Site selection criteria, sample collection and analysis

Joe K Kizhakudan, Shoba Joe Kizhakudan, Kaladharan P, Thirumalaisevan S, Poovannan P, Mohan S and Sitaramacharyulu V.

The selection of suitable sites for the deployment of artificial reefs is a very important phase in the success of the reef functioning and efficiency. Following the primary and secondary stakeholders' meetings, on the proposed dates of field sampling, the team of experts with the SCUBA team, ARSC members and active fishers with GPS sail on the pre-identified country boats or vessels, carrying all the sampling gears and anchors. They sail towards locations suggested by the fishermen, at distances of 2-5 km from the shore with a water depth of 7-25 m. The tidal parameters, amplitude, wave heights, wind speeds and local weather details are noted before sailing. A Global Positioning System (GPS) and sonar can be used along with scuba gear to locate the reef sites. The coordinates of the sampling sites are duly recorded separately by the fisher ARSC team members and the sampling team and corroborated to fix the sampling site.

The sites for artificial reefs should be installed in coastal waters adjacent to fishing villages where non-destructive gears suitable for fishing in reefs, like hooks and lines are available and regularly used. It is necessary to ensure that the fisher stakeholders are involved in the sampling and site-fixing process. The sampling locations must be located at a suitable distance from outfalls, barmouths, river discharge points, mangroves, mud flats, mud banks, coral reefs, seagrass and seaweed beds, industrial installations, and industry/urban effluent discharge points. Sites proximal to thermal, saline, and chemical effluent discharge points are to be strictly avoided. No-take zones such as MPAs/Sanctuary/National parks/NHS are to be avoided unless specified for the conservation program. Locations with hard and sandy sediments are preferred while locations with soft sediments and turbid eddies are to be avoided. The site should fall within MFRA limits of the respective state allotted for the traditional sector and should not be in violation of the Coastal Admiralty or CZM rules, and should not be a priority area for fishing by other fishers or gears; it should be located within the geographical grid and limits of the village and not conflict with adjacent fishing zones of other villages. Any natural conflicts/reasons that may interfere with selecting the site for artificial deployment must be addressed prior to sampling and site fixing. A local fishery- and ground-based resource availability is ascertained through questionnaire surveys for benchmark analysis.

The notable parameters of utmost relevance to the performance of the AR are -

A. Depth and distance from the shoreline
B. Turbidity/visibility/productivity
C. Sea bed texture and nature/sediment characteristics

D. Current speeds and upwelling
E. Vicinity to barmouths/discharges /installations
F. Proximity to natural reefs and habitats/mud flats /mangroves
G. Proximity to AR and fish corridors

A. Depth: The water depth is measured using scuba diving computer and/or using the boat's depth finder; alternatively, a deadweight lead sinker tied to a marked rope can also be lowered for depth soundings. The preferred zone for the performance of production-based reefs is beyond the frequent surf zone and between **7-25** m depth range, while the perfect one would be in a **10-20** m zone, as this leaves enough space for the light penetration, causing less obstruction to drift gillnets, and is sufficiently away from the frequent surf beaten zone, depending on the sea bed slopes. The distance of the site could be anywhere within the MFRA approved limits of each coastal state, allowing for the traditional fishers while keeping in mind that the visibility and activity within sight range from the shore should make it more convenient for management and safety; hence ideally, the site should be at a distance of 2-5 km from the shoreline. The distance may vary depending on the depth profile of the sea in the village. For deployment in shallow sites, sufficient surface clearance is to be left for vessels and boats to navigate without obstruction.

Table 4. The fishing limits of traditional fishermen in coastal states of India (distance from LLT /depth)

State	Artisanal	Mechanized	AR deployment zone
Maharashtra	10-20 m depth	>20 m depth	within 20 m depth
Goa	<5 km	>5 km	<5 km
Orissa	<5 km	>5 km	>5 km
Karnataka	<6 km	OAL<15 m	>6 km
		OAL>15m	>20 km
Kerala	<10 km	GRT<25	>10 km
		GRT>25	>23 km
TN	<5 km	>5 km	>5 km
AP	<10 km	OAL <20 m	>10 km
		OAL >20 m	>23 km

B. Turbidity/visibility/productivity: The 8" Secchi disc readings should be sufficiently more than 1.5 meters and the observed turbidity should not be related to silt or clay suspension indicating heavy current speeds or bottom swells. However, reduced light penetration due

to increased plankton production or reduced sunlight should be negotiable based on the local enquiry on the general trend in that zone.

