English translation for「改訂　有性生殖によるサンゴ増殖の手引き」

A guidebook for coral propagation through asexual reproduction (revised version)

March 2019
Fisheries Agency - Fisheries Infrastructure Department

有性生殖による サンゴ増殖の手引き

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Table of Contents: Case Studies

1.4 Geographic distributions of corals

((*After the world map))

Figure I.1-7 shows the geographic distribution of Japanese coral reefs and coral populations.

Coral reefs in Japan are mainly distributed in the Ryukyu archipelago which span from 24° N to 29° N (Okinawa prefecture and Amami Islands in Kagoshima prefecture) and the Ogasawara Islands (Tokyo prefecture) and are considered the northern limit of world-wide distribution of coral reefs. Coral reefs are also found in Senkaku Islands, Daito Islands, Iou Islands, Izu Islands, Minami-tori Island, Okino-tori Island, and others. The Okino-tori Island which is located at 20 °N is the southern limit of coral reef distribution within Japan. Coral populations are found in the Tateyama Bay in Chiba prefecture on the Pacific side, and around Sadogashima Island on the Japan Sea side.

The main reason why there is a higher diversity of reef-building corals in Japan despite the relatively higher latitude can be attributed to the Kuroshio Current (Japan Current). Kuroshio Current is the largest warm current, which flows along the Ryukyu archipelago and towards north. This results in warmer sea temperatures relative to other regions of the same latitude, and because the coral larvae are supplied from the south, especially the Ryukyu archipelago has a high biodiversity, a greater connectivity of the reef-scape, and larger reef area despite being the northern limit for coral reefs distribution (茅根 et al. 2004).

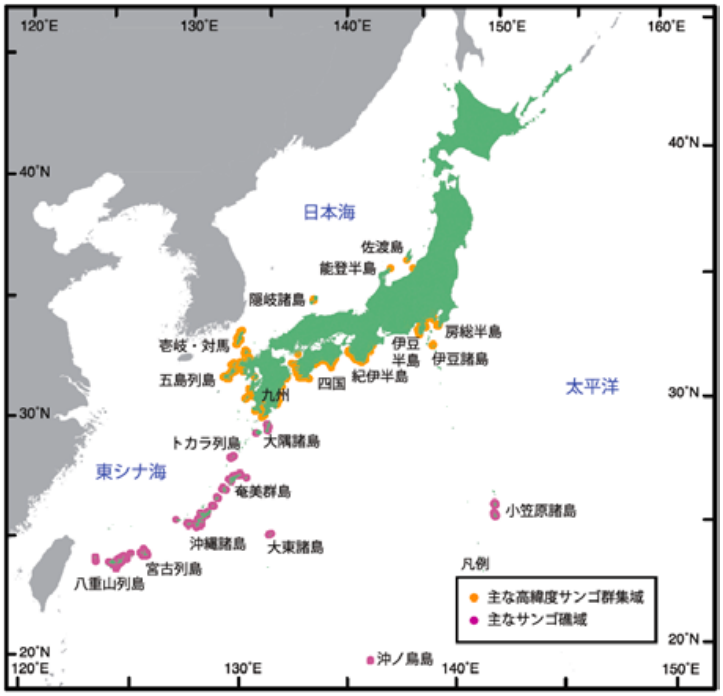


Figure I.1-7 Geographic distribution of Japanese coral reefs and coral populations (Ministry of the Environment, 2016)

2. Coral propagation plans

To repair and regenerate coral reefs, a plan that is efficient and practical, based on a mindset that reflects purposes such as national land conservation, environment & ecosystem conservation, and maximising fishery resources is required. Specifically, it is important to identify the cause of growth inhibition of corals, then plan countermeasures to remove or reduce such obstacles. Here, we will describe the basic approach to coral propagation, planning for coral repair and regeneration, and common countermeasures.

2.1 Basic approach

[When planning for coral propagation, one needs to not only understand the characteristics of the natural environment of planned sea area, but also choose the coral species appropriate for the purpose of propagation and understand their ecological characteristics. Especially the cause of growth inhibition of corals tends to be a complex entanglement of many factors, and therefore it is best to move forward with an accommodative management plan.]

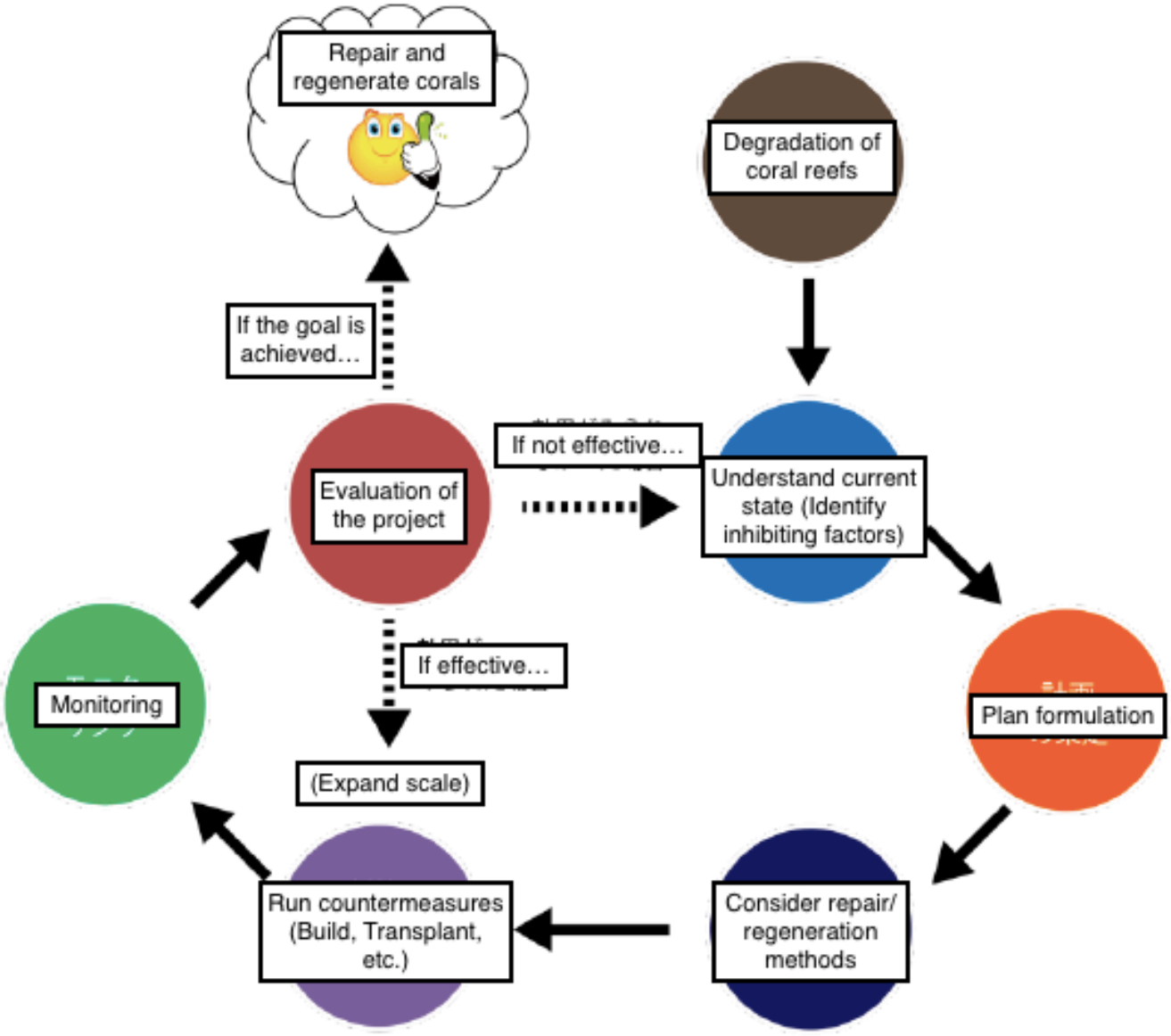


Figure I.2-1 PDCA Cycle for coral reefs regeneration

2.2 Coral reefs repair and regeneration approaches for maximising fishery resources

[When repairing and regenerating coral reefs with the purpose of maximising fishery resources, it is necessary to plan based on the idea of creating a favourable living environment for the given life history of aquatic life.]

<Explanation>

Coral reefs provide for many diverse organisms with habitat, shelter, and breeding ground. Such important resource also functions as a fishing ground for humans however it is under threat due to degradation of the reefs (read more in Chapter I 1.6). “Fishery environment maintenance” is promoted in the sea area where fishing ground function has declined, with the goal to raise the overall productivity in the ecosystem. It considers the movements and life history of the fishery organisms and aims to create a favourable aquatic environment for them.

It is therefore important to understand the targeted fishery organisms’ life history and their arena, as well as the roles the coral reefs play (breeding grounds, nursery ground, adult habitat, feeding ground) to set a coral propagation plan based on the basic approach stated above.

The reports are currently limited, however we now know that some fishery species utilise branching coral colonies as crucial habitat. For example, *Epinephelus ongus*, one of the biggest total catch for groupers, is known to use branching *Acropora* as their main adult habitat and bottlebrush branching *Acropora* as their nursery ground (Nanami et al. 2013). Therefore, the establishment of a restoration method for branching *Acropora* that would otherwise would be less likely to regenerate by themselves, is a top priority from a fishery resource recover stand point (Suzuki et al. 2011).

Figure I.2-2 is an illustrative guide of the habitat and nursery ground for the grouper mentioned above.

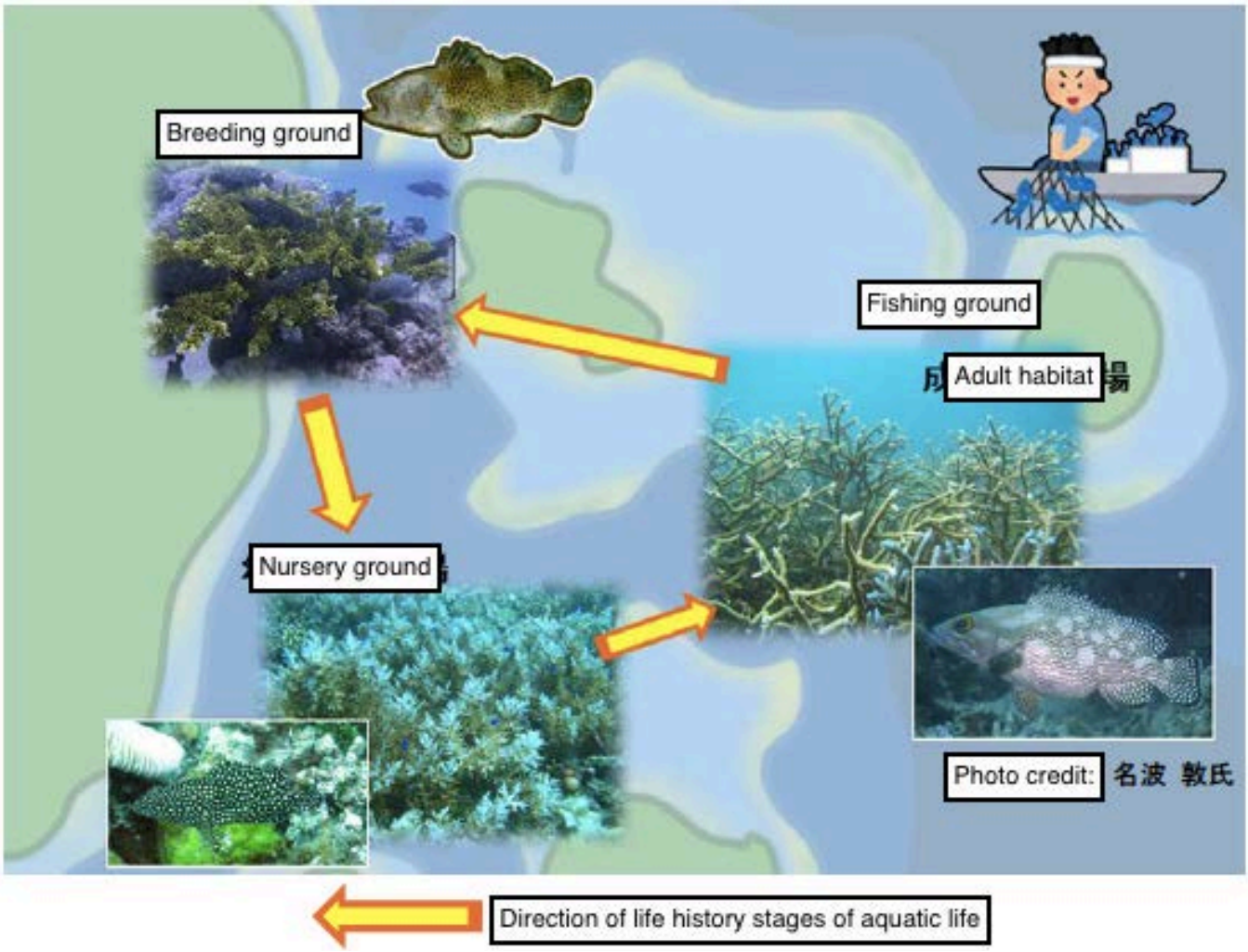


Figure I.2-2 (Example) Establishment of aquatic environment around the coral reefs area

2.3 Understanding the current state

[When understanding the current state, one should conduct a survey to acquire accurate information on the ecology and environmental requirements of corals in the planned sea area, then evaluate those results to utilize for planning project trajectories or repair and regeneration of coral reefs.]

TableI.2-1 Contents for current state assessment (survey)

Content	Pre-assessment
Coral distribution	○
Depth (Bathymetry)	○
Water temperature	○
Benthos	○
Suspended matter (or water visibility)	○
Wave action / Current flow	○
Predatory organisms (COTs, fish etc)	△
Coral recruits	△
Salt nutrient	△
Sand movement	△

Legend: ○ Survey required △ Survey as seen necessary

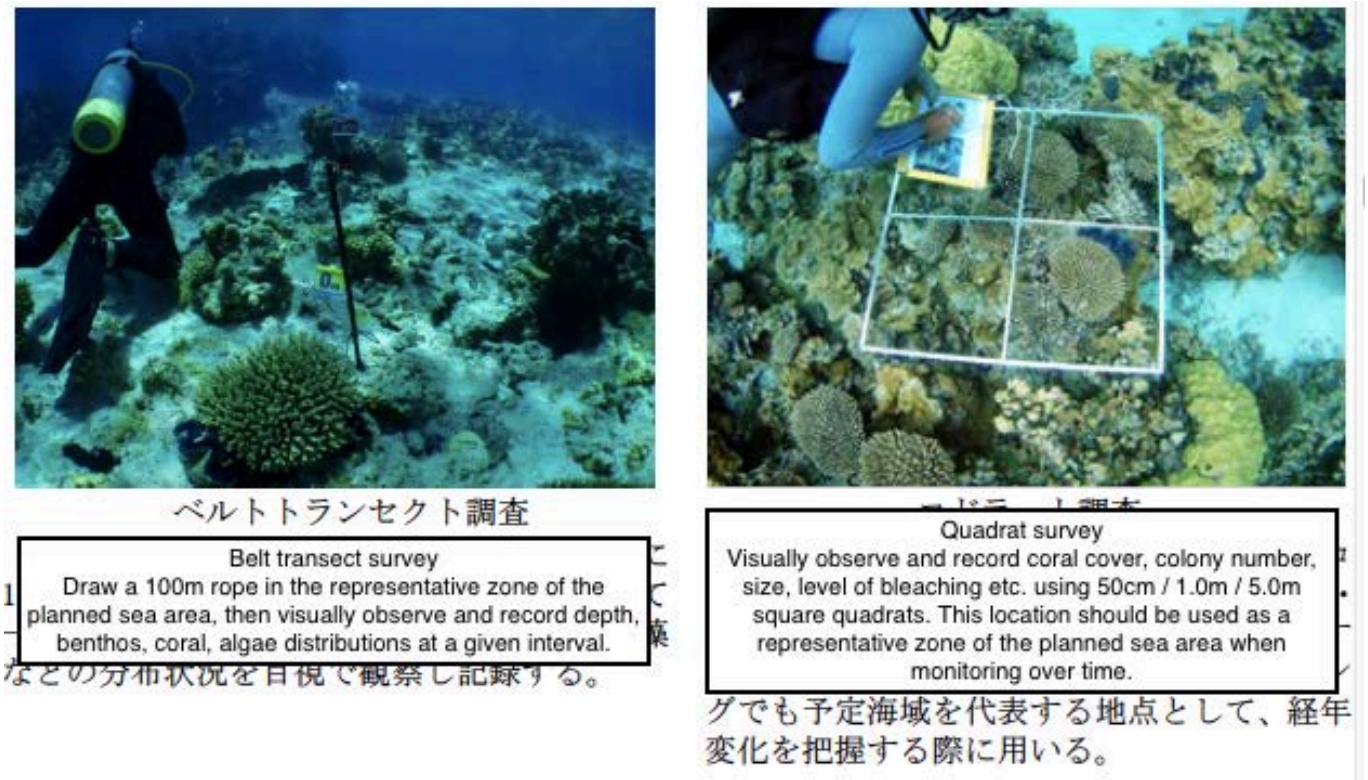
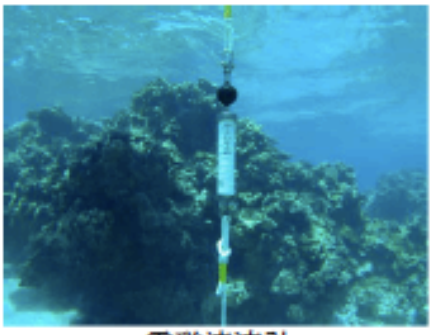


Figure I.2-3 Coral distribution survey

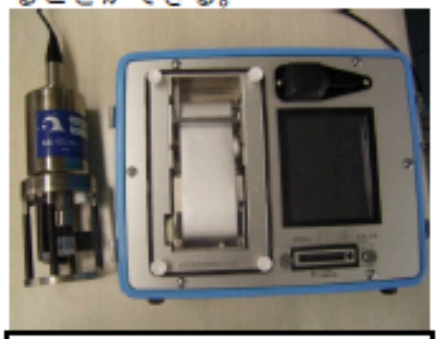
Wave meter
Install on sea floor to monitor wave height



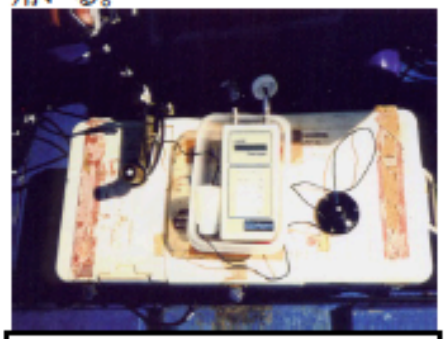
Electromagnetic flow meter
Install on sea floor to monitor flow speed. Install on multiple depths to monitor depth-specific flow

Drifting buoy
Float on sea surface and track surface water flow. Used to monitor spawning slick movement

Temperature logger
Install on sea floor to monitor sea temperature. Can record more than a year

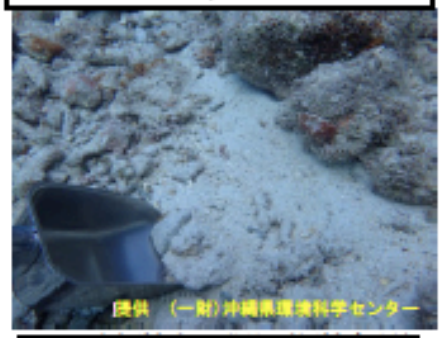


Multi-item water quality analyser
Measure sea temperature, salinity, turbidity etc. at different depths



Quantameter
Measures light required for photosynthesis by the symbionts. Photometer works too

Recruitment tiles
Observe coral recruitment by placing a 10cm square slate on the sea floor. Many ways to place these



SPSS (suspended solid in benthic sediment)
Collect substratum and measure red soil accumulation per 1msq. See Okinawa Prefecture (2017b) for details

Horizontal visibility
Use a Secchi disk perpendicular to the sea floor and measure horizontal visibility at a relatively clear reef flat

Figure I.2-4 Main equipment for environmental monitoring surveys

2.4 Plan formulation

[Using the results from current state surveys, evaluate the planned sea area for coral reef repair and regeneration and set a project goal. Then create an practical plan by assessing the appropriate coral species and place, as well as methods and processes of repair and regeneration. However, to move forward we need to agree with the fisheries manager and sea area users and respect their ideas and needs.]

<Explanation>

1) Set a project goal

Evaluate the planned sea area based on current state surveys and consider where and how in the planned sea area the coral reef repair and restoration can be achieved. Consider not only the available techniques but also limitations due to legislations and budget and set an achievable goal. In addition, ecosystems surrounding the corals are unpredictable and often susceptible to change, which means that it takes years for a stable coral community to develop. Therefore, it is ideal to estimate the length of time (in years) required to achieve the goal.

2) Assemble the implementation system

The implementation bodies and associates which will promote the coral reef repair and restoration are diverse; local governments, research institutes, fishery managers and unions, diving shops, residents and companies. Therefore, it is important to understand the thoughts and needs of each party and set the expectation of participation from the planning stage. It is also critical to share the rationale behind the project with all participants to effectively execute the plan.

To achieve consensus, both long-term and broad, short-term and narrow goals need to be shared. In addition, it is encouraged to create an atmosphere which ideas and creativities are welcomed for setting up infrastructure, conservation activities, and maintenance schedules. There is no concrete method or technique to achieve consensus, however in other projects occasional meetings and workshops with the aid of experts are often utilised (Tables I.2-2, 2-3).

3) Selecting target species

Select from the prioritised coral species based on environmental factors and topographic characteristics of the planned sea area. Prioritised coral species are the ones considered to be favourable for the current environmental state and have higher propagation potential. However, even if it is a prioritised species, placing a delicate branching *Acropora* in a high wave energy environment or a corymbose species in a calm area will probably not maximise the growth potential. Therefore, take into account the specific environmental and topographic factors of the planned location.

If sexual reproduction propagation method is going to be used for nursery stock production, it is necessary to choose the species which is currently known as reproducible.

4) Identify inhibiting factors and narrowing down planned site

Identify or estimate the inhibiting factors based on the current state survey results.

If the factors are identified, choose the location where the target species can grow based on the planned sea area habitat map, inhibiting factors, and topographic characteristics. For example, draw different zones within the planned sea area (e.g. Figure I.2-5) and identify the inhibiting factors within each zone. Discuss the repair and restoration methods which can deal with each factors and narrow down the effective and realistic site.

The zone with healthy corals can be considered as a favourable environment for recruitment and growth, and therefore should be protected. On the other hand, zones where they used to have corals but no longer have or they do but unhealthy can be chosen as suitable sites in condition that there are executable countermeasures. If the zone is difficult to evaluate, or the countermeasure is difficult to execute, it is best not to choose as suitable sites.

There are different ways to narrow down the suitable sites. For example, you can run a transplant experiment of a target species fragment and decide on a location with better survival and growth rate. Alternatively, you can layer a simulation map of wide scale environmental parameters (wave and wind, water quality, etc.) onto a coral habitat map, then choose areas where (1) Bommies exist but new recruits are less, or (2) sand movement is minimal even during typhoon season (refer to Chapter II.2-4).

After narrowing down the restoration suitable site, consider the repair and restoration methods (Chapter I 2.5).

Table I.2-2 Roles of each organisation

Prefectures/Municipalities	Fisheries managers/Dive operators
• Organise project • Funding support • Provide information • Organise and run workshops • Nurture core leaders (fishery managers)	• Production and nurturing coral nursery stock • Monitoring • Coral reefs conservation activities • Participate in workshops
Researchers/Research institute professionals	**Local residents/Companies**
• Provide information on the state of coral reefs • Prevention of coral reefs degradation, research and development of conservation techniques • Participate in workshops	• Cooperate in monitoring • Cooperate in coral reefs conservation activities • Participate in workshops

Table I.2-3 Websites of testing and research organisations of corals

Japanese Coral Reef Society	http://www.jcrs.jp/
Seikai National Fisheries Research Institute, Fisheries Research Agency	http://snf.fra.affrc.go.jp/
International Coral Reef Research and Monitoring Center	https://www.env.go.jp/nature/nats/ntr/11/03.html
Kyushu Regional Environmental Office, Ministry of the Environment Government of Japan	http://kyushu.env.go.jp/list.html
Sesoko Station, University of the Ryukyus Tropical Biosphere Research Center	http://www.tbc.u-ryukyu.ac.jp/sesoko/home
Okinawa Prefectural Fisheries Research and Extension Center	http://www.pref.okinawa.jp/fish/
Akajima Marine Science Laboratory	http://www.amsl.or.jp/
Biological Institute on Kuroshio	http://www.kuroshio.or.jp/

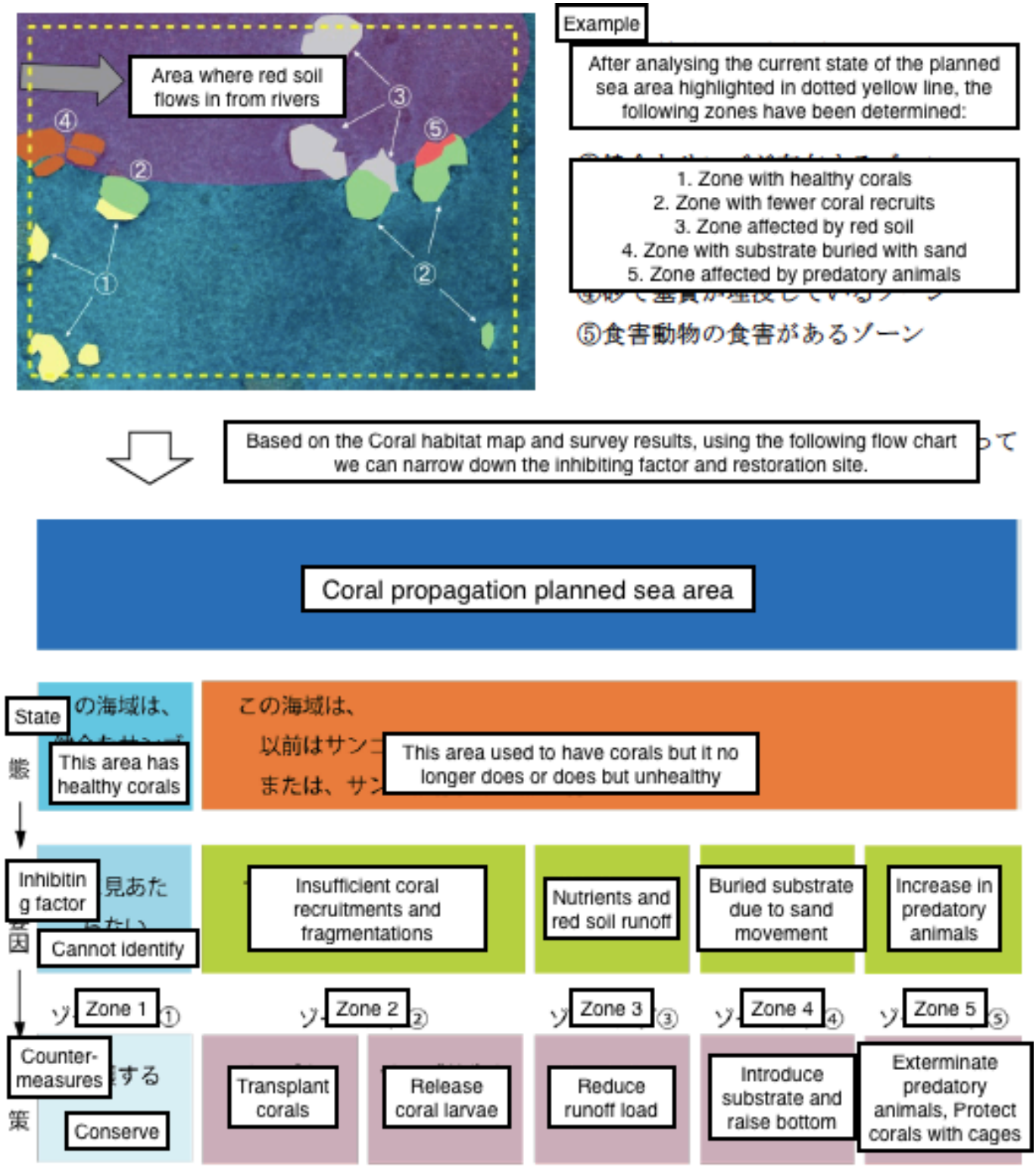


Figure I.2-5 Examples of identifying growth inhibiting factors and narrowing down potential restoration sites

2.5 Consideration for repair and regeneration techniques

[Choose methods appropriate for eliminating or reducing inhibiting factors in the planned sea area which were narrowed down from the current state surveys. However, if there are multiple inhibiting factors, combine different methods and execute effectively.]

<Explanation>

There are two scenarios which corals cannot grow: there is a smaller adult coral population that new recruits cannot be expected, or the growth environment for the target coral has deteriorated. It is also possible to have these two scenarios happening at the same time, and one of the underlying causes is water temperature increase due to global warming which leads to mass bleaching events or ocean acidification. This is a global issue which local efforts only cannot solve. Therefore, it is crucial for the society as a whole to reduce carbon dioxide emissions.

On the other hand, issues such as loss of shoal area, substrata decrease, coral damage due to rubble movement, red soil/nutrient soil/pesticide run-offs, wave height change, predation, competition, disease, overfishing, excessive aquaculture and tourisms are all small scale problems which can be dealt with local efforts. It is then possible to aid the resilience of corals through taking countermeasures. Loss of shoal area and terrestrial run-offs are problems associated with other projects and terrestrial industries, and therefore cooperation with corresponding government and administration is crucial. Overfishing can be prevented by setting marine protected areas (MPA), no-take season, and limits to catch size for managing fishery resources. Excessive aquaculture requires a systemic measure to promote environmentally friendly practices. Other issues can be dealt with specialised techniques listed in Figure I.2-6.

Coral reef repair and restoration methods can be categorised into biological methods (reinforce the reproduction capacity of corals) and engineering methods (improve the environment). The latter can be further divided into facility establishment and conservation activities (e.g. COTs killing). Some of these methods already have evidence of positive outcome, and others such as temperature increase and disease outbreaks rely on future technological development. Table 1.2-4 lists the methods proven to be effective so far. If multiple inhibiting factors have been identified, different methods need to be combined to run countermeasures. For example, if there is a lack of substrate for new coral recruits, as well as lack of larvae influx, the establishment of coral propagation reef and transplantation would be ideal countermeasures.

