Chapter 1

Effect of flow rates on physico-chemical parameters in the seawater re-circulating system
1.1 Introduction

Over the past three decades shrimp culture industry with its great potential has generated high export earnings, which has resulted in expansion of shrimp culture in many countries. The explosive growth of the industry has been characteristic by a trend towards intensification of shrimp farming in the form of semi intensive, intensive and in recent years super intensive shrimp aquaculture systems. In general, the objective of intensive aquaculture is to obtain the highest possible production from unit volume of water and this is accomplished by proper management methods such as fertilization, artificial feed enhanced stocking density and aeration. In aquaculture venture, the discharge of nutrient and organic waste produced by intensive shrimp ponds comprise solid matters, mainly uneaten feed, fecal matter and phytoplankton and dissolved metabolites such as ammonia, urea and carbon dioxide.

Sea is a dynamic ecosystem where physical and chemical conditions in water are always subjected to changes and variations. These changes affect all the living things in it. Organisms which can adapt and withstand the range in the variations live grow and proliferate. Others migrate to suitable and safer places or perish. But the natural environment being very large, these changes are brought back to the normal equilibrium though a complex and well organized chain of actions such as air-sea interaction, turbulence, water currents and other biological and chemical changes brought
about by microorganisms. Thus the conditions suited to the organisms are maintained. Or the organisms select the different layers and niches in the water column best suited to them.

But this is not possible in the case of closed culture systems in which the animals grown have no choice to select the physico-chemical and biological conditions or to migrate to other areas of their choice. They are at the mercy of the farmers for such conditions. They either survive and grow or die. Success of a culture system basically depends in providing optimum conditions for the growth of the cultured species. This is made possible by the common method of periodic water exchange in the culture ponds. Water exchange is very effective in removal of suspended organic particles, ammonia and nitrite, in increasing the dissolved oxygen and decreasing carbon dioxide levels and in regulating the physical parameters such as temperature and salinity. But in areas where good quality water is scarce, water exchange becomes a big problem to the farmers.

Therefore an alternate system to overcome these difficulties, especially in intensive culture system needs attention. The recycling of water in most economic way becomes essential and with this idea the present study was undertaken so that the same water could be recycled for reuse in the same system by adapting the technology of recirculating sea water through biofilters.

1.2 Materials and Methods
The experiments on water recirculation were carried out in Fibre Reinforced Plastic (FRP) Tanks available in the hatchery Laboratory of the Central Marine Fisheries Research Institute, Cochin. The sea water re-circulating system was installed in these tanks through an airlift water recirculation using one biological filter for one set of three tanks (Plq.1.1)

1.2.1 Experimental tanks

Experiments were carried out in twelve 400 litre capacity Fibre Reinforced Plastic (FRP) Tanks and another three larger tanks provided with bio filters. These tanks were thoroughly cleaned, dried and filled with clean and fine filtered sea water having salinity 25 parts per thousand (ppt.). The volume of water kept was about 300 liters in each tank. All the tanks were provided with PVC pipes and pieces of roof tiles at the bottom of tank to provide shelter to post larvae. Aeration was provided in each tank to supplement oxygen.

1.2.2 Bio filter

Bio filter unit used comprised rectangular, Fibre Reinforced Plastic (FRP) Tanks having dimension 1.7m length, 0.50 m breadth and 0.30 m width with an outlet of 5 cm above the base. The filter bed platform was fabricated from acrylic sheets perforated at every 5 cm² using 5mm drill bits. This platform 5 cm less than the length and breadth of the FRP tank bottom and it was raised from the bottom of the tank by fixing 5cm perforated acrylic pieces on four sides of the sheet and by fixing another two pieces in parallel through the centre lengthwise. Two holes of 25mm size were cut at the two corners of this platform and 25 mm size rigid PVC pipes were fixed using PVC male and female connectors, so that the PVC pipe stood erect on the plat form.
elbow was fitted on its top. A hole was made at the top of the elbow through which a flexible aeration tube was inserted to the PVC pipe and an air diffuser was fixed at the end of the tube positioning it 5 cm above the acrylic platform. The length of the pipe was adjusted so that it will stand one inch above the water level. This acted as the air lift system of the bio filter. Gravels of above 5mm sizes were washed thoroughly, sun dried and spread above the acrylic platform in 10 cm thickness. The tanks were then filled by slowly pouring the sea water collected and kept in the tanks up to the required level and the upper end of the PVC tube for air lifting stood 2 cm above the water level. Care was taken not to disturb the gravel bed while filling the water. Air from a blower was allowed to pass through the tube and diffuser stones. The upward lift of air brought water to the upper surface thereby developing a suction effect from the bottom of the acrylic stand below the sand filter. The tanks with water and the bio filter were kept undisturbed for a period of 10 days for maturation for the settlement of beneficial nitrifying and denitrifying bacteria in sufficient numbers. These matured tanks were used for the experiments described in chapter 1 and chapter 2.

