

Closed Sea Water Circulating System for Cultivation of Marine & Estuarine Organisms in the Laboratory

K. K. C. NAIR, T. C. GOPALAKRISHNAN, M. GEORGE PETER & T. S. S. RAO

Regional Centre, National Institute of Oceanography, Cochin 682018

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A closed sea water circulating system has been developed for the cultivation of marine and estuarine organisms under laboratory conditions. The system consists of 2 large fibre glass tanks, one for the storage and supply of purified water and the other for collecting impure water from the rearing tanks. The latter tank functions also as a biological filter. A set of PVC tubes supply the purified water to individual rearing tanks and another set drains the impure water from the rearing tanks for filtration. This flow is maintained by siphon system. The impure water is filtered through a biological filter and then passed through a physico-chemical filter and pumped up where it is well aerated and circulated again. In this system many crustaceans, polychaetes and molluscs have been successfully grown. Complete life cycle of 2 species of amphipods *Quadrivisia bengalensis*, *Melita* sp. and isopod *Cirolana fluviatilis* are worked out. The bivalve *Meritrix* sp., alpheid *Alpheus rapax*, many decapod shrimps, tube dwelling polychaete *Diopatra naeopolitana* and amphipod *Corophium trilaonyx* have been kept alive for several weeks for experiments. Changes in the dissolved oxygen and ammonia in the water in individual rearing tanks are estimated at fixed intervals to assess the efficiency of the system.

BOTH marine and estuarine animals have gained considerable importance in the field of aquaculture and efforts are being made to further develop and refine the existing techniques in culture methods¹⁻⁷. The ultimate goal in the cultivation of marine organisms are mainly: (1) commercial, to find out alternatives for the hunting of sea food and (2) basic, for the purpose of research to (a) acquire basic knowledge on living systems, (b) study the ecosystem, (c) produce food for the other culture organisms and (d) understand multi-species assemblage and species interrelationship⁷.

Sea water as a medium is very complicated because of its unique physico-chemical characters. Its life supporting capacity gradually decreases when used in water systems where heterotrophs are present. The major problems encountered are the increase in ammonia, particulate matter and dissolved organic compounds, and the changes in pH and dissolved oxygen. Accumulation of ammonia is detrimental to life and its concentration must always remain below 0.001 mg l⁻¹ (ref. 7). Biological, mechanical or physico-chemical water treatment can solve most of the problems.

Various types of water systems are used in the field of aquaculture: (1) Still water systems used for culturing bacteria, unicellular plants and small invertebrates. (2) Running water systems used for multicellular plants and animals. The running water systems are of various types: (a) open sea water systems where it is fed by the natural sources, (b) semi-open systems where some technological principles are employed and is a combination of open and closed systems and (c) closed sea water systems where the sea water is monitored and recycled. Closed circulation can effectively be used in

mariculture because it affords control over temperature, salinity, dissolved oxygen, etc. In this paper a closed sea water circulating system developed for continuous culture for research is described.

Methods

Oxygen was estimated by Winkler method as described by Strickland and Parsons⁸ and ammonia by indophenol blue method of Koroleff⁹.

Description of the system—The system consists of 2 large fibre glass tanks (90 × 60 × 60 cm) of 324 l capacity each (Fig. 1a and b); tank *a* functions as a reservoir for the purified water and tank *b* as a filter bed reservoir, 10 rearing tanks of 10.25 l capacity each, set of PVC tubes for the supply and draining of water, a biological and a physico-chemical filter and a pump. From tank *a* which is placed on an elevated platform purified water flows to the main distribution tube (Fig. 1c) made of acrylic plastic (90 cm long and 20 mm diam.), which has 10 outlets (2 mm diam.) each at equal intervals, through which water is supplied to the 10 rearing tanks (Fig. 1, *d*1 to *d*10) by PVC tubes of 3 mm diam. (Fig. 1, *h*1 to *h*10). This flow is regulated by 10 separate valves (glass tubes of 2 mm diam. with ground glass valves at the centre) made of glass (Fig. 1i). The rearing tanks (glass troughs of 21.5 cm diam. and 29 cm high; 10.2 l capacity) are kept at a lower level to tank *a*. From the rearing tanks water is drained (375 ml/min) by another set of PVC tubes of 3 mm diameter each (Fig. 1, *j*1 to *j*10), also regulated by ten separate valves, to a main collecting tube (Fig. 1e) similar to the main distribution tube, from where the water flows to the filter bed reservoir kept at a still lower level. The impure