C. **Sea bed texture and nature/sediment characteristics**: The composition of the ocean bottom is an important factor that could affect the length of time a reef will remain productive. If the material sinks into the sediments or is covered by them, the reef loses its effectiveness. Information about the bottom type and depth can be obtained with bottom sampling equipment like grabs, direct diver inspection, depth recorder, sounding lead, information from national ocean survey charts, state fisheries departments, game agencies, local colleges and universities with marine science programs, commercial fishermen, or oil company geologists. Natural reefs and rock patches should be completely avoided as they are natural ecosystems which support a unique ecosystem. Muddy bottoms experience shifting of sediments, hence sinking of structures and increased sediment deposit over the surfaces cause choking of invertebrate settlers. Plain hard sea bed floors are ideal for module deployments and longer life of AR sites.

D. **Current speeds and upwelling (Dutchman's log):** Another important instrument used can be a float buoy in the size of an orange along with a digital watch to measure current speed. It could be released to measure the distance it travels in a minute. This is done three times at each site to minimize error (0.8 x float velocity). This is an easy way to determine the average surface current at each site. Temperature is measured in two ways, either by an underwater thermometer attached to a sampler or by the dive boat's computerized sensor. All readings, surface to bottom, are taken at the same position and time. A current meter will be more accurate.

E. **Vicinity to barmouths/discharges /installations**: The AR sites should ideally be away from any barmouth or discharge point by at least 3 km on either side and should not be in a zone of influence of increased sedimentation rates or sinking. Any industrial or infrastructural installation also should be avoided due to other legal issues. Navigational and admiralty routes, and shipping and tug channels are to be strictly avoided.

F. **Proximity to natural reefs and habitats/mud flats /mangroves**: The experience so far in our waters shows that if the site is at a distance of 500 m from any natural reef or rock patch, the performance is very good and fish corridors are instantly built. However, if they are adjacent to mud flats and mangroves the logic of keeping a 3 km distance on either side is safer, to avoid soft sediment settlements and increased turbidity. Using underwater videotapes and photographs of the sea bed, the fouling coverage and diversity are estimated in the lab, using a monitor.

G. **Proximity to AR and fish corridors**: Keep a distance of 500 m and develop a subsequent unit, as resident and settling communities spread well and extend to a 300 m plus boundary. The extended satellite corridors help in increasing mobility, forage and shelter and escapement routes.

Table 5. Optimal parameters for selection of suitable sites for artificial reefs.

No.	Parameter	Range	Optimal	Remark
1	Depth (m)	7-25m	10-20m	Depending on the site and availability
2	Transparency (m)	1-5m	>1.5m	Effluent discharge points and loose sediments could be giving turbidity.
3	Current velocity	1-10cm/s	2-6cm/s	Bottom currents, particularly at estuary or river flow points, it will exceed
4	Wave heights	0.5-4m	0.5-2m	During monsoon could be the max
5	Soil texture sand: silt + clay	85-99: 15-1%	98: 2	Fine sand and organic sediments to be carefully identified and quantified
6	Proximity to barmouths, discharges outfalls	Away by >3 -5km	Away by >5	Avoid pollution, sedimentation and sinking, plastic debris accumulation
7	MPA/Coral reefs	500-1000 m away	At least 500 m away	Avoids conflicts and violations
8	Proximity to AR /natural reefs	300-500 m distance	500 m from the AR	Helps in creating fish corridors and reduces exits and transit losses
9	Dissolved orthophosphate (PO_4-p)	ideal range: 1-3 micro mols /l	2-3 micro mols /l	Indicates the nutrient wellness of the site for primary production
10.	Reactive silicate (SiO_4-Si)	4-8 micro mols /l	4-6 micro mols /l	Indicates the nutrient wellness of the site for primary production
11	Nitrate (NO_3-N)	1-5 micro	1-3 micro	Indicates the nutrient wellness

		mols /l	mols /l	of the site for primary production
12	Chlorophyll a	Chl a. 1-4mg/m^3	1-3mg/m^3	Indication primary production levels
13	DO	1.5-5 mg /l	2-4mg /l	Anoxia prevails during upwelling

