1. Brackishwater aquaculture in India: An overview

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It has been increasingly recognized that future of food security largely depends on the aquaculture production. With the world's population predicted to increase to 10.9 billion people by 2050, where in India itself would expected to be 1.6 billion, the need for increased food production is a major challenge, particularly in areas that have high rates of food insecurity. Aquaculture is well acknowledged as one of the few options that contribute significantly at global and national level to food security and economic growth, if responsibly developed, practiced and maintained in a sustainable way. The aquaculture is therefore pinned with great hopes and expectations. Although modern aquaculture enterprise has been criticized for its unsustainable way of development, it is the mainstay for the growth of aquaculture. This article provides an overview of present status of Indian brackishwater aquaculture, and unveils the hidden potential ecosystem approach for Indian brackishwater aquaculture, through economically feasible, environmentally friendly and socially acceptable approach.

1.1 Brackishwater resources

The brackishwater resources in India were delineated in late 1970s by conduct of micro survey. Indian coastal areas have nine states, two island territories with a coastline of 7516.6 km. It has 97 major estuaries with a total area of 3.9 million ha and backwaters of 3.5 million ha. The total mangrove area is 6740 km^2 and of these 57% of mangrove ecosystem are at east coast and 23% are at west coast, and 20% are at Andaman and Nicobar islands. About 1.2 million ha has been identified as potentially suitable for brackishwater aquaculture, whereas only 0.17 million ha (14.8%) has been utilised for the culture. West Bengal and Gujarat have the majority of the potential area for brackishwater aquaculture owing to the high tidal amplitude. Andhra Pradesh developed almost 57% of area available for shrimp culture where as Maharashtra and Gujarat utilized only 1.2 to 0.6% of the available area. Hence there is vast opportunity in the country to expand the bracksihwater aquaculture sector in area and productivity, with production of finfish and shellfish for food, employment and income generation.

1.2 Shrimp Aquaculture in India

Brackishwater aquaculture in India is almost synonymous to shrimp farming in India, hence, unsurprisingly, the history of brackishwater aquaculture is the history of shrimp farming. In early 1950s, juvenile shrimps were extensively fished from the paddy fields bordering the backwaters and estuaries of Kerala (pokkali), West Bengal (bheries), Karanataka (Ghazan) and Goa (Kazhan), and were exported to Myanmar to market as a shrimp known as 'prawn-pulp'. Later at the advent of frozen shrimp industry in India, the demand for larger shrimps has

increased considerably, and, therefore it was essential to grow the shrimp in the farm field to meet the demand of export industry. Thus the paddy field shrimp fishery has been evolved into a primitive form of aquaculture where, the naturally immigrating shrimp seeds from coastal waters are entrapped and prevented from returning to sea, and reared for few months, without any feed or aeration. Later, to augment the production, farmers started the practice of stocking the ponds with wild caught seeds, and thereafter, when commercial hatcheries started, with hatchery reared seeds. This form of improved extensive type of shrimp culture is still prevailing in Kerala with a production of about 400 kg/ha to 600kg/ha for a short period of culture without supplementary feeding, where it can be understood that this type of culture is a form of ecosystem based culture or an organic shrimp aquaculture, in perennial farms and 'pokkali' rice farming fields.

Although extensive production system of shrimp started as early as 1960s, the industry only really began to intensify in the early 1990s, after the successful demonstration of commercial tiger shrimp hatchery in AP, through an MPEDA and DBT project, by TASPARC, with help of foreign technological support, which triggered the establishment of commercial hatcheries in private sector. However, this development has not happened in the already existing traditional shrimp farming regions: Kerala, West Bengal, Karnataka and Goa, and the modern shrimp aquaculture development largely centred in the areas where shrimp aquaculture did not have any prior history, such as Andhra Pradesh and Tamil Nadu. This can be attributed to the entrepreneurship of the local people, seasonal and geographical advantage. What followed is a spectacular growth of shrimp aquaculture system, during 1990-1995 with commercial hatcheries and farms with the use of desired seeds, formulated feeds and life supporting systems such as aerators. Farmed shrimp production showed a remarkable growth during this period of early 1990s with tiger shrimp *Penaeus monodon*, and thereafter production stagnated from 1996 to 2000, mainly due to WSSV pandemic, and related crop failures. From 2000 to 2006 shrimp farming gradually increased and peaked with a maximum production of about 1.4 lakh tonnes in 2006, but production reduced drastically in 2008, due to the multiple issues of seed quality, disease problems, poor water and pond environment, and the situation warranted a shift in the farming systems and the species itself.