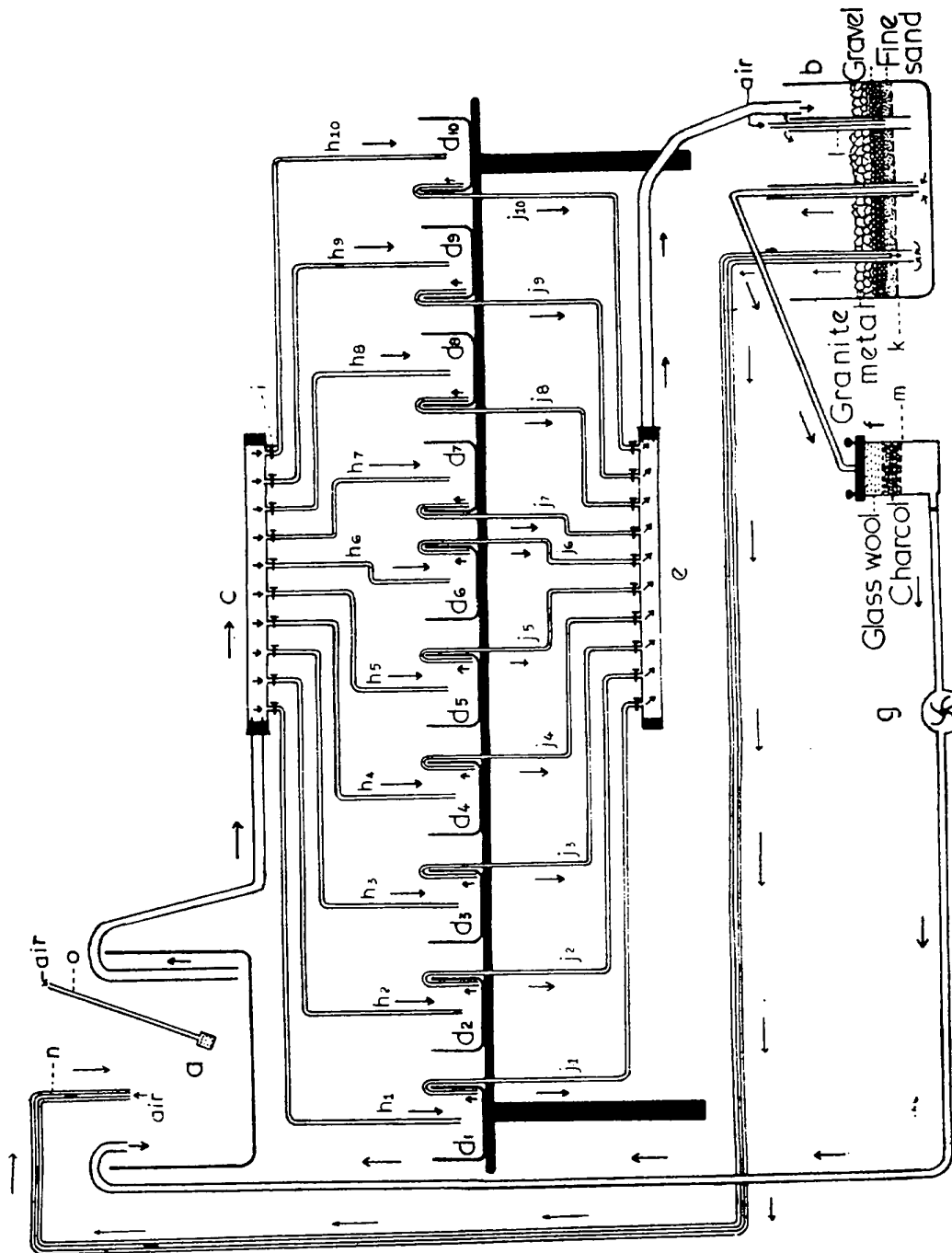


Fig. 1 — Schematic diagram of the closed sea water circulating system

water collected from the rearing tanks is poured over a filter bed inside tank *b*. A porous asbestos sheet (90×60 cm) fixed inside tank *b* (Fig. 1k) at about 10 cm above the bottom of the tank with a piece of 300 μ bolting silk spread over it, a layer of fine sand (1/10 to 1/4 mm), a layer of gravel (1-2 mm) and a layer of granite metal (15-20 mm) constitute the biological filter. The impure water filtered through this filter bed (5400 cm²) gets collected at the bottom of the tank where it is well aerated (Fig. 1l) and passed through the physico-chemical filter (Fig. 1f) and pumped up to tank *a*. Inside the physico-chemical filter (27 cm high and 16.5 cm diam.) there are a layer of glass wool and a layer of activated charcoal placed over a perforated PVC sheet (Fig. 1m) and the filtered water gets collected below this before being pumped up. The lifting of water from tank *b* to tank *a* is done by a pump (Fig. 1g; centrifugal pump attached to 1/8 H. P. motor, 3 l/min), as well as by air lifting (Fig. 1n) using aerators (1500 ml/min). In tank *a* the filtered water is again well aerated (Fig. 1o) to make sure about the optimum dissolved oxygen conditions. While the pump is operated the replacement of water in each rearing tank is comparatively faster (375 ml/min) when compared to air lifting (200 ml/min) which gives a gentle flow. The flow of water from tank *a* to tank *b* is by gravity. The salinity is kept almost constant 21-23‰ by adding fresh water to compensate the loss due to evaporation and the temperature is around 27-28°C which is the room temperature in this area. The estimated cost for setting up the system is around Rs 5000.

Maintenance—The various tubes in the distribution and collecting systems and the regulating valves get clogged periodically by dirt, dead algae, sometimes by smaller organisms and their moults. Closing of the free end of the tubes to stop the passage of organisms from one rearing tank to another, so as to keep one rearing tank exclusively for one species caused faster clogging and slowed down the flow of water very often and hence it could