Water and plankton sample collection

A portable GPS set and lead weight (200-500gm) depth sounder with a rope having measured markings are carried on board. The GPS coordinates and reading units of the fisher members are confirmed with the gadgets available with the team. The boat/vessel/canoe is anchored at the site and then the depth sounding is done to confirm the depth and the sediment nature. The SCUBA team then dives with the sampling equipment and containers (the sediment scoop, two wide-mouthed 1000 ml PPE water containers, camera, torch, and high-density polythene sealing type bags of 2 kg capacity). They take photos and videos of the sediment, habitat and fauna and also provide information on the topography and mound formation of the sea bed and slope. They collect bottom water and sediment samples.

Once the diving team comes back on board, the surface water and plankton sampling are done. Both the surface and bottom samples are to be collected to verify the site-specific parameters. Water samples collected for nutrient and chemical parameters are stored in PE containers and refrigerated till further analysis. Dissolved oxygen (DO) is sampled in BOD glass bottles (Winkler method) and fixed using the Winkler's solution on-site and analyzed in the lab. A portable field thermometer is used for temperature, a salinometer for salinity and a pH meter for pH. The sediments collected for benthos and meiobenthos are preserved in 10% formalin mixed with Rose Bengal stain.

Sample analysis

Zooplankton: A zooplankton net of 50 cm diameter x 3 m length with 40 µ mesh in the main area of the net and with 150 µ at the collars, and with a collection cup secured to a 500 g lead weight is lowered at the site. Three samples are collected from each site by a still net at natural flow in the current direction at the bottom, for an hour, followed by horizontal tow in the mid-water column and surface by driving the boat for 10 minutes each, noting the flowmeter readings**.** The bottom anchored collection gives a good account of the fish eggs, larvae, and fry at the sea bottom. Samples are filtered and preserved in 10% formalin with Rose Bengal stain in a 250 ml PP bottle. The net volume is calculated by the volume displacement method and sub-samples made through a Folsom splitter are used for quantitative and qualitative analysis under

a microscope. Larger plankters would be counted and for the rest, a subsample taken on the Sedgwick Rafter cell would be observed under a microscope for identification and count. The total number would then be estimated. Species diversity indices, Margalef's Species richness (d) Pielou's Evenness (J'), Shannon Weiner Diversity (H') and Simpsons Dominance Index can be calculated using the PRIMER-E software. The total numbers are represented as numbers /10 m^3.

Phytoplankton: Bottom and surface samples are collected using a phytoplankton sampler which has 5 µ mesh size net and 30 cm diameter. Horizontal tow for 10 minutes with flow meter readings is done for the collection. The samples are preserved in 5% formalin with 0.1% Lugol's Iodine. The subsamples are observed under a microscope and the counts are taken later using a haemocytometer counting chamber. The total number would then be estimated. Species diversity indices, Margalef's Species richness (d) Pielou's Evenness (J'), Shannon Weiner Diversity (H') and Simpsons Dominance Index can be calculated using the PRIMER-E software.

Dissolved nutrients:

1. **Dissolved orthophosphate (PO_4-p)** is determined quantitatively by the spectrophotometric method (885 nm) using the Ascorbic acid method (Murphy and Riley, 1962). Merck Spectroquant method with instant reagent kits is more convenient and accurate (ideal range: 1-3 micro mols /l).
2. **Reactive silicate (SiO_4-Si)** is determined in seawater in the dissolved form mainly as orthosilicic acid $Si(OH)_4$ estimated by the Ascorbic acid method using a spectrophotometer at 810 nm as given by Mullin and Riley (1955) and modified by Strickland and Parson (1968). Merck Spectroquant method with instant reagent kits is more convenient and accurate (ideal range: 4-8 micro mols /l).
3. **Nitrate (NO_3-N)** is estimated following the method described by Morris and Riley (1963) and modified by Grasshoff and Wood *et al*. (1967) using a spectrophotometer. Merck Spectroquant method with instant reagent kits is more convenient and accurate (ideal range: 1-3 micro mols /l).
4. **Chlorophyll a**, **b, and c** are estimated from at least 1000 ml water samples filtered first through a 0.2 mm filter to remove all the particles, and then filtered through a 47 mm Whatman No 1 GF/C filter paper. The pigments are extracted from the paper by adding 90% v/v acetone 10 ml in a tube. The resultant pigments are calculated from UV spectrophotometer readings at 750, 664, 647 and 630 nm by applying the formula given by Gaarder and Gran (1927) (ideal range: Chl a. 1-3mg/m^3).
5. **Primary Productivity**: is estimated by light and dark bottle method. The changes in dissolved oxygen levels in the bottles after a suitable time are expressed in g C/unit vol/h (Winkler method).