1.3 Introduction of *Penaeus vannamei*

The Taiwanese, being the leaders in scientific shrimp farming in Asia, witnessed the initial set back in 1988. The reasons for Taiwanese production losses are still unexplained, although causes of mortality are attributed to degradation of farming environment, pollution, and disease problems due to bacterial and viral pathogens. Thailand, the second successful shrimp farming nation, also faced crop failures and large scale production losses, mainly due to viral disease such as yellow head virus, but these production losses had only limited impact on world shrimp

supply, and little impact on shrimp aquaculture in India. Whereas, the catastrophic and widespread shrimp mortality caused by white spot syndrome virus (WSSV) and subsequent crop losses occurred in 1995, in all Asian shrimp farming nations including India, and later in Americas. WSSV pandemic cast doubt about the economic feasibility and sustainability of shrimp aquaculture in India and across the globe.

No therapeutic options available for the control of viral pandemics such as WSSV and the only management way out is to adopt preventive strategies. The use of post larvae generated from the specific pathogen free (SPF) broodstocks along with strict biosecurity measure are the most effective management option to ensure successful crops. Although development of SPF broodstock is time consuming and extremely difficult, it is essential pre-requisite for selective breeding. The US was successful in the selective breeding, which they initiate much earlier, resulted in the production of SPF *P. vannamei*, although the scale of shrimp farming was only limited in Americas. Following the success of Taiwan, *P. vannamei* was introduced into several South American and South East Asian countries including India. In India, from 2010, a dramatic growth of farmed shrimp production due to the introduction of *P. vannamei* was recorded, with 90,000 MT in 2010 to 4,06018 MT in 2015-16. This was possible due to the superior aquaculture traits of *P. vannamei*, for example: high survival rate, fast growth rate, tolerance to high stocking density, lower dietary requirements, more efficient utilization of plant protein in the formulated diet and stronger adaptability to low salinity, make this species as the most preferred species for aquaculture. Also, the biological advantages such as column feeding habits, and captive reproduction, contributed in the successful growth of *vannamei* farming.

1.4 Production and export statistics

The first recorded data for farmed shrimp production in India were 20 mt in 1970 and first major change became obvious in 1991 when it reached 40000 MT. Farmed shrimp production showed a remarkable growth during early 1990s. Rapid growth of shrimp aquaculture induced an increase in area of shrimp farming and production (Table 1). Andhra Pradesh contributed more than half of the farmed shrimp production in India (Table 2). This growth occurred in spite of the set-back caused by white spot syndrome virus (WSSV) in the late 1994. The disease impacted aquaculture industry severely, and it caused the exit of almost all corporate investors by 1997. A recovery and moderate growth happened in the post WSSV era, from 2000 to 2006, where shrimp farming gradually increased and peaked with a maximum production of about 1.4 lakh tonnes in 2006, but production reduced drastically in 2008. Again, after the introduction of P.*vannamei,* the country has witnessed a remarkable upsurge of farmed shrimp production with production of 5,00,000 MT of farmed shrimp in 2015.

Table 1. Area (ha) under shrimp farming in coastal states

State	1990	1994	1999	2016
West Bengal	33815	34400	42525	51980
Odisha	7075	8500	11332	8991
Andhra Pradesh	6000	34500	84269	42437
Tamil Nadu	250	2000	2670	8024
Kerala	13000	14100	14595	8328
Karnataka	2500	3500	3540	2281
Goa	525	600	650	10
Maharashtra	1800	2400	970	1359
Gujarat	125	700	997	4552
Total	**65090**	**100700**	**161548**	**127962**

Table 2. State-wise area under shrimp farming and production during 2015-16

State	Area under farming	Production (MT)	Percent of total production
West Bengal	51980	68774	14.1
Odisha	8991	28432	5.8
Andhra Pradesh	42437	299071	61.4
Tamil Nadu	8024	45556	9.3
Kerala	8328	3564	0.7
Karnataka	2281	1727	0.4
Goa	10	33	0.0
Maharashtra	1359	6124	1.3
Gujarat	4552	34189	7.0
Total	**127962**	**487470**	