not be done effectively. Once in 6 months all the tubes were cleaned in fresh water and thoroughly washed. The physico-chemical filter was cleaned once in a month, using a stepney. The glass wool and charcoal used inside the filter were dried before using again. They were replaced whenever possible. The glass troughs used as rearing tanks were not cleaned after the system started functioning because the close association of the thick bed of diatoms attached to the walls of the tanks and the animals reared inside was found to be beneficial for the animals. The biological filter was found to function effectively without cleaning it for nearly a year. To avoid mass contamination, each rearing tank was provided with independent valves to regulate inflow and outflow which proved to be essential when compared to a serial connection starting from one end and draining from the other end. The pump and the air lifting systems were operated alternatively thereby giving sufficient rest to each.

Results and Discussion

This simple closed sea water circulating system described above functioned very well especially for

rearing animals in the laboratory when compared to large and complicated systems. There was a rich growth of diatoms and algae in the medium and this provided a suitable environment for the growth of small crustaceans, bivalves and polychaetes. Two species of amphipods *Quadrivisia bengalensis*, *Melita* sp., and isopod *Cirolana fluviatilis* collected from the estuary were reared successfully through their complete life cycle in this system. The alpheid *Alpheus rapax*, bivalve *Meritrix* sp., many decapod shrimps, tube dwelling polychaete *Diopatra naeopolitana* and amphipod *Corophium triaeronyx* were able to live in this system for several weeks. The basic idea in having such a system is to have a clear unpolluted medium to keep a viable culture of animals alive in the laboratory for extended time for experiments on their behaviour, breeding, life history and studies on biochemical composition.

In general, the major problems encountered in the circulation system are the increase in ammonia, particulate matter, dissolved organic compounds and the changes in pH and dissolved oxygen.