6. **Total Suspended Solids (TSS)** are calculated by filtering a known volume (500-100 ml) of the sample using a vacuum pump with 47 mm GF/C paper and drying the residue; the dry weight gives an estimate of the TSS.
7. **Total Dissolved Solids (TDS)** are estimated by evaporating a known volume of the filtrate obtained during TDS extraction, in a crucible kept in an oven; the resulting residue gives an estimate of the total dissolved organic and inorganic matter in the sample.

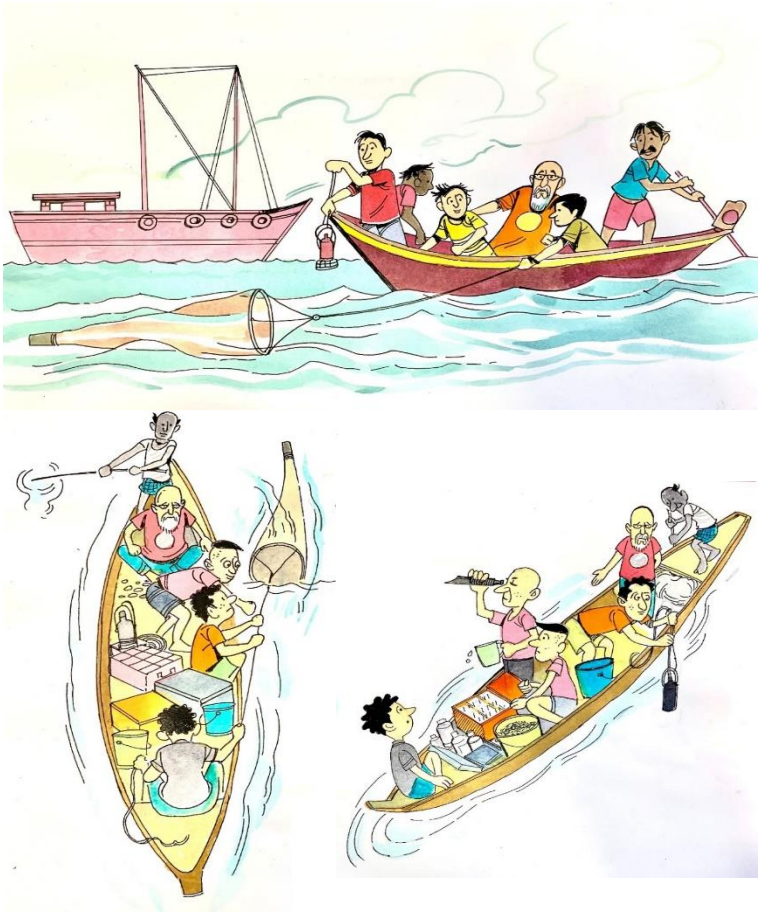
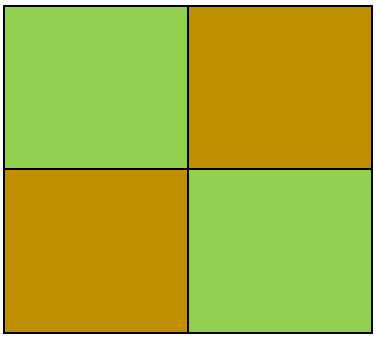


Fig.23. Illustration of water and sediment sampling for site selection

Sediment studies, benthos, and sampling procedures

The samples are to be collected at least from two random sites for the sediment textural classification and the benthos and meiobenthic status observations. The samples can be obtained using a Van-veen grab or any similar equipment or collected through scuba divers. A quadrant of 20 x 20 cm is usually taken for the divers' collection methodology and the top 2" layer of soil is collected into a high-density sealing type polyethene bags (2 Kg).