1.2.3 Air lift pumps

Air lift pump consisted of open ended pipe or tube in which air is injected. The mechanism involved in operation is to lift the water column by this air. Through this lift the bottom water of the tank was brought into the filter bed (Pic Notd)

This airlift water recirculation was fabricated by making use of a rigid PVC pipe of 25 mm diameter, rigid elbow, socket to fix the pipe to tanks and aeration tubes. Air from an air blower is injected in pipe by connecting flexible aerator tubing at 10 cm above the bottom of the pipe by making a bore and ensuring it airtight. Airlift pump is kept in position of fixing it to tank with help of sockets.
The different rate of water flow through the airlift pump was maintained by regulating the incoming air pressure inside pipe. Three different flow rates for water recirculation were calibrated and maintained. They were 0.5 l/min, 1.5 l/min & 2.5 l/min in set II, Set III and set IV. The control tanks were not provided with the airlift system, but were provided simple aeration.

1.2.4 Experiment set up

Experiment was carried out in 4 sets and each set was in triplicate. Each bio filter tank was connected to the homogenous set of experimental tanks having the same flow rate tanks. They were connected in such a way that the re-circulated water drawn from these tanks would flow back to the bio filter tank automatically. Water from the bottom of bio filter tank was brought up by airlift pumps. This enabled the bottom water to pass through the bio filter bed for filtration. It was then distributed to tanks through PVC pipe attached at the bottom of bio filter. The mouths of the connecting PVC pipes were provided with pieces of nets to prevent escape of shrimp larvae from one tank to another. A common bio filter was used for each set, which helped to maintain identical conditions in all the three tanks of a set.

1.2.5 Aeration

An air blower was used for aeration and to airlift the water and aeration was distributed to the rearing tanks through flexible aeration tubes, connected with air diffuser stones. Uniform air pressure was maintained in each set of tanks through regulator valves.
1.2.6 Source of Water

Saline water required for the experiment was obtained from the clean and unpolluted coastal waters off Cochin having salinity 30±2 ppt and were fine filtered and kept in large dark storage tanks.

1.2.7 Experimental Animals

Post larvae of *P. indicus* having an average size of 20 mm weighting 50±3 mg were used in the experiments. They were procured from a local hatchery. For transportation to the lab the post larvae were packed in 5 litre capacity oxygen packed polythene bags containing 2 litres of seawater. In the hatchery the animals were maintained at a salinity of 25 ppt and a temperature of 28°C. In the laboratory the animals were transferred to a 30 litre fiberglass tank containing seawater at a salinity of 25 ppt and temperature of 28°C for acclimatization. The acclimatization was done by floating the bags containing the post larvae in the tank and adding small volumes of water periodically until both temperature and salinity within the bag and outside became same. After acclimatization the next day the animals were segregated according to their size and were transferred to 5 litre plastic jars.

1.2.8 Stocking of Post larvae

The acclimatized and conditioned post larvae were stocked in the experimental tanks having 1000 liters water after gradual acclimatization. Each tank was stocked with 30 *P.indicus* juveniles almost of the same weight the weight being 50±3 mg.

1.2.9 Experimental Diet

A commercial feed “Higashi Starter A 3000” was used for all the 12 experimental tanks through out the experiment.
1.2.10 Physico-chemical parameters:

The physico-chemical parameters such as water temperature, pH, dissolved oxygen, salinity, ammonia, nitrite, nitrate and phosphorous were regularly recorded at an interval of five days.

i) Temperature:

The temperature of water affects the growth and metabolism of prawns. The temperature of various tanks was measured with a mercury thermometer having an accuracy of 0.1°C.

ii) pH:

The pH measures the hydrogen ion concentration in water. It indicates the quality of water. If the pH value is less than neutral value of 7, it denotes that the water is acidic while pH above 7 indicates that the water is alkaline. pH values in 7-8 are considered optimum for prawn culture. Above and below this range, cause stress on prawn and consequently results in heavy mortality. The pH values were measured by a pH meter.

iii) Oxygen:

The dissolved oxygen (DO) is the most important parameter among all, which affects on growth of prawn through its indirect influence on optimum feed consumption and metabolism. Oxygen also helps in maintenance of favorable chemical and hygienic environment of culture water. The desired DO level was maintained through efficient aeration and airlift systems. The DO was measured by using Winkler’s Titration Method.
iv) Salinity:
The salinity of rearing tanks was measured by a refractometer (range 0-50 ppt). Salinity was maintained between 28±2 parts per thousand (ppt) throughout the experiment, by addition of freshwater whenever needed to adjust salinity.

v) Ammonia (NH₃-N):
Ammonia reacts with phenol and hypochloride in an alkaline solution to form idophenal blue; the reaction is catalysed by nitroprusside. The resulting absorbance is proportional to the ammonia present and is measured using spectrophotometer at 635nm.