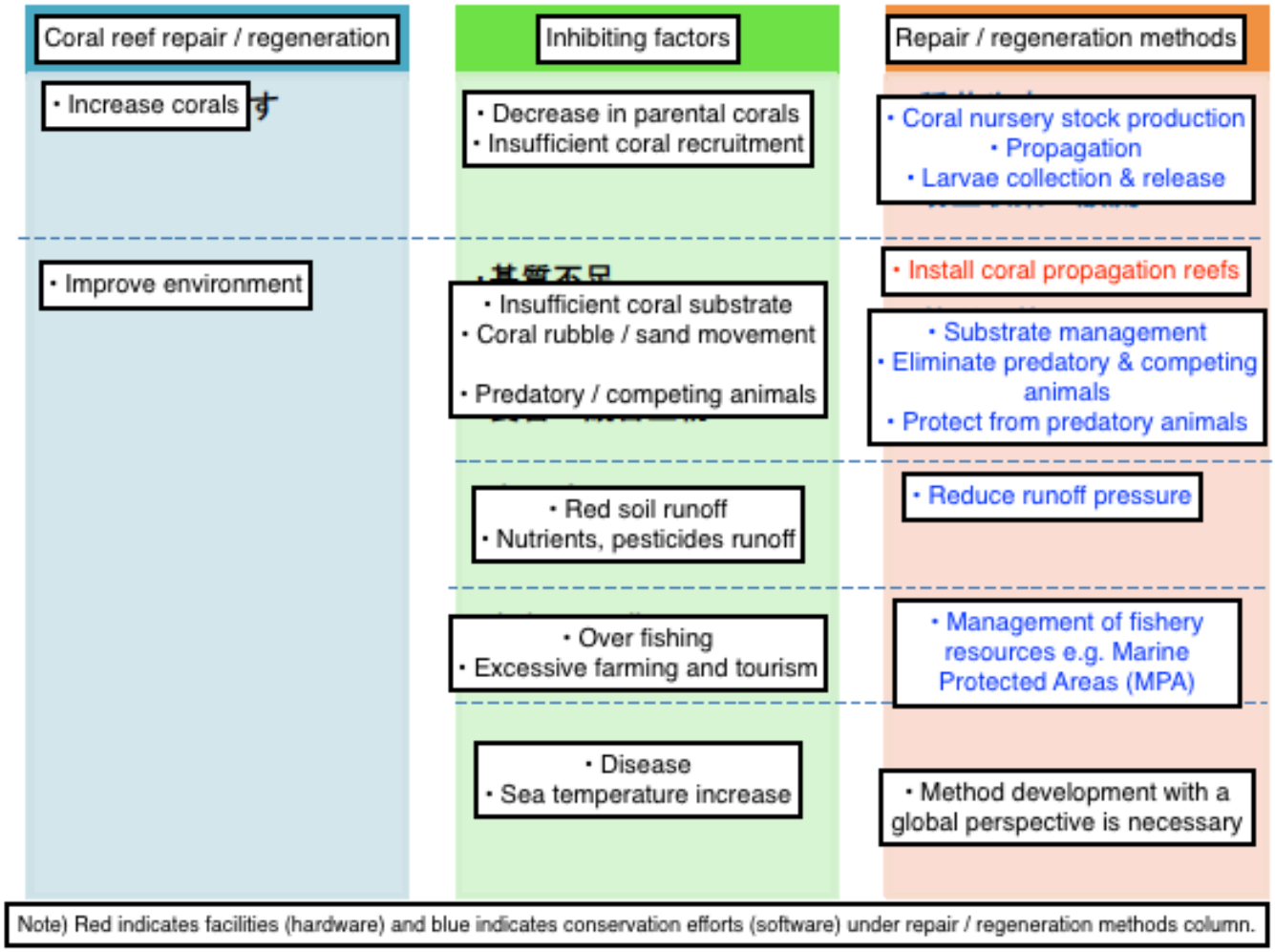


Figure I.2-6 Main coral inhibiting factors and coral reef repair and regeneration methods

Table I.2-4 Overview of coral repair and regeneration methods

Countermeasure techniques		Overview	Notes
Increase corals	Direct transplant	Break off a branch from parental colony (fragmentation), transplant on a base with underwater glue.	• Make sure not to cause stress to the parental colony during fragmentation. • Need a permit from local municipalities when using natural coral colonies. Not possible in Okinawa prefecture.
	Transplantation after mid-term rearing via asexual reproduction	Rear fragments acquired through aquaculture or natural harvest and transplant to base.	• Requires a mid-term rearing facility to rear nursery stock in the sea. • Fragmentation is possible with any species. • The bigger the fragment is, the less stress caused by transport or transplant. • Labour and cost is greater than direct transplant, but less than sexual reproduction method.
	Natural settlement of planula larvae	Set a settlement equipment in the trajectory of planula larvae, let them naturally settle, and transplant	• Can maintain biodiversity. • Settlement rate and post-settlement growth rate are so far low.
	Mass production of larvae and release	Produce larvae at the on-land nursery stock facility and harvest/rear larvae from spawning slicks, then release them towards settlement equipment.	• Spawning slicks may not be available depending on the weather. • Requires rearing of the collected larvae in aquaria until they are ready to settle.
	Transplantation after mid-term rearing via sexual reproduction	Rear nursery stock produced and transplant to base.	• Possible to produce nursery stock in thousands. • Does not harm parental coral colony. • Can maintain genetic diversity. • Requires expertise and human power in nursery stock production. • Requires a mid-term rearing facility to rear nursery stock in the sea.
Improve environment	Establish substrate	Establish a coral propagation reef in case there are no favourable substrate for larvae to settle.	• The substrate needs to be a favourable environment for corals. • It has to be structurally safe with regards to wave and current.
	Remove suspended sediment	Raise substrate to the height which sediment is less likely to settle, or choose the right shape.	• Needs an *in situ* assessment for sediment height. • The grid-shaped substrate is less like to have sediment, but will attract more algae. Requires periodic removal maintenance.
	Counter predatory animals	Remove crown-of-thorns-starfish, *Drupella cornus* etc.	• Requires periodic removal of predatory animals. • Costs to dispose of the removed animals.
	Maintain competitions	Remove algae and benthic organisms which can compete for substrate.	• Requires periodic removal of competing animals.
	Prevent red soil etc. run-offs	Execute prevention measures for terrestrial run-offs (multing, green belt, sand pond etc.)	• Need to work with other organisations to reduce the run-off amount. • Need to raise awareness on why it is necessary.
	Limit overfishing and excessive aquaculture	Establish a closed season for fishery or MPAs to manage fishery resources.	• Need to establish effective protected areas. • Promote aquaculture methods with less influence on the environment.
	Limit tourism	Limit the number of people entering the area and/or the area open for public use.	• Need to set protected areas and general use areas. • Install community mooring lines. • Conduct workshops and community beach clean-ups to raise awareness.

Note) Mid-term rearing facility is to rear the nursery stock corals to the size ready for transplantation.

Coral propagation reef is to let the coral grow on its own and does not require transplantation to elsewhere afterwards.

2.6 Execution of countermeasures

[When executing the countermeasures, pay attention to the environment and be flexible while following the original intention. When there are multiple inhibiting factors, combine countermeasure techniques.]

<Explanation>

1) Conservation efforts

Refer to「サンゴ礁保全活動の手引き」(Guidebook for coral reefs conservation methods, Fishery Agency 2015a) for conservation effort method details, necessary equipment, and things to consider.

2) Establishing infrastructure

(1) Mid-term rearing facility design and establishment

Refer to Chapter II 2.3.1 or 3.2.1 for mid-term rearing methods which utilises rearing facility establishment (Chapter II 1.1.2) to maintain coral nursery stock via sexual reproduction or collected fragments (asexual reproduction) until they grow to sizes suitable for transplantation.

(2) Coral propagation reef design and establishment

Install a coral propagation reef when there is a lack of substrate for corals to settle. For design, refer to「漁港・漁場の施設の設計の手引」(Guidebook for ports and fishing ground facility establishment) (Fishery Agency 2015b) Chapter 16 section 2 'Settlement substrate' 2.2 Algal reef.

For establishment, consider the surrounding environment, establishment methods, and timing. The methods are more-or-less similar to establishing fishery reef or settlement substrate. However, it is likely that the target sea area is shallow and/or has bommies and therefore establishment plan must be carried out carefully (refer to Chapter II 2.3.1). In addition, if transplantation is happening at the same time, schedule control is necessary to ensure propagation reef is ready by transplanting time. Keep in mind that different permits (e.g. special collection permit) and special equipment need to be prepared as well.

2.7 Rearing management

[For rearing management, monitor the growth of transplanted corals and changes in the environmental parameters. If there are any problems, provide feedback to the plan, design, or countermeasure that is causing the problem and proceed to reducing the problem.]

<Explanation>

Monitoring need to be carried out periodically. It takes several years for corals to grow and become sexually mature. In the meantime, the environment is not always guaranteed to be favourable, and the transplantation site may be overgrown with algae or buried in sand and rubble. Therefore, a periodic monitoring is necessary to keep the corals in check. Conduct surveys at least once a year and compare results, share among the associates and utilise the data for further planning or considering countermeasures.

1) Monitoring

Monitoring is required to accurately understand the current state of the target and qualitatively assess the level of achievement. To ensure accuracy, not only monitor the surviving coral individual numbers and transplanted coral cover, but also record the surrounding environmental parameters and other corals. The following points are obstacles that often people face and therefore it is important to understand the difficulty of monitoring, how specialised it is, repeatedly conduct monitoring surveys, ensure the surveyors are adequately experienced, and the results can be statistically analysed (have enough replicates).

- Coral distribution changes over space and time.
- The visual area that can be covered geographically and surveyors' individual ability is limited.
- Difficult to identify individual coral colonies.
- The surveyors with adequate experience and knowledge are limited.
- Sampling of wild corals are often regulated by law.

2) Rearing maintenance

The results of monitoring and level of achievement should be evaluated with the help of an expert. It should be evaluated collectively based on the transplanted coral survival rate and growth over time, as well as predatory animals' existence, sediment accumulation, and fish aggregation presence.

If the evaluation concludes that the goal is achieved, the project can be terminated. If the goal is yet to be achieved, if there are some progress made, continue the project and work towards achieving the goal. If there is no progress, provide feedback and re-evaluate the plan. Note that even if the goal is achieved, it is not guaranteed that the positive outcome will continue longer term. Therefore, it is ideal to conduct further monitoring at least once a year.

No positive progress can be seen in different ways: (1) corals not growing, (2) expected coral cover not achieved, or (3) timing of evaluation was too soon. Feedback should be provided to the planning group for (1) and (2) to reconsider the plan. There could be cases where the project direction itself was not wrong but the suitable site, transplantation timing, frequencies of countermeasures put in place, or all those combined could result in an unfavourable outcome. Identify the true cause and realign the plan. For (3), keep in mind that it takes several years for corals to grow and continue to monitor. During this time, sea temperature increase or typhoon could have happened, so it is best to consult an expert if these factors are possibilities.

During monitoring, conduct algae and other encrusting organisms removal and predation protection net exchange or maintenance to promote coral survival and growth (refer to Chapter II 2.3.4 and 2.4.3).

Chapter 2 Whole-area propagation techniques for corals

1. Whole-area propagation techniques for corals

1.1. Basic approach

[Whole-area propagation techniques for corals mean to mass-produce coral nursery stock via sexual reproduction, conduct mid-term rearing, transplant them, then propagate them in a wide-area scale.]

Table II.1-1 Characteristics of sexual and asexual reproduction methods for coral propagation techniques

Sexual reproduction method for coral propagation technique (whole-area propagation technique)		Asexual reproduction method for coral propagation technique	
Merit	Demerit	Merit	Demerit
• Mass production of the transplantation nursery stock enables larger scale transplantation. • Does not harm parental coral colony. • Can produce genetically diverse corals which may improve the fertilisation rate in the natural environment.	• Requires highly specialised techniques. • Currently the nursery stock production techniques are developed for only a limited few species of the genus *Acropora*. • Takes time to rear until a transplantable size.	• Fragmentation (stock production) is possible with any species. • Cost effective than sexual reproduction technique. • Larger fragment will grow to a transplantable size much quicker than sexual reproduction method.	• Fragmentation damages the parental coral colony. Cannot harvest too much. • Even if the fragments spawn, if the parental colony is the same, they will not fertilize. • Harvesting wild coral colony is regulated under law.

Table II.1-2 Flowcharts of whole-area coral propagation techniques via sexual reproduction with different nursery stock production methods

Nursery stock production		Mid-term rearing	Transplantation
On-land facility	Using an on-land nursery stock production facility, keep the corals in the aquaria, wait until they spawn and produce nursery stock.	Transport the settled nursery stocks to a mid-term facility where the environment is favourable, and management is relatively easy. Keep until they grow to size ready for transplantation.	After deciding on the optimum transplantation site, transplant the nursery stock to the location. After transplantation, periodically maintain to maximise coral growth and survival.
Larvae collection equipment	Using a larvae collection equipment in sea, collect eggs and larvae, let the larvae settle on the settlement equipment and produce nursery stock.		

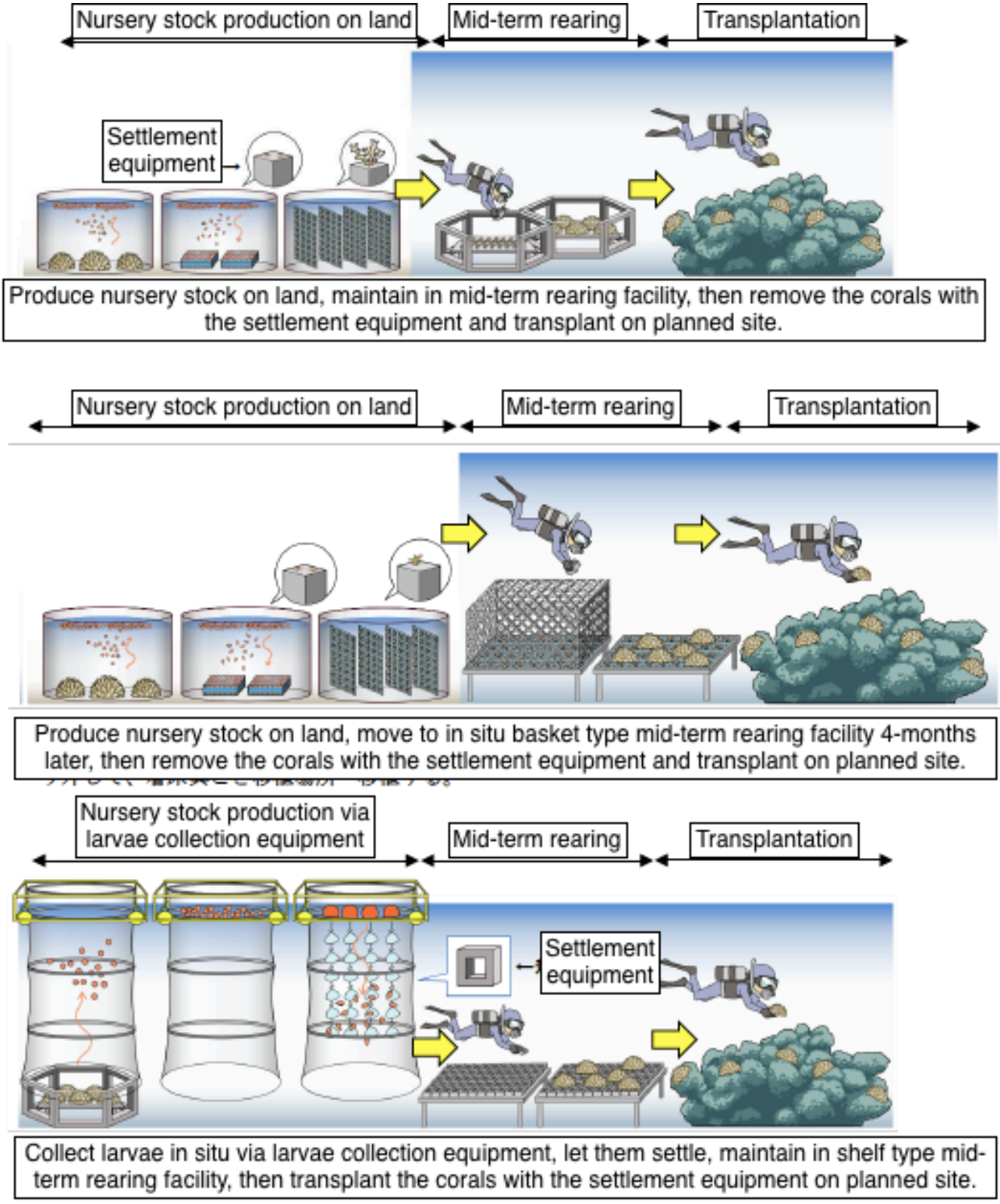


Figure II.1-1 (Example) Whole-area coral propagation via sexual reproduction with different nursery stock production methods

1.1.1. Nursery stock production

[Nursery stock production via sexual reproduction is a technique to fertilize eggs and sperms using synchronous spawner corals, let the planula larvae settle on settlement equipment, then care for them until they grow to the sizes ready for mid-term rearing. It can be separated into two methods: nursery stock production in a terrestrial facility and nursery stock production in the actual sea area.]

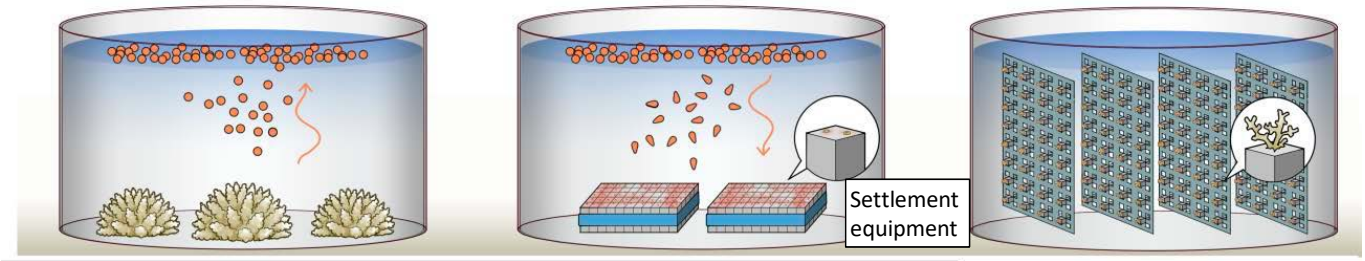


Figure II.1-2 (Example) Coral nursery stock production using an on-land facility

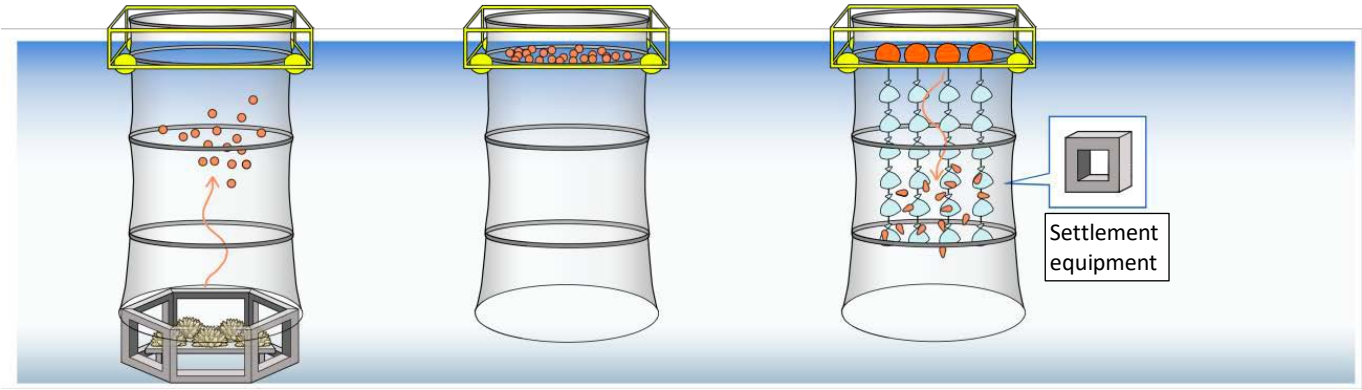
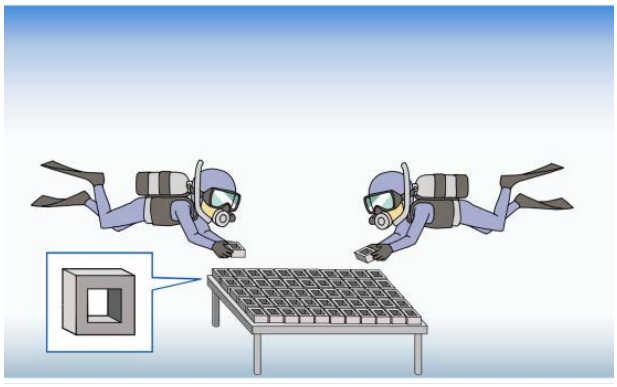


Figure II.1-3 (Example) Coral nursery stock production using a larvae collection equipment

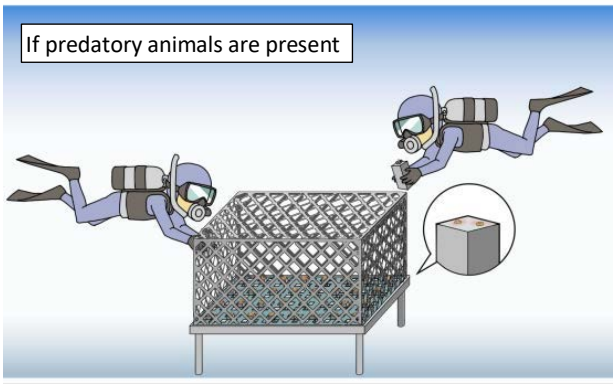
1.1.2. Mid-term rearing

[Plant and rear the coral nursery stock in a mid-term rearing facility in the actual sea area where a favourable environment for corals to grow can be achieved, until they grow to the sizes appropriate for transplant.]

Figure II. 1-4 (Example) Mid-term rearing facility where wave actions and water flow are greater



Shelf type mid-term rearing facility



Basket type mid-term rearing facility

Figure II. 1-5 (Example) Mid-term rearing facility where wave actions and water flow are smaller

1.1.3. Transplantation

[When transplanting, remove the corals from the mid-term rearing facility, transport, then plant in the appropriate transplant area. In addition, periodically maintain the area to ensure high survival and growth rates.]

Figure II.1-6 Transplanting

2. Whole-area propagation techniques by nursery stock production in on-land facility

(Contents same as the table of contents)
Figure II.2 -1 Flowchart of whole-area coral propagation techniques in Okinotori Island

2.1. Harvesting and transporting parental colony
[When collecting parental coral colonies, plan and decide on the number of colonies necessary for nursery stock production (6 colonies or more if possible), keeping in mind to choose ones that are healthy and close to spawning. The collected colonies must be carefully transported to a large shipping vessel while being kept in an optimum growing condition (e.g. light, water temperature, flow), then delivered to an on-land rearing facility.]

(Contents same as the table of contents)
Figure II.2-2 Collection and transportation of parental colonies

2.1.1. Harvesting parental colony
[When collecting parental coral colonies, plan the timing, decide on the colony size and number in advance, and be careful not to cause stress to the colonies. Moreover, instead of transporting the colonies immediately, leave them in the sea temporarily to relieve the collection stress.]

Figure II.2-3 Section of a coral (Pink parts are the eggs ready to be spawned)

Equipment for harvesting


Harvest by hammer and chisel


Harvest by stone-cutting chisel


Collected coral colony

Figure II.2-4 Parental coral colony collection

Figure II.2-5 Temporary placement of the parental coral colonies

Table II.2-1 Resources and equipment necessary to collect parental coral colonies

Category	Resources / equipment
Exploration / Collection	Small boat, diving equipment, depth gauge, underwater watch, thermometer, GPS, hammer, chisel, stone-cutting chisel, underwater camera, underwater paper, gloves
Temporary placement	Diving equipment, stable base, zip ties, rope, net (for predatory animal prevention), shading net, underwater epoxy (glue), gloves

2.1.2. Transporting parental colony

[Collected parental coral colonies are first transported in a small shipping vessel from the temporary placement to a larger shipping vessel. Then they will be placed in the aquaria on the ship to be transported long-distance to the on-land nursery stock production facility. During the long-distance transport, manage an optimum growing condition (e.g. light, water temperature, flow).]

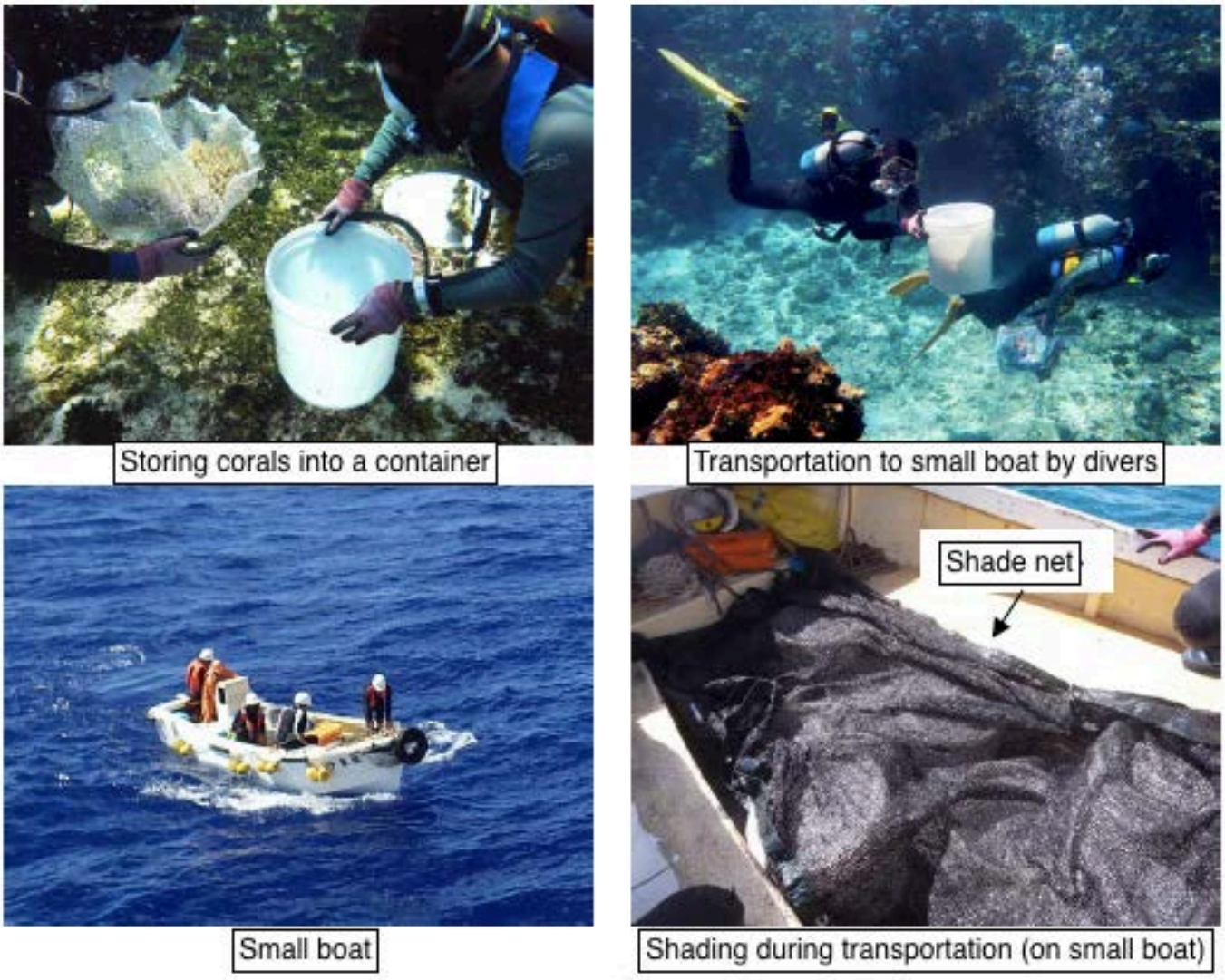


Figure II.2-6 Transportation of the parental coral colony to the shipping vessel

1.Overview of the on board aquaria

2. Lids and clips

3. Shade net 4. Sprinkler

5. Water pump

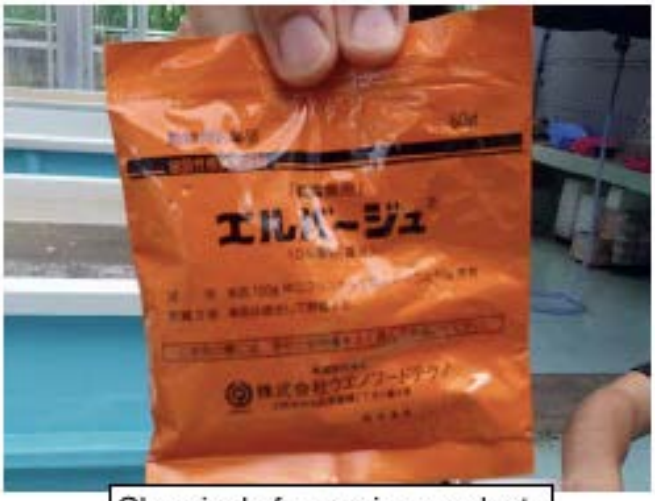
Figure II.2-7 Aquarium facility on the shipping vessel

Figure II.2-8 Conducting temperature acclimatisation

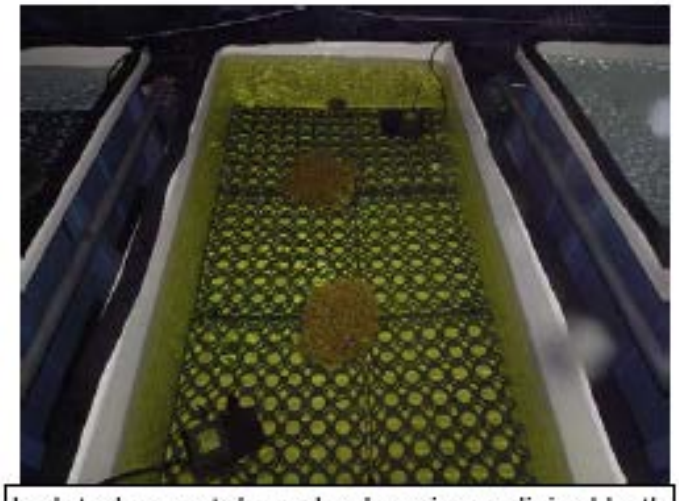
Figure II.2-9 Placement of parental coral colonies

Table II.2-2 Activities for on-board rearing management

Category	Frequency	Notes
Seawater exchange	3 times per day	Change 1/3 of the total water volume each time
Observation of coral nubbin	3 times per day	Take photos
Temperature check	3 times per day	
Sprinkler	Always (24 hrs)	Not necessary when it rains
Clean tanks	Occasionally during seawater exchange	If organic material is present



Chemicals for marine products



Isolated parental coral colony in medicinal bath

Figure II.2-10 Medicinal therapy for parental coral colonies using chemicals for marine products

Table II.2-3 Resources and equipment necessary for parental coral colony transportation

Category	Resources / equipment
Transportation from temporary placement to large ship	Small boat, diving equipment, gloves, container (e.g. 30 cm diameter bucket with lid), cushion, shading net
Maintain parental coral colony	Tank, transparent lid for tank, cushion tape (waterproof), clips to secure lid, coral securing mat (mat for the tank bottom), zip ties, scaffold, shading net, hose, pump, flow pump, thermometer, notes and pencils, chemicals for marine products (Sodium Nifurstyrenate), syringe, gloves

2.2. Nursery stock production of Acroporidae in on-land facility

[Any parental coral colonies collected from a different region will be kept in aquaria under optimum growth conditions. Nursery stock production will be conducted using the eggs spawned by these parental corals, however fertilization must be conducted under the appropriate sperm concentration and time. Eggs develop into planula larvae which can settle in about 4 days after spawning, therefore it is necessary to let them settle on settlement equipment which is coated with bacteria and crustose coralline algae that induce settlement and metamorphosis. Once it metamorphose to primary polyps, maintain them in the aquaria under optimum growth conditions.]

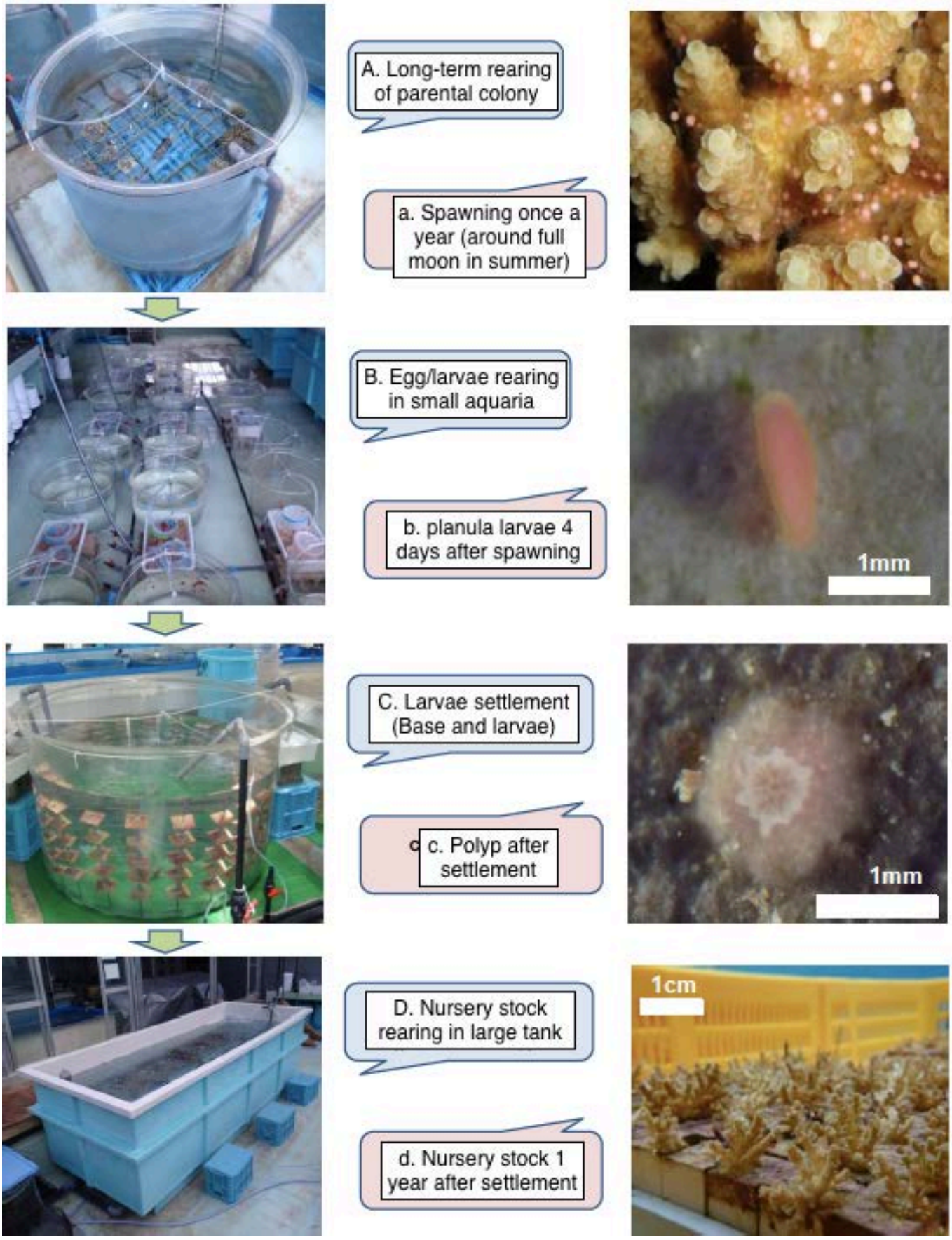


Figure II.2-11 Steps for coral nursery stock production via sexual reproduction

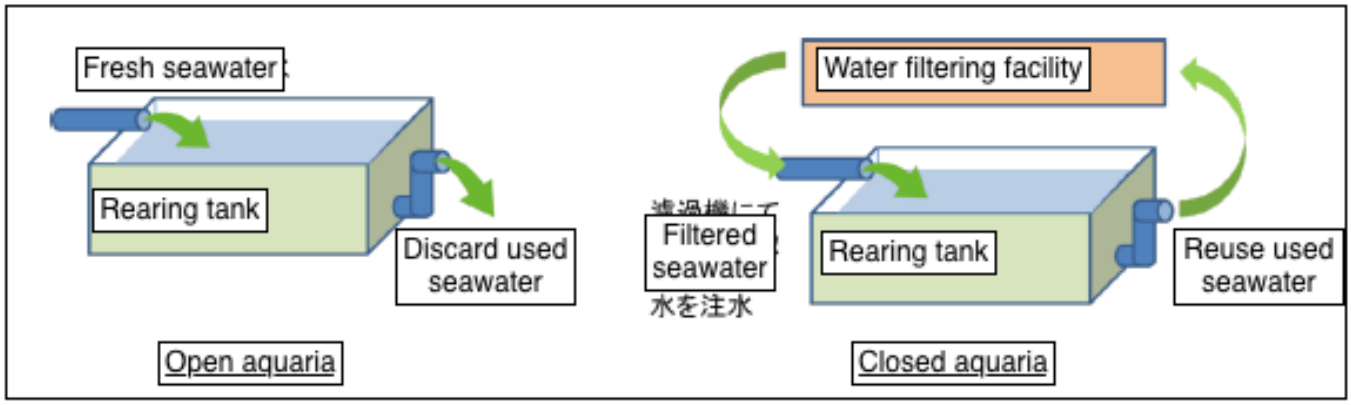


Figure II.2-12 Open and closed aquaria schematics

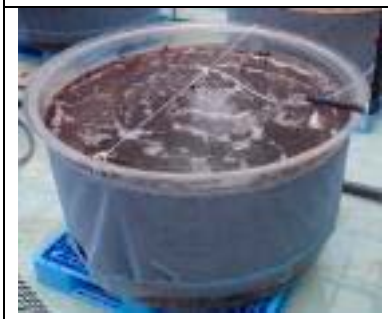
2.2.1. Nursery stock production facility

[Coral nursery stock production needs to be conducted in a facility where an optimum growth conditions especially with sea water and light can be maintained. Furthermore, resources and equipment, as well as number of staffs need to be secured.]