A 100 m x 100 m area is marked where the site is identified and fixed the sediment samples are to be taken and the samples for the textural analysis are to be mixed between two diagonally opposite station samples (brown)and then prepared for drying and sieving, while the samples for benthos and meiobenthos are treated separately with formalin and sieved and samples collected and then the average numerical abundance is computed (green).

The samples are taken back to the lab, dried and then passed through a series of five nested sieves. From this, the relative amounts of different particle sizes are determined by weight. The particle types and percentages are then determined and labelled according to the Wentworth classification. The collected bottom samples are dried and sieved through a series of five nested test sieves to separate the different grain sizes and each size class is weighed.

Table 6. Soil classification based on grain size.

Particle Size Class	Grain Size (mm)
Gravel	> 2.0
Very coarse sand	> 1.0 < 2.0
Coarse sand	> 0.5 < 1.0
Medium sand	> 0.25 < 0.5
Fine sand	> 0.125 < 0.25
Very fine sand	> 0.0625 < 0.125
Silt/clay	< 0.0625

The composition of the ocean bottom is an important factor that could affect the length of time a reef will remain productive. If the material sinks into the sediments or is covered by them, the reef loses its effectiveness. Information about the bottom type and depth can be obtained with bottom sampling equipment grabs or direct diver inspection, depth recorder, or sounding lead, information from National Ocean Survey charts, State fisheries dept, local colleges and universities with marine science programs, commercial fishermen, or oil company geologists.

Natural reef and rock patches should be completely avoided as they are natural systems which support a unique ecosystem.

Collecting representative samples in a marine area requires prior knowledge about the sea bed. Initially, some guidance can be obtained from bathymetric maps, knowledge of tidal currents, and information about the likely exposure to high-energy current forces such as waves from major storms. In areas with heavy use of bottom trawls for fishing. A great deal of information about bottom morphology and regional patterns of sediment texture can be obtained by using bottom imaging techniques such as side scan sonar also.

The wet samples collected are immediately preserved in a 10% aqueous solution of borax-buffered formalin mixed with Rose Bengal stain and brought to the lab and in a week's time sorted for the microbenthic fauna and later for the meiobenthos. They will remain in this solution for a minimum of 24 hours and a maximum of 7 days to allow proper fixation of the animal tissue while minimizing the loss of calcium carbonate structures (e.g., molluscan shells, echinoderm spicules). All sample-processing activities (including rescreening and sorting)

Sample Sorting: Sorting is the process of removing all faunal material from the sediment sample. All whole macroinfaunal invertebrates and fragments of organisms that were alive at the time of preservation are to be removed from the sample and sorted into the following taxonomic groups: Annelida, Arthropoda, Mollusca, Echinodermata, and miscellaneous phyla (counted separately).

Meiofaunal organisms such as nematodes and foraminifera will not be removed from the sample. Colonial organisms such as hydrozoans, sponges, and bryozoans will be removed completely from the sample. This includes all colony fragments and all parts of colonies attached to hard surfaces such as worm tubes, shells, or rocks (the substrate may be included in the vial with the organisms). Organisms will be stored in vials or jars containing 70% ethanol.

The sorting process is accomplished as follows: Identification and enumeration of sorted organisms will be performed to the lowest taxonomic level possible, usually to species-level. The identifications will be done by in-house taxonomists, using minimum 10X magnification dissecting light microscopes and compound light microscopes equipped with 10X, 20X, 40X, 63X, and 100X magnification objective lenses. Organisms should be sorted into the major phyla: Annelida, Arthropoda, Mollusca, Echinodermata, and miscellaneous phyla. All organisms will be sorted into vials containing 70% ethanol and tightly sealed with polyseal caps. The total number would then be estimated. Species diversity indices, Margalef's Species richness (d) Pielou's

Evenness (J'), Shannon Weiner Diversity (H') and Simpsons Dominance Index can be calculated using the PRIMER Vers(5).The total numbers are represented as numbers /M3

