diameter of 4-5 mm).

air conditioning units and water coolers. Light control is provided by customised light armatures and screens. The facility as a whole, is custom-built into a number of sea containers in order to be mobile and readily deployable to various project sites (Figures 3 and 4).

Figure 4 shows the ReefGuard interior in more detail. Each container has its own specific role in the breeding process. The important feature of the ReefGuard is that it is designed to be scaled-up. An example is the basins in the patio area – ReefGuard's capacity can easily be enlarged by increasing the number of basins.

REEFGUARD FIELD TESTS: LEARNING FROM FIELD EXPERIMENTS

After the design and construction of ReefGuard was completed in 2013, it was applied in four field experiments. The four pilot projects – two on Ningaloo reef in Australia (2014 & 2015), and two in the Bahamas (2015 & 2016) were successfully executed. The following section highlights some of the lessons learned from these experiments.

Experiment 1: Delivering proof-of-concept of ReefGuard functionality (Coral Bay, Western Australia, 2014)

The main aim of the Coral Bay experiment in 2014 was to put the ReefGuard to the test under field conditions and deliver proof-of-concept that the facility could be used to perform all the required coral breeding steps. As Ningaloo is a pristine reef, rehabilitation was not a goal in this trial. In fact, this reef was selected to increase the likelihood of acquiring sufficiently large amounts of gametes that could be assumed to produce healthy larvae. This was a prerequisite for the proof-of-concept objective. Teaming up with experienced international specialists, Van Oord investigated how the facility could be best used to produce and culture as large as possible amount of coral juveniles, settled on pre-conditioned settlement tiles.

The gametes of two coral species were collected ex-situ: *Acropora millepora* and *Acropora digitifera*. After successful fertilisation of both species, the larvae of the *Acropora millepora* were settled on approximately 10,000 pre-conditioned aragonite plugs. A subset of these plugs was placed back in the field (in-situ nursery). Simultaneously, the remaining plugs were cultured further in the ReefGuard facility (ex-situ nursery).

After a period of approximately six weeks – two months after spawning – the survival rates of both the in-situ and ex-situ juveniles were compared. The remaining survivors were placed back on the reef (in-situ nursery) for a period of approximately six months, which is eight months after spawning, after which all plugs were retrieved and the final survivors were counted (Figure 5).

There was some variation between survival rates at different in-situ sites and between the in-situ and ex-situ cultured juveniles. Algal overgrowth and sedimentation could readily be identified as key factors influencing survival rates in-situ (Figure 6). Clearly, the absence of these stressors inside the ReefGuard enhanced the likelihood of survival allowing the recruits to grow to a larger size before being reintroduced onto the reef.

It is important to note that the setup in this first pilot was not designed to draw any scientific conclusions; in fact, it was designed to gain familiarity with the requirements and difficulties associated with each step. Additional lessons were learned from the difficulties associated with maintaining controlled conditions. The ReefGuard crew were faced with serious challenges controlling temperature fluctuations due to the climate

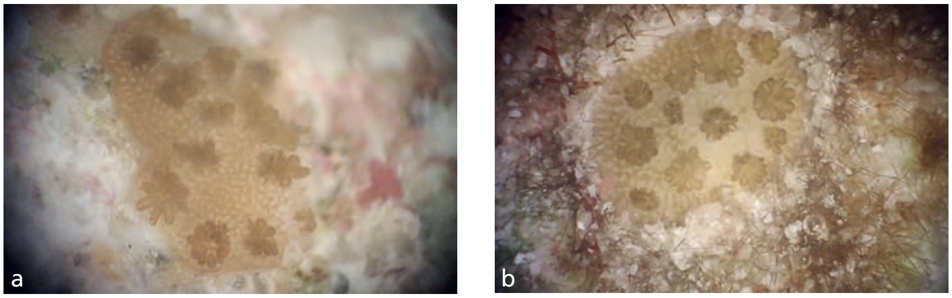

Figure 6. (a) Coral juvenile cultured for a period of approximately 6 weeks ex-situ (2 months after spawning) inside the ReefGuard. (b) Coral juvenile cultured for a period of approximately 2 months in-situ (2 months after spawning). The additional in-situ pressure of algal overgrowth on the young coral is clearly visible in the right panel. Absence of such pressures inside ReefGuard (a) will increase survival rates.

around Coral Bay. During the day, temperatures could easily reach levels of over 40 degrees Celsius and during the night, temperatures could drop below the ideal level of approximately 26 degrees Celsius. Besides the variations in temperature, there were high concentrations of dust around the area. In the end, the ReefGuard systems proved they could withstand these harsh conditions and consistently provide the corals with controlled conditions to develop.