Table II.2-4 One example of coral nursery stock production facility

[Location]	500-1 Maja, Kumejima, Shimajiri-gun, Okinawa (Kaiyo-shinsosui laboratory)
[Area]	Rearing facility 348 m^2, Laboratory and shed 82 m^2
[Usable sea water volume]	Surface water 261,486 t per year (intake from 15 m depth)
[Main facility]	Parental colony rearing tank (1 t cylinder FRP, 3 sites)
	Parental colony rearing tank (1 t cylinder polycarbonate, 5 sites)
	Nursery stock rearing tank (5 t FRP, L400 x W150 x D100cm, 5 sites)
	Nursery stock rearing tank (3.5 t FRP, L520 x W160 x D35cm, 1 site)
	Water distribution and aeration piping

Table II.2-5 List of equipment and resources for coral nursery stock production

Resources / equipment	**Purpose**
A) Parental colony rearing	
Cylinder tank (polycarbonate and FRP, 1 t)	Rear parental coral colonies
Air stone	Create water flow
Shading net	Adjust amount of light
Water heater	Temperature regulation in winter
 1 t cylinder polycarbonate tank  1 t cylinder FRP tank  Water heater	
B) Collect eggs, larvae rearing	
Cylinder tank (polycarbonate 30 – 100 L)	Rear eggs and larvae
Scoop net (mesh, 100 µm)	To collect eggs and larvae for washing and water exchange
Plastic cups (200 ml – 5 L)	Temporary placement and transportation of eggs and larvae
Glass equipment (pipette, beaker, slide glass)	Observation and counting of eggs and larvae


Cylinder polycarbonate tank

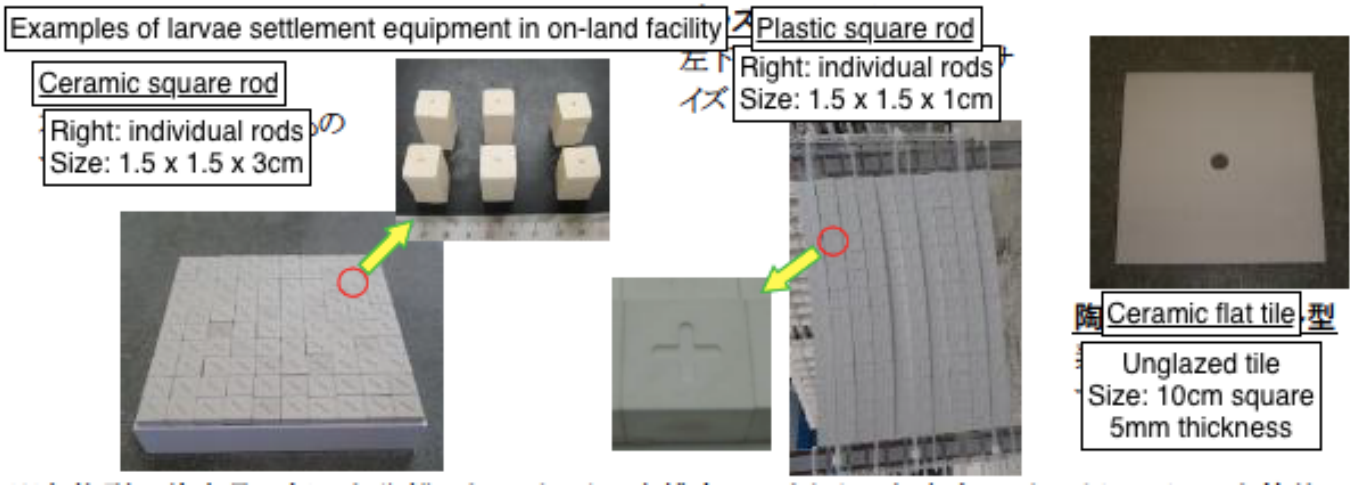

Scoop net (100 um)


Plastic cups


Glass equipment

C) Settlement of larvae

Tank (cylinder polycarbonate 1 t, square 500 L)	Larvae settlement tank
Settlement equipment (terracotta tiles, square rod)	Base to let the larvae settle
Scoop net (mesh, 100 µm)	To collect larvae for washing and water exchange
Air stone	Create water flow
Plastic cups (200 ml – 5 L)	Transportation and distribution of larvae

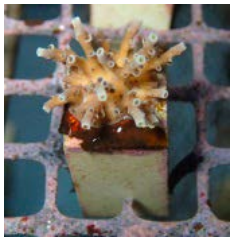


* Square rods can be separated individually. In the aquaria they can be stored in chunks to save space and when transplanting they can be separated to maintain space between corals. Flat tiles make it easier to observe under microscopes, therefore better for different experiments.

D) Nursery stock rearing

Tank (FRP 5 t)	Nursery stock rearing tank
Settlement equipment holder	To hold settlement equipment inside tank
Shading net	Adjust amount of light


5 t FRP tank


Settlement equipment holder equipped with plastic net

*For a few months after settlement, keep the settlement equipment closed to each other, and as the corals grow, move them to the Settlement equipment holder equipped with plastic net (to maximise tank space)

E) Common materials

Stereoscopic microscope and	Eggs / sperm / larvae / polyp observations
Counter	Eggs / sperm / larvae / polyp counts
Measuring equipment (photometer, salinity, dissolved oxygen, pH, nutritive salts)	To check tank environment, water quality check
Thermometer, temperature logger	Temperature monitoring and recording

2.2.2. Handling and feeding of parental colony

[The important environmental conditions for parental coral colony management are water temperature, light, and water flow. To enable long-term rearing and annual spawning, follow the optimum growth conditions tested in the past.]

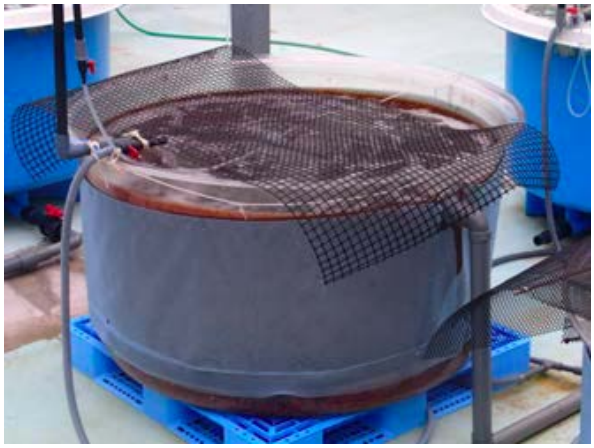


Figure II.2-13 Example of shading the parental colony rearing tank

Shade the tank by covering the top of the tank with a plastic net (20mm mesh size). Reduces light by 30%. Cover the sides with minnow nets (2mm mesh size) and reduce the direct sunlight.

Figure II.2-14 Strong aeration of the parental colony rearing tank (1 t circular polycarbonate tank)

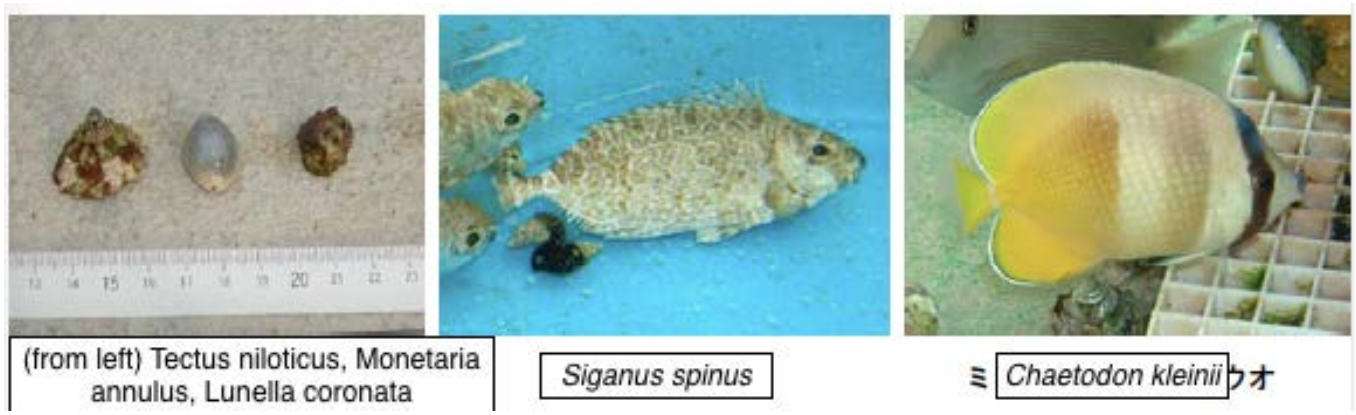


Figure II.2-15 Bivalves and fishes utilised for algae and anemone removal

2.2.3. Gamete collection • Larvae nurturing and recruitment

[It is critical to maintain sperm concentration to 10^5-10^7/ml and longer fertilization time for a successful coral fertilization. In addition, the embryos becomes very fragile once they undergo cleavage and therefore they need to be maintained in a calm space. Install settlement equipment in the aquaria after 4 days as the larvae will have the ability to settle by that time. The larvae will then settle within 1 or 2 days and metamorphose to primary polyps. It is critical to coat the settlement equipment with bacteria and crustose coralline algae that induce larval settlement.]

<Explanation>

1) Ecology of coral reproduction

Many Acroporids are hermaphrodites and they cross-fertilize. Therefore, for nursery stock production for each coral species, eggs and sperms collected from more than two colonies with different genotypes are required. There are cases when high fertilization rate can be achieved with limited number of colonies, however it is best to use as many colonies as possible to achieve nearly 100% fertilisation rate and to increase genetic diversity of the nursery stock. Report from Iwao et al (2014) shows that eggs and sperms collected from more than 6 colonies have over 95% fertilisation rate and higher genetic diversity.

In general, corals spawn around full moon in summer (mid-May to August; Figure II.2-16 and 2-17). Each coral species has a month they spawn. The actual date they spawn differs according to the sea area (with similar environmental factors) and they tend to synchronise within the same area. Presently it is difficult to predict the

date of spawning. There are spawning inducing methods developed by Hayashibara et al (2004), however implementation requires caution as it harms the parental colony.

The time of spawning is relatively determined for each coral species (for example, *Acropora tenuis* spawns around 19:30, *A. humilis* around 22:00; Hayashibara 1995; Fukami et al 2003). It takes approximately 30 minutes to spawn.

Corals spawn multiple eggs per polyp. *Porites* and *Dipsastraea* spawn singular eggs while many *Acropora* species spawn 'bundles' of eggs and sperm together (Figure II.2-18). There are 10 eggs in a typical bundle of *Acropora* (Kitada 2002). 1-2 hours prior to spawning, bundles can be seen at the mouth of the polyps (called 'bundle set'; Figure II.2-19) and can be visually confirmed that it is very close to spawning.

Acropora fertilisation is known to have a higher success rate when the sperm concentration is $1x10^5$ – $1x10^7$ spermatozoa/ml, and is highest around $1x10^6$ spermatozoa/ml (Nozawa et al 2015). It is important to maintain the sperm concentration when fertilising the eggs. Number of sperm can be counted using hemocytometer.

2) Preparation

A base is required for coral larvae to settle (settlement equipment). Choose a settlement equipment according to the maintenance and transplant methods and the environment and topography of the transplantation site (Table II.2-5).

Coral larvae settlement and metamorphosis are known to be induced by crustose coralline algae and bacteria (Morse DE et al 1988, Morse ANC et al 1996, Negri et al 2001, Tebben et al 2015, Kato et al 2016). To let the settlement- and metamorphosis-inducing bacteria colonise the settlement equipment, immerse the settlement equipment in the shallow sea area (Figure II.2-20). The longer they are immersed, the quicker the larvae settle and metamorphose and the higher the settlement rate (number of individual settled/added larvae). However, it also means many other organisms would have colonised the equipment at this point. Therefore the optimum immersion time is 1-2 months.

The shallow sea area to immerse the settlement equipment is best if it is above coral rubble or on rocks where suspended sediment or sand is less likely to accumulate. Once immersed long enough, remove the settlement equipment a few days prior to larvae settlement and brush the surface algae and mussels off. Be careful not to brush too much as it can scrape off the biofilm. Brush off a right amount just to leave a little bit of microalgae on the surface (Figure II.2-20, right).

3) Steps from egg harvesting to settlement

(1) Egg harvesting and fertilisation

Move the parental coral colonies with the bundle set at the polyp mouth into one tank and let them spawn (Figure II.2-21). After spawning, move the parental colonies out of the tank and break up the bundles with pipettes and spatulas in the water to encourage fertilisation. Be careful not to have too much water volume in the tank if the number of eggs spawned were fewer, as this method makes the sperm concentration lower. Fertilisation time should take about an hour.

(2) Maintaining eggs and larvae

After fertilisation, put the fertilised eggs into a scoop net with mesh size 100 μm and wash with fresh sea water, then move to a 100 L polycarbonate rearing tank.

Coral eggs do not form fertilisation membrane, so it is impossible to tell whether they indeed fertilised or not until cleavage starts. The egg cleavage of *Acropora tenuis, A. humilis*, *A. hyacinthus* starts approximately 2-3 hours after fertilisation. Eggs that started cleavage are delicate and are easily broken apart, therefore the above method (1) needs to be done before cleavage starts. Once the eggs are moved into a rearing tank, take caution not to shake or disrupt the tank. In addition, only check fertilisation success after more than 3 hours have passed since fertilisation.

Eggs develop into embryos then to larvae. During this time, open circuit tanks (water exchange 4 cycles/day) can be used to reduce water exchange work (Figure II.2-22). Eggs and embryos tend to float at the water surface, which means expelling the water out from the bottom of the tank reduces the risk of eggs and embryos escaping. However, as they develop into larvae by the next night after spawning and start swimming around, stop water flow during the night and set up a scooping net at the water outlet during the day to put the larvae back into the tank. If using a 100L polycarbonate tank with an open circuit setup, you can keep up to

50,000 fertilised eggs per tank. There are risks of embryos sticking to the wall and die, or embryos sticking to each other if you go over this number. Maintain larvae below room temperature and up to 4 days after spawning.

According to Fukuda et al (2003), larvae rearing "can be done in a bowl if it is for a smaller scale experiment. Larvae can be moved with a pipette into a fresh bowl once per day to maintain a clean environment." In this case, the number of larvae should be kept at a density of 2,000 individuals/L or below. On the other hand, if a larger tank such as 100L polycarbonate tank with open circuit setup is going to be used, to maintain a clean water environment, the larvae density of 500 individuals/L or less is ideal.

For an easier rearing setup, you could also keep the larvae in a closed-circuit tank and use a scoop net to exchange water once per day.

(3) Settlement

4 days after spawning, larvae will start to sit up straight on the bottom of the tank or 'hop' around the bottom to find a place to settle. These are the signs for settlement.

Set the settlement equipment in the settlement tank (e.g. 1t polycarbonate tank) prior to introducing larvae (Figure II.2-23). If the water temperature is higher, embryo development and larvae growth are accelerated, and settlement/metamorphosis can happen sooner. In this case, move the larvae 3 days after spawning.

Settlement rate of larvae differ between species and the use of the equipment, but generally it is around 50-60%. It is also known that if larvae settle at over 1 individual/cm^2 density, it has a higher mortality rate. Therefore, decide on the number of larvae introduced per tank with the optimum settlement density, available area, number of equipment, and success rate in mind. Settlement day 1 should start with fewer larvae and add more the following days as you observe the settlement rate.

During settlement, exchange water at about 1 cycle/day. Water exchange method is the same as the open circuit setup for larvae rearing.

Figure II.2-16 *Acropora humilis* spawning

Figure II.2-17 *Acropora tenuis* after spawning – pink bundles floating on the surface

Figure II.2-18 *Acropora tenuis* bundles

Figure II.2-19 *Acropora globiceps* bundle sets

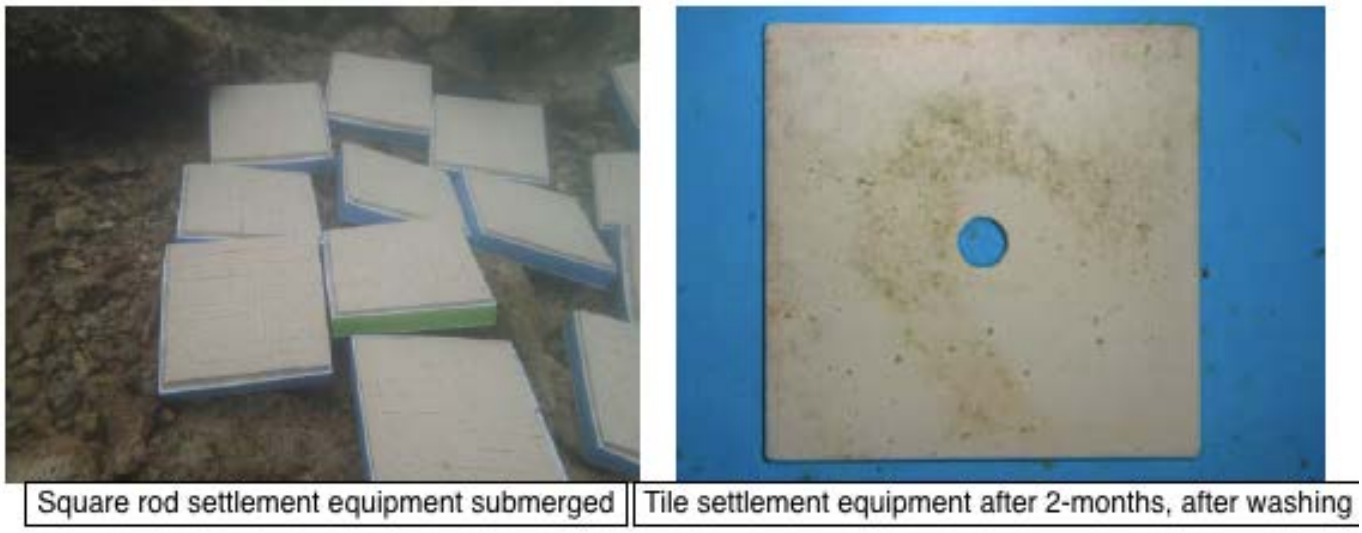


Figure II.2-20 Immersion of settlement equipment in the sea area

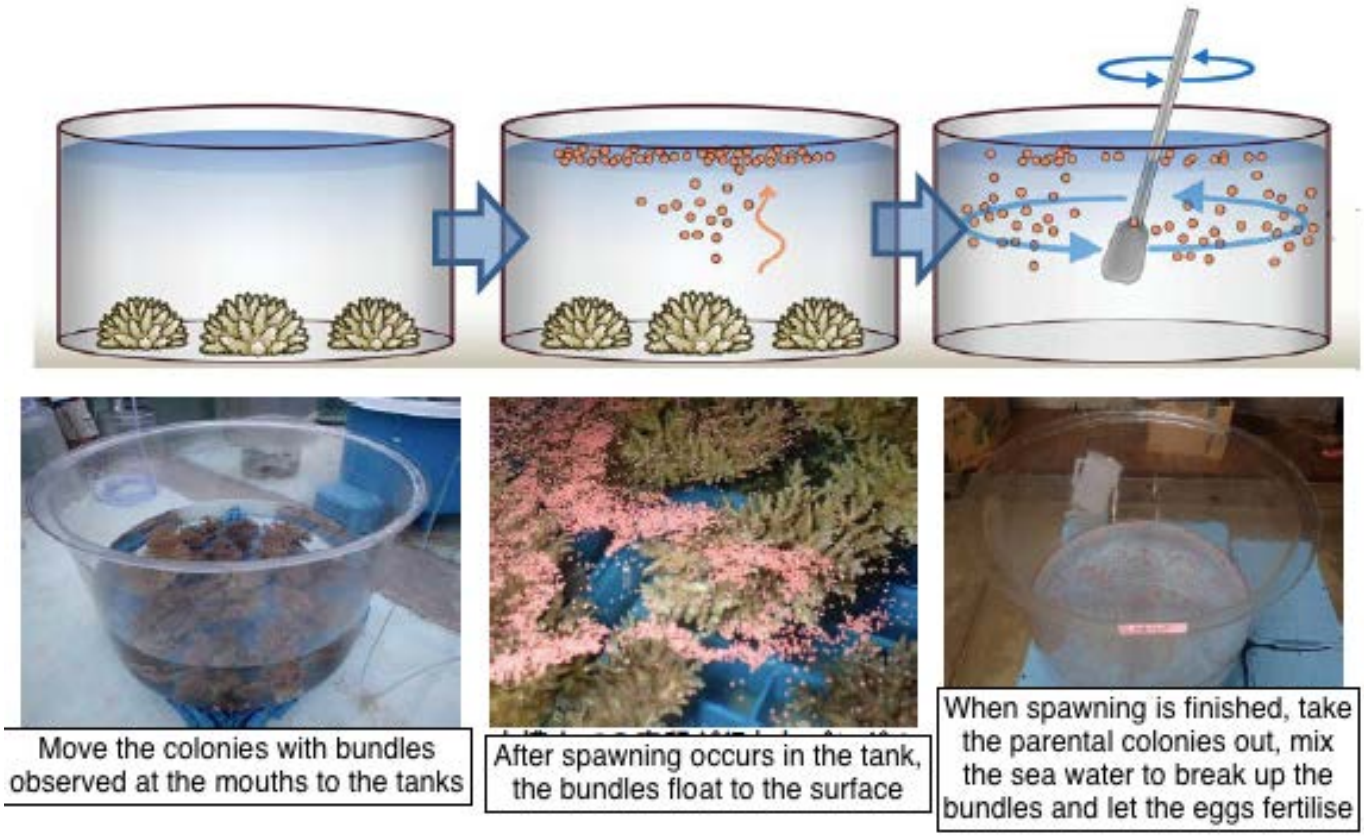


Figure II.2-21 Steps for coral spawning collection and fertilisation

Figure II.2-22 Open circuit growth tank for eggs and larvae
A: Vinyl tube water feeder, B: vinyl chloride spout, C: scoop net (100 um mesh size)

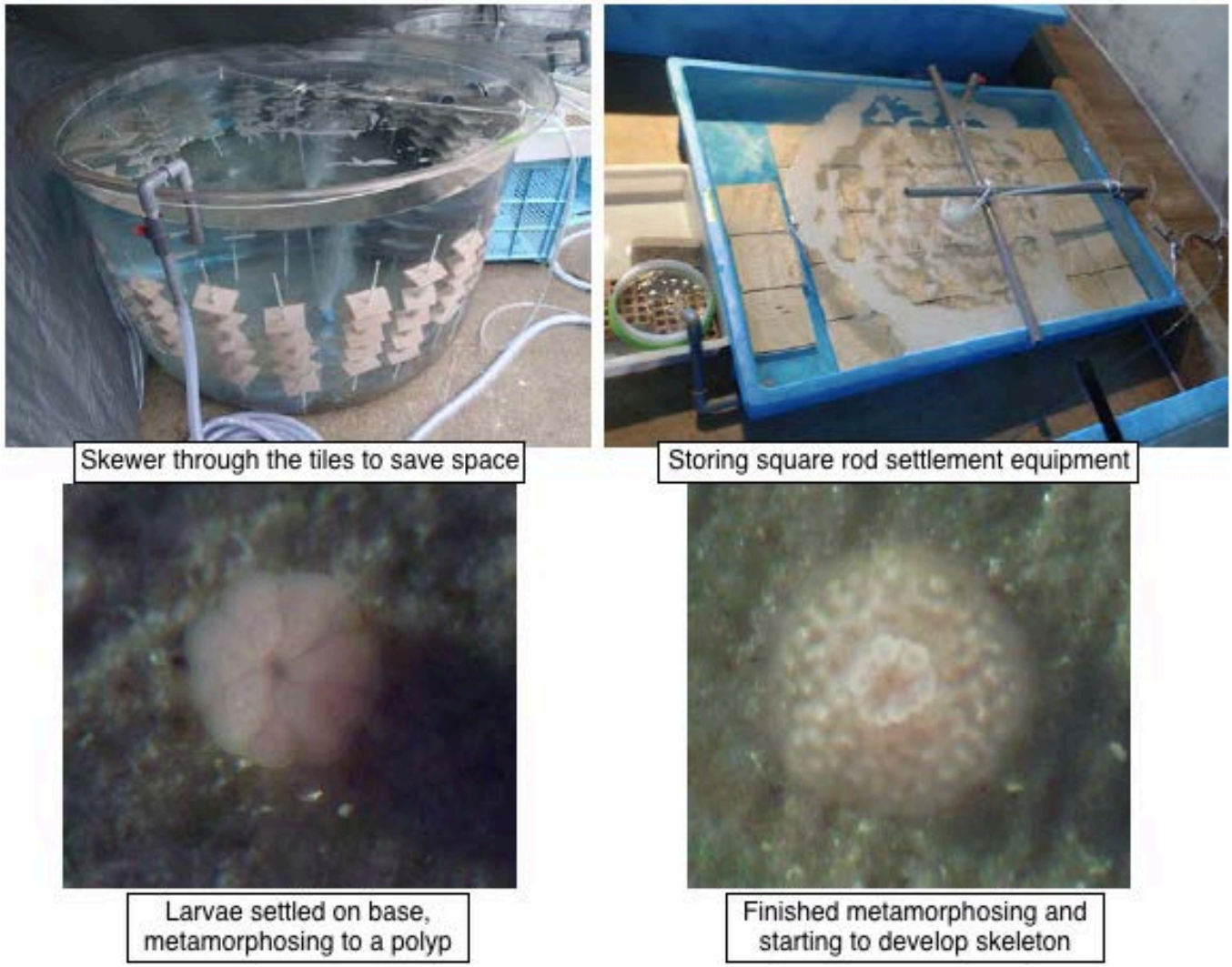


Figure II.2-23 Recruitment tank with the settlement equipment placed and primary polyps immediately after settlement

2.2.4. Coral recruit nurturing

[Move the coral recruits which developed enough skeletal structures to the recruit growth tanks. While caring for these recruits, maintain optimum growth conditions as well as mix-cultivate herbivorous organisms in the tank to prevent overgrowth of algae.]

<Explanation>

1) Transport the nursery stock to rearing tanks

Once the polyps develop enough skeletons (approximately 4 days to a week after settlement), move them to the nursery stock rearing tanks.

2) Rearing conditions for the nursery stock

(1) Water temperature

In Okinawa, when you keep the nursery stock in the outdoor tanks, water temperature ranges from 30°C in summer to 20°C in winter. This is an acceptable environment for maintaining corals. In addition, even at 30°C, as long as the water temperature is kept consistent, the corals will be fine. The important thing is that polyps die when temperature changes rapidly. For example, before and after the typhoon when water temperature changes by over 2°C or the low-high of the daily temperature difference is more than 1°C.

When the water temperature goes over 30°C, coral polyps survival rate tends to decrease. In this case, consider using a water cooler machine etc. to cool the sea water down. For locations like Okinotori Island where it is lower latitude than Okinawa, better survival rate in winter times was observed when sea water was heated up. For corals originally from around Okinotori Island, a stick type titan in-water heater was used to maintain water temperature above 24°C (lowest monthly average).

(2) Light

Use shading net to adjust the amount of light, just like the parental coral colonies (Figure II. 2-4). Even when you use the same shading net, the amount of light changes depending on the seasons and the angle of the sun, so keep in mind to use a higher shading rate net in summer compared to winter.

Figure II.2-25 shows the relationship between light (x-axis) and survival rate of polyps (y-axis) between 2014 and 2016. From this test result, the optimum light for nursery stock rearing tank was concluded to be around 20% of ambient light. In this study, the light emission on a sunny day around noon was 300-400 $\mu mol/m^2/s$.

(3) Water flow

Create water flow by installing a vinyl nitrate tube with small holes at the bottom of the tank and send air through (Figure II.2-26).

The relationship between strength of aeration and survival rate of nursery stock has been studied between 2014 and 2016. There was a trend which a higher survival rate (y-axis) was seen at low to medium aeration strength (Figure II.2-27, two grouped bars in the middle). The flow rate around the polyps at those strengths were 3-5 cm/s, which has been concluded to be the optimum flow rate.

(4) Other rearing conditions

We tested the influence of rearing tank sizes to nursery stock survival with 100L, 1.4t, and 3.5t tanks, and found that the larger the better survival. However, considering the daily clean ups, ease of general work around the tank, and a better water circulation within the tank, the optimum size would be 1-5 t volume. 5t tanks normally keep the water temperature stable and are easy to work with.

3) Countermeasures for competing organisms

Similar to parental coral maintenance (refer to Chapter II 2.2.2), utilise the herbivorous bivalves (e.g. *Tectus niloticus*, *Monetaria annulus*, *Lunella coronata*) and fishes (*Siganus*, *Chaetodon*, Monacanthids) for algae and anemone removal. In a 5t tank, generally about 1,000 bivalves and 1,2 fishes are adequate, however adjust the numbers according to the degree of algal growth. Algae and other encrusting organisms that these bivalves and fishes cannot eat must be removed by hand. The above mentioned fish and bivalves will not harm the polyps or eat them, and therefore there is no issue with moving them together into a larger tank.

4) Tank maintenance

Similar to parental coral colony maintenance, exchange water once a week and clean the bottom of the tank.

Figure II.2-24 Shading of the coral recruit growth tanks (windbreak nets that are 50% light shielding)

Figure II.2-25 Relationship between light emission and survival rate of coral recruits (*Acropora tenuis*) 6 months after settlement

Figure II.2-26 Aeration of coral recruit growth tank

Figure II.2-27 Relationship between flow rate and survival rate of coral recruits (*Acropora tenuis*) 6 months after settlement

2.2.5. Disease countermeasures

[There are cases where parental coral colony experience partial or total mortality due to diseases and colony size becomes smaller. For coral recruits, mass mortality can happen due to diseases. Diseases are typically bacterial. Therefore it is important to keep them in a good environment and keep the corals healthy to prevent them from catching the disease. If they are infected, use antibiotics to cure them.]

<Explanation>

1) Coral disease

(1) Parental coral disease

Parental coral colony could get Rapid tissue necrosis (RTN) which could lead to total mortality or partial mortality to the size smaller than a sexually reproducible size (Figure II.2-28).

The cause is believed to be bacteria normally existing within coral tissues (*Vibrio harveyi*, *V. coralliilyticus*, *Vibrio* sp. nov. undescribed). It has been suggested that these bacteria cause the host corals to show symptoms when they are stressed and become unhealthy (Luna et al 2007).