Analysis of sediment texture at artificial reef sites before and after deployment indicated varying patterns at each site with a tendency for an increase in coarser sediments at the sites, after deployment of artificial reefs indicating more sediment porosity, molluscan and crustacean fauna and hence more shell grits and carbon and calcium deposits. The macrobenthos in the sediment increases by 10-fold in numbers over the sediment from a non-reef area. Annual patterns in phytoplankton, zooplankton and benthos composition indicated higher species diversity and density in artificial reef sites, compared to adjacent non-reef sites. The diversity indices observations indicate higher values for richness in the reef sites amongst the phytoplankton, Zooplankton, meiobenthos and macrobenthos and fish fauna (Species Richness, Pielou's Species Evenness and Shannon-Weiner Diversity Index). The rich nutrient profile gives rise to developing diatoms and microalgal populations and in turn supports the recruiting larval forms of shrimps, oysters, mussels, clams, crabs, fish larvae and echinoderm larvae and the filter-feeding organisms. The composition at all reef sites remained fluctuating between similar groups indicating uniform performance in terms of the reef output in Tamil Nadu (Kizhakudan, 2019).

Fig. 24. Benthos

Fig: 25. An artist's impression on the productivity channels -sediment benthos, zooplankters and the periphyton and plankton from the artificial reef habitat.

Fig. 26. Processing of sediment sample and microscopic analysis of benthos in a sediment sample

Fig. 27. Microscopic view of benthos in a sediment sample

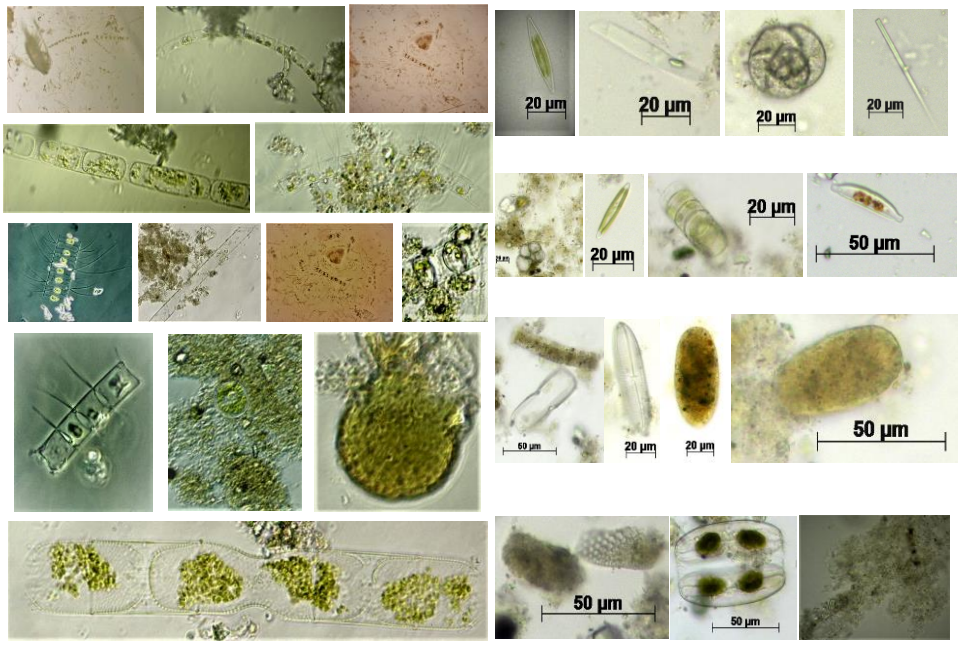


Fig. 28. Microscopic view of the periphytons and immediate phytoplankters around the reef modules after incubation