vi) Nitrite (NO₂-N):
Nitrite was analysed by Strickland and Parsons method (1968). Sulphanilamide 7 Diamine was used as reagent which gives sample pink colour complex. The light absorption of which was measured using spectrophotometer at 543 nm.

viii) Nitrate (NO₃-N):
The nitrate is buffered and reduced by means of buffer reagent (phenol and sodium hydrochloride solution) and reducing reagent (copper sulphate and hydrazine sulphate solution). Sulphanilamide and diamine added to sample to yield a red azo dye. The light absorption of which was measured by spectrophotometer at 543nm.
ix) Phosphate (PO$_4$ -P)

Phosphate content was estimated by adding ammonium molybdate to 50 ml of sample and allowed to rest for 10 minutes. Then 0.2 ml stannous chloride was added and resultant solution was estimated with help of spectrophotometer at 690 nm.

1.3 Results

The results of the experiment over a period of 40 days are presented below. The readings were taken at 5 days intervals. The effect of flow rates on various physico-chemical parameters are given in tables 1.1 to 1.4.

1.3.1 Temperature

There was no control on the temperature in the water of the experimental tanks as they were exposed to the normal room temperature. The mean temperature values varied from 24.7°C to 27.6°C in set I (control), 24.7°C to 26.5°C in set II, 24.6°C to 26.3°C in set III and 24.8°C to 26.3°C in set IV. A slight noticeable decrease in temperature values could be seen in the tanks with more re-circulation.

1.3.2 pH

The rearing medium was alkaline throughout the experiment except for the control tank (Set I), which did not have the water re-circulating system. The mean values of pH ranged from 6.8 - 8.1 in set I, 7.5 - 8.5 in Set II, to 7.8 - 8.5 in Set III and 7.9 - 8.4 in Set IV. An increase in the pH values was noticed in all the tanks between the 10th and 15th day of the experiment (Tables 1.1 to 1.4).
1.3.3 Dissolved Oxygen

The average initial values of the Dissolved Oxygen levels in the tanks were recorded as 9.2 ppm. The average dissolved oxygen level varied from 7.5 to 9.0 ppm in set I (control), 8.2 - 10.8ppm in set II, 8.22 to 11.2 ppm in set III and 8.2 - 11.4 set IV, respectively.

1.3.4 Ammonia (NH₃)

During initial phase the ammonia level in the tanks was recorded and found to be 1.7 mg/l. This level decreased gradually during the experiment time in tanks of set II, II and IV. However, in the control tank an increase in this level was observed. The values ranged from 1.70mg/l to 2.36mg/l in set I, 0.44 mg/l to 1.62 mg/l in set II, 0.32 mg/l to 1.52mg/l and 0.18 to 1.70mg/l during the experiment period.

1.3.5 Nitrite (NO₂)

The nitrification rate was observed to be higher in tanks with recirculation system. The rate of nitrification showed an increase with increasing water flow rates. All sets of tanks showed an increased rate of nitrification in the first 10 days and then gradually declining before becoming steady (Table 1.1 – 1.4). The observed values were in the range of 0.32 mg/l – 1.22 mg/l in set I, 0.30 mg/l – 0.38 mg/l in set II, 0.28 mg/l – 0.38 in set III and 0.22 – 0.37 mg/l in set IV, respectively.

1.3.6 Nitrate (NO₃)

The initial level of nitrate concentration was estimated as 0.20 mg/l in the experimental tanks. The values recorded during the 40 days experiment time ranged from 0.19 mg/l – 0.24 mg/l in set I, 0.23 mg/l – 0.78 mg/l in set II, 0.20 mg/l – 0.91 mg/l in set III and 0.26 mg/l – 1.12 mg/l in set IV, respectively.

1.3.6 Phosphate (PO₄)
The initial phosphate level in the tanks was 1.2 mg/l. An increase in the concentration was observed during the experiment time. The values ranged between 0.6 mg/l - 1.2 mg/l in set I, 1.2 mg/l - 2.5 mg/l in set II, 1.2 mg/l - 3.0 mg/l in set III and 1.2 mg/l - 3.4 mg/l in set IV.

1.4 Discussion

Physico-chemical conditions such as temperature, pH, dissolved oxygen, ammonia and other nitrogen compounds decide the quality of water making water suitable for the growth of animals living in it. (Boghen and Castell, 1979). Temperature plays a very significant role in the survival and growth of juvenile shrimps (Aziz and Greenwood, 1981). Effect of salinity, ammonia and nitrite on P. chinensis was reported by Chen et al., (1990). Salinity and temperature effects on larval development of the crab *Panopeus herbstii* was described by Costlow et al., (1962). Huang (1983) studied the effect of physico-chemical factors in the successful culture of *P. stylirostris* and *P. vennamei*. Denitrifying and nitrifying bacteriae are responsible for the recycling of nitrogenous wastes and their role in the prawn culture system was reported by Ninawe and Paul Raj (1993) and Sharma and Ahlert (1977). Scope of water reuse systems and water quality requirement for intensive culture were studied earlier. Aeration and water circulation help in the reduction of dissolved organic wastes, regulation of pH and increase in the dissolved oxygen levels. (Muir, 1994, Wickins, 1980).