1.5 Issues in current brackishwater aquaculture

Currently *P. vannamei* has been facing several problems globally in the maturation and spawning (deterioration of male reproductive quality), disease issues in the larviculture (Zoea 2 syndrome), production system (early mortality syndrome and uncharacterized disease such as rapid mortality syndrome), and presumed inbreeding depression due to the large scale use of farm raised broodstock. Shrimp aquaculture in India and other south East Asian countries seems to be followed a natural progression form a fishery based aquaculture to full-fledged aquaculture using shrimp seed from domesticated stock. However, it can be seen that the progress of shrimp farming is due to non-native domesticated stock. In global aquaculture scenario, non-native species positively contributed for the growth of aquaculture, although the problems of non-native species have been well documented.

1.6 Way forward

The key for the successful development of sustainable aquaculture is multi-fold. It should address each component of supply chain starting from the ecosystem to market. Several strategies to be adopted to increase the sustainable and economically viable productions:

- Development of new management practices that increase control over the production system incorporating selective breeding program,
- Development of new species and optimization of its production procedures,
- Better aqua feed and new feed ingredients, improved health and environment management,
- Expansion of new inland and coastal areas,
- Development of non-fed aquaculture (shell fish and seaweed).
- Ecosystem approach to aquaculture

The present day shrimp aquaculture is able to thrive even under severe environmental, physical and biological stresses which are manipulated based on the understanding of experiences of successful management practices adopted by different culturists over the years. A pond with good soil and water quality will produce healthier shrimp and poor environmental conditions in pond bring in a state of stress that is unfavourable for the cultured animals but favourable for the disease causing agents. Even if the site is good with optimum soil and water characteristics, problems may still crop up by high stocking densities and use of large quantity of feed and other inputs, which lead to excessive phytoplankton production, low dissolved oxygen, high ammonia, poor bottom soil condition and other problems. Most of these problems can be avoided by proper management practices during pond preparation and culture period. Water treatment is an important step during pond preparation for the maintenance of good water quality at later stage. The well-designed and implemented BMPs should increase the efficiency and productivity by improving the soil and water quality, reducing the risk of shrimp health problems, reduce or mitigate the impacts of farming on the environment. More in-depth studies are required for the development of location and system specific and cost-effective BMPs incorporating principles of eco-based management and bio-security protocols.

1.7 Conclusion

Brackishwater aquaculture represents an important activity for economic development and social cohesion in coastal India. It provides valuable export earnings, family wage jobs and food and social security if it developed responsibly. The modern aquaculture should adopt an environmental approach integrating aquaculture, environment and society. That provides basis for new social contract involving all the stake holders and decision makers. In order to achieve the goals of economically viable, environmentally sustainable socially acceptable brackishwater farming, institutions such as CIBA, developmental agencies such as RGCA-MPEDA, fisheries universities, regulatory organizations of state and central Government should come together.

2. Biology of shrimps with special reference to aquaculture

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Aquaculture is the farming of fish crustaceans, molluscs and aquatic plants in aquatic environment, sometimes it is referred to as aquatic agriculture, as aquatic counterpart of terrestrial agriculture. Here farming implies some sort of intervention in rearing process, such as regular stocking, feeding or protection from the predators. Aquaculture can be categorized into several ways: For example, the environment used for aquaculture (Marine aquaculture or mariculture, coastal aquaculture or brackishwater aquaculture, freshwater aquaculture), based on organisms used for aquaculture (shrimp culture, molluscan culture, finfish culture etc), based on structure used for aquaculture (cage culture, pen culture etc).

Shrimp is one of the most traded seafood commodities, and aquaculture of shrimp is considered to be a success story of modern aquaculture. Shrimps had been raised as an incidental crops in coastal ponds/or coastal low lying ecosystems including India. The advent of sophisticated refrigeration facilities provided by artisanal farmers access by international markets. Thus traditional coastal aquaculture shifted to an export oriented or industrialized aquaculture. Although many crustaceans attract lucrative markets, shrimp has become the single most successful crops, and mainstay of the brackishwater coastal aquaculture in India and mainly Asian countries. Farmed shrimp production has shown a remarkable growth during the last 25 years, from almost 50000 mt in 1990 to 600000 mt in 2016. The present lecture note is intended to provide a basic biological knowledge with regard to the shrimp farming