Experiment 2: Test influence of environmental factors on survival rates (Coral Bay, Western Australia, 2015)

The second trial in Coral Bay (2015) was focused on a scientific experiment to determine the influence of environmental conditions on the survival rates of coral recruits – corals that are less than one year of age.

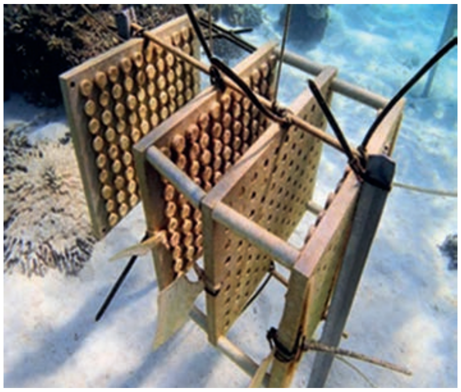

Figure 7. Typical setup of an outplacement rack with three replicate plates, each filled with 99 settlement plugs with microscope confirmed settlement of recruits, and one dummy plate.

For this, gametes of two coral species were collected: *Acropora millepora* and *Acropora digitifera*. A total of 36,000 plugs were preconditioned and *Acropora digitifera* larvae were exposed to these plugs for settlement. The aim was to achieve an amount of 12,600 plugs minimum, with confirmed corals settled on top, to be used in a scientific experiment. The experiment tested survival of recruits for up to fourteen different outplacement locations spread over an area of approximately 100 km with varying environmental conditions such as water depth, sedimentation rates,