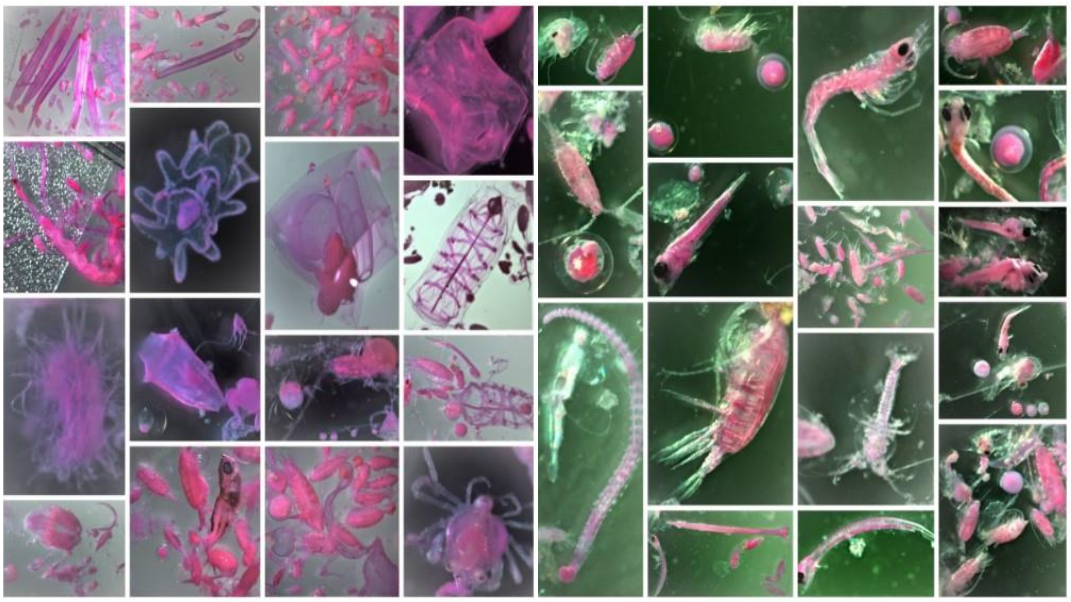


Fig. 29. Zooplankters surrounding the reef waters in the benthic realm.

Fig.30. Benthic engineers and recyclers of the reef sediment habitats.

Fig. 31. Microbenthic forms which add on to the coarser sediment and shelled forms