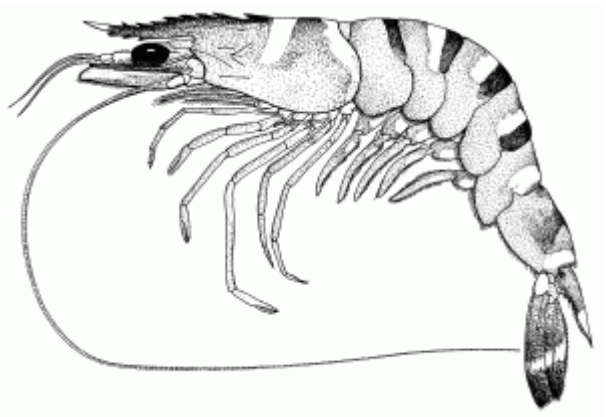
2.1 Biology of shrimp

Shrimp versus prawns: These two words are used synonymously in many literature, despite the consensus arrived at the world conference on biology and culture of shrimps and prawns held in Mexico City 1967 to restrict the term ‘prawn’ to freshwater forms and ‘shrimps’ to marine and brackishwater counterpart. There are no technical difference between shrimp and prawns.

Species used for culture: All the farmed shrimps used for the aquaculture belong to the genus *Penaeus*, and comes under the family penaeidae. The species coming under this family is generally called peaneids. Although there are 30 species are reported under the genus *Penaeus* only less than 10 species are used currently in aquaculture. Further among these species aquaculture production is dominated by two species: *Penaeus monodon* and *Penaeus vannamei*. Additionally at the Indian context, Indian white shrimp, *P. indicus* is designated as a priority species for further development of shrimp aquaculture.

***Penaeus monodon*:**

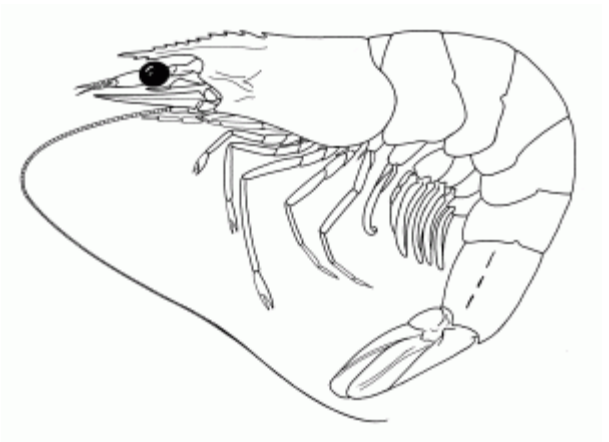
Penaeus monodon is found at depths from 0 to 110 m, inhabiting bottom mud and sand. Giant tiger prawn live in brackish, estuarine (juveniles) and marine (adults) environments. In its natural range, this species are found to occur in salinities 2 to 45 ppt and it can tolerate a temperature from 18 to 34. 5°C. In culture environment this species can be reared from 1 to 45 ppt. *Penaeus monodon* appears to select muddy mangrove channels and often associates with marginal or floating vegetation. This species can easily be identified by the transverse bands on the body with reddish brown flagella without bands.



Penaeus monodon

Penaeus vannamei

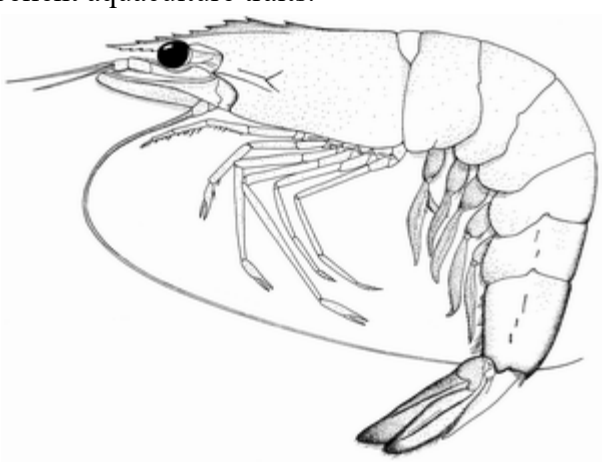
This species is a native to the Western Pacific tropical coast of Latin America from southern Mexico in the north to northern Peru in the south (Lat 32°N and 23°S). This penaeid is highly abundant along the coast of Ecuador to the Esmeraldas (the province of Columbia). Although this species was introduced into Asia from 1978 to 1979 experimentally, it was commercially introduced in 1996. India officially introduced this species in 2009, and since then the aquaculture production of shrimp grown exponentially. The commercial success of introducing *P. vannamei* into Asia can be attributed to its superior aquaculture traits compared with Penaeus monodon, the most popular cultured Asian penaeid. These include higher availability of genetically selected viral-pathogen-free domesticated broodstock, high larval survival, faster growth rate, better tolerance to high stocking density, lower dietary protein requirement, more efficient utilization of plant proteins in formulated diets, stronger adaptability to low salinity, better tolerance to ammonia and nitrite toxicity, and lower susceptibility to serious viral pathogens infecting *P. monodon.*