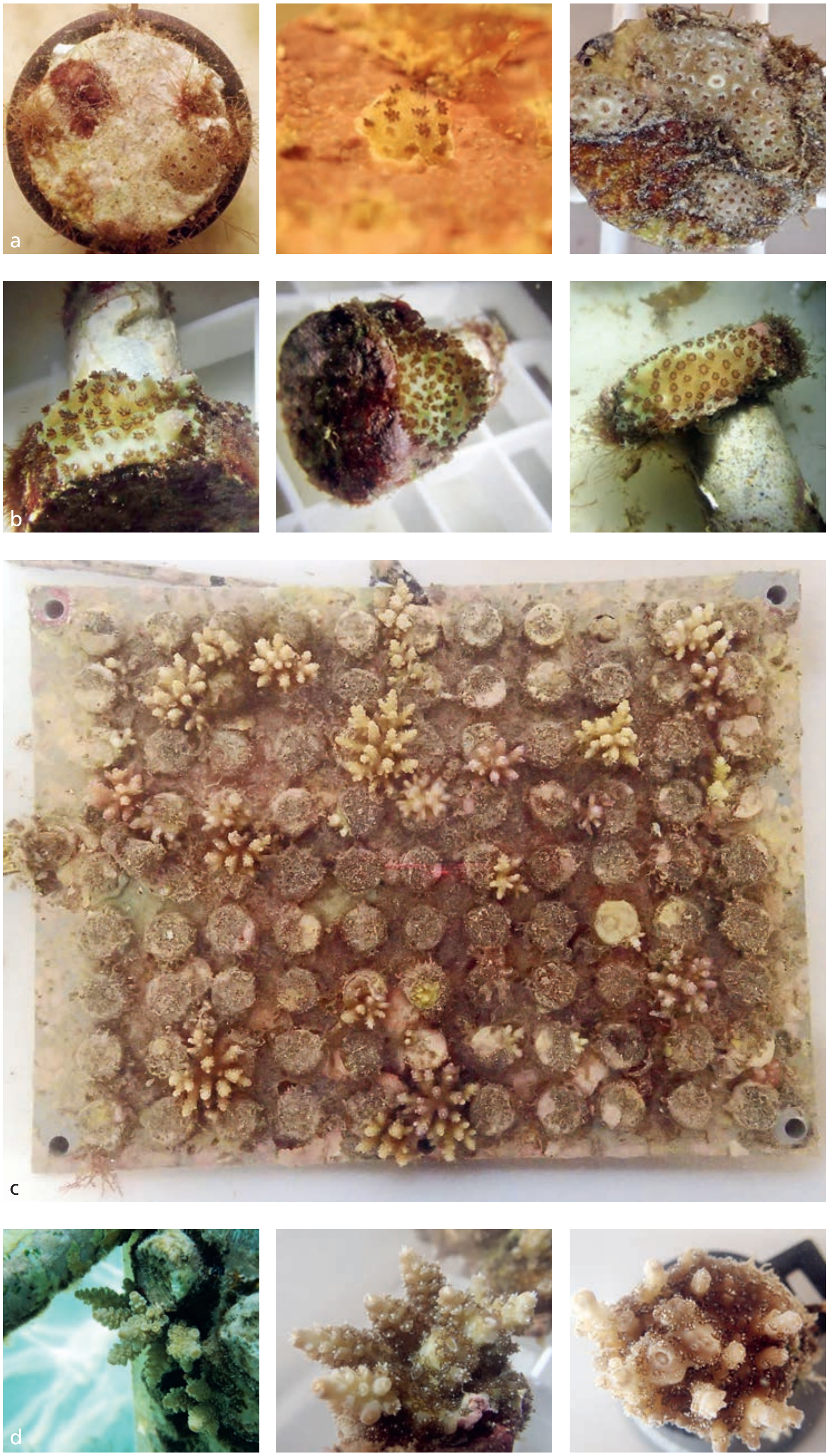

Figure 8. (a) Three close-ups of corals after 4 months of in-situ culturing. (b) Three close-ups after 8 months of in-situ culturing. (c) Overview of plate with survivors after 15 months of in-situ culturing. (d) Three close-ups of corals after 15 months of in-situ culturing.

Figure 9. *Acropora palmata* reef in the Bahamas. View just before sundown at one of the sites that was used for spawn collection.

algal growth, and wave energy.
For each outplacement location, three sites (Figure 7) were selected with three replicates, each containing 99 settlement plugs. Survival was monitored during three campaigns, 4, 8 and 15 months after the culturing period. Each monitoring step involved the retrieval of one plate per site, which was then inspected in the lab for survivors. The variations in survival could then be statistically related to environmental variations.

The experiment as a whole was a big success (Figure 8) as after 15 months of in-situ growth, the surviving corals were now approximately 3-4cm in size. They had escaped the size bottleneck and can safely be outplaced on the reef. The data from the experiment is currently being analysed and will be published at a later date. The conclusions from the data can be used to objectively select favourable outplacement locations that are likely to yield higher survival rates. This will serve as an important contribution to one of ReefGuard's main objectives.

The information gathered from the experiment was not limited to in-situ coral survival rates. During the spawning event the ReefGuard location was hit by Category 3 cyclone, Olwyn. This triggered an emergency evacuation of all non-critical team members, leaving just a skeleton crew behind to take care of the facility and the corals. The ReefGuard facility was designed to withstand cyclones of this level, but the actual occurrence of Olwyn proved the design and emergency procedures to be effective. The in-situ experiment was subsequently hit by Category 4 cyclone, Quang, about six weeks later. The results of at least part of the outplacement locations are expected to be affected by this. But the in-situ nursery setups could withstand this kind of conditions.

Experiment 3: Expand capability of in-situ spawn collection with Caribbean coral species (Coral Harbour, Bahamas, 2015)

After the two experiments in Australia, ReefGuard was shipped to Coral Harbour, New Providence, Commonwealth of the Bahamas, to be operated in the context of a port upgrade project that was executed by Van Oord for the Royal Bahamas Defence Force.

The main aim of the third experiment conducted in the Bahamas in the second half of 2015 was to gain experience with an in-situ gametes collection campaign with a different coral species – the highly endangered Caribbean reef-building coral *Acropora palmata* (Figure 9) – than was used in Australia. Also, it was to investigate the settlement rates on various types of substrate such as aragonite plugs, various types of rope, steel cable, and even PVC pipes and under different conditions like substrate orientation and incubation times.