In the present study, effect of aeration in combination with water recirculating system of sea water in the laboratory conditions was studied. It was seen from the
results that the two components help significantly in regulating pH an important physical parameter. Salinity was maintained at constant level for the experiment period. There was no control on temperature. But still little variations were observed in different tanks with different flow rates. However, in the case of chemical parameters all of them were seen to have significant correlation with respect to different flow rates (Wickins 1976, 1980), Wickins and Arnstein (1977). The conditions were found to be better with increasing order of flow rates and was optimum in set IV which had the maximum flow rate of 2.5 litres per minute (Table 1.1 to 1.4). The control tanks without water recirculation had the lowest values. The filter bed and the gravel size have increased the surface area for the settlement of the beneficial bacteria and the efficiency of the biofilters in combination with the aeration seems to have a noticeable role in the recycling of sea water.

Results given in the tables 1.1 to 1.4 indicate that the rate of water circulation has direct relationship in the control of physico-chemical parameters. Flow rates were found not to have affected salinity and temperature. The salinity levels in the tanks were almost maintained more or less same throughout the experiment so that the growth of juvenile shrimps should not be affected. The tanks were exposed to the ambient temperature. However there was a slight change noticed during the period, which is not much significant. However, in the case of ammonia, dissolved oxygen, nitrite nitrate and phosphate levels, which are very important in the culture systems the flow rates had a direct control on these parameters thus maintaining the system in optimum conditions (Wickins 1976, 1980), Wickins and Arnstein (1977). The present study shows that the effect of flow rates had a good impact in maintaining the water quality in controlled culture systems.
1.4 Summary

Effect of flow rates on various physico-chemical parameters in the experimental tanks was studied. The flow rates in four sets of three tanks each were regulated by controlling the airflow. The flow rates were 0 for control tanks (set I), 0.5 litre per minute (Set II), 1.5 litres per minute (Set III) and 2.5 litres per minute (Set IV). The period of experiments was for a period of 40 days. Observations were made at 5 days intervals. The flow rates were noticed to have significant influence on the physico-chemical parameters. Water quality during the period of study was the best in the tanks having the maximum flow rate and the quality decreased with decreasing flow rates. In intensive and super intensive culture systems the quality of water could be improved using more improvised filtration and recirculating system. This in turn will be helpful in the use of sea water in a more economically viable manner.
Table 1.1  AVERAGE PHYSICO-CHEMICAL PARAMETERS OF SET I without re-circulating system(CONTROL) TANKS

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Table 1.2 AVERAGE PHYSICO-CHEMICAL PARAMETERS OF SET II WITH 0.5 L/MIN. WATER RECIRCULATION
Table 1.3  AVERAGE PHYSICO-CHEMICAL PARAMETERS OF SET III WITH 1.5 L/MIN. WATER RECIRCULATION

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Fig. 1.1 Effect of Flowrate on Water Temperature

- Control
- 0.5 L/min
- 1.5 L/min
- 2.5 L/min

Temperature (°C)

Number of days
Fig. 1.2 Effect of Flowrate on pH

Number of Days

pH

Control
0.5 L/min
1.5 L/min
2.5 L/min

Number of Days
Fig. 1.3 Effect of Flow Rate on Dissolved Oxygen (DO)
**Fig. 1.4 Effect of Flow Rate on Ammonia (NH₃)**

![Bar chart showing the effect of flow rate on ammonia levels over time.](image)

- **Control**
- **0.5L/min**
- **1.5L/min**
- **2.5L/min**

**Number of days**

**Ammonia (mg/l)**
Fig. 1.5 Effect of Flowrate on Nitrite Concentration

Control | 0.5L/min | 1.5L/min | 2.5L/min
Fig. 1.6 Effect of Flowrate of Nitrate (NO₃)

Number of Days

Control
0.5L/min
1.5L/min
2.5L/min

Nitrate (mg/L)

0 0.2 0.4 0.6 0.8 1 1.2

0 5 10 15 20 25 30 35 40

Number of Days
Fig. 1.7 Effect of Flowrate of Phosphate (PO4)

- Control
- 0.5L/min
- 1.5L/min
- 2.5L/min

Number of Days

Phosphate (mg/l)
Pic. 1.1 Biofilter with airlift system
Fig. 1.2 Diagrammatic representation of the sea water re-cycling system