Penaeus vannamei

Penaeus indicus

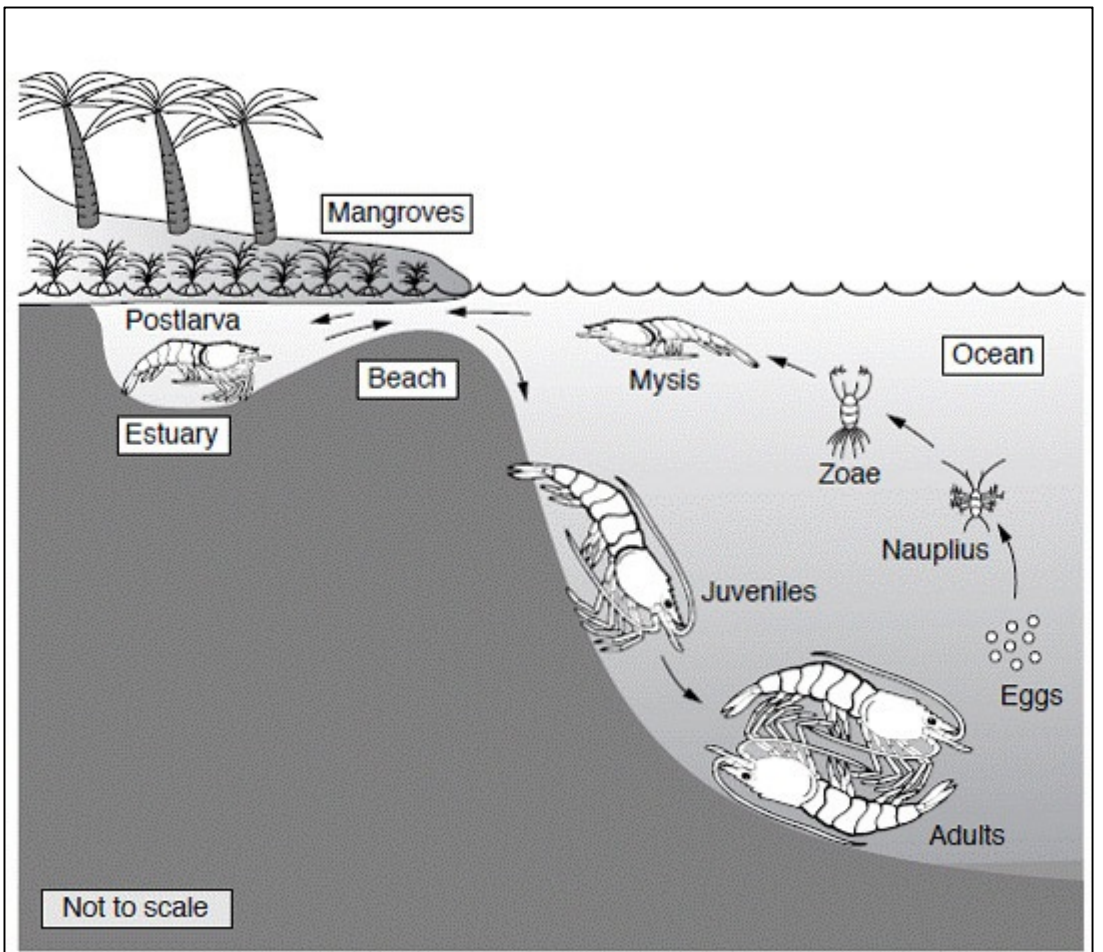
Penaeus indicus is widely known as Indian white shrimp, is found at depths of 2 to 90 m, inhabiting bottom mud or sand. It is most abundant in shallow waters of less than 30 m depth, on sand or mud. The adults are marine and breed offshore, while postlarvae and juveniles are estuarine. This species is second major species among native shrimps. This species has been identified as a priority species for domestication and genetic improvement owing to its ease in captive breeding and excellent aquaculture traits.