The move towards the Bahamas and the shift to *Acropora palmata* moved the ReefGuard efforts from a pristine reef in Australia towards a Caribbean reef that is much more under pressure and a coral species that is endangered. It was anticipated that the gamete collection for corals under these circumstances would be significantly harder than on the pristine Ningaloo reef. Furthermore, literature stated that the mortality rates in the subsequent breeding steps were likely to be higher. Thus, the experiment on the Bahamas closely resembled the conditions that one would expect for reefs that actually could benefit from rehabilitation efforts.

Due to the endangered status and the sheer size of *Acropora palmata,* the gametes had to be collected in-situ. *Acropora palmata* colonies spawn on a reasonably predictable date. However, not all colonies may in fact participate in the spawning each year. In addition, a colony that spawns does not always spawn in its entirety – only one or a few branches may spawn. Although technically it is feasible to check the gravidity (i.e. presence of maturing gametes) of a coral branch by snapping off a (small) piece, this invasive method is not preferred for an endangered coral species such as *Acropora palmata*. As a consequence, the best and safest way to collect gametes is to do so while scuba-diving in-situ using so-called spawn collection nets at the night of the spawning (Figure 10). This presented the Van Oord crew with an important challenge, including the setup and execution of additional health and safety protocols. Results of this pilot are currently being analysed and a publication is in preparation (Robijns, 2016; Robijns et al., in prep.).

Figure 10. Night diving team that successfully collected the gametes using spawn collection nets during the spawning event in the Bahamas

As from the previous experiments, the lessons learned were not limited to in-situ spawn collection and settlement success on different substrates. Aside from biological factors, technical aspects of setting up shop in remote locations also came into play. It was concluded that in order to maintain controlled conditions 24/7, a high level of power management (including an automatic alerting system) was going to be of critical importance. Consequently, following this pilot an additional power control unit was added to the ReefGuard setup.

Experiment 4: Test influence of aqua culture treatments on survival rates (Coral Harbour, Bahamas, 2016)

The fourth field application was focused on executing a scientific experiment on the following: the effects of variations in aquaculture length and aquaculture treatments on ex-situ growth; and the survival rates and in-situ survival rates after outplacement to the reef. This experiment would thus provide important information related to the issue of aquaculture length and the range of sizes at which the recruits could or should be outplaced as described in the previous sections.

An experiment was setup to test the effect of four different aquaculture treatments:

- regular ambient sea water
- regular ambient sea water + feeding
- sea water with increased levels of total alkalinity
- sea water with increased levels of total alkalinity + feeding

After spawn collection, fertilisation and settlement, the presence of recruits on each settlement tile was verified using the characteristic green fluorescence of newly settled coral larvae when illuminated using blue light (BlueStar flashlight package, NIGHTSEA). Settlement tiles with confirmed presence of recruits were assigned to nine batches of equal size consisting of six replicate plates and accommodated in the different aquaculture basins. During the first four weeks, all recruits were allowed to establish symbiosis by exposing them to coral fragments that served as symbiont donors. Batch one was outplaced after successfully establishing symbiosis, after four weeks in ambient treatment. Batch two to five were outplaced after nine weeks of receiving one of the above mentioned four aquaculture treatments, and batch six to nine were outplaced after 14 weeks of receiving one of the four aquaculture treatments (Figure 11). After another 13 weeks, all plugs were retrieved from the field to assess the survival rates. The data will be used to statistically analyse the effect of each of the treatments, as well as the duration for which each of the treatments was applied on growth and ultimately survival rates.

The entire experiment was conducted successfully as planned. Due to a slightly lower than anticipated spawning intensity in this particular year, the experiment was scaled down a little using six rather than nine