Among the potentially life endangering substances accumulating in the culture water, ammonia is of primary importance. Animals excrete ammonia, urea, uric acid and other nitrogenous substances as end products of their nitrogen metabolism. In addition dead and decomposing organisms and waste food rapidly release ammonia. Biological filtration, which can be defined as the removal of unwanted substances, primarily ammonia from the water by bacteria is solely the work of bacteria attached to the surface of the gravel in the filter bed⁴. Phytoplankters have shown to use ammonia in preference to nitrate⁷. In Table 1, two sets of ammonia estimations at 4 months interval are given. The low values for ammonia in the 2nd set may partly be attributed to the rich phytoplankton and algal growth in the rearing tanks and the bacteria attached to the filter bed. When this system got 'conditioned' as defined by Spotte⁴ the maximum ammonia level of 7.79 μ g/litre was brought down to 2.375 μ g/litre. The fluctuation in the ammonia values in the closed tank in the 1st set of experiments

TABLE 1—CHANGES IN DISSOLVED OXYGEN AND AMMONIA IN THE REARING TANKS AT FIXED INTERVALS

Time (hrs)	Saturation (%) of dissolved oxygen		Ammonia μ g/litre	
	Closed tank	Open tank	Closed tank	Open tank
20-5-1977				
1130	90.46	90.46	7.41	7.41
1230	86.15	99.07	5.70	7.6
1330	86.15	90.46	4.56	7.51
1430	87.23	94.76	10.36	7.6
1530	80.76	92.61	4.85	7.79
1630	84	92.61	8.06	7.32
25-9-1977				
1000	75.38	75.38	1.805	1.805
1200	68.92	71.07	4.465	1.995
1400	53.84	73.23	4.275	2.375
1600	47.4	71.07	4.655	2.185

on 20-5-1977 may be due to the fact that the system was not 'conditioned' at that time. In one rearing tank, with 50 to 60 adult amphipods in it, when cut off from circulating system the ammonia level went up from 1.805 to 4.655 $\mu\text{g}/\text{litre}$ in 6 hr. Minimizing the increase of ammonia and decline of pH in other recirculating sea water systems using filters have also been reported by previous workers^{1-3,5,6}.

The dirt, dead algae, smaller organisms and their moults result in an increase in the particulate matter in the medium. The particulate matter here is completely removed by mechanical filtration while the water passes through the filter bed.

The metabolic activities of the animals increase the amount of dissolved organic matter which is undesirable because they increase the oxidative activities of the heterotrophic bacteria, gradually lower pH of the water and use up an excessive amount of oxygen. Only part of the dissolved organic matter is removed in the filter bed by the activity of the heterotrophic bacteria. The only alternative is chemical filtration⁴. Most of the dissolved organic matter in the water that collects below the filter bed will be removed by adsorption while it passes through activated charcoal in the physico-chemical filter. Foam separation, as stated by Kinne⁷ is an effective method to remove excess dissolved organic material, secure proper gas exchange and to stabilize pH by removing weak acidic substances from solution. The air lifting of water from tank *b* to tank *a* in this system, described above, might be functioning as an effective foam separator.

Constant aeration in tank *a* and tank *b* provided optimum dissolved oxygen concentrations. It could be maintained between 71.07 and 99.07% saturation when the system was in operation. It went down to 47.4 from 75.38% in 6 hr in one rearing tank when cut off from the system.

The system was found ideally suited for gammarid amphipods, isopods, some harpacticoid copepods,

polychaetes and bivalves. Both amphipods and isopods took 3 to 4 months to complete their life cycles. Once in 4 months 50 to 60 adult animals could be removed from each tank, which was found to be the maximum carrying capacity of each tank, leaving behind in the tank sufficient number of juveniles to develop into a viable population. The amphipods were fed with fresh water algae *Utricularia* sp. and the isopods with fresh flesh of bivalves. Benthic polychaetes, bivalves, decapod crustaceans and sergestid shrimps like *Acetes*, *Lucifer* and alpheidids could be kept alive for several weeks for experiments.

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