Penaeus indicus (FAO)

2.2 Life cycle of shrimp

All the penaeid shrimp shares a common life cycle. The adult lives and breeds in the sea, and completes the larval development in the sea. The post larvae drift towards the coast and enter into brackishwater creeks and estuaries, which provides shelter and food to them. They grow in this environment and after 4 to 6 months they migrate to sea, where they attain sexual maturity and spawn and complete life cycle.

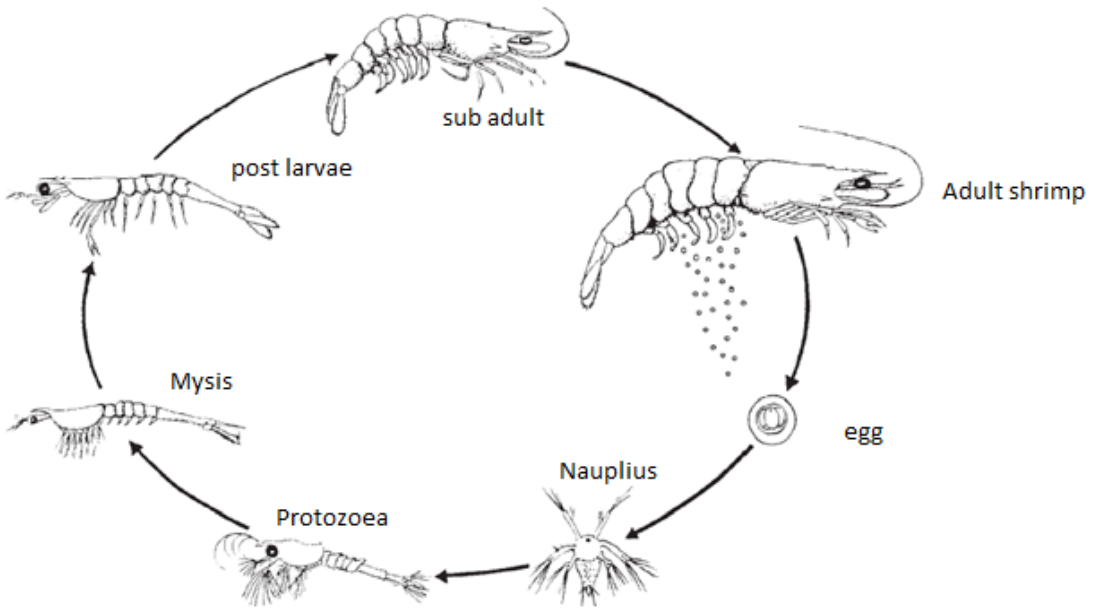


Penaeid life cycle (source: Rosenberry, 2009)

Adult females release the eggs directly in to the water, and sinks to the bottom of the sea and lie loose in the substratum. Eggs hatch within 8 to 10 hours, and newly release larvae are called Nauplius. The nauplius swim up wards, attracted by the light on the surface of the sea, this larval stage lacks mouth or alimentary canal, and survives by the maternal yolk present in the body. After 36 to 48 h this stage metamorphoses to the next larval stage called protozoea, after three to four days this stage metamorphoses to the next stage called mysis. Mysis metamorphose to post larvae after three to four days.

2.3 Food and feeding

Penaeid shrimps are generally omnivores feeding on small bottom living organisms and detritus that settle on the bottom.



2.4 Growth

As the body of the shrimp is covered with a chitinous shell, it can grow only after shedding the shell. The process of shedding the shell is called moulting, and shrimp grows by moulting periodically. The young shrimp moult more frequently than the larger shrimp. Therefore younger shrimp grows faster than the larger shrimps. The shrimp hardens one or two days after moulting. As the moulting is the directly linked to the shrimp growth it is most important aquaculture traits. When animals are infected or less fed or water quality is not conducible, animals fail to moult.

2.5 Hatchery production

Hatcheries are the place where animals are brought to the reproductive potential and spawned. The resulting young ones are raised to the point where they can stock into the grow-out ponds. Health and performance of farmed species largely depends on quality of juveniles stocked. Therefore