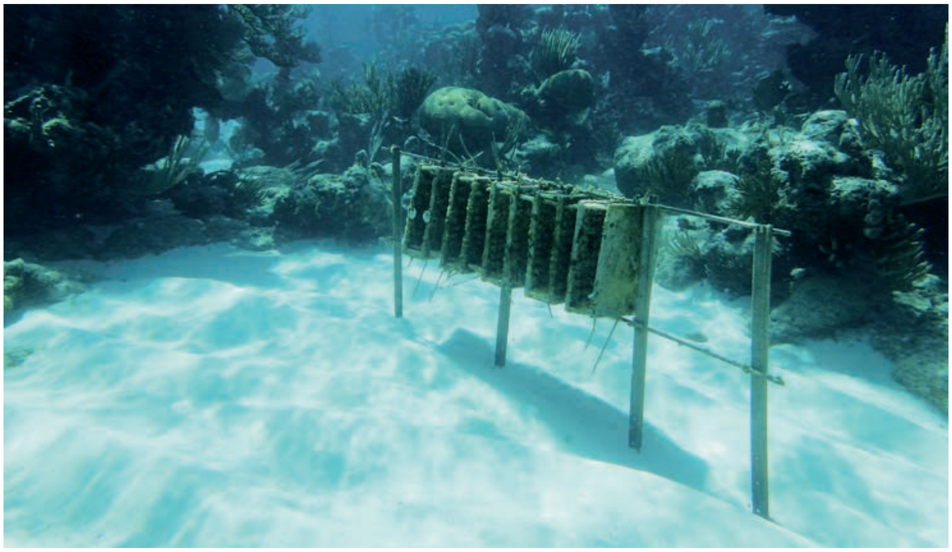

Figure 11. One of the six replicate outplacement racks containing nine plates with recruits from all three outplacements. (From right to left) Each rack contains: one dummy plate; one plate with recruits outplaced after 4 weeks of ambient aquaculture treatment (first outplacement); four plates with recruits outplaced after 9 weeks of each of four aquaculture treatments (second outplacement); and four plates with recruits outplaced 14 weeks of each of four aquaculture treatments (third outplacement).