(2) Nursery stock disease

During rearing period, there are cases where the nursery stocks experience mass mortality, to the extent that nearly the entire tank content dies off.

It has been suggested from the diseased coral and infection trials that *Thalassobius mediterraneus* is the possible cause. This bacterium is found in healthy coral tissues as well, which has led to the conclusion that this is an opportunistic infection.

Coral polyps mass mortality tends to happen from one settlement equipment spreading out to neighbouring ones and triggering a chain reaction throughout the tank. From this, it is suggested that the cause is bacterial.

2) Outbreak control

(1) Prevention

For both parental and nursery stock corals not to get sick, the best way is to keep them healthy. Follow the guidelines from 2.2.2 and stick to the maintenance protocol to keep the rearing environment favourable for the corals. Moreover, water quality deterioration or salinity decrease could also contribute to disease outbreaks. Therefore, in addition to the above mentioned tank maintenance, periodically and/or after heavy rain, conduct water quality checks (salinity, phosphorus, nitrogen) and if anything is out of the normal range, identify the cause and rectify immediately.

(2) Cure

[1] Parental coral cure

Below medicine (chemical) has been proven effective towards *Vibrio* sp.

- Sodium nifurstyrenate
- Ampicillin
- Oxolinic acid
- Florfenicol

These chemicals are commercially available as fishery organisms care products. When tested on parental coral colonies, some individuals with symptoms either recovered or were kept alive longer. However, there were still many cases of death without any effect, and the recorded recovery rate is at 40% as of 2016. Further investigation for effective treatment is required.

Moreover, it is important to immediately isolate the sick colonies once identified, as RTN rapidly infects other colonies.

[2] Nursery stock coral cure

Below medicine (chemical) has been proven effective towards *Vibrio* sp.

- Sodium nifurstyrenate
- Ampicillin

Similar to parental colonies, when tested on the nursery stock, toxicity was not observed however recovery rate was also at 40% (Figure II.2-29). In addition, if mass mortality is observed in a large tank such as the 5t one, the chemicals will be added to the tank with suspended water flow for 1-4hours, however only half of the time the spread of infections could be halted. Moreover, relapse has been observed many times, which so far leads to the conclusion that chemicals itself is not enough to stop the spread of disease. The best way is to maintain the rearing environment and only rely on chemicals in case of emergencies.

Figure II.2-28 Corals infected by RTN (Rapid tissue necrosis)

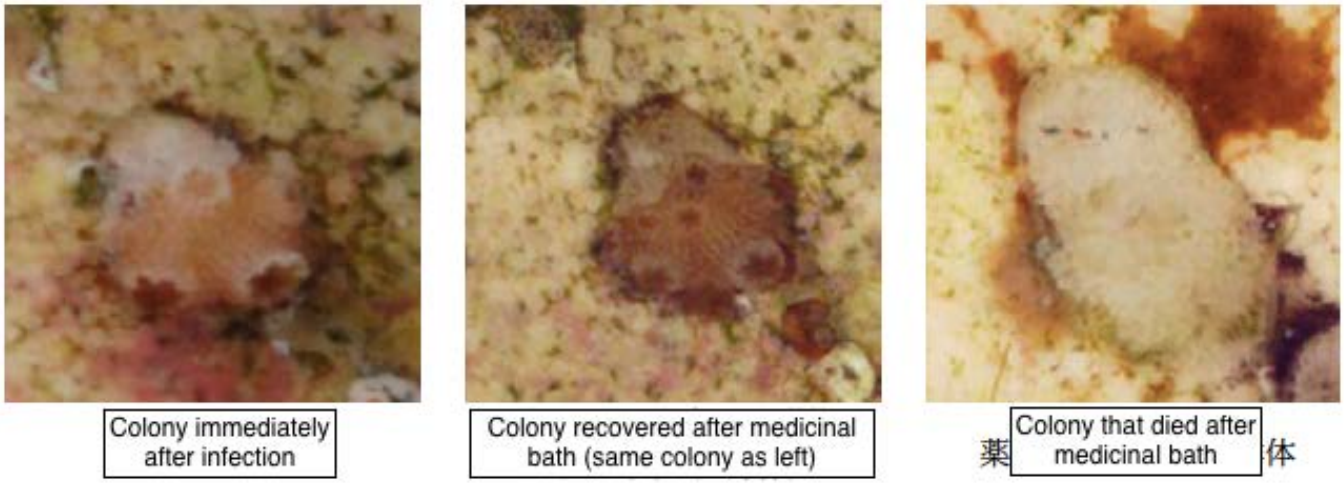


Figure II.2-29 Coral recruits infected by diseases

2.3. Mid-term rearing

[Mid-term rearing in the sea area involves planting the coral recruits transported from the on-land production facility on a concrete slab mid-term facility and managing them until they grow to the size appropriate for transplanting.]

Figure II.2-30 Mid-term rearing using intermediate nets (Rinkevich 2006)

(Contents same as the table of contents)

Figure II.2-31 Flowchart of mid-term rearing tasks

2.3.1. Establishment of mid-term rearing facility

[Where wave actions are stronger and areas are harder to manage, install concrete slab as mid-term rearing facility which in the long run has less influence from coral growth inhibiting factors.]

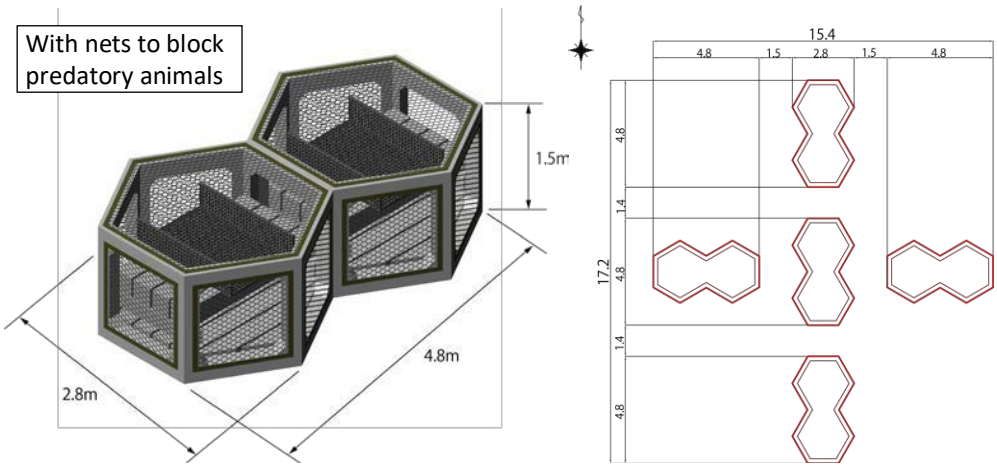


Figure II.2-32 Mid-term rearing facility sketch and positioning in Okinotori Island

Figure II.2-33 Mid-term rearing facility schematics in Okinotori Island

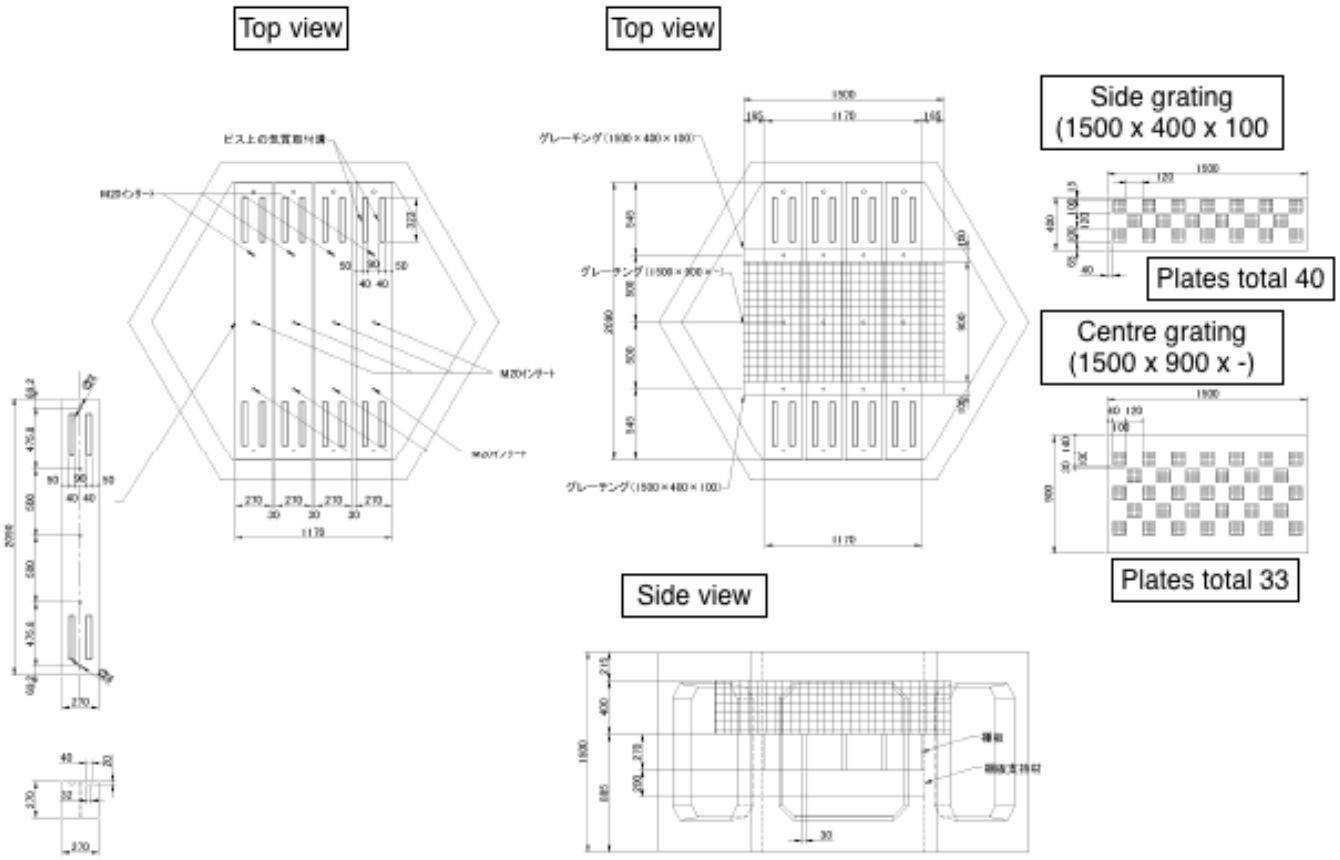


Figure II.2-33 (typo-34) Lattice for water flow and prevention of sludge accumulation

Figure II.2-34 (typo-35) Showing height of planting area

Figure II.2-35 (typo-36) Net for preventing predatory animal entry

Figure II.2-36 (typo-37) Spacious internal area

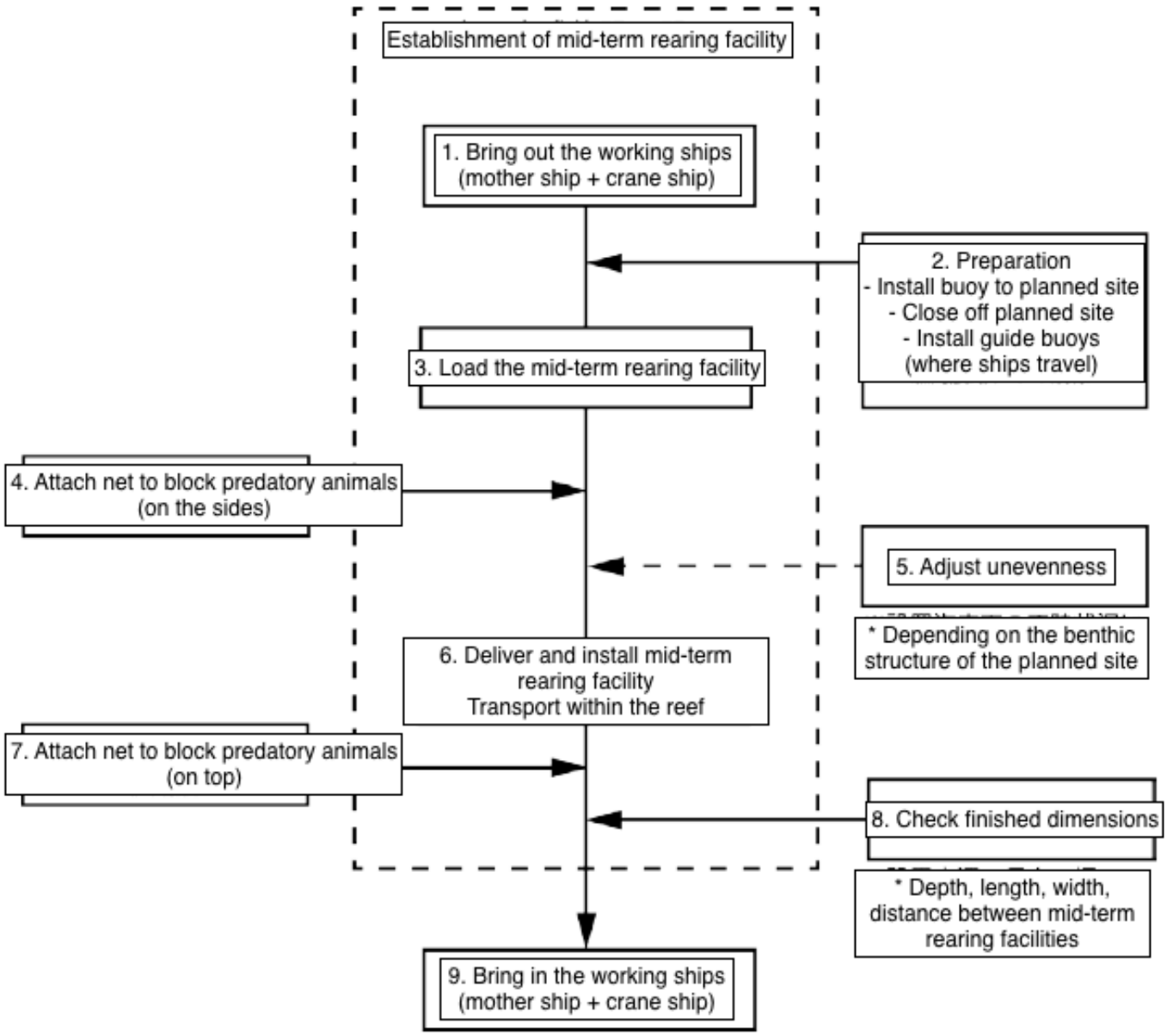


Figure II.2-38 Construction flow of mid-term rearing facility in Okinotori Island

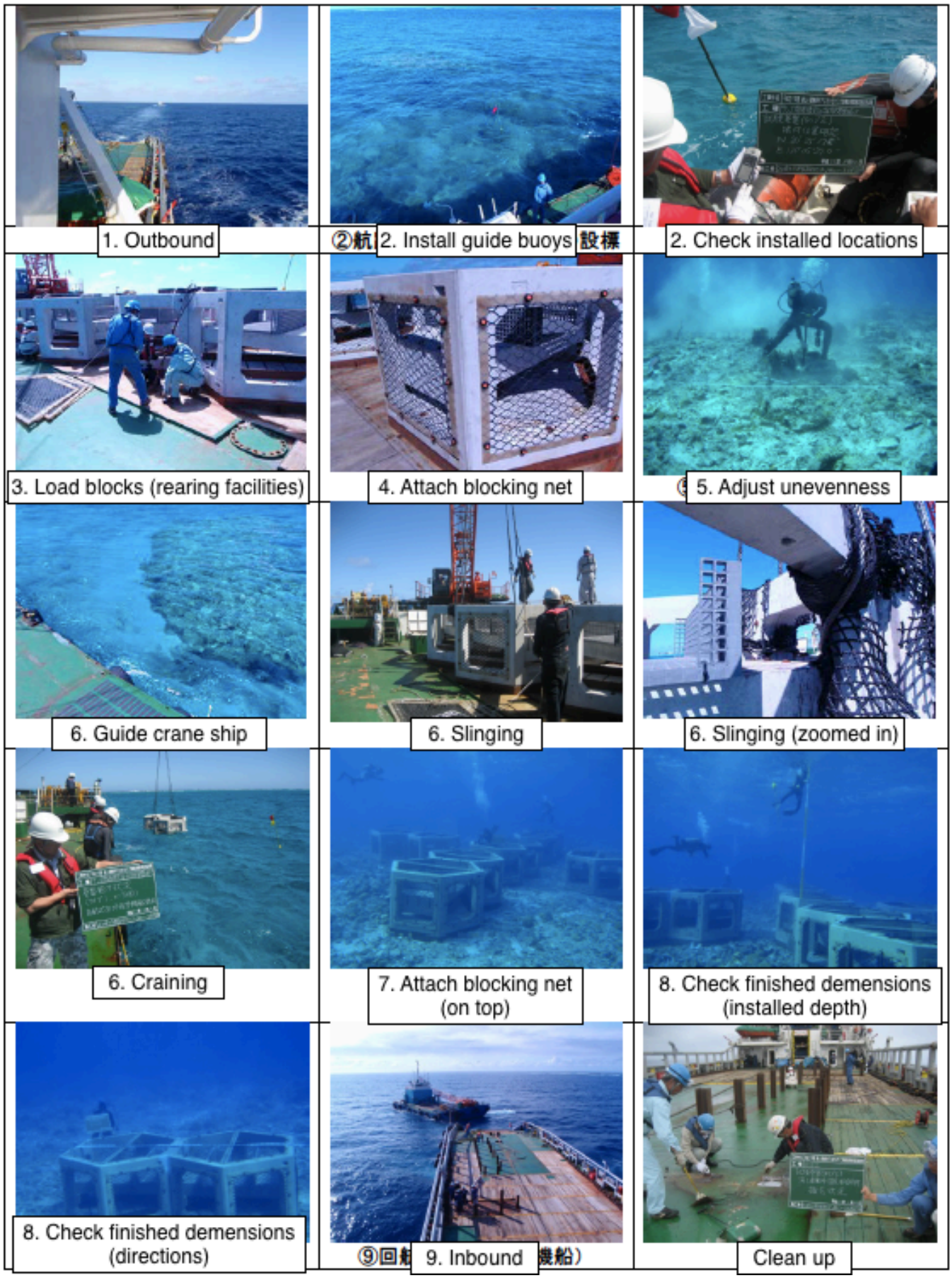


Figure II.2-39 Progress of mid-term rearing facility construction in Okinotori Island

2.3.2. Selection and transportation of corals

[Select produced nursery stocks that are favourable, maintain them on the aquaria facility on the shipping vessel and transport them to Okinotori Island, then temporarily place them to help acclimatise to the local environment.]

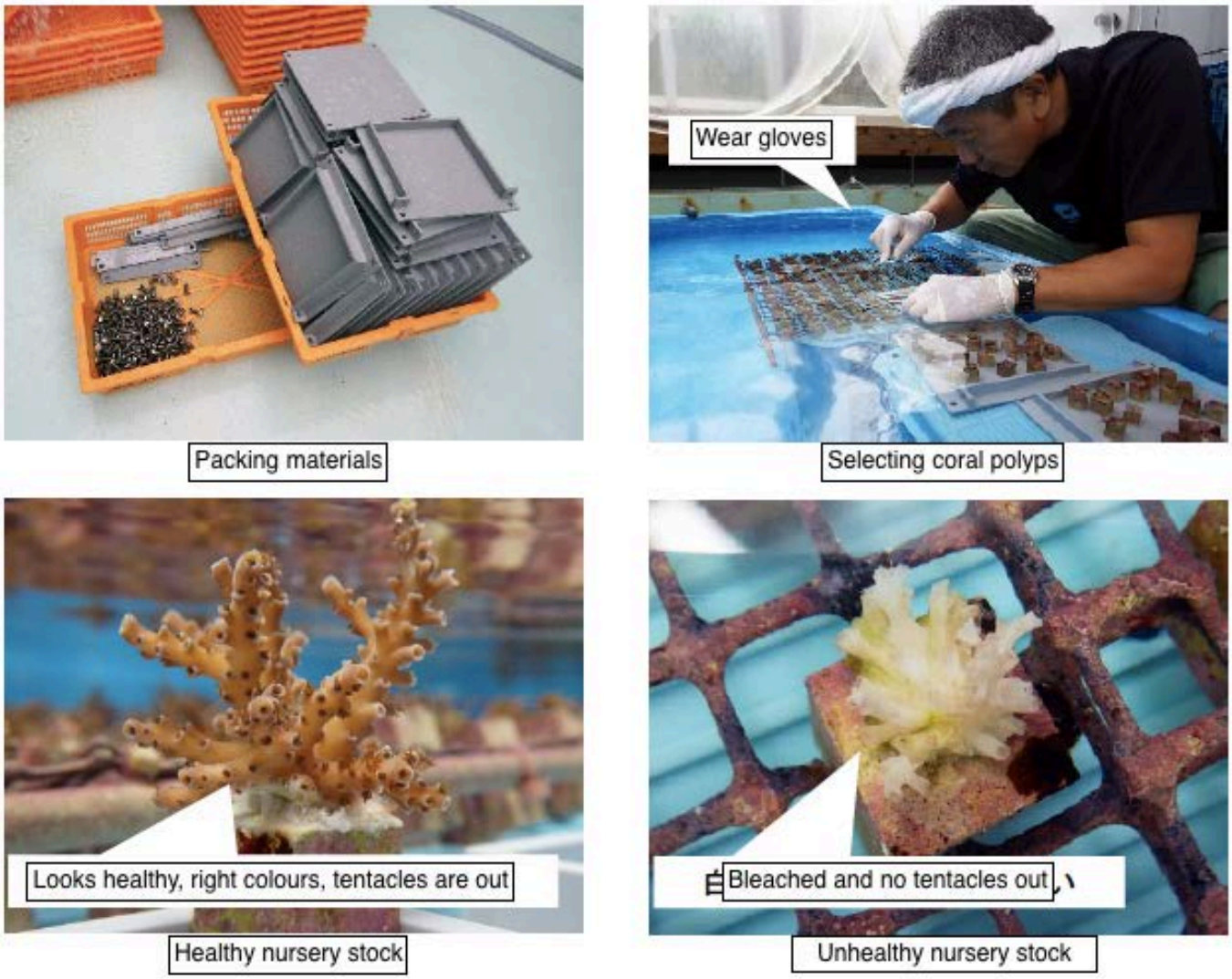


Figure II.2-40 Checking and selecting coral nursery stocks

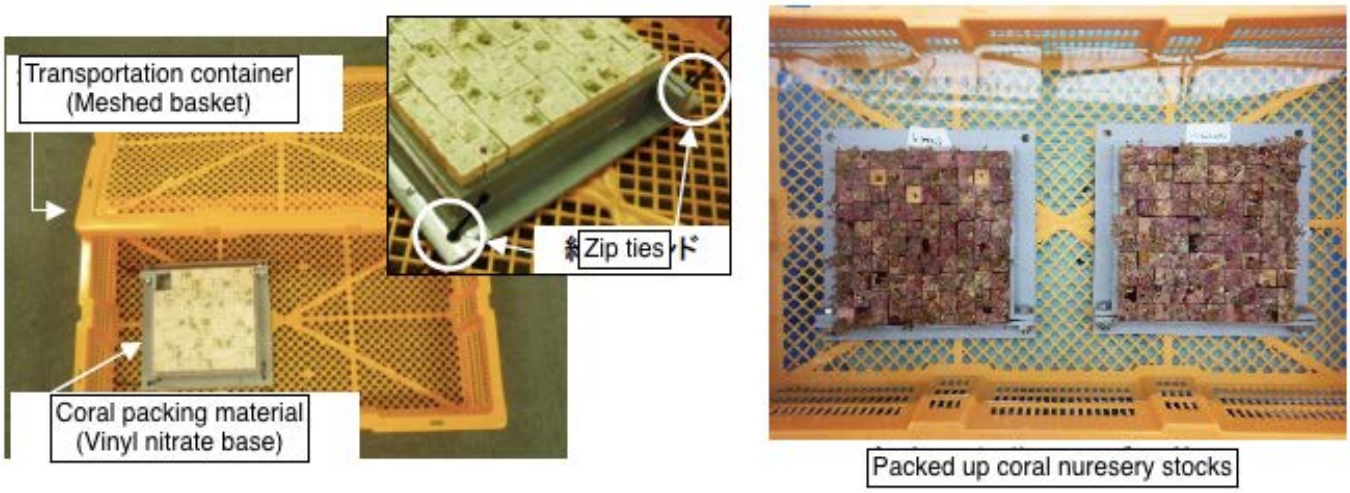


Figure II.2-41 Packing example of the coral nursery stock

Table II.2-6 Resources and equipment necessary for selection and packing

Category	Resources / equipment
Choosing	Field notebook, pencils, tweezers, gloves

Packing	Transportation container that water can go through (e.g. meshed basket with lid), coral packing material (e.g. vinyl nitrate base), zip ties, tweezers, gloves

Figure II.2-42 Transportation of coral nursery stock to the port

Figure II.2-43 Management of coral nursery stock

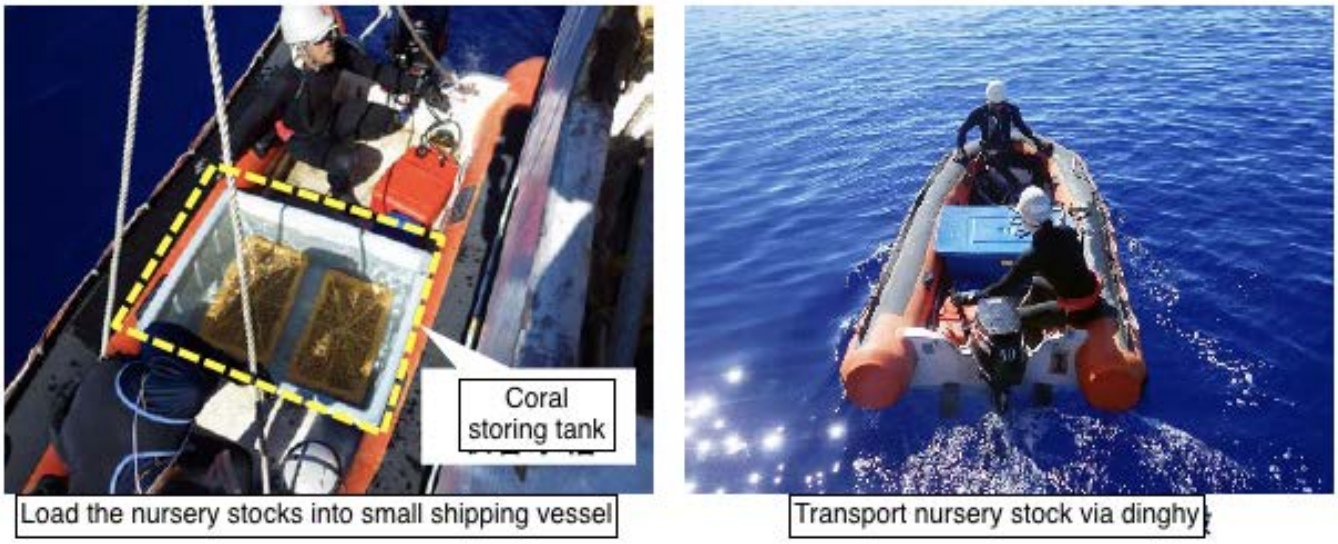


Figure II.2-44 Transportation of coral nursery stock to the mid-term rearing facility

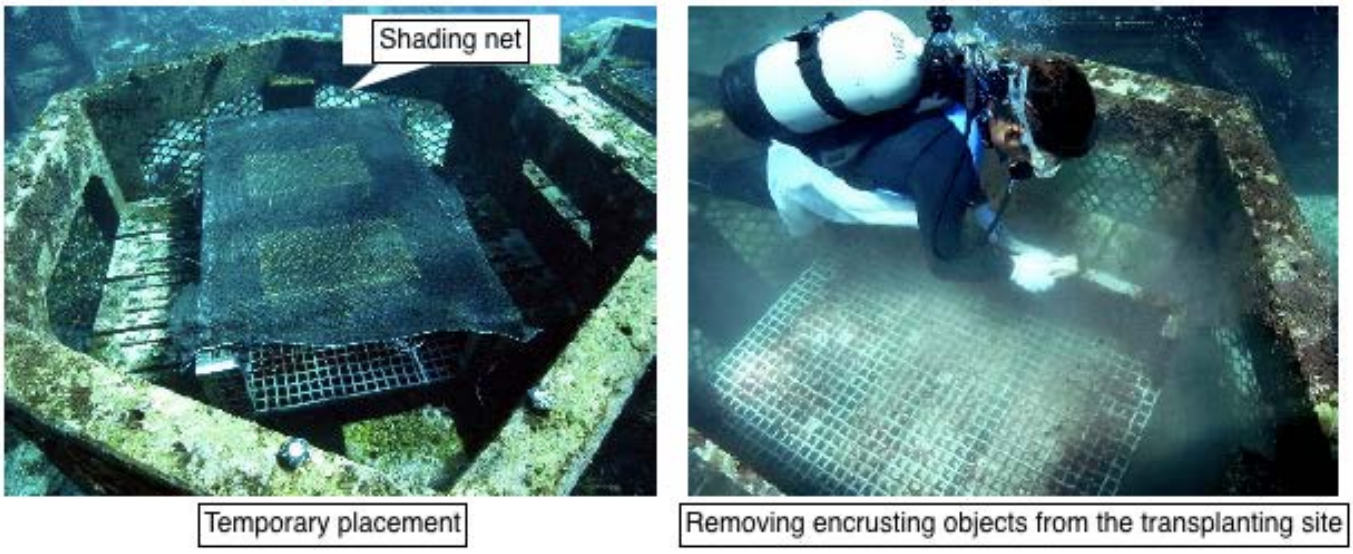


Figure II.2-45 Temporary placement of coral nursery stock and removal of accretions

Table II.2-7 Resources and equipment necessary for transportation

Category	Resources / equipment
Transport from nursery stock production facility to port	Coral storing container (e.g. Styrofoam), vehicle
Management of nursery stock during long-distance transportation	Tank, lid for tank, cushion tape (waterproof), clips to secure lid, material to fix coral (bottom mat for tank), zip ties, scaffold, shading net, hose, pump, thermometer, notebook and pencils, chemicals for marine products (Sodium Nifurstyrenate), syringe, gloves
Transport to mid-term rearing facility	Small shipping vessel (dinghy), diving equipment, coral storing tank, shading net, bucket
Temporary placement	Rope, shading net
Removing encrusting objects	Brush, wire brush, scraper

2.3.3. Coral planting

[When planting the nursery stocks on the mid-term rearing facility, use the planting method that minimises the influence of many growth inhibiting factors and keep in mind to not cause stress to the nursery stocks as you work.]