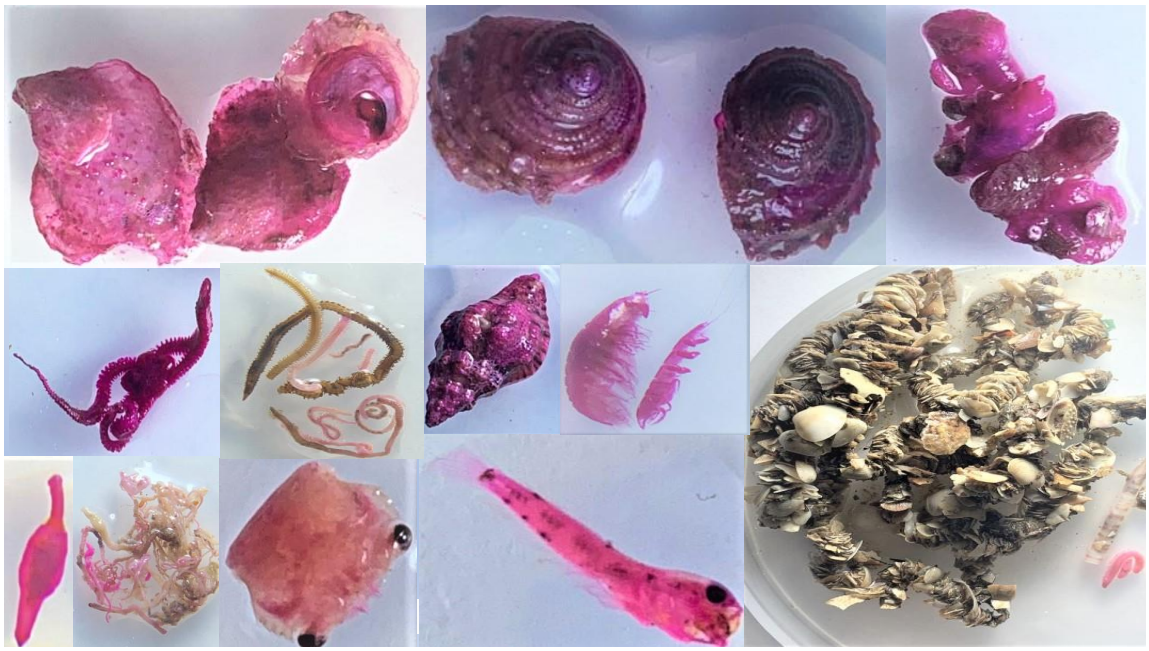


Fig. 32. Macrobenthic forms which are large-level converters

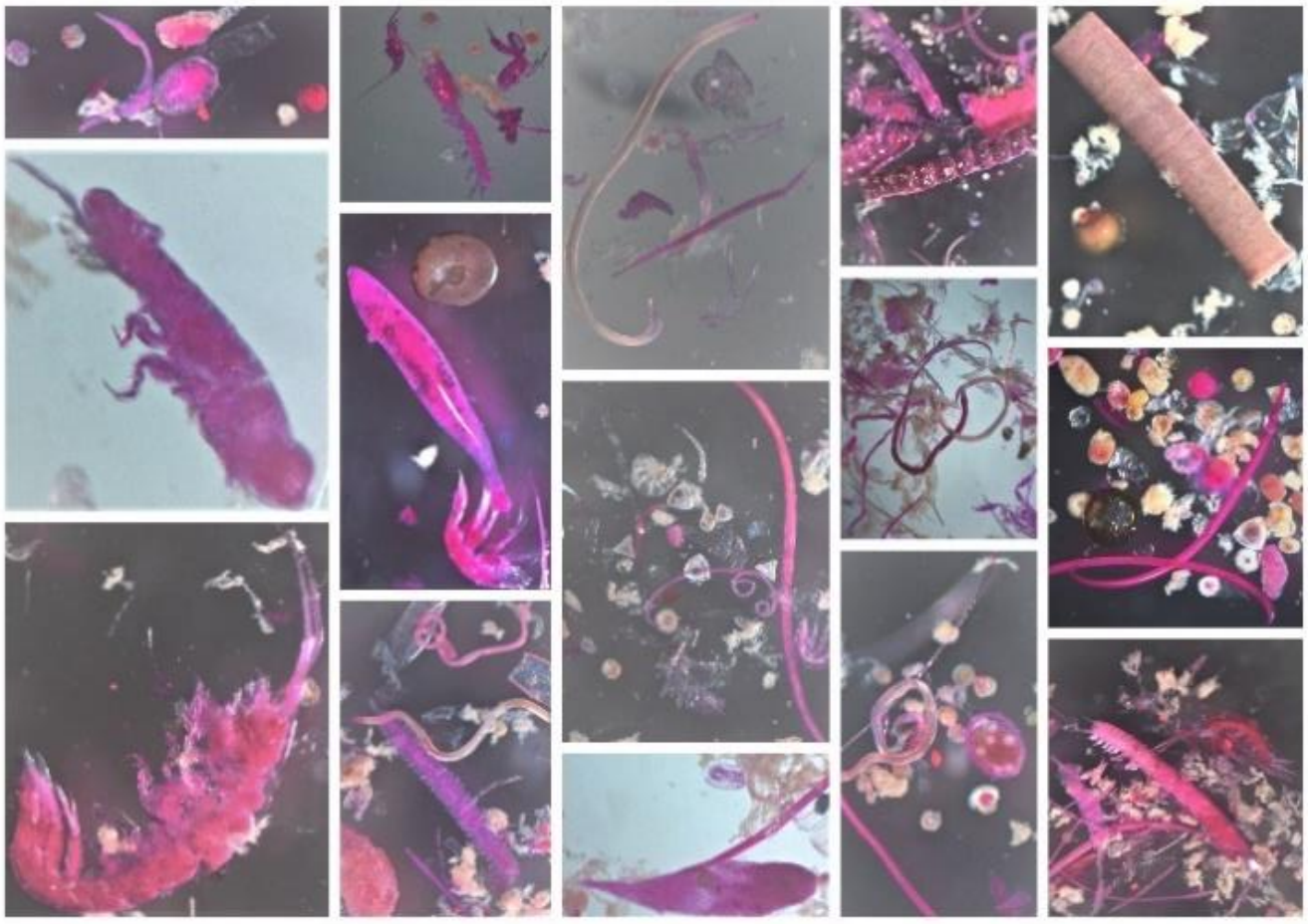


Fig. 33. Meiobenthos of the sediments around reefs - microlevel engineers of recycling