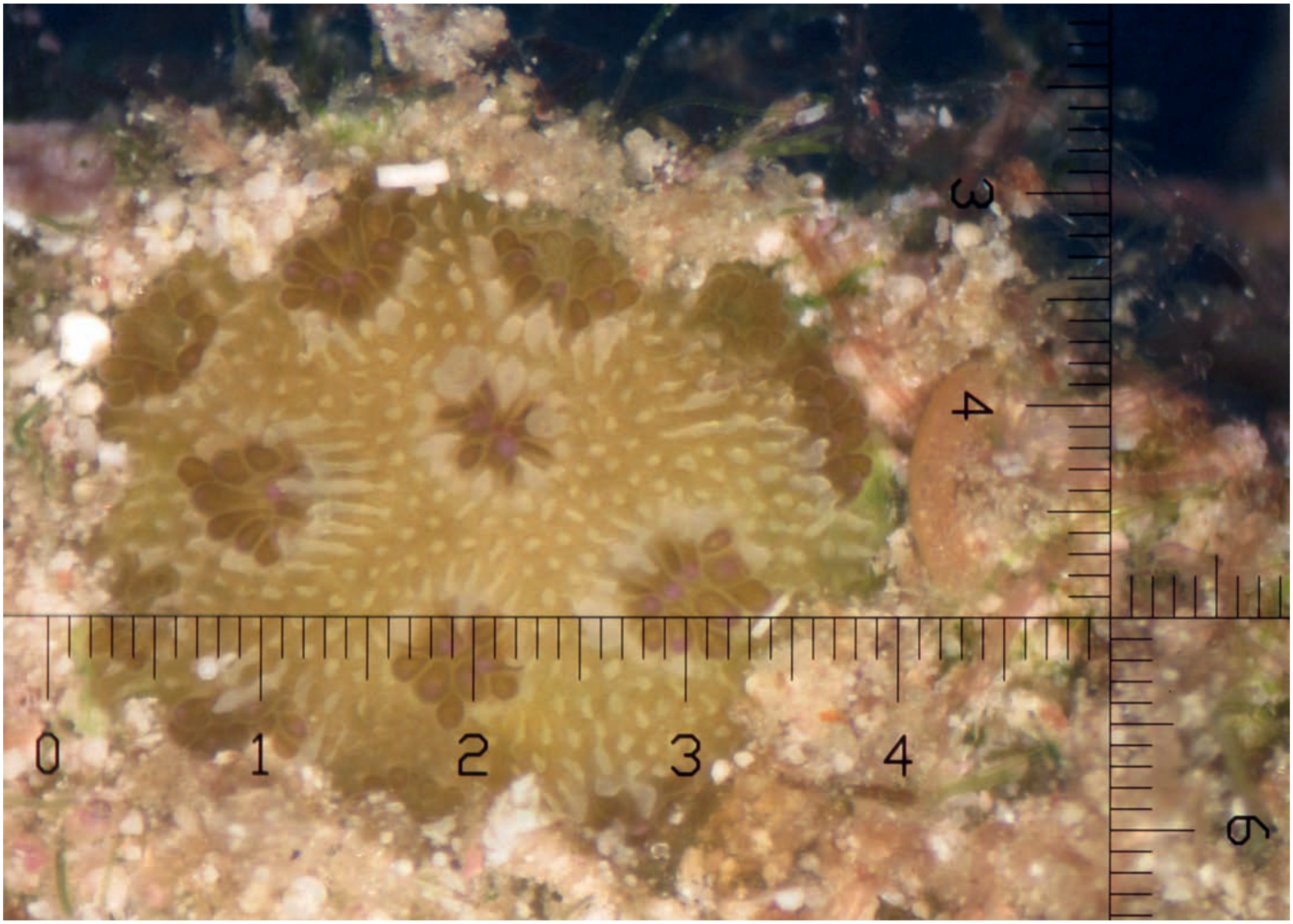

Figure 12. A healthy *Acropora palmata* recruit consisting of 13 polyps photographed at six months after settlement (diameter of ~ 4 mm, 10x magnification)

replicates and having less recruits settled per settlement tile. However, the experiment was still one of the largest of this kind ever conducted. At six months after larval settlement, 1,514 living coral recruits (~17%) on 1,242 settlement tiles (~30%) were counted (Figure 12). This is a great achievement and an import step forward for the endangered coral species *Acropora palmata,* which is currently suffering severe recruitment limitation (i.e. near absence of natural recruitment). The results of this pilot are currently being analysed and a publication is in preparation (Bloemberg, 2017; Schutter et al., in prep.).

The information and experience garnered from this experiment was not limited to in-situ spawn collection and the testing of different aquaculture treatments on growth and survival rates. In October 2016, the Bahamas and also where the ReefGuard was located, were struck by Hurricane Matthew at a near Category 3 strength. Again, the ReefGuard survived these conditions. The island itself, however, was quite severely impacted and for a period of time it was hard to reach the ReefGuard site. Luckily, however, the newly added power management system was stable, resulting in stable survival rates of the coral inside ReefGuard. The hurricane struck just before the second outplacement of Batch two to five. The already outplaced Batch one took a severe beating from the storm. The survival data of this batch is assumed to be influenced by this event. A lesson to take away from this experiment was that an ex-situ culturing period inside the ReefGuard facility should be considered for at least as long as is necessary to make it out of the hurricane season. Furthermore, the fact that three major tropical storm events happened during the four experiments suggests that storm impact is not an over-estimated risk.

PRACTICAL GUIDELINES AND LESSONS LEARNED

The ReefGuard experiments provided the experiment crew with the essential knowledge and practical know-how on how to perform: controlled coral breeding experiments at quite remote locations as dictated by the presence of coral reefs, and of an ecologically significant scale (tens of thousands of surviving juveniles to work with).

The fact that the researchers focused on tens of thousands settlement substrates was mainly influenced by their ambition to conduct robust scientific experiments.

This involved a visual microscope inspection and recruit count of every single tile at various stages of the experiment. The size of the experiments was thus to a large extent limited by available time and counting capacity. For large scale rehabilitation projects such detailed counts will not be necessary, allowing the process to be scaled up drastically. Note that an evidence-based approach is still recommended in order to monitor/demonstrate effectiveness of the approach as stressed by Young (2000).

To make sure the lessons learned are recorded properly and easily transferred to the next professional applications, two practical guidelines of Van Oord are actively maintained and updated:

- ReefGuard – Operating Manual
- ReefGuard – Book of Protocols

The guidelines (Figure 13) contain checklists and photo references for steps to be taken where possible.