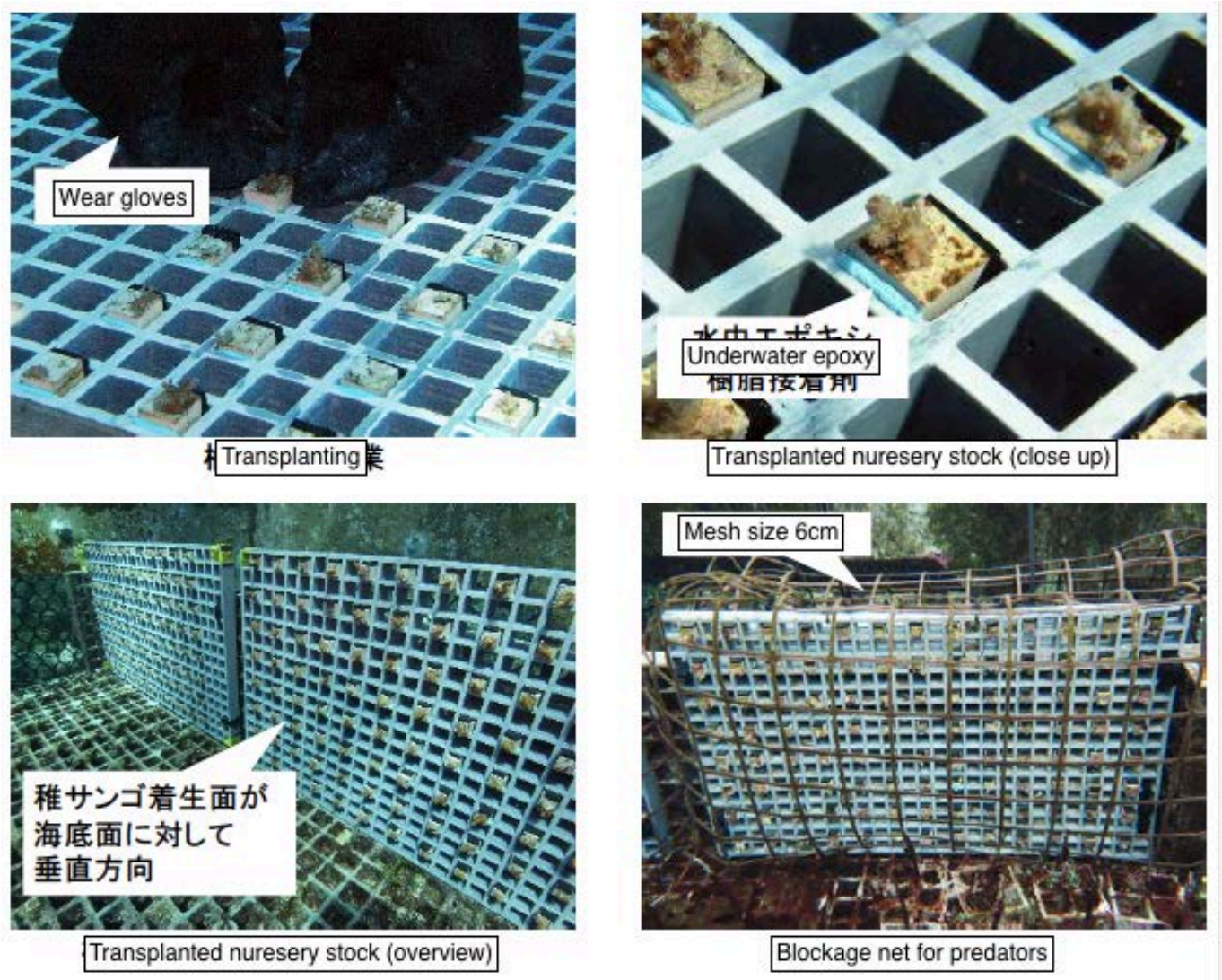


Figure II.2-46 Planting coral nursery stock

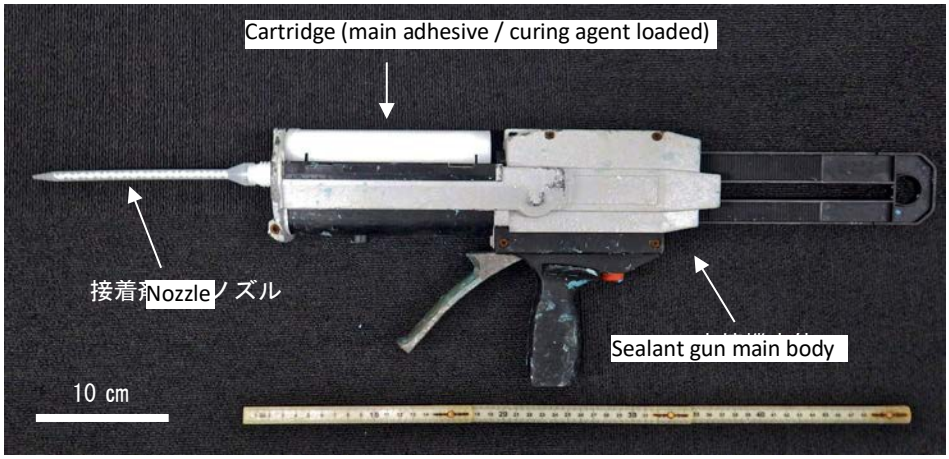


Figure II.2-47 Adhesive filler gun (sealant gun)

Table II.2-8 Resources and equipment necessary for planting

Category	Resources / equipment
Planting nursery stock	Gloves, underwater epoxy

Attaching predator blockage net	Net (mesh size 6cm), zip ties

2.3.4. Rearing management

[Monitor the coral nursery stocks planted on the mid-term rearing facility periodically and record coral survival, growth, and any change in the surrounding environment that may influence the corals. If frequent monitoring is difficult, utilise monitoring equipment. If any problem is found, take necessary measures.]

Figure II.2-48 Coral monitoring

Figure II.2-49 Installed water temperature logger

Figure II.2-50 Continuous recording of the corals by a long-term interval camera

Table II.2-9 Monitoring items of planted corals

Category / Content		**Method**
1. Check for survival / mortality (of the overall colony)		
a. Alive	Whole colony is alive	Observation
b. Partial mortality	Part of the colony is alive (ideally record the range as much as possible during observation)	
c. Total mortality	Bleached and only skeleton remaining, or skeleton disappearing	
2. Check for health (health state of the overall colony)		
a. Healthy	Whole colony is alive	Observation
b. Weak	Pale, excreting mucous, partially overgrown by algae	
c. Dead	*) same as 1. Check for survival c. Total mortality	
3. Measure colony size (check growth rate)		
a. Diameter	Length of live portion	Quick measure
b. Area	Area of live portion	Observation, Image analysis*
4. Check for predation (evaluate the effectiveness of blockage net)		
a. Fish	Bite marks or breakage	Observation
b. Mussels	Tissue loss, shells and live mussels found nearby	
c. Starfish	Same as above	
5. Check for algae overgrowth (evaluate the necessity of competition intervention)		
a. None	No algae overgrowth	Observation
b. Some	Some algae overgrowth (ideally record the range as much as possible during observation)	
c. All	Covered	
6. Measure (monitor growth environment)		
a. Water temperature	Check for fluctuations (especially during summertime)	Continuous monitoring

*To check for growth rate, it is best to be done on the spot via observation and quick measures. (Find more information about image analysis in Chapter III technical note 1)

Table II.2-10 Monitoring items of the surrounding sea area environment

Environmental factors	**Content**	**Method**
Coral cover	Natural coral cover and species Change in predation	Observation via diving Image analysis*
Water temperature	Water temperature temporal changes in locations and depths Estimate annual DHW from water temperature data	Continuous temperature monitoring
Climate	Route and severity of typhoons Wave and current	Bureau of meteorology data Wave and current monitoring
Predatory animals	Change in predatory animals species and number of individuals within the reef	Observation via diving

Competing animals	Change in algae cover that may compete with corals	Observation via diving Image analysis*

*Change in coral cover and algae cover can be acquired via image and video analyses. (Find more information about video analysis in Chapter III technical note 3)

Figure II.2-51 Removal of accretions around the planted corals

Table II.2-11 Resources and equipment necessary for rearing management of planted corals

Category	**Resources / equipment**
Removal of encrusting animals	Brush, scraper, tweezers
Check for predation prevention	Blockage net spare (mesh size 6cm), zip ties
Removal of dead corals	Chisel, hammer

2.4. Transplantation

[Select an appropriate transplantation site, then transplant the corals kept in the mid-term rearing facility. Conduct rearing management on a regular basis to maximise the transplanted coral growth and survival rate.]

(Content same as table of contents)
Figure II.2-52 Workflow of transplantation

2.4.1. Selection of optimal transplantation site

[Appropriate transplantation site should be chosen based on the following criteria: the transplanted corals will grow healthy; following the future spawning the larvae will settle on coral reefs on a wider range; all activities lead to coral reefs repair and restoration.]

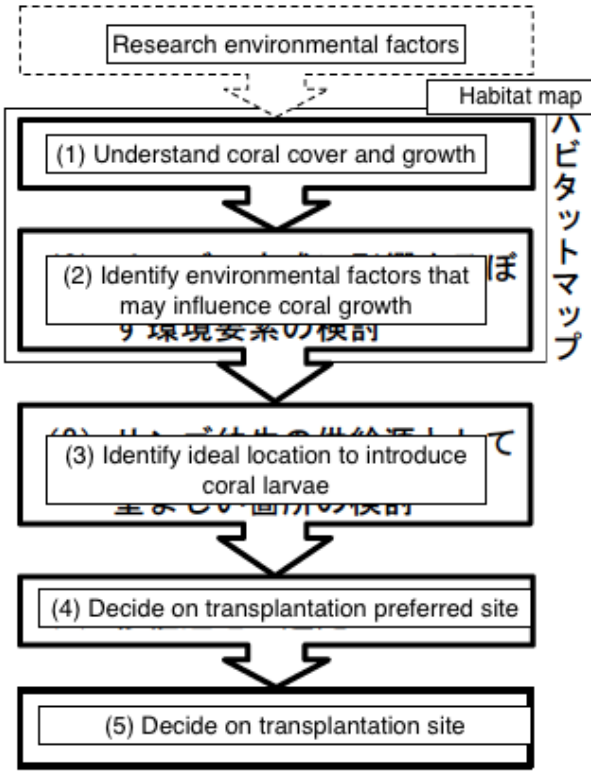


Figure II.2-53 Flowchart of appropriate transplantation site selection

Table II.2-12 Research and analysis items for selecting sites in a remote location

Environmental factors	Research and analysis content
(1) Understand coral cover and growth	
Coral distribution	Coral cover
Coral species	Main coral species at a representative point
(2) Identify environmental factors that may influence coral growth	
Topography, substrate	Depth and benthic substrate of the reef
Water temperature	Water temperature at a representative point
Wave and current	Wave, current, and sand/rubble movement within the reef
Predation	Predatory animals (COTs, mussels, fish) presence within the reef
(3) Identify ideal location to introduce coral larvae	
Larvae supplement site	A location where the coral larvae will stay within the reef rather than flow out of the reef due to current and dispersal actions

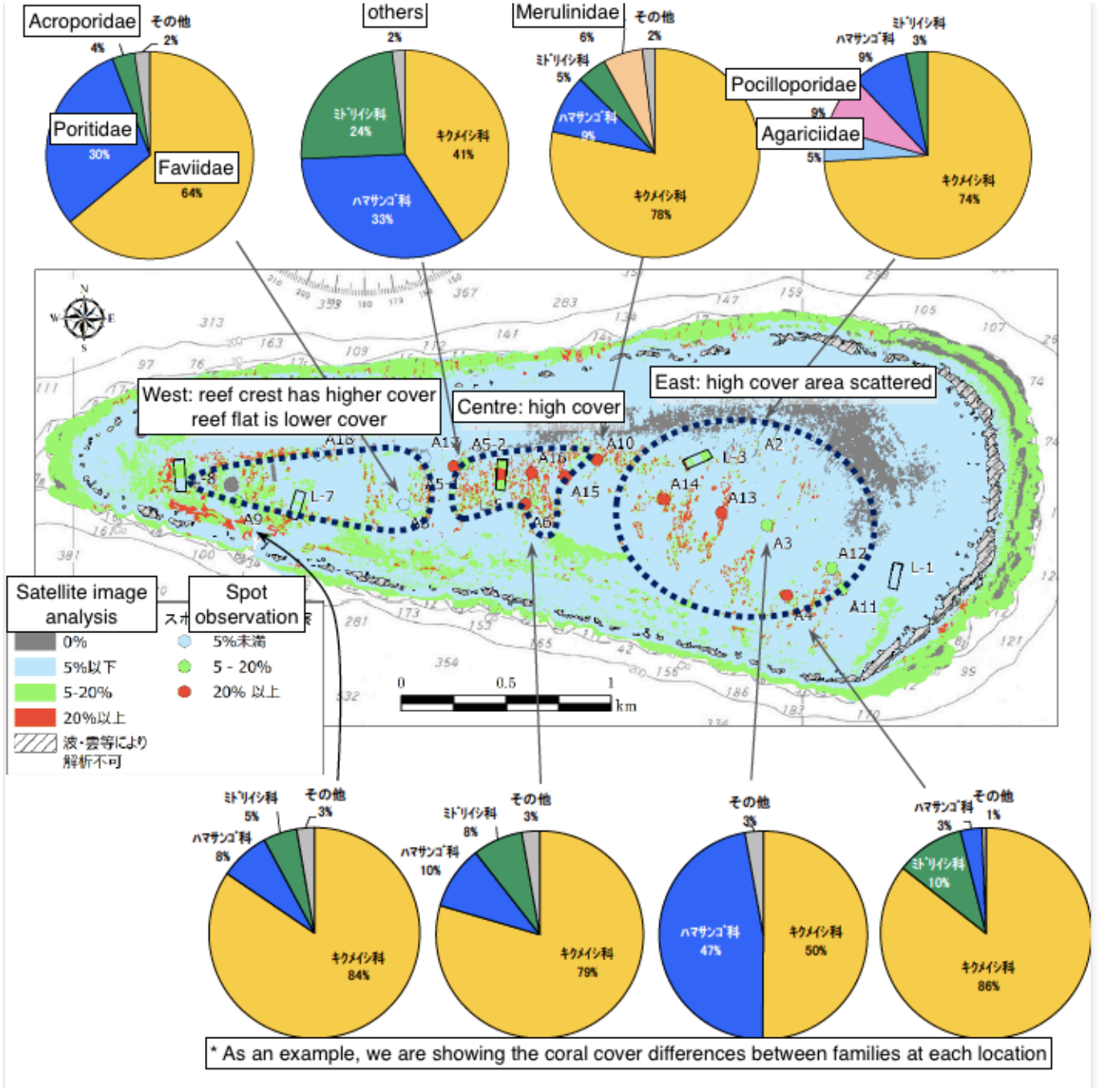


Figure II.2-54 Coral distribution map in Okinotori Island based on satellite image analysis and *in-situ* diving surveys (Using sea map W49 by Maritime Safety Agency as a base map)

Table II.2-13 Coral cover standards

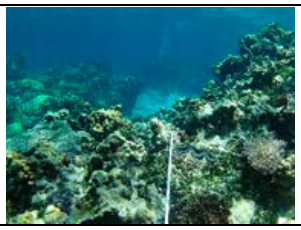
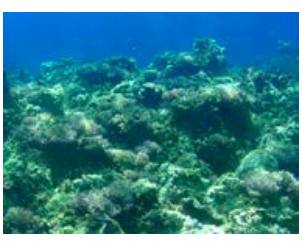
	0% cover No corals		<5% cover Very scattered		5-25% cover Scattered
	25-50% cover Can see benthos more than corals		50-75% cover Can see corals more than benthos		>75% cover Almost cannot see benthos

Figure II.2-55 Examples of understanding topography

Figure II.2-56 Water temperature distribution map in Okinotori Island (Annual average length of days with over 30°C water temperature between 2007 and 2013) (Using sea map W49 by Maritime Safety Agency as a base map)

Table II.2-14 Example of simulation analysis of a place with high wave action

Overview	Necessary data	Reproducibility	Things to consider
Choose the model based on the characteristics of the targeted sea area. Wind wave prediction by energy balance equation is common.	Topography (nautical charts published by Japan Hydrographic Association or bathymetric maps by other organisations), Offing waves (GPV data from the Meteorological Agency or local monitoring data)	Compare against local monitoring data of wind wave.	Choose high wind wave state as the condition to damage corals. Choose normal state (annual average wave energy) as the condition suitable for corals.

*GPV data: Grid Point Value data

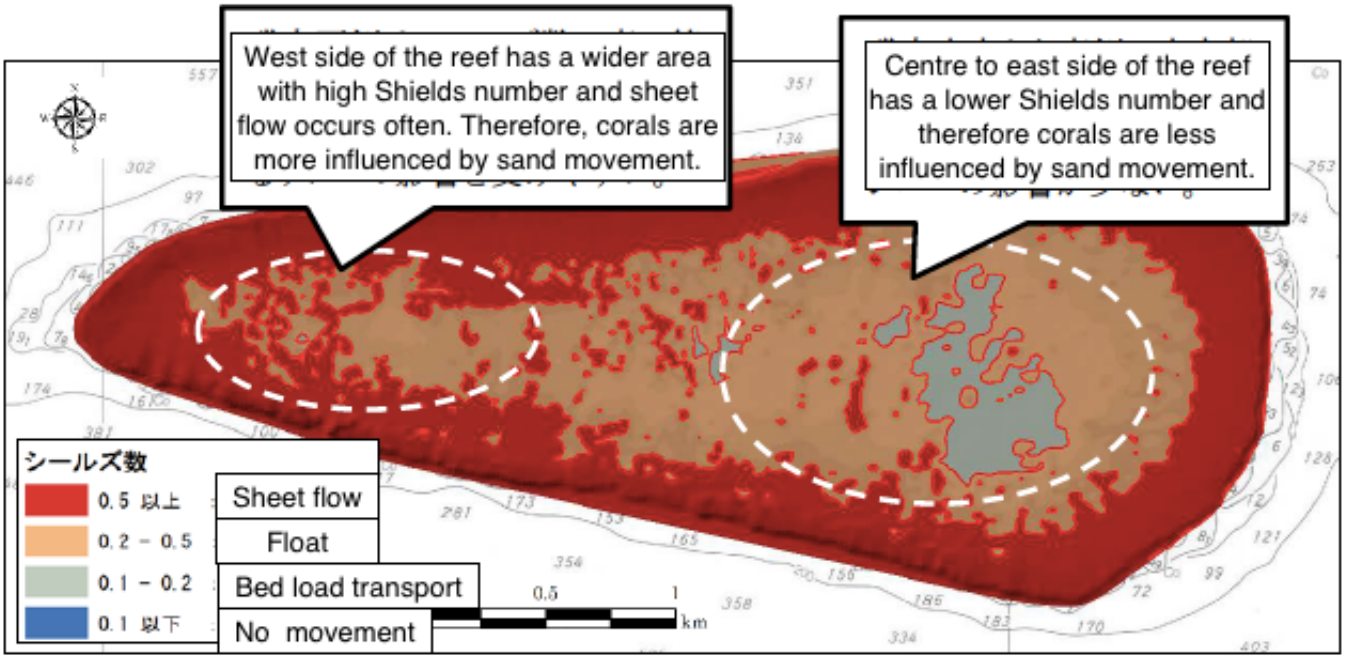


Figure II.2-57 Example of sand and rubble movement (Shields number) influence map in Okinotori Island. Calculation criteria: annual wave height (5-7m offing wave), characteristic sediment diameter 1mm (Using sea map W49 by Maritime Safety Agency as a base map)

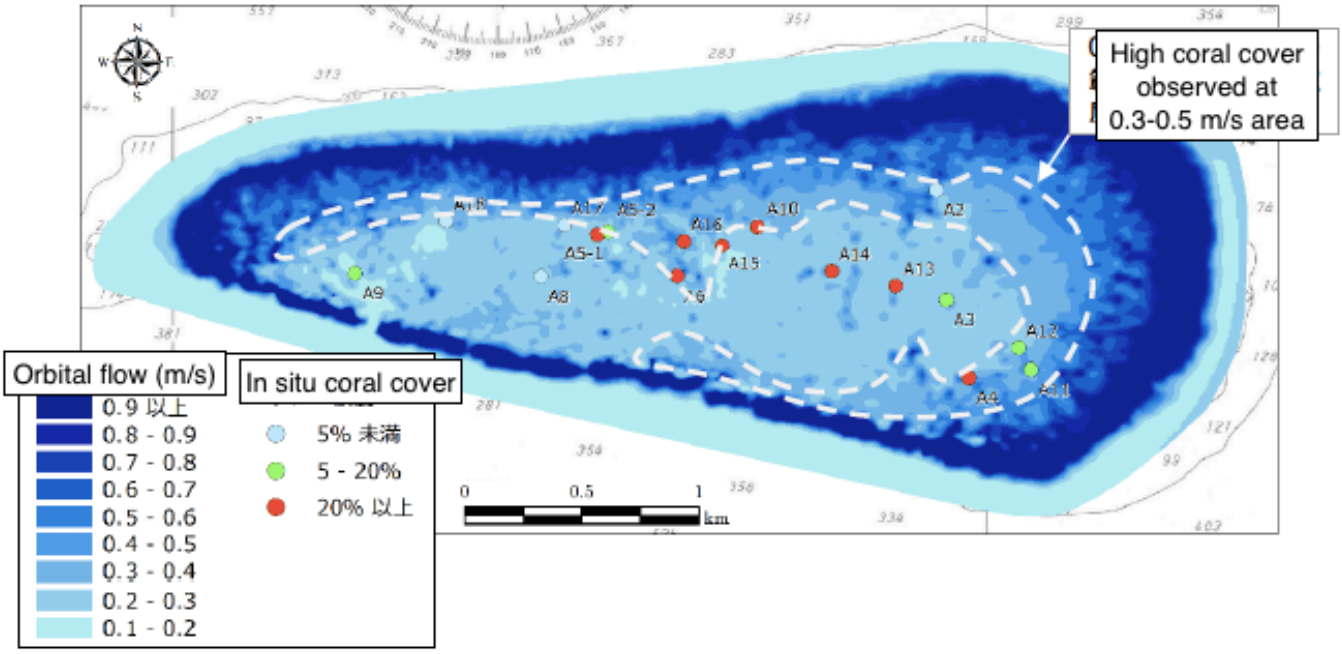


Figure II.2-58 Example of water flow rate distribution at 1m depth in Okinotori Island. Calculation criteria: dimensional flow rate (average effective wave energy) (Using sea map W49 by Maritime Safety Agency as a base map)

Table II.2-15 Representative fish and animals that affect coral growth

Name	Influence on corals	Name	Influence on corals
Fish		**Blenniidae**	
Chaetodontidae		*Exallias brevis*	Corallivore
Chaetodon unimaculatus	Corallivore	**Scaridae**	
Chaetodon bennetti	Corallivore	*Scarus frenatus*	'Biters'
Chaetodon plebeius	Corallivore	*Bolbometopon muricatum*	'Biters', Corallivore
Chaetodon speculum	Corallivore	*Calotomus* spp.	'Biters'
Chaetodon ornatissimus	Corallivore	**Balistidae**	
Chaetodon baronessa	Corallivore	*Balistoides viridescens*	'Biters'
Chaetodon lunulatus	Corallivore	*Balistapus undulatus*	'Biters', Corallivore
Heniochus chrysostomus	Corallivore	*Pseudobalistes flavimarginatus*	'Biters'
Chaetodon trifascialis	Corallivore	**Monacanthidae**	
Chaetodon melannotus	Corallivore	*Cantherhines dumerilii*	'Biters', Corallivore
Chaetodon rafflesii	Corallivore	*Cantherhines pardalis*	'Biters', Corallivore
Chaetodon citrinellus	Corallivore	*Oxymonacanthus longirostris*	Corallivore
Chaetodon ulietensis	Corallivore	**Acanthuridae**	
Chaetodon ephippium	Corallivore	*Acanthurus dussumieri*	'Biters'
Chaetodon auripes	Corallivore	*Acanthurus olivaceus*	'Biters'
Chaetodon auriga	Corallivore	*Acanthurus pyroferus*	'Biters'
Chaetodon vagabundus	Corallivore	*Ctenochaetus striatus*	'Biters'
Chaetodon kleinii	Corallivore	**Tetraodontidae**	
Pomacentridae		*Arothron hispidus*	'Biters', Corallivore
Cheiloprion labiatus	Corallivore	*Arothron meleagris*	'Biters', Corallivore
Plectroglyphidodon dickii	Corallivore	*Arothron nigropunctatus*	'Biters', Corallivore
Plectroglyphidodon johnstonianus	Corallivore	*Arothron reticularis*	'Biters', Corallivore
Oplegnathidae		*Arothron stellatus*	'Biters', Corallivore
Oplegnathus punctatus	'Biters'	*Canthigaster amboinensis*	'Biters', Corallivore
Labridae			
Coris aygula	'Biters'	**Other predatory animals**	
Labrichthys unilineatus	Corallivore	*Acanthaster planci*	Corallivore
Larger Labrids	'Biters'	*Reishia* spp.	Corallivore
Gobiidae		*Drupella fragum*	Corallivore
Gobiodon sp.1	Corallivore	*Morula spinosa*	Corallivore

*'Biters': herbivores that coincidentally bite off coral chunks as they eat the algae on the substrate. References: Iwao (2010), Kanda (1996), Nishihira (1995)

Table II.2-16 Example to calculate the direction to which coral larvae will flow

Model	Overview	Necessary data	Reproducibility	Things to consider
Flow model	Choose the model based on the characteristics of the targeted sea area. Models which consider the wind wave flow and drift current are common.	Topography (nautical charts published by Japan Hydrographic Association or bathymetric maps by other organisations), Wind (Meteorological Agency AMeDAS or local monitoring data), Tides (Meteorological Agency or local monitoring data)	Compare against tidal oval based on tidal surveys and data from JCOPE.	Choose the flow model for target transplant species' spawning seasons. For example, for *Acropora tenuis*, consider the wind conditions from May to June.
Larvae dispersal model	A method to consider dispersal effect using random numbers on Euler-Lagrange equation. Can periodically track larvae until they settle.	Benthic structure distribution (nautical charts published by Japan Hydrographic Association or bathymetric maps by other organisations), coral cover, larvae size, density	Larvae movement surveys, recruitment surveys	Spawner corals' gametes float about for a few days after spawning, metamorphose to planula larvae, then settle. The model allows to predict where the settlement will occur. Settlement sites of brooders need to be monitored immediately after spawning.

*JCOPE: Japan Coastal Predictability Experiment

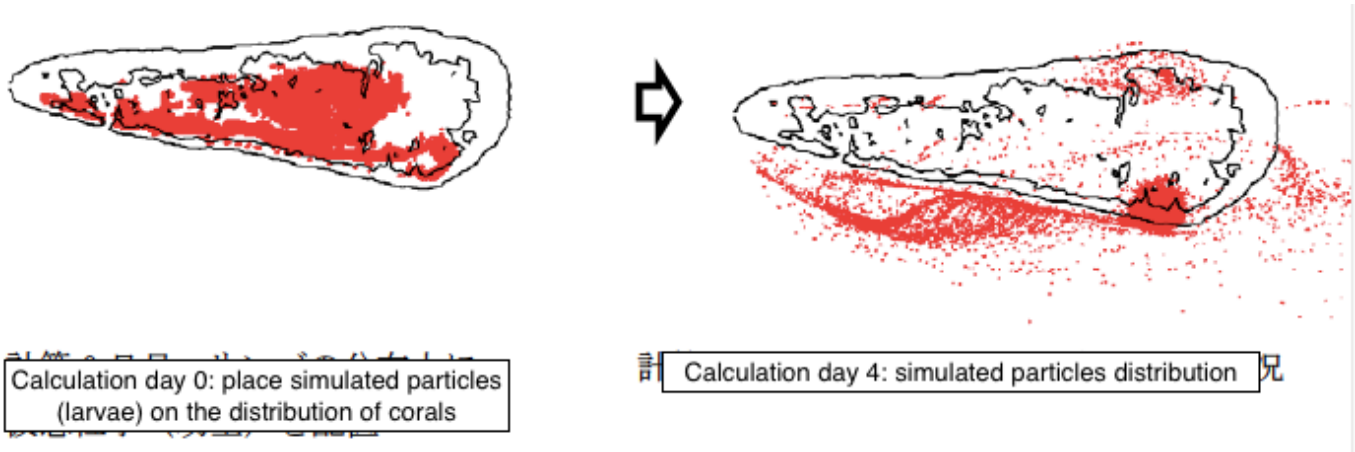


Figure II.2-59 Modelling example of larval dispersal in Okinotori Island

Figure II.2-60 Ratio of larvae existence within each reef location 4-5 days after spawning in Okinotori Island (Using sea map W49 by Maritime Safety Agency as a base map)

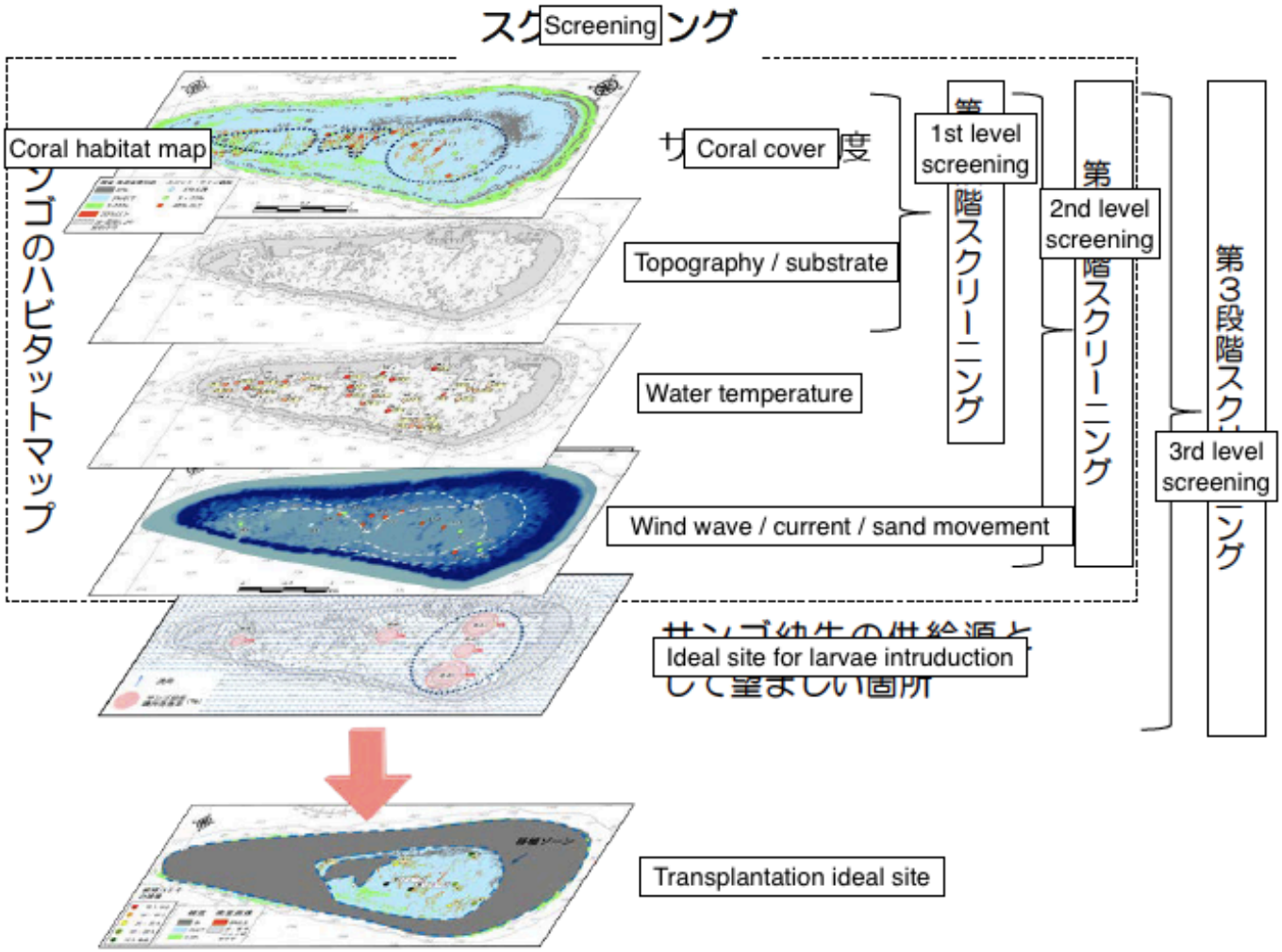


Figure II.2-61 Conceptual scheme of narrowing down appropriate transplantation site

Figure II.2-62 Result of appropriate transplantation site selection in Okinotori Island (Katayama et al. 2014)

Table II.2-17 Research items for appropriate transplantation site selection

Category	Overview	Survey method
Transplantation base	Position, depth, basal shape	*In situ* observation (or underwater video)
Coral and other organisms	Coral cover, colony number, bleaching category, algae and other predatory animals presence	

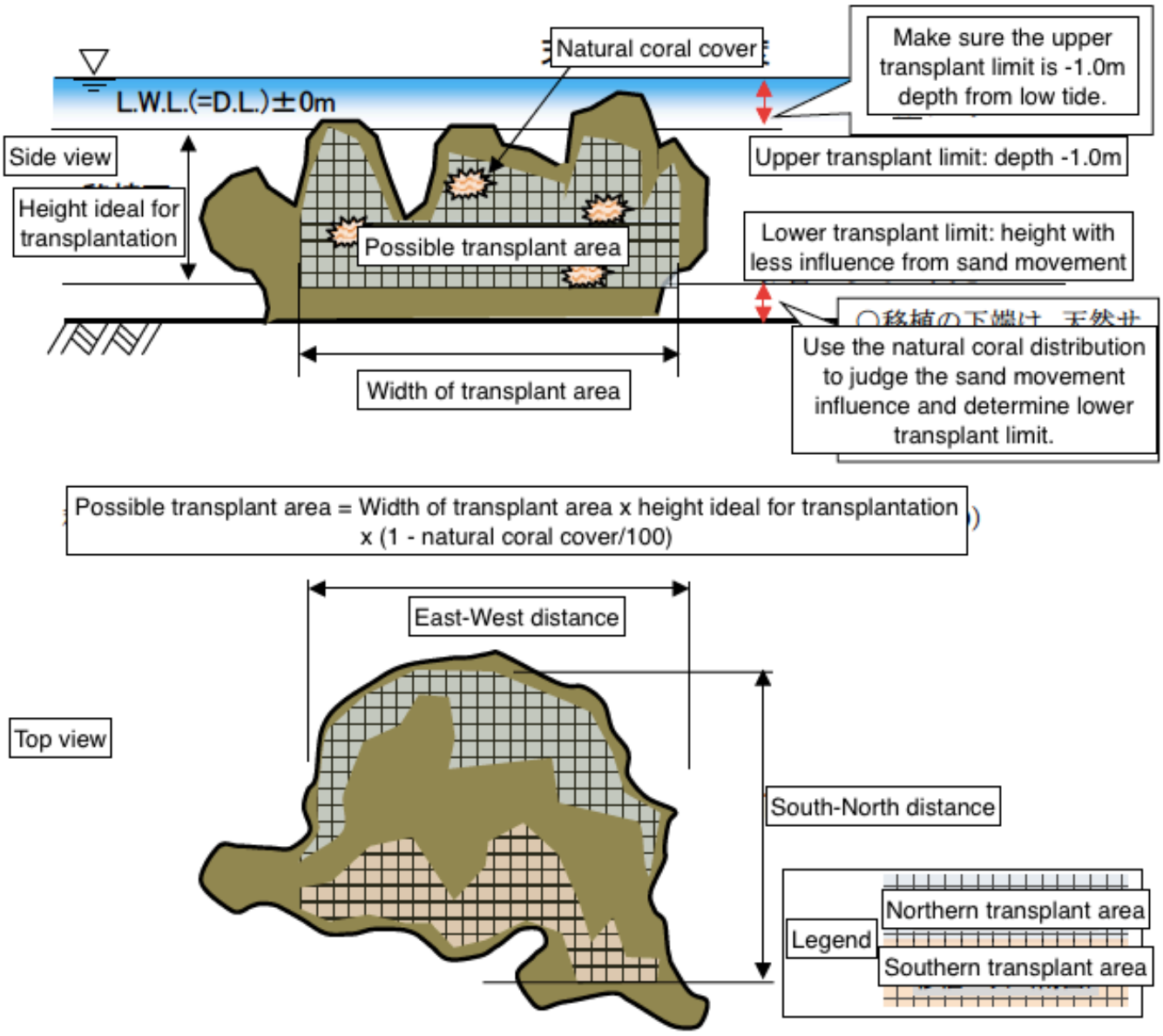


Figure II.2-63 Example of appropriate transplantation site selection

2.4.2. Transplantation

[Remove the corals in the mid-term rearing facility that has grown to the appropriate size for transplanting, transport them to the transplantation site and plant.]