Overall it can be concluded that the initial objectives of the Coral Rehabilitation Initiative were successfully achieved:

- The ReefGuard crew were are able to mobilise a highly controlled environment for coral breeding activities to basically any project site in the world (including the acquisition of required permits).
- ReefGuard can maintain this controlled environment under the most extreme conditions (heat, dust, hurricane force winds), provided that all equipment can function up to specifications.
- Gametes can be successfully collected both in-situ (with a diving team at night) and ex-situ (both on a jetty and inside the ReefGuard).
- The collected eggs can be successfully fertilised, yielding > 1 million larvae consistently that can be nurtured up to the stage that they are ready to settle.
- The coral larvae can be settled on substrates of a researcher's choice (tens of thousands aragonite settlement tiles, multiple types of rope and other substrate types).
- In Australia and the Bahamas, the ReefGuard crew were able to outplace substrates in nurseries on the reef and still have significant number of survivors after several months ready to be used for active reef rehabilitation.

Furthermore, the ReefGuard crew built up the in-house expertise to do all of the above, while working constructively and professionally with coral scientists and local stakeholders.

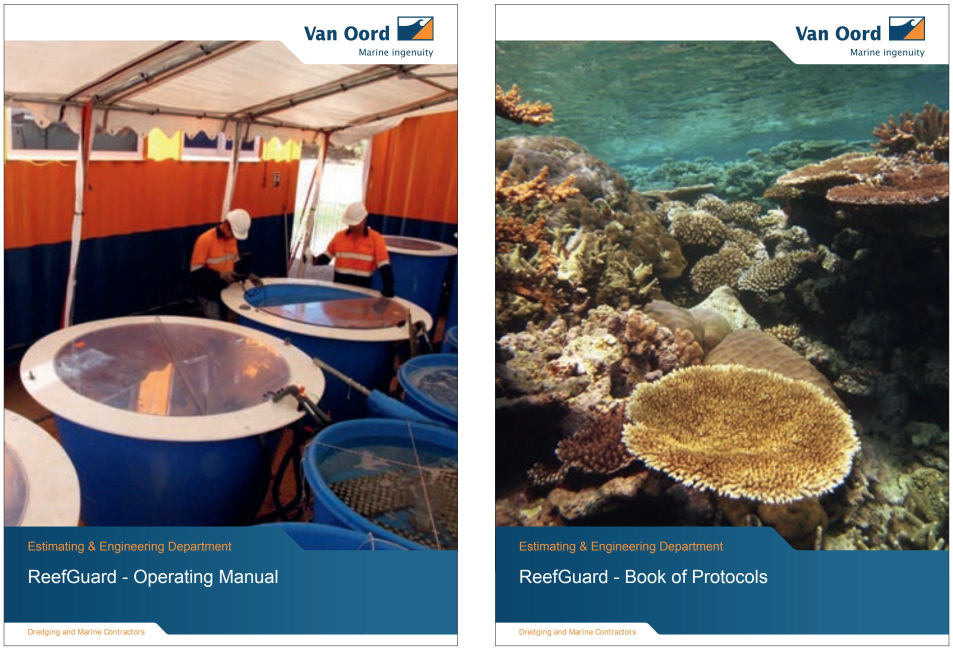

Figure 13. Practical guidelines for ReefGuard operation and a *Book of Protocols* for coral breeding.

CONCLUSIONS

The Coral Rehabilitation Initiative and mainly the ReefGuard, in combination with four field experiments in Australia and in the Bahamas have conclusively demonstrated that active reef rehabilitation can indeed be a viable part of marine and coastal infrastructure development. A successful campaign requires a substantial level of knowledge on coral ecology as well as on aquaculture techniques.

Worldwide, a huge coral community is working on the challenge of progressing effective reef restoration. An important lesson learned with ReefGuard is that a close interaction between academic researchers, non-profit conservation organisations and marine contractors significantly increases the potential to achieve successful coral rehabilitation. This is especially so when it comes to the actual improvement of existing knowledge and making it applicable around the world utilising the momentum that is associated with large marine infrastructure development projects.

An interesting observation is that pro-active solutions, as described in this paper, are rarely addressed in environmental impact assessments. As a consequence, these techniques are equally rarely recommended as potential mitigating measures, which makes their actual implementation in infrastructure developments so much harder. Hopefully, the Coral Rehabilitation Initiative, in combination with the wider trends for more sustainable solutions – 'Building with Nature', 'Working with Nature' and 'Engineering with Nature' – will also boost the attention for pro-active solutions such as promoted by ReefGuard.

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