Table II.2-18 Resources and equipment necessary for removing corals from mid-term rearing facility

Category	Resources and equipment
Selecting and marking transplant corals	Underwater camera, notebook and pencil, ribbon
Removing marked corals	Gloves, scraper, hammer, container, zip ties
Transportation	Container, shading net, bucket, brush

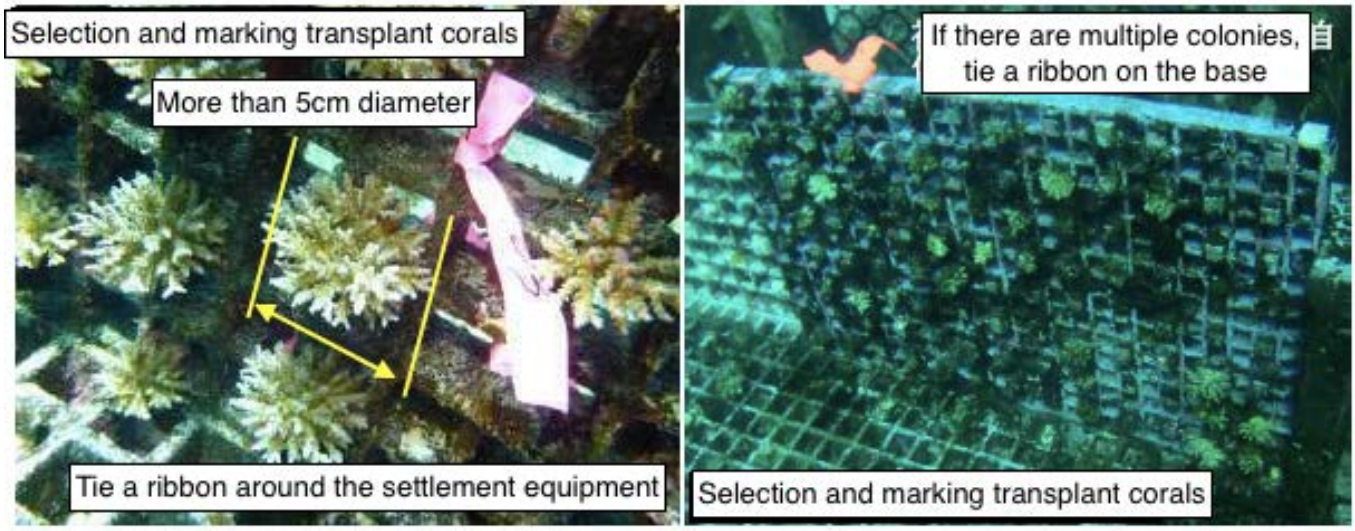


Figure II.2-64 Progress shots of selecting and marking target corals to transplant

Figure II.2-65 Relationship between diameter of transplanted corals and next year mortality (Example from Okinotori Island)

Figure II.2-66 Progress shots of removing marked corals and transportation

Table II.2-19 Items for status check

Contents to check	What to check
Topography and benthic structure	• The benthic or coral structures could have changed due to typhoon or high waves. Compare with how it was when the site was selected.
Competing species (for space)	• Presence of algae etc. that could inhibit coral growth • Presence of new natural coral recruitment
Predation species	• Corallivorous fish and COTs predation pressure presence (Refer to Table II. 2-15 for predation species)

Table II.2-20 Resources and equipment necessary for transplanting

Category	Resources and equipment
Check current status	GPS, marker buoy, underwater camera, notebook and pencil, previous year data
Transplantation	Scraper, underwater epoxy, wire, predation blocking basket, weights and ropes for temporary placement, tags, gloves, underwater camera, notebook and pencil, ruler

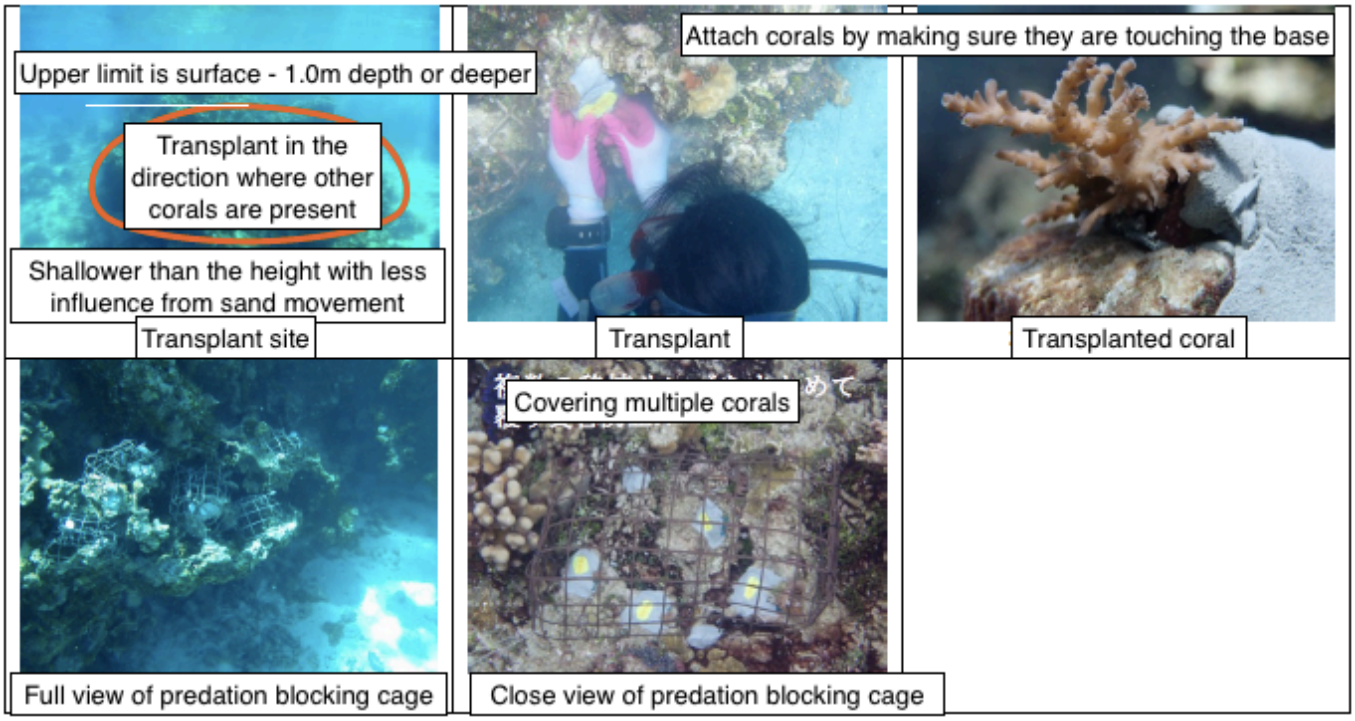


Figure II.2-67 Progress shots of transplantation

2.4.3. Rearing management

[Monitor the transplanted corals and maintain as necessary.]

Table II.2-21 Monitoring items for transplanted coral survivorship and growth

Category / Content		Method
1. Record transplanted corals' identities and location		
a. ID	Check the ID tag from when it was transplanted	Observation
b. Transplanted date	Year, month, date of transplant	
c. Transplant base	Transplant location and base	
d. Species	Transplanted coral species	
e. Depth	Depth from surface to transplant location (m)	
f. Height	Distance from sea floor to transplant location (m)	
g. Direction of transplant from centre of base	Direction of transplant from centre of base (East-west-south-north, top)	
2. Check for survival / mortality (of the overall colony)		
a. Alive	Whole colony is alive	Observation
b. Partial mortality	Part of the colony is alive (ideally record the range as much as possible during observation)	
c. Total mortality	Bleached and only skeleton remaining, or skeleton disappearing	
3. Measure colony size (overall growth rate)		
a. Short/long diameters, height	Size of the live portion	Measure on site
4. Check for health (health state of the overall colony)		
a. Healthy	Whole colony is alive	Observation
b. Weak	Pale, excreting mucous, partially overgrown by algae	
c. Dead	*) same as 1. Check for survival c. Total mortality	
5. Attachment to base		
a. Attachment to base	Weather the coral fully attached to the base	Observation
6. Check for predation (evaluate the effectiveness of blockage net)		
a. Fish	Bite marks or breakage	Observation
b. Mussels	Tissue loss, shells and live mussels found nearby	
c. Starfish	Same as above	
7. Check for algae overgrowth (evaluate the necessity of competition intervention)		
a. None	No algae overgrowth	Observation

b. Some	Some algae overgrowth (ideally record the range as much as possible during observation)	
c. All	Covered	
8. Installing protection cage		
a. Cage for blocking predatory animals	Check state of cage	Observation
9. Water temperature check		
a. Water temperature	Check for fluctuations (especially during summertime)	Continuous monitoring

Table II.2-22 Resources and equipment necessary for rearing management of transplanted corals

Category	Resources and equipment
Algae removal	Brush, scraper
Reinforce coral and protection cage	Underwater epoxy, wire
Protection cage removal	Hammer, scraper, pliers

Figure II.2-68 Progress shots of rearing management of transplanted corals

3. Whole-area propagation techniques by nursery stock production using larvae collection equipment

Figure II.3-1 Workflow of whole-area propagation techniques in the Okinawa sea area

3.1. Nursery stock production using larvae collection equipment

[Nursery stock production using larvae collection equipment means to collect parental coral colonies, let them spawn *in situ* and collect the eggs and sperm and fertilize, maintain the developed larvae in an incubator until they can settle on a substrate, and help them settle on a settlement equipment placed inside the incubator.]

<Explanation>

1) Function of larvae collection equipment and steps

This method uses the larvae collection equipment (Figure II.3-2) which has the following functions in the sea area: <Functions of larvae collection equipment>

1 – 'Collection and fertilisation' of eggs and sperm spawned from parental colonies

2 – 'Maintenance' of larvae acquired from function1 until they are ready to settle

3 – 'Settlement' of larvae maintained in function2 onto settlement equipment

This equipment can collect more than 1 million coral eggs per $1m^2$ and can maintain the larvae with over 90% survival rate until they are ready to settle (4-day old).

The workflow of nursery stock production using this equipment is described in Figure II.3-3.

2) Larvae collection equipment structure and materials

The overview of structure and materials of this equipment are described in Figure II.3-4 and Table II.3-1.

(1) Equipment main body and external frame

The overall structure of the main body should be cylindrical to minimise the restriction of water flow, and to maintain the structure, attach an external frame to the body. Hardened plastic tube should be used as the material of the external frame for flexibility and durability. To be able to attach the external frame with ease but also sound strength, sew a durable net (eye-mesh: see Figure II.3-4, mesh size 2-3cm) as a belt around the main body every 1 m.

Use a nylon mesh for the material for main body, which is often used for plankton catch nets. Fertilised coral eggs and early stage embryos are very fragile and can disintegrate with the slightest physical shock. As long as the nylon mesh size is about 30 µm, there is minimum effect of the mesh rubbing against the larvae, and a 90% or more survival rate up until 4-day old can be maintained (Okada et al 2016).

If you were to store a 4cm square settlement equipment in the scale of thousands, the main body should be at least 9 m^3 volume. If the main body has a large diameter but short, or too tall, the flow resistance becomes greater. The ideal size is around 1.7 m diameter and 4 m tall. With this size, the maximum number of larvae that can be collected and maintained is about 3 million.

(2) Equipment ceiling

The ceiling should be sealed to prevent eggs and larvae escaping from the surface. However, ceiling access is required for larvae number sampling (refer to section 3.1.4) and settlement equipment installation (refer to section 3.1.5). Therefore, attach a zipper (Figure II.3-5) and close it at all times except for when it is necessary.

Ideally the environmental factors during fertilisation should be kept as close as possible to a natural surrounding environment. Therefore, use the external frame and surface buoys to lift the main body and let it float on the sea surface (to minimise the wave and wind effects, 20cm below surface is ideal). Attach buoys or lift bags that are stable around the external frame. If using the same dimensions as (1), the required buoyancy is 4kg x 16 buoys = 64kg.

(3) Equipment bottom

To secure the main body to the ocean floor, attach a rope on either the external frame or onto the eye-mesh belts. In addition, to prevent the equipment from breaking due to rubbing against the sea floor, attach a canvas-like strong material on the bottom and around the sides (about 30cm off the bottom).

The bottom also needs to open when collecting the eggs. Attach a zipper around, and when collecting the eggs, open the zipper, set the placement shelf with parental colonies inside, and close after spawning to prevent eggs and larvae from escaping.

3) Parental colony placement shelf structure and materials

Set up a shelf on the sea floor to place parental coral colonies.

The shelf is made of bar steel with legs that will raise the top of the shelf at least 50cm high to prevent damage from sand and rubble movement, as well as invasion of predatory animals such as COTs. The top of the shelf should be a grid which allows water flow and minimises sediment accumulation.

An example shelf is shown for a calm sea area and to be able to use as a mid-term rearing facility as well (Figure II.3-6). The materials and dimensions are described in Table II.3-2.

4) Settlement equipment structure and material

Nursery stock production via larvae collection equipment uses the settlement equipment directly in the collection equipment and continues to mid-term rearing. This means that the nursery stock is at a vulnerable stage and requires prevention measures on the settlement equipment against fish predation and sediment accumulation that would otherwise inhibit the coral growth.

Ceramic plugs used to be utilised a lot for settlement equipment, however recently a square-tubular one (40mm x 40mm x 40mm, thickness 4mm; Figure II.3-7) has been developed.

The square-tubular settlement equipment not only allows good waterflow and less accumulation of sediment, but also prevents predation from fishes, which results in better survivorship than the flat tiles. In fact, 66% survival rate (settlement maintenance rate) of nursery stock after 15 months have been recorded (Figure II.3-8; Suzuki et al 2011). Moreover, the size is easy to handle and therefore higher efficiency can be expected.

Figure II.3-2 Larvae collection equipment

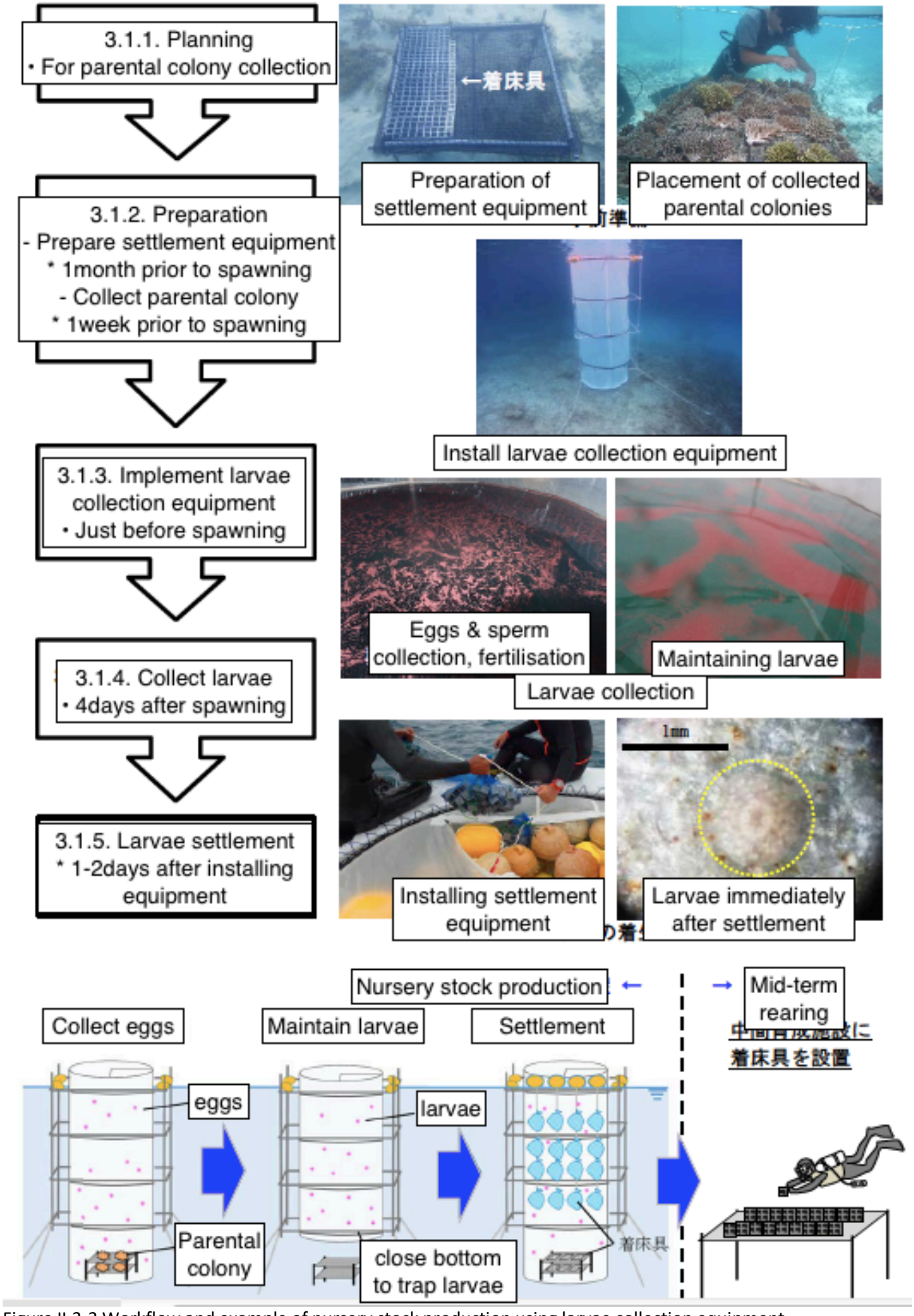


Figure II.3-3 Workflow and example of nursery stock production using larvae collection equipment

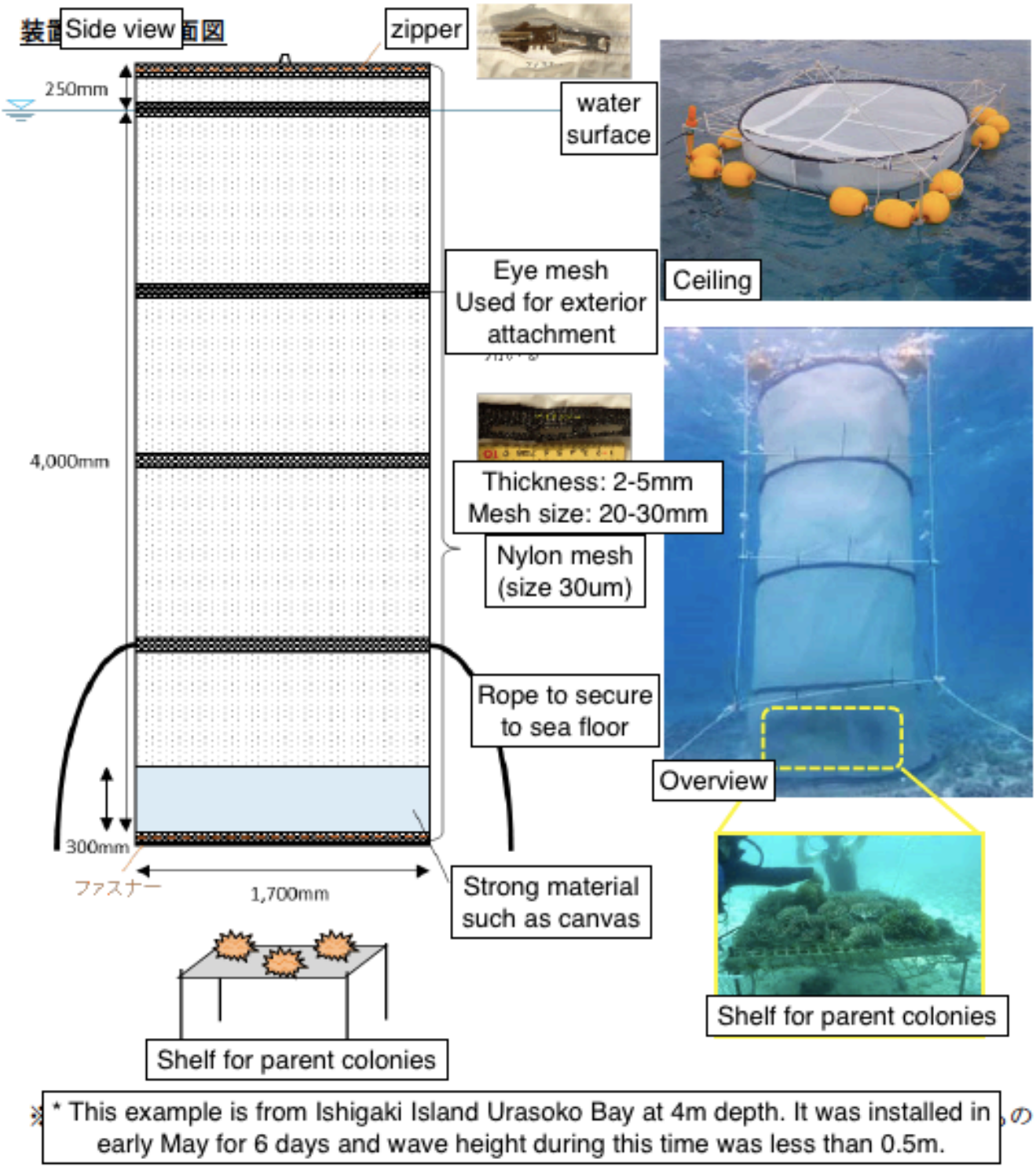


Figure II.3-4 Larvae collection equipment (overview)

Table II.3-1 Structure and material overview of larvae collection equipment

Section	Structure / material
Main body	• Body: Nylon mesh, mesh size 30um (φ1.7m) x 3.8m • 6 bits of main body: Eye mesh, mesh size 2-3cm • Bottom+bottom area: canvas • Ceiling+bottom: zippers
Exterior frame	• Hardened plastic pipe (Outer diameter φ14mm, inner φ4mm) • Others: connecting pipes
Float (water surface)	• Buoyancy 4kg x 16pieces = 64kg
Rope (to secure to sea floor)	• φ10mm x 5m x 4 ropes = 20m

Figure II.3-5 Zippers on the equipment ceiling and bottom

Figure II.3-6 Frames for parental coral colonies

Table II.3-2 Material and specs of shelf frame

Section	Structure / material
Top	(1) FRP grating Dimensions: width 100cm, length 100cm, thickness 4cm (2) Resin net + Vinyl nitrate pipe (8: 4 sides x above and under the net) Dimensions: width 100cm, length 100cm
Legs	Bar steel (D13) x 4 Dimensions: outer diameter 12.7mm, length 100cm

Figure II.3-7 Square tubular settlement equipment (rigid polyvinyl chloride)

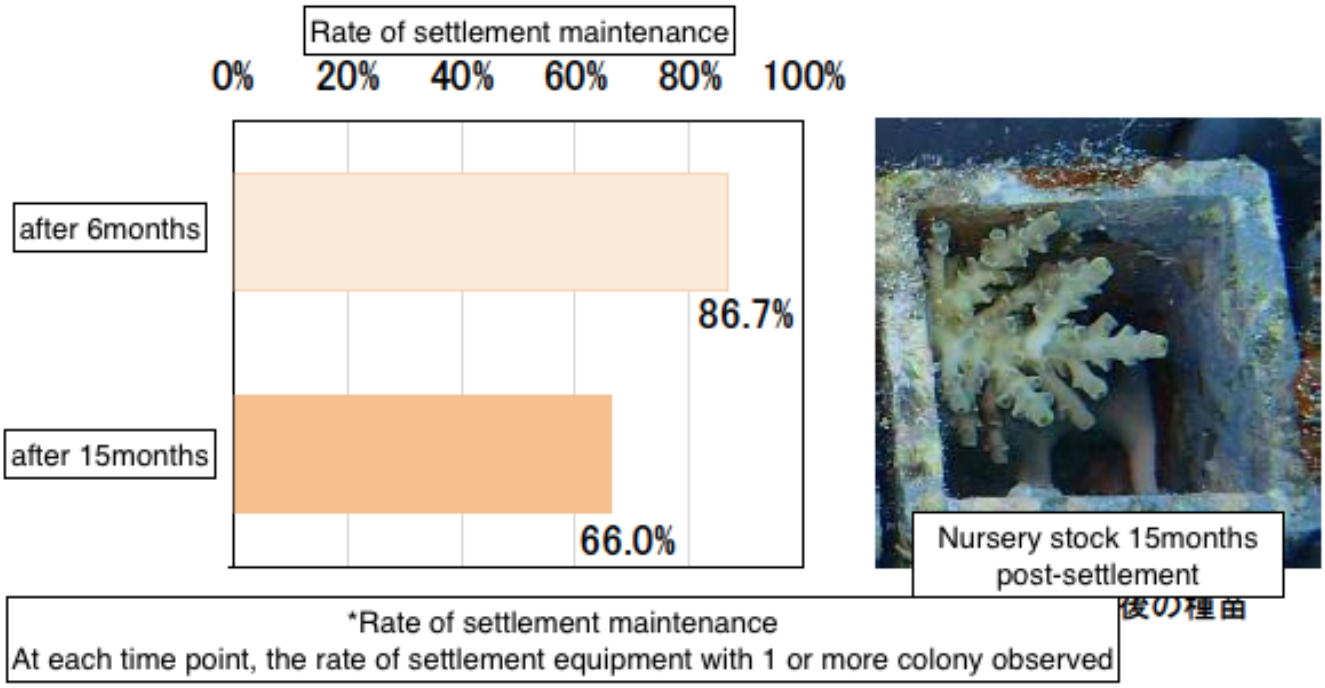


Figure II.3-8 Rate of steady settlement on the settlement equipment

3.1.1. Planning

[Depending on the nursery stock production scale, plan the number of settlement equipment required. Parental coral colonies spawn in masses, however high concentration of settlement could lead to mass mortality of the recruits. Therefore keep in mind to maintain an appropriate settlement concentration of the recruits and adjust the amount of larvae supply.]

<Explanation>

1) Number of parental colonies to collect

As we said in Chapter II 2.1, the number of parental coral colonies required for nursery stock production should be more than 6 to ensure genetic diversity. To be considerate, minimise the collection number as much as possible.

2) Larvae production

For *Acropora tenuis*, a colony size of 30 cm diameter can roughly spawn 240,000 eggs (Kitada 2002). Following this, 6 colonies can theoretically produce approx. 230,000 larvae that will eventually settle.

The fertilisation rate, survival rate, and settlement rate used here are based on developmental testing for larvae collection equipment conducted off the coast of Okinawa.

Number of eggs spawned by 6 parental colonies

240,000 x 6 colonies = 1,440,000 eggs

↓

Number of eggs fertilised and successfully becoming larvae

1,440,000 x 90% fertilisation success = 1,296,000 larvae

↓

Surviving larvae until settlement period (4-day old)

1,296,000 x 90% survivorship = 1,166,400 larvae

↓

Number of larvae successfully settling (= larvae production)

1,166,400 x 20% settlement rate = **233,280 larvae**

3) Plan for number of settlement equipment

A large number of larvae can be acquired from 6 parental coral colonies, however high density settlement for larvae can cause mass mortality. Therefore, when planning for the number of settlement equipment production, consider the optimum settlement density of the square-tubular equipment (internal area 50cm^2 per equipment) and adjust the amount of larvae introduction. According to Suzuki et al (2012), when larvae settle on a grid shaped plate in the ocean, mortality rate significantly increases at settlement density higher than 0.5 individual/cm^2 and the optimum density was 0.1 individual/cm^2.

For example, if the production goal for settlement equipment is set to 10,000, with the optimum density condition for larvae settlement, 50,000 larvae is required. Therefore, the larvae production from the example given in 2) above is excessive.

10,000 settlement equipment x (0.1 individual/cm^2 x 50 cm^2 /equipment) = **50,000 larvae**

As such, if the larvae production is far greater than the production goal for the settlement equipment, decrease the number of larvae before you install the settlement equipment (3-4 days old, before the larvae are ready to settle), or only collect the fragment of the parental coral colony (i.e. less than 30cm diameter colony size) and reduce the number of eggs spawned. As a rough guide, for 10,000 settlement equipment, you need 1/4 of each 6 colonies. On the contrary, if the production goal for the settlement equipment is greater than the larvae production, increase the number of parental colonies collected.

A few notes for production planning:

• Larvae collection equipment (1.7m diameter, 4m height, 9m^3 volume) can hold approximately 10,000 settlement equipment. This is based on the developmental stage testing of this equipment, and therefore a larger collection equipment or multiples are required.

• Parental coral colonies will be at different maturity levels and spawning can be varied. Make sure to count the actual number of larvae before installing the settlement equipment (refer to section 3.1.4) and adjust accordingly.

At the developmental stage of the larvae collection equipment, we observed 80% successful settlement on the settlement equipment (Table II.3-3). With this outcome, if you were to install 10,000 settlement tubes, roughly 8,000 nursery stock can be obtained.

Table II.3-3 Achieved stable settlement rate 3 days after settlement in the larvae collection equipment

Number of settlement equipment	**Number of nursery stock (*number of settlement equipment with at least one larvae settled)**	**Maintained settlement rate**
7,348	6,152	83.7%

3.1.2. Preparation

[Submerge the required number of settlement equipment in sea water 1-month prior to spawning and allow crustose coralline algae and biofilm growth on the equipment to induce coral larvae settlement. In addition, collect parental coral colony 1-week prior to spawning.]

<Explanation>

1) Preparation of settlement equipment (immersion in sea area)

The larvae settlement is determined by not the material of the equipment but the crustose coralline algae and biofilm presence on the surface of the equipment (Omori 2016). Therefore, a preconditioning of the equipment by leaving it in the sea area for more than a month is required to let the algae and bacteria colonise the surface.

The effects of depth and length of immersion have been investigated by comparing the settlement density on the square-tubular settlement tubes. There was no significant difference between 3m and 13m depths and between 1 month and 3 months periods.

Therefore, up to 10m depth is good for immersion and 1month is more than enough (Figure II.3-10).

2) Parental colony collection

Collect colonies 1-week prior to predicted spawning time at each sea area.

For sexual reproduction to be successful, eggs and sperms from genetically different colonies are required. It is more likely that the same species collected from the same location are clones, and thus it is best to collect colonies from multiple locations.

It is ideal to collect not the whole colony but a fragment using a hammer and chisel considering the natural recovery of the colony (refer to Chapter II 2.1). Note that the parental colony is expected to be put back to where it came from after spawning.

Collected colonies should be kept on a temporary placement for efficiency and to check for damage during collection. If the sea area is calm and the weather forecast is clear, keep the parental colonies directly on the shelf where the larvae collection equipment will be installed. Where strong wave action or bad weather is forecasted, use a zip tie to secure the colonies onto the shelf.

Application for permits is required to relevant agencies when collecting coral colonies (see Chapter III).

Figure II.3-9 Immersion of settlement equipment (lining them up on the shelf frame)

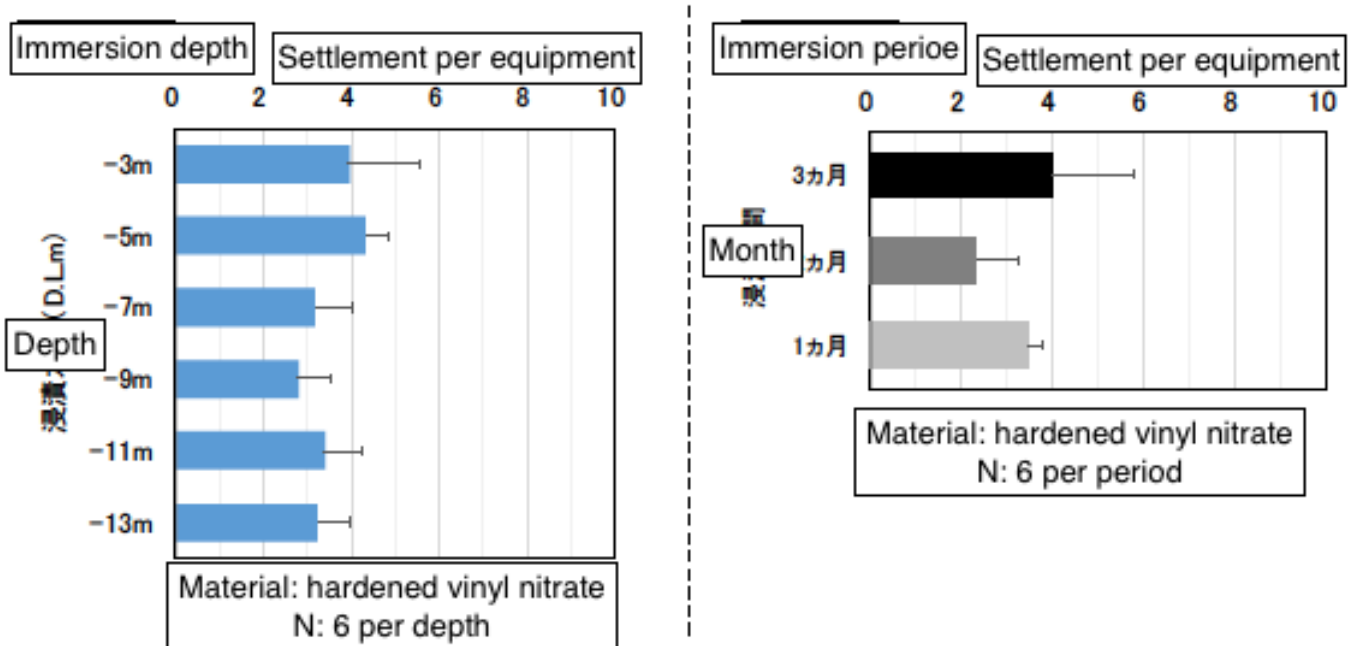


Figure II.3-10 Immersion conditions (depth, length of time) and number of recruits on square tubular settlement equipment

Figure II.3-11 Temporary placement of parental coral colonies on shelf frame

3.1.3. Implementation of larvae collection equipment

[Decide on the location of larvae collection equipment installation based on the equipment height, depth, and benthic composition. Keep in mind that the equipment is easily influenced by water movement.]

<Explanation>

1) Choosing the site

Choose a site to install larvae collection equipment. Considering the volume of the equipment, the height should be about 4m, and therefore choose a location where at high tide the equipment will not be submerged (roughly 4m depth).

Keep in mind that bar steel needs to be hammered into the sea floor for the shelf frame and securing ropes, therefore sandy bottom is ideal (Figure II.3-12).

2) Steps

The steps for installing the equipment is as follows:

1. Set up the shelf frame where the larvae collection equipment will be installed and place the parnetal colonies on top
2. Hammer in the bar steel in four directions from the site to secure ropes
3. Load the equipment onto the boat with everything folded and transport to site
4. Unload directly above the site
5. Divers open up the folded equipment underwater. Ideally have 3-4 divers to open up downwards at the same time to minimise the effect of current
6. Adjust the equipment so that the top of the shelf frame is level with the bottom of the equipment, then secure four corners with ropes. Maintain same pull from all directions

Figure II.3-12 Example of larvae collection equipment installation site

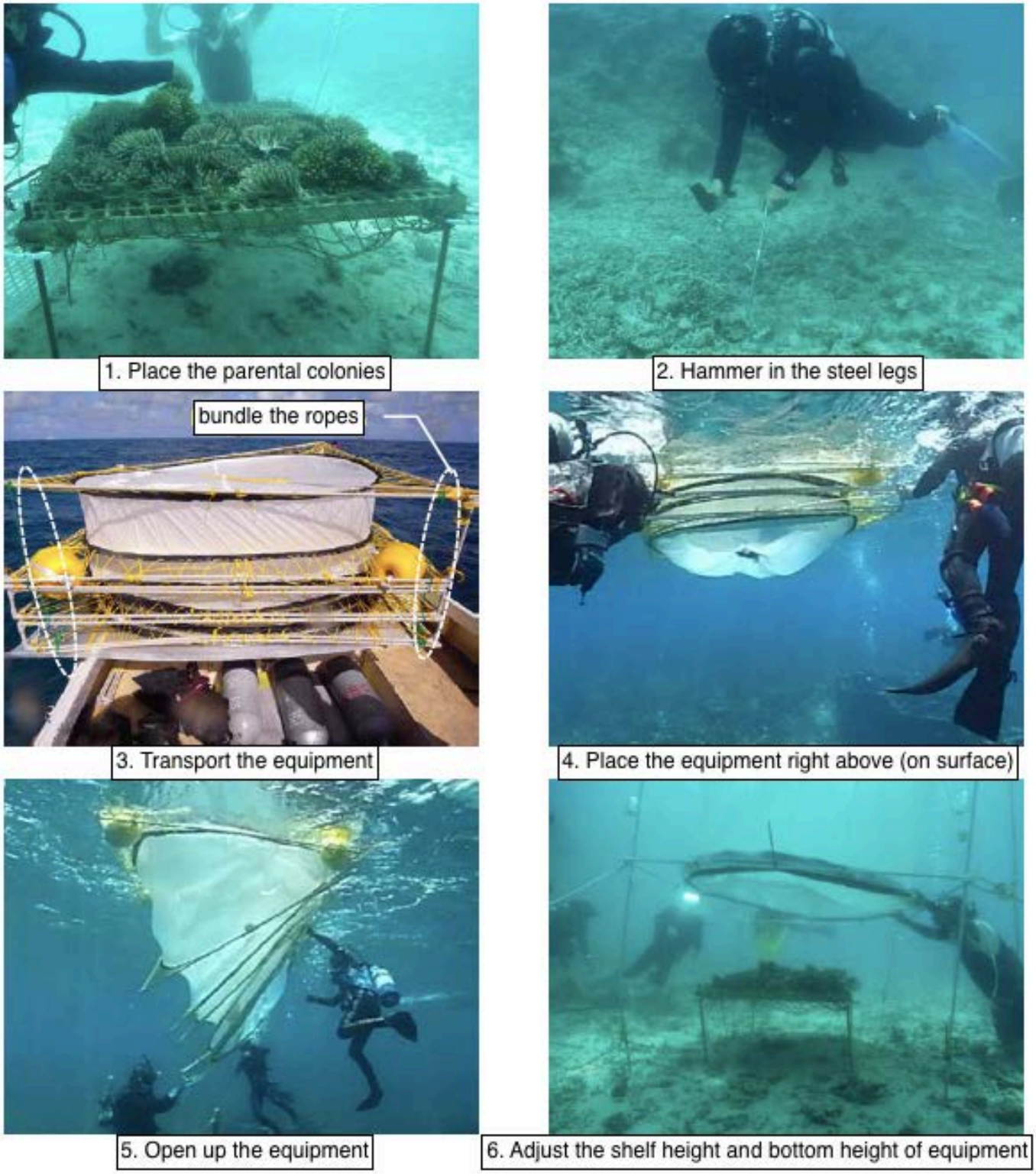


Figure II.3-13 Workflow of equipment installation

3.1.4. Collecting larvae

[Confirm that the parental coral colony has spawned inside the equipment. If confirmed, close the bottom of the equipment to prevent eggs and larvae from escaping. If not, conduct spawning inducing protocols. After collecting the larvae, count the number of larvae to determine the number of settlement equipment required.]

<Explanation>

1) Collecting eggs and sperm

During the predicted spawning season, check every morning for spawning. For species like *Acropora tenuis* or *A. yongei* which spawn around 19-20:00, you can check for bundle set around 18:00 and can judge whether they are likely to spawn that day.

If the corals don't spawn after their predicted times, spawning induction method can be used. Spawning induction is done by using hydrogen peroxide to the mature corals (Hayashibara et al 2004). The timing and steps are described below and Figure II.3-14.

Timing

- The collected fragment is mature and ready to spawn
- The method has a high success rate, and can be done in the afternoon of the day before you want it to spawn

Steps

1. Bring up the parental colony that was placed on the shelf frame in a bucket
2. Add hydrogen peroxide into the bucket so the final concentration will be 1mM. Leave it for 2 hours
3. Move the corals into a clean bucket with fresh sea water and bring it back into the water and set it up on the shelf frame

2) Closing off the equipment bottom

Once spawning is confirmed, close the bottom zipper to prevent the eggs and larvae from escaping.

3) Count the number of larvae

Spawning number prediction is theoretical, so always count the actual number of larvae before installing the settlement equipment (when larvae is 3-4 days old).

Methods:

1. Open the equipment ceiling zipper, use the Kitahara method plankton net (mouth φ200m, filtering mouth φ400m, mesh size 100um) and vertically pull up the net from the bottom of the equipment to the surface and sample larvae.
2. Decant the sample into a bucket with a scale, add sea water to it so it becomes 10L, then sample 1L and return the remaining 9L to the collection equipment. Sample three times.
3. Bring the sample to the lab, add more sea water to make it to 2L, then take 50ml each and count the number under a microscope. Take the average from three samples and adjust the settlement equipment numbers accordingly.

A few notes for larvae collection:

• Cover the parental colonies and collected larvae with shading net on the shipping vessel and pour fresh sea water occasionally to prevent temperature increase.

• Before you count the larvae, make sure to agitate the water up inside the equipment for better accuracy. Insert air flow system from the bottom and blow are for a few minutes and create a current (Figure II.3-16).

• Put the parental colonies back to where you collected from after harvesting eggs.

Figure II.3-14 Example procedures of spawning inducing protocols

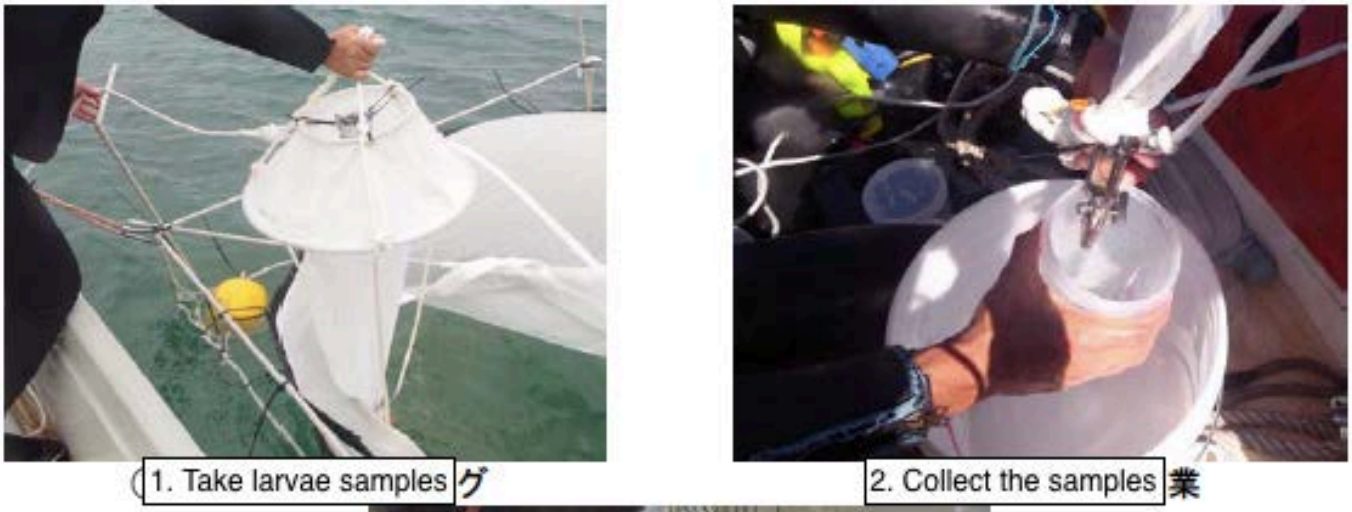


Figure II.3-15 Methods to count number of larvae

Figure II.3-16 Method to stir larvae for even distribution

3.1.5. Settlement of larvae

[To allow the larvae to settle, the larvae must be at right before recruitment stage (66 hours after larval development), as well as vertically evenly distributed. Install the settlement equipment and retrieve in 1-2 days. The installation is done by vertically hanging the equipment off a buoy.]

<Explanation>

1) Timing to install settlement equipment

Settlement equipment will be installed utilising all the space inside the collection equipment, and therefore has to be immediately before the settlement phase of larvae and also when larvae are evenly distributed across the internal space.

For *Acropora tenuis*, 66 hours after larvae development, it is known that they distribute themselves evenly in the given space (Iwao et al 2014). A study done in Ishigaki island also showed that 3-day old *A. tenuis* larvae sampled from top, middle, and bottom bands of the equipment were evenly distributed among each other (Table II.3-4). This timing can differ between species and it is recommended to refer to other studies for actual timeline.

2) How to install settlement equipment

Put 50 or so settlement tubes into a mesh bag with mesh size 1-2cm, hang it off of a buoy at 50cm depth and repeat every 50cm until 1m above the shelf frame. This is one set unit, and hang as many sets as necessary until you reach the number of settlement tubes required (Figure II.3-17).

3) When to collect settlement equipment

Collect the settlement equipment 1-2 days after hanging them from the buoy. At the developmental stage of the collection equipment, at every depth of the mesh bag, settlement of larvae has been confirmed (Okada et al 2016).

Table II.3-4 Example of number of larvae (3-days old) distribution within the collection equipment

Collection band (depth band)	Number of larvae (individuals per band)	Distribution ratio (%)	Number of larvae (n/base)
Top (0-133cm from surface)	95,332	37.7	252,704
Middle (134-266cm from surface)	72,634	28.8	
Bottom (267-400cm from surface)	84,739	33.5	

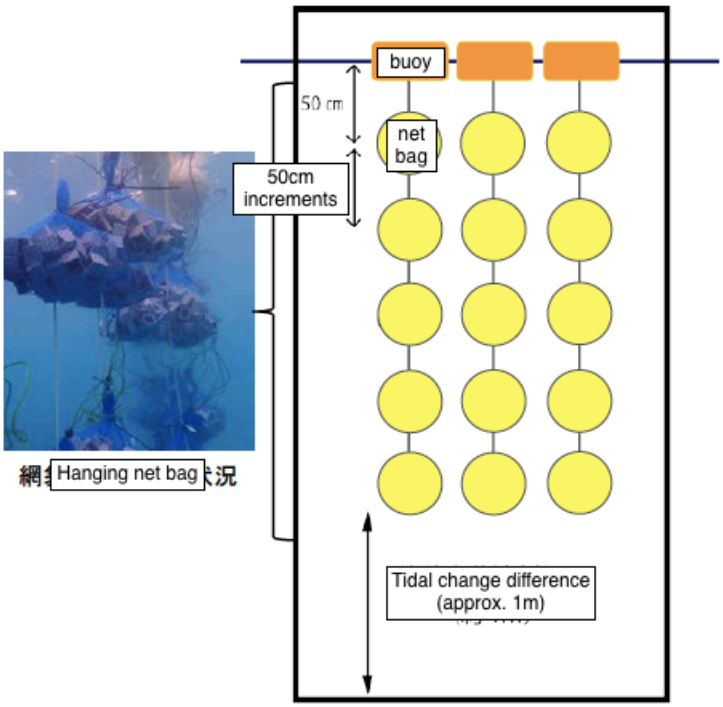


Figure II.3-17 Example of settlement equipment installation

3.2. Mid-term rearing

[Mid-term rearing involves installing the settlement equipment from the larvae collection equipment into the mid-term rearing facility and managing them until they grow to the size appropriate for transplanting.]

(Same as table of contents)
Figure II.3-18 Workflow of mid-term rearing

** Below is more or less repeat from Chapter II Section 2.3 and onwards.

3.2.1. Establishment of mid-term rearing facility

[Typhoon-prone areas such as coastal Okinawa main island, use the shelf type mid-term rearing facility which you bolt down on the sea floor or the basket type mid-term rearing facility.]

Figure II.3-19 Shelf type mid-term rearing facility

Table II.3-5 Material and specs for shelf type mid-term rearing facility

Figure II.3-20 Basket type mid-term rearing facility

Figure II.3-21 Installed bivalve-proofing

Table II.3-6 Material and specs for basket type mid-term rearing facility

Figure II.3-22 Ideas to maintain horizontality in a mid-term rearing facility

3.2.2. Installing recruitment (nursery stock) equipment

[Place the square tubular settlement equipment with 0-year old recruits settled onto the mid-term rearing facility.]

Figure II.3-23 Placing the settlement equipment and the coral recruits

Figure II.3-24 How corals will look like after growing via mid-term rearing (15-months post settlement)

3.2.3. Rearing management

[It is ideal to conduct monitoring and rearing management frequently on coastal Okinawa main island.]

3.3. Transplantation

[Select an appropriate transplantation site, then transplant the corals kept in the mid-term rearing facility. Conduct rearing management on a regular basis to maximise the transplanted coral growth and survival rate.]

3.3.1. Selection of optimal transplantation site

[Appropriate transplantation site should be chosen based on the following criteria: the transplanted corals will grow healthy; following the future spawning the larvae will settle on coral reefs on a wider range; all activities lead to coral reefs repair and restoration. Especially on the coastal mainlands, pay attention to anthropogenic disturbances (red soil runoff and eutrophication) when choosing the site.]

Table II.3-7 Research and analysis items for selecting sites on the main island coast line

3.3.2. Transplantation

[Remove the corals in the mid-term rearing facility that has grown to the appropriate size for transplanting, transport them to the transplantation site and plant.]

3.3.3. Rearing management

[Monitor the transplanted corals and maintain as necessary.]

Technical note 1: Simple calculation method of growth area of corals during mid-term rearing

Traditionally, monitoring of coral growth after transplantation has been done by divers' observations recording the cover (%). However, this method has individual differences, and if there were many transplanted corals more labour and time will be required. Therefore, a development of an efficient, accurate monitoring method to record data is required.

We summarised the monitoring methods using photography and post-image analyses to measure coral area below.

Field observation and analysis steps

The steps are shown in Figure III.1-1; 1. Photography, 2. Visual observation, and 3. Image analysis. 1. Photography entails underwater photography of the target corals by divers. 2. Visual observation records the coral survivorship which can be difficult to tell from image analysis later. 3. Image analysis calculates the area of corals (cm^2) from the images obtained.

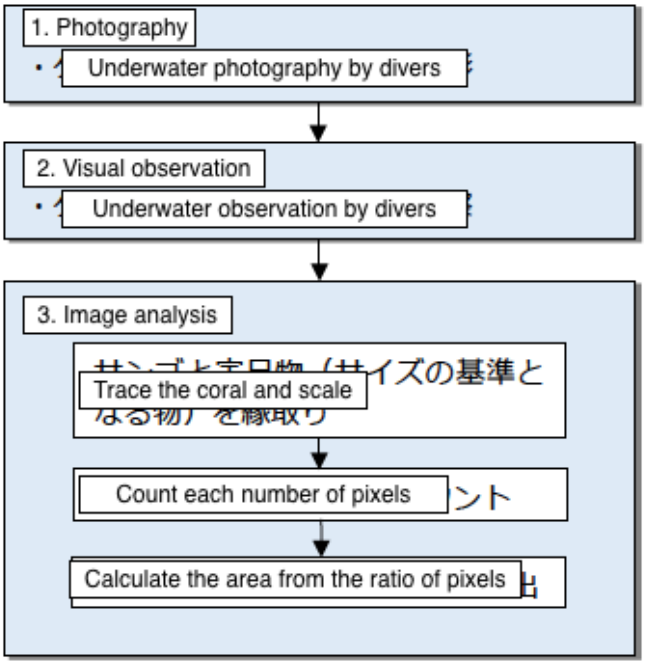


Figure III.1-1 Image analysis steps to calculate coral area

1. Photography
   Place a scale within the view shed at the same surface as the coral and take a photo of the coral parallel to the surface of settlement tube. From the example in Okinotori Island, we used one the grids from the grid base of the mid-term rearing facility (4x4cm). Use a stick to maintain a constant distance from the coral, which also allows you to check whether you are standing parallel to the coral, as well as to be efficient with multiple photo shots.

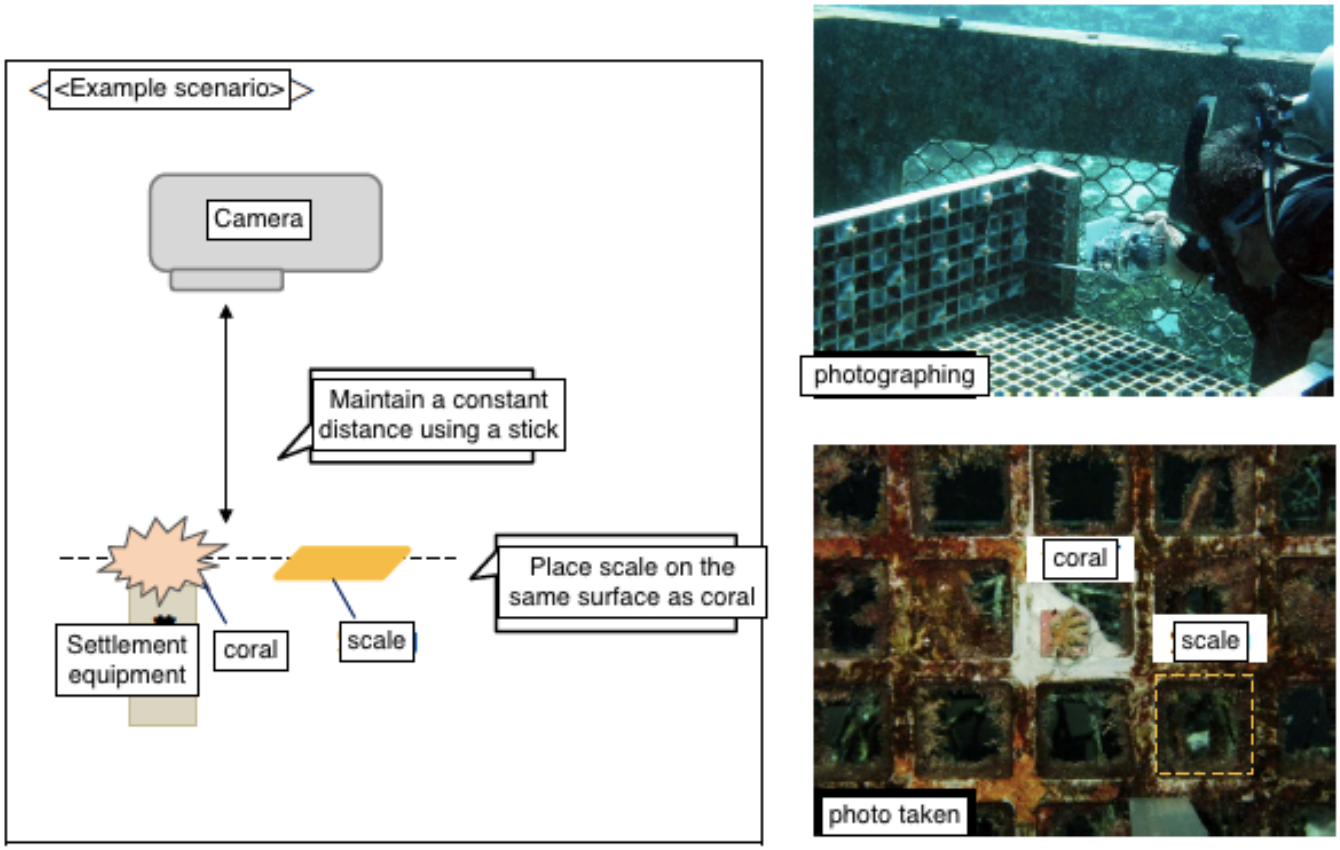


Figure III.1-2 Photographing corals

2. Visual observation
   It is difficult to tell the survivorship of corals from photographed images and therefore conduct visual observations on the spot and record survivorship and any external changes and use as additional information for image analysis. Refer to Chapter II section 2.3.4 Table II.2-9 for survivorship monitoring.

Figure III.1-3 Visual observation of corals (checking survivorship)

3. Image analysis
   Use image analyses software (such as Adobe Photoshop) and compare the number of pixels of the coral and the scale in the photo.
   Digital images are aggregations of colour information (tone and level). Each point is called 'pixel' and the image analyses software allows you to count the number of pixels within the selected region. With the number of pixels counted for both coral and scale, with the information of the area of scale, you can calculate the area of coral based on ratio.
   Calculation steps for coral area using image analysis software is shown below.

   [Calculation steps]
   1. Open the image analysis software and open the photo.
   2. Trace the outline of coral and scale, and calculate the number of pixels inside the line (the method of calculation depends on the software)
   3. Calculate the coral area based on the ratio equation below:
      Coral area = coral pixels / scale pixels x scale area

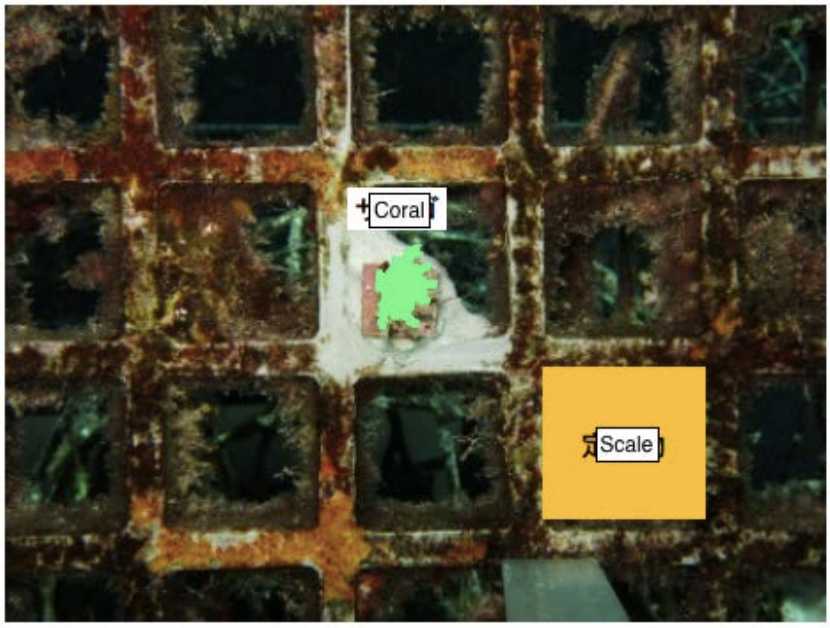


Figure III.1-4 Image analysis example of a coral photograph

Technical note 2: Method for coral cover analysis using satellite images

Divers' observation and ROV surveys have been the standard methods to record coral growth and cover, however if the survey needs to be conducted over a greater scale of reefs extending for over 10 km, it is labour and time costly for the divers. There is a clear need to develop efficient monitoring methods for widescale coral cover.

Here, we summarise the methods for coral cover analysis using satellite images and consider these techniques for practical use.

1) Steps to analyse satellite images

The steps to analyse satellite images are described in Figure III.2-1. They can be separated into '(1) Acquire multi-spectrum satellite images' and '(2) Analysis of coral cover distribution'. '(1) Acquire multi-spectrum satellite images' requires the right season and resolution necessary for analysis captured in the images. '(2) Analysis of coral cover distribution' requires white balancing, calibration to account for water evaporation, and distortion for each image before analysing for coral cover.

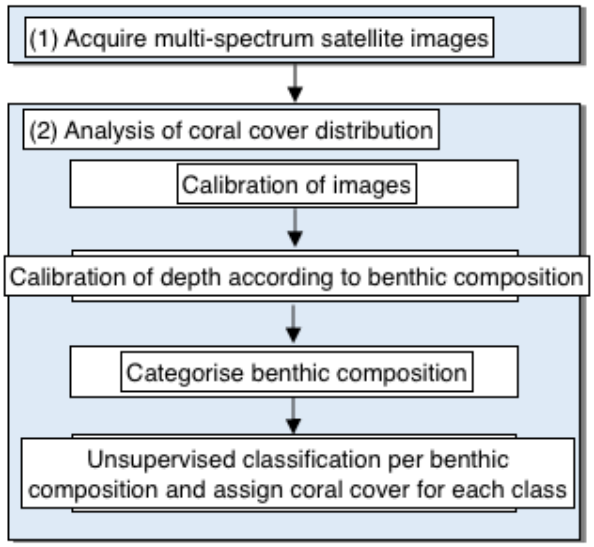


Figure III.2-1 Steps to analyse satellite images

*Unsupervised classification means to not use classification data (data to tie the image to categories of choice) and use similar characteristics within the data instead.

(1) Acquire multi-spectrum satellite images

First you need to acquire satellite images from the location and time of interest. If you only need one time point, choose the image from the same time when local surveys were conducted. If there are multiple time points, make sure to include at least one time point that coincides with local survey time. Table III.2-1 shows the popular satellites that the imageries can be used from. For analyses such as to detect individual bommies, a higher resolution (1m or less) is better, while for analyses to grasp the general distribution, a moderate resolution (approx. 10m) can be used. Note that such analyses can also utilise aerial photography, which can have better resolution than satellite images. Either utilise the aerial images from the Geographical Survey Institution or other organisations, or take your own new aerial photographs and run high resolution image analyses.

Points to consider when choosing and purchasing satellite images are listed below.

Table III.2-1 Popular satellites to acquire images from

Type of satellite	**Panchromatic-sharpening image[*2] (4 bands) resolution and price examples**	
	Resolution	**Price [*4]**
IKONOS	0.8m [*3]	4,500 yen
GeoEye-1	0.5m [*3]	6,000 yen
WorldView-2, WorldView-3, WorldView-4	0.5m [*3]	6,000 yen

Pleiades	0.5m	2,400 yen

*2 Fuses Panchromatic image, which is high resolution than multi-spectrum image, and multi-spectrum image and creates orthographs.
*3 These resolutions are common examples, images with other resolutions are also available.
*4 Minimum purchase area for satellite image data is 25km^2. These prices are based on archived images that have been divided into 1km^2 (as of 7 Dec 2017).

[Points to consider when choosing and purchasing satellite images]
• Choose images with less clouds to be able to analyse the entire target area.
• Where wind and wave are generally stronger, choose the time when waves are smaller to minimise halation and white waves (these make analyses difficult or almost impossible).
• Satellite images can be used for coral image analysis only when the sea floor is visible and properly photographed. Therefore the general depth and visibility of the local area must be considered to decide whether image analysis is realistic or not. In the Okinotori Island example, the depth ban was between 0-5m.
• Analyses can be done with images from different satellites. If analysis spans across multiple years, keep in mind the above points and choose the images.

(2) Analysis of coral cover distribution

Coral cover distribution analysis can be done after photo calibration and depth calibration using bottom index. These analyses are normally done using software such as ERDAS IMAGINE, ArcGIS, and SIS. This study used ERDAS IMAGINE.

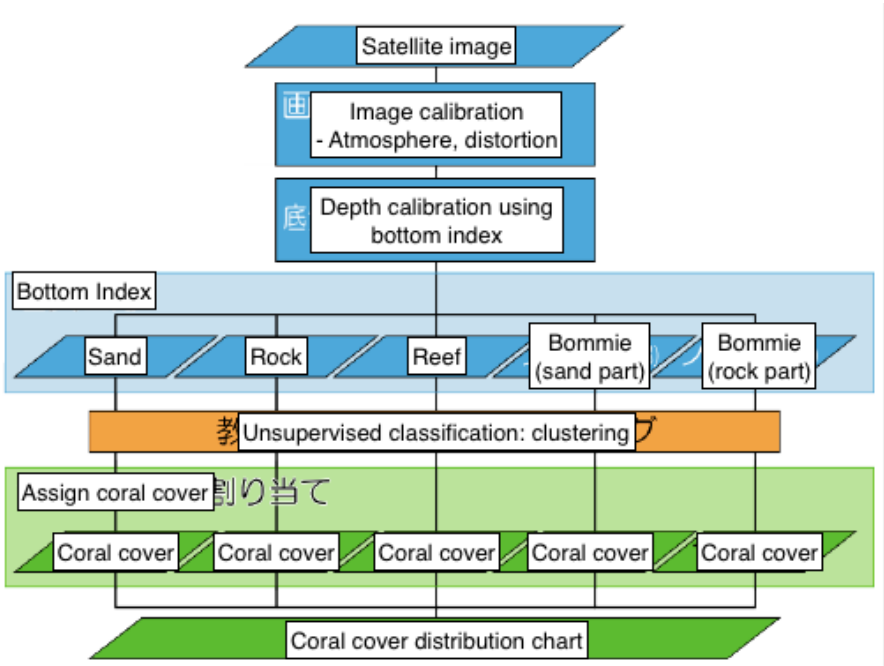


Figure III.2-2 Steps to analyse coral cover distribution

1. Image calibration

Satellite images require 'atmospheric correction' to reduce the influence from water evaporation and 'geometric correction' to calibrate for distortion.

Atmospheric correction can be done by 'Dark Object Subtraction', a method to remove atmospheric light scattering. Geometric correction removes the elevation point error and uses a control point (a structure that does not change over the years) to provide geographic coordinates.

[Points to consider for image calibration]
• When using Ground Control Point (GCP) for geometric correction, distribute GCP evenly across the target area.

2. Depth calibration using Bottom Index

Satellite images which include water bodies are always influenced by depth. To remove the depth effect, we use Bottom Index (Lyzenga 1978). This Bottom Index is based on the theory of "if the bottom composition is the same, the bottom scattering (reflection) ratio of two bands will be constant" (Luy et al 2008), and uses the digital number (DN) included in the satellite image data in the equation below:

$$BI_{ij} = LN(DN_i - DN_{si}) - kLN(DN_j - DN_{sj})$$

Where BI_{ij}: Bottom index from bands i and j; DN_i, DN_j: DN values of bands i and j; DN_{si}, DN_{sj}: DN values of deeper depths of bands i and j; k_{ij}: water dissipation coefficient

Water dissipation coefficient can be calculated from the slope of a regression line drawn between the bands' natural log of DN of pixels from the same bottom index (Ikema et al 2003). The dissipation coefficient can then be used against the reflection ratio between two band widths and derive the bottom index which calibrate for the water depth effect. If the analysis focuses on the shallow waters, some cases are better without the Bottom Index.

3. Benthic composition categorisation

Layer the bottom index chart and satellite image and categorise the image based on benthic composition categories. In the Okinotori Island example, the image of the reef lagoon was categorised under sand, rock, reef, bommie (sand), bommie (rock).

[Points to consider for benthic composition categorisation]

• It is ideal to conduct a field survey for benthic composition distribution at the target site beforehand so that the benthic composition categorisation will have better accuracy.

• The Bottom index calculations are based on Lyzenga (1978). This method uses two band widths*, which requires you to choose the right bands appropriate for the calculation. In this study, we used blue and green which are common.

• Sample points used to calculate the bottom index should be from the same benthic composition.

*Bands mean the bandwidths of the image and generally red, blue, and green are common.

4. Unsupervised classification of benthic composition category and assigning coral cover for each class

Run clustering (unsupervised classification), which is a method to automatically categorise sections of the image based on specific characteristics, on the images with benthic composition categorised. After the clustering, assign the coral cover to each class based on the field survey data, and create a coral cover distribution map.

In the Okinotori Island example, we used the images from 2006 which we also had coral cover survey data to create the coral cover distribution map. We also used satellite images from 2000, 2011, 2012, and 2017 after calibrating with artificial structure, and ran unsupervised classification to assign coral cover (based on the 2006 field data). Figure III.2-3 shows the coral cover distribution maps created with these steps. This method also allows analysis on coral cover change over time (Figure III.2-4; Katayama et al 2014).

Figure III.2-3 Coral cover distribution analysis results (example from Okinotori island)
(note: H followed by numbers are Japanese way of calling the years)

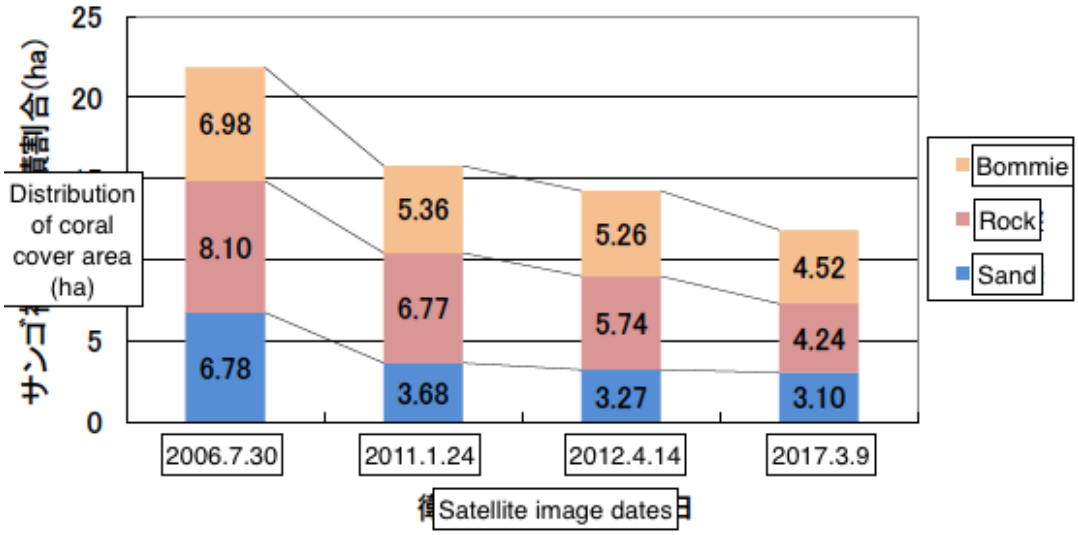


Figure III.2-4 Change in coral cover area of each benthic composition categories in Okinotori Island

2) Accuracy of satellite image analysis

The accuracy of satellite image analyses was tested using satellite image from 2012 and local field survey data from 2013-2014. The field survey for coral cover was conducted on the seven sites in Okinotori Island shown in Figure III.2-5. The accuracy test results of image categorisation are shown in Figure III.2-6, 2-7, 2-8 and Table III.2-2.

The accuracy of coral cover analysis results from satellite images against local field data was approximately ±3% RMSE, total precision was 80%, and Kappa coefficient was 0.7 (high level match). However, as shown in Figure III.2-7, where local field surveys marked 5% for coral cover, class "2.5-12.5%" was used as the automatic categorisation on satellite image which is a maximum 7.5% error. Therefore, care should be taken when interpreting the results.

Figure III.2-5 Local field survey sites used to test the accuracy of satellite image analysis

Figure III.2-6 Comparison between local field survey and satellite image analysis (local field survey: 2013, 2014; satellite image: 2012)
Left: local field survey (shown on satellite image), Right: Image analysis results (shown with local field survey line)

Figure III.2-7 Comparison between local coral cover data and image analysis results (satellite image from 2012, local surveys from 2013-2014)

Figure III.2-8 Error (RMSE) from image analysis results (satellite image from 2012, local surveys from 2013-2014)
(Overall RMSE = 3.3%)

• RMSE (root mean squared error) for analysis error check

RMSE (root mean squared error) is an accuracy index to show how much the image analysis coral cover is different from the true cover (local field survey) and can be calculated as below:

RMSE = $\sqrt{}$ (Σ (image analysis value – true value)^2 / number of data)

• Classification precision test (Kappa coefficient, total precision)

Classification precision test can statistically test the probability of the image analysis result. We used the Kappa coefficient which is commonly used in the remote sensing discipline.

Kappa coefficient: values between -1 and 1, the closer it is to 1, the better prediction, and anything less than 0 is considered a coincidental match. According to the Landis and Koch (1977) standard, 0.41-0.60 is moderate match, 0.61-0.80 is high level match, and 0.81-1 is almost perfect match.

Total precision: The ratio of target area precisely categorised under the satellite image (correctly categorised area / total area)

Table III.2-2 Classification precision test results (satellite image: 2012, local field survey: 2013-2014)

Total precision	Kappa coefficient
80%	0.7 (high level match)

3) Conditions of application of satellite image analysis

We now know that coral cover categorisation on satellite images can be automatically done at approximately 80% precision.

In addition, as shown in Table III.2-4, compared to visual observation methods by divers, satellite image analysis method becomes more cost-effective when the survey area is above 0.5km^2, and the greater the area, the cost difference becomes evident.

Here, we summarised the characteristics and precision of each method for coral cover surveys and when it is appropriate to choose which.

Table III.2-3 Comparison between satellite image coral cover distribution analysis and underwater observation

	Satellite image coral cover distribution analysis	Underwater observation
Survey area	A few km to 10s of km	A few hundred meters
Observation categories	Coral cover	Coral cover, species
Observation timing	If one time point allows to match the local survey data and satellite image, other time points do not need the local survey data	Only when observation is conducted

[Survey content to which satellite image coral cover distribution analysis can be applied]

• Understanding the trend of coral cover increase/decrease (coral cover, area)

• Understanding coral distribution and its change

[Survey sea area and scale]

• A few km to 10s of km; effective for wide scale coral distribution surveys. Also effective for understanding coral cover change as long as you can acquire satellite images from previous years.

[Note]

• When choosing and obtaining satellite images, be aware of the weather and climate (clouds, halation, white waves etc.).

• When using Ground Control Point (GCP) for geometric correction, distribute GCP evenly across the target area.

• It is ideal to conduct a field survey for benthic composition distribution at the target site beforehand so that the benthic composition categorisation will have better accuracy.

• The Bottom index calculations are based on Lyzenga (1978). This method uses two band widths*, which requires you to choose the right bands appropriate for the calculation. (In this study, we used blue and green.)

• Sample points used to calculate the bottom index should be from the same benthic composition.

• The precision of coral cover classification using image classification with satellite image is about 80%. Keep in mind that the results contain errors. In addition, understanding species composition is almost impossible, and therefore underwater surveys should be used as needed.

Table III.2-4 Cost comparisons according to survey area sizes between satellite image coral cover distribution analysis and underwater observations

		Survey area	(km^2)	0.1	0.5	1	10	100
		Length (example)		0.5 x 0.2km	0.5 x 1km	1 x 1km	2.5 x 4km	10 x 10km
Diver observation and classification	**Local surveys**	**Survey length**	(km)	0.5	2	3	24	210
		Survey time	(day)	2.2	8.7	13.0	104.3	913.0
		Boat hire	(day)	3	9	14	105	914
		Cost	(yen)	455,000	1,625,000	2,470,000	19,305,000	168,610,000
	Organise results	**Labour time**	(day)	1	4	6	48	420
		Cost	(yen)	30,300	121,200	181,800	1,454,400	12,726,000
	Total cost		(yen)	**485,300**	**1,746,200**	**2,651,800**	**20,759,400**	**181,336,000**
Satellite image coral cover distribution analysis	**Local surveys**	**Survey time**	(km)	0.5	1	1	2	5
			(day)	2.2	4.3	4.3	8.7	21.7
		Boat hire	(day)	3	5	5	9	22
		Cost	(yen)	455,000	845,000	845,000	1,625,000	4,030,000
	Organise results	**Labour time**	(day)	1	2	2	4	10
		Cost	(yen)	30,300	60,600	60,600	121,200	303,000
	Image purchasing	**Cost**	(yen)	87,500	87,500	87,500	87,500	350,000
	Calibration & Analysis	**Labour time**	(day)	4	4.5	6	8	15
		PC hours	(day)	4	5	6	8	15
		Cost	(yen)	126,400	142,200	189,600	252,800	474,000
	Total cost		**(yen)**	**699,200**	**1,135,300**	**1,182,700**	**2,086,500**	**5,157,000**

Notes:

- Diver visual observation hours are based on 230 m standard takes 6 hours
- Local survey cost = 3 staffs (2 divers, 1 boat person). Diver = 46,400 yen/day (Minister of Land, Infrastructure, Transport and Tourism base price; depth<10m), Boat person = 26,800 yen/day (Tokyo prefecture public labour base cost), boat hire = 65,000 yen/day. Boat is assuming a research boat (FRP D model 70PS, 3.0t, 51kW), diesel A 52.5yen/L
- Organisation of results are assuming skilled labour base cost 'C' = 30,000 yen/day
- Local surveys for satellite image coral cover distribution analysis is for supervised classification data and test data acquisition, the total length assumes 1 transect x100m.
- Satellite image assumes 1 scene of high resolution (0.5m; 4 bandwidths). Minimum 25km^2 area purchase which is 87,500 yen, and 3,500 yen for every additional 1km^2.
- PC hours assumes software license use of 7,200 yen/day.
- All cost does not include transportation and accommodation.
- All cost is only the base cost and any other miscellaneous cost is not included.

Technical note 3: Method for automated coral identification using underwater video clips

1) Survey and analysis steps

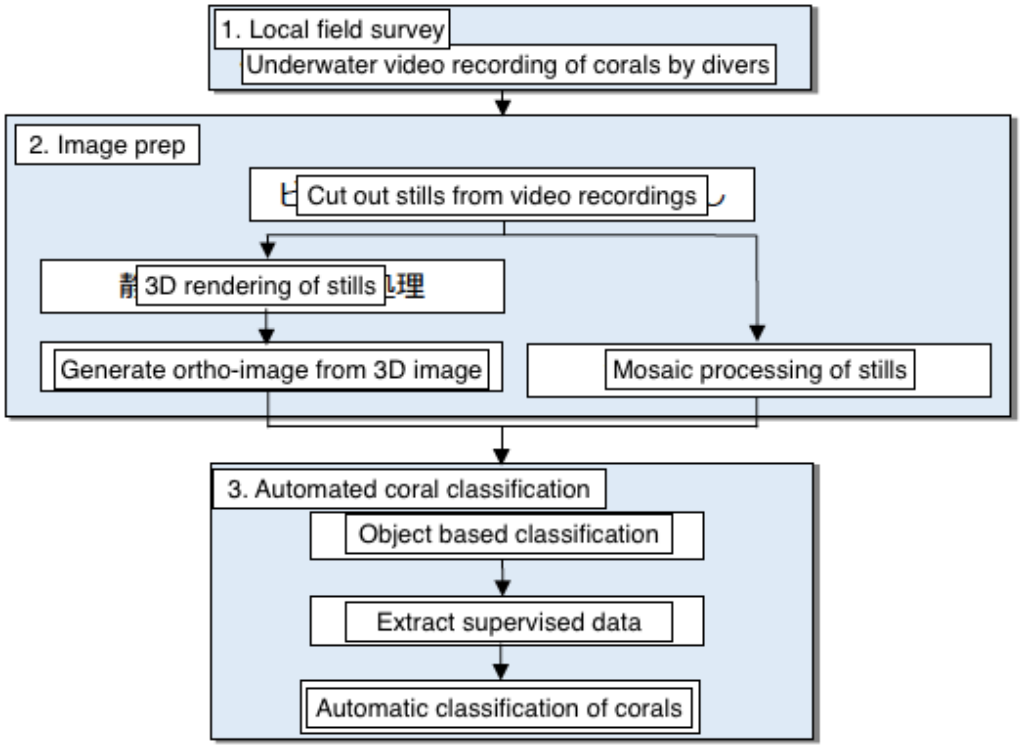


Figure III.3-1 Flowchart of survey and analysis

(1) Local field survey

Figure III.3-2 Underwater photography example route taken by a diver

(2) Image preparations

Figure III.3-3 Example of mosaic processed image

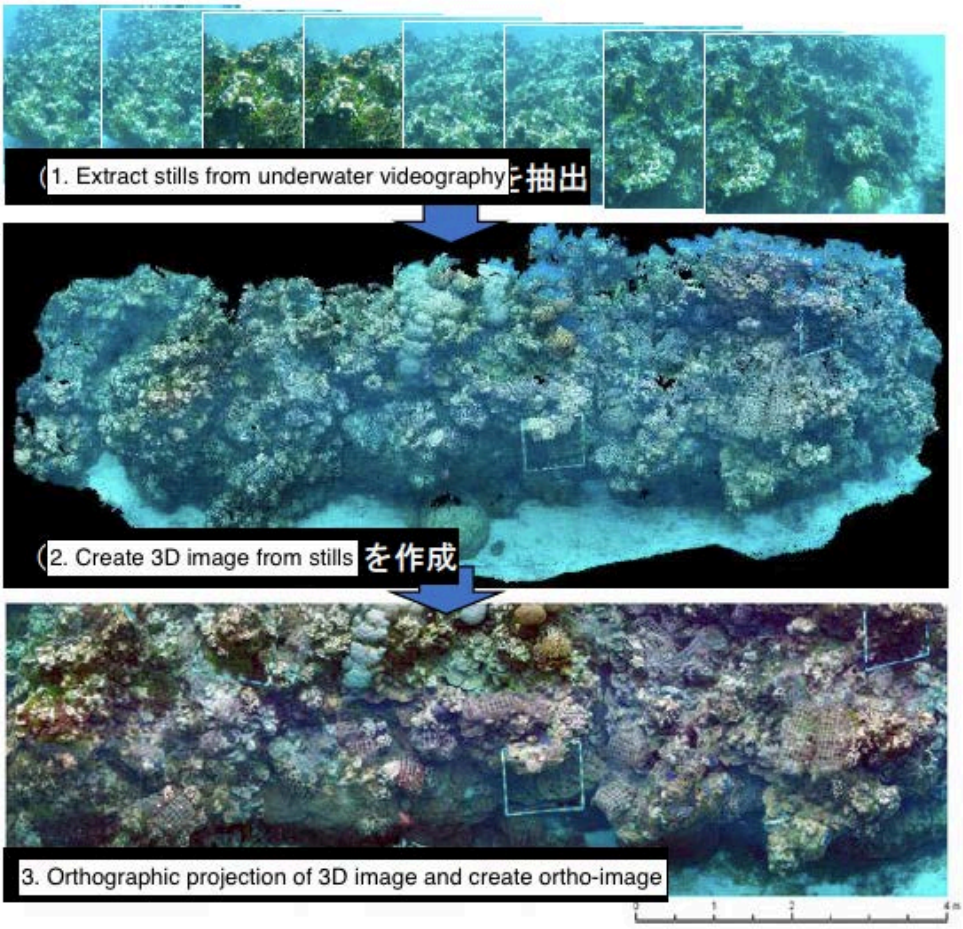


Figure III.3-4 Example of ortho-image creation

(3) Automatic coral classification

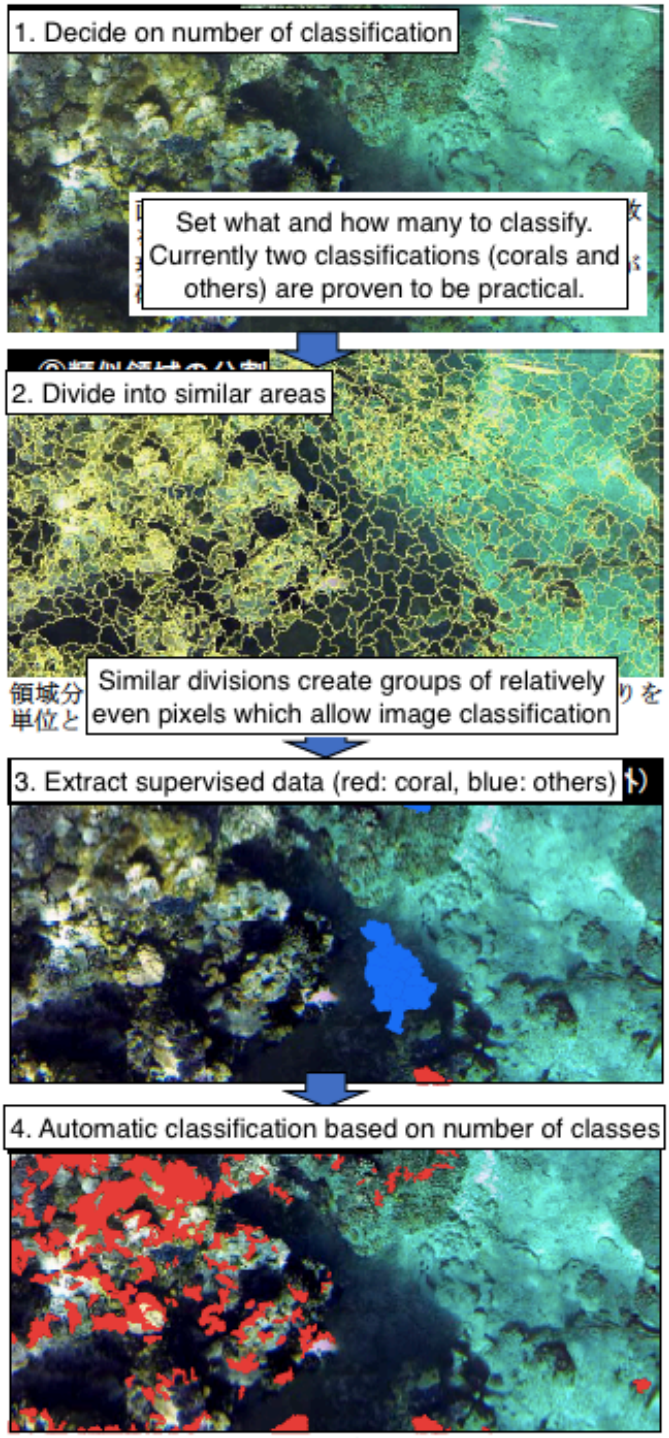


Figure III.3-5 Steps for automatic coral classification

2) Precision of automatic coral classification method using underwater videography images

Table III.3-1 Precision testing results (to improve precision)

Table III.3-2 Precision testing results (when images are analysed using supervised data from other sites)

Figure III.3-6 Changes in automatic classification precision of two coral groups over time

3) Application conditions of automatic coral classification method using underwater videography images

Table III.3-3 Cost comparisons according to survey area sizes between underwater video recording classification and underwater observation by divers

Larvae collection equipment

1) Overview

Larvae collection equipment can gather over 1 million coral eggs per 1 m^2 and maintain larvae at over 90% survival rate until they are ready to settle. With this equipment, the entire process of nursery stock production can be conducted within the same sea area (refer to Chapter 2 Section 3. Whole-area propagation techniques by nursery stock production using larvae collection equipment).

The main body of the equipment is cylinder shaped made of nylon net. If the target parental coral colony is on a substrate, you would go with the sea floor fixed type, whereas if there is no substrate available or there is too much depth, you can use a floating type which sets the parental colony inside the equipment bottom. Table III.4-1 summarises the characteristics of both types.

Table III.4-1 Characteristics of sea floor fixed- and floating- larvae collection equipment

	Sea floor fixed type	**Floating type**
Pros	• Stable against wave and current because it is fixed on the sea floor • Can target a parental colony that is attached to a substrate	• An improvement from the sea floor fixed type • Depth is not an issue when installing • No need to consider tidal change and submergence is not a concern
Cons	• If fixing to a deeper sea floor, the cylinder must be longer • Tidal change needs to be considered so as not to let the equipment entirely submerge	• Less stable against wave action and current • The equipment must be altered to enable parental colony to sit at the bottom

Figure III.4-1 Larvae collection equipment in use (left: sea floor fixed, right: floating)

2) Materials and costs

Table III.4-2 shows the materials required for the sea floor fixed type larvae collection equipment and Table III.4-3 for their costs.

Table III.4-2 Materials for larvae collection equipment (sea floor fixed type)

Materials	**Details**
<Equipment>	
Main body	• Body: Nylon mesh (mesh size 30μm) ϕ1.7m x 3.8m • 6 sections of body: Eye mesh (mesh size 20-30mm) • Bottom + bottom surrounds: Canvas • Ceiling + Bottom: Zipper
<External frame>	
Reinforced plastic composite pipe (F-14)	• External frame: (Outer diameter, inner diameter = ϕ14mm, ϕ4mm x 1680mm) x 4 pipes x 6 sets • For raising ceiling: (Outer diameter, inner diameter = ϕ14mm, ϕ4mm x 260mm) x 4 pipes
Vinyl nitrate pipe (VP13)	• Bottom frame: (Outer diameter, inner diameter = ϕ18mm, ϕ13mm x 1300mm) x 4 pipes • Connector sockets x 4 *connect 4 in a circle
Reinforced plastic composite pipe – 3 mouth elbow	• For connecting external frame: (Inner diameter = ϕ14mm) x 8
Reinforced plastic composite pipe – 2 mouth elbow	• For connecting external frame: (Inner diameter = ϕ14mm) x 16
Buoy (float)	• For connecting external frame (water surface): (EVA float SHE-40: buoyancy 4kg) x 16 • For hanging settlement equipment: (EVA float SHE-40: buoyancy 4kg) x as required
<Fixing component>	
Screws	• For connecting external frame and elbows: (3.5mm x 12mm) x 56
Cable ties	• For connecting external frame and main body: (4.8mm width x 300mm) = approx. 100 • Vertical guide bar: (4.8mm width x 300mm): 5 x 4 sets = 20
Rope	• For folding equipment: (ϕ5mm) 3m x 4 ropes = 12m • For securing equipment: (ϕ10mm) 5m x 4 ropes = 20m

	• For hanging settlement equipment: (ϕ8mm) 2-4m (number of ropes as required)
Steel bars	• For securing bottom: (4sets / ϕ12mm x 1m): 8 bars (cross two and hammer in)
Small marker lamp	• Sea light CH-2000B LED automatic nighttime flash, waterproof: 1

Table III.4-3 Costs for larvae collection equipment (sea floor fixed type)

Materials	Cost (yen)
<Equipment>	
Main body (net)	350,000
<External frame>	
Reinforced plastic composite pipe (1680mm cuts)	24,000 (1,000 x 24 pipes)
Buoy (float: buoyancy 4kg)	64,000 – 144,000 (For securing: 4,000 x 16 + For dangling: 4,000 x 10-20)
Others (connectors, vinyl nitrate pipes)	4,000
<Fixing component>	
Rope	10,000 – 20,000 (ϕ5mm x 12m, ϕ10mm x 20m, ϕ8mm x 40-80m)
Small marker lamp	3,000
Others (cable ties, steel bars)	5,000
Total	**460,000 – 550,000**

3) Blueprint

Figure III.4-2 shows the larvae collection equipment blueprints for both sea floor fixed type and floating type.

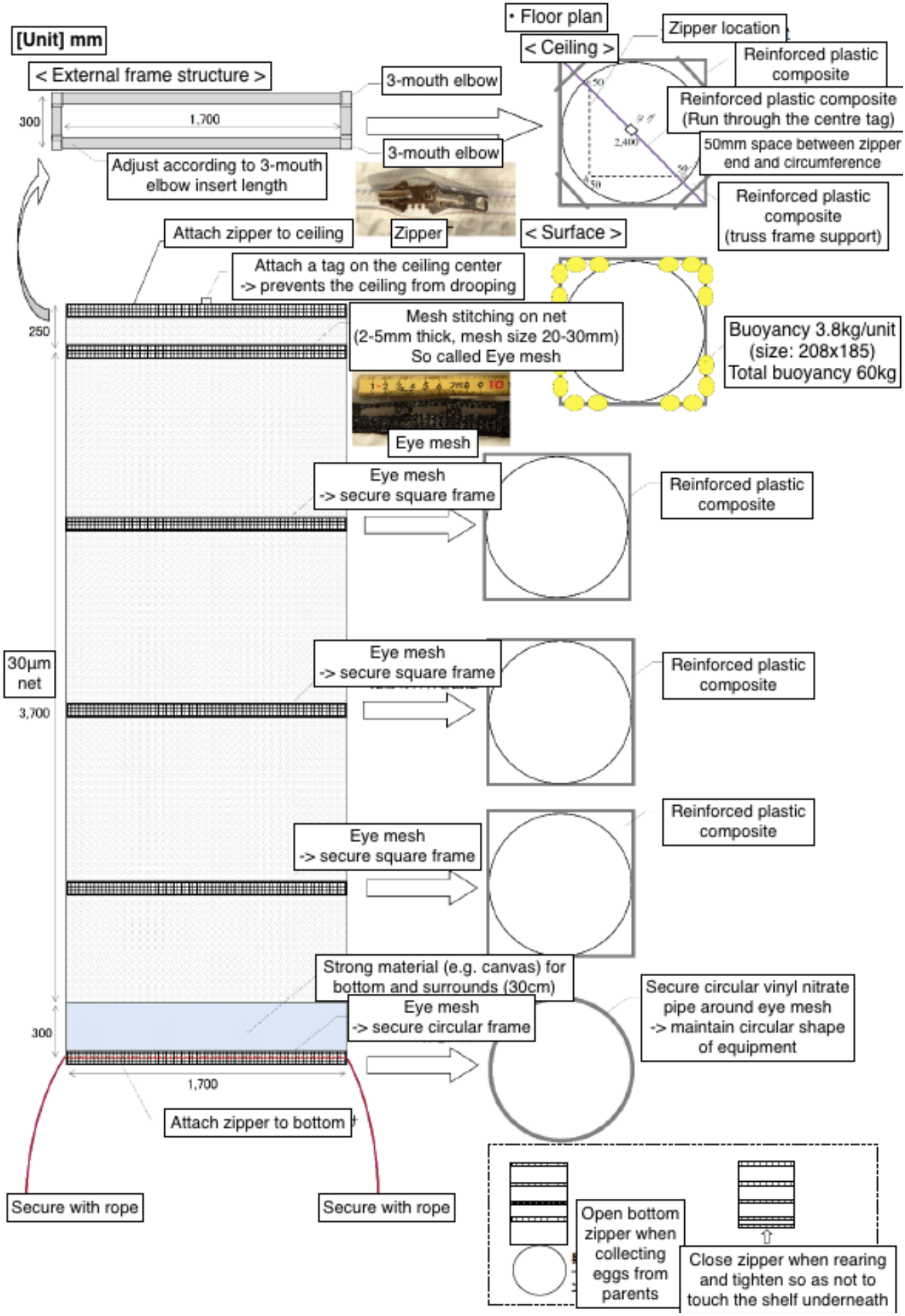


Figure III.4-2 Blueprint of larvae collection equipment (sea floor fixed type)

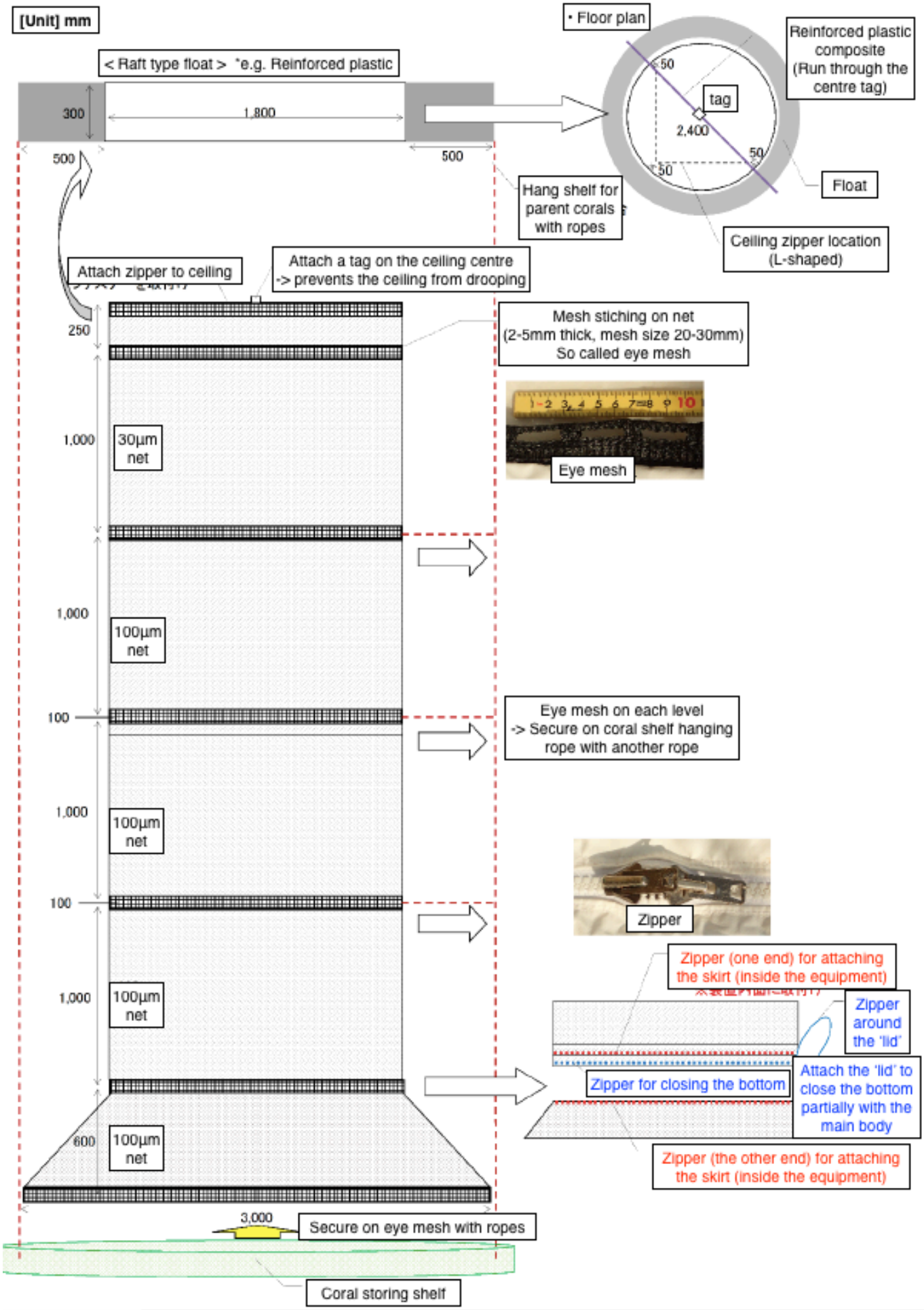


Figure III.4-3 Blueprint of larvae collection equipment (floating type)

Paperwork for field work

1) For surveys in shipping channel and surrounding sea areas

Figure III.5-1 Permit application format

2) For surveys outside of shipping channel and surrounding sea areas

3) For surveys within fishing right areas

4) Laws regarding surveys (excerpt)

5) Special harvest permit
(1) Overview
(2) How to apply
(3) Regulations and laws regarding special harvest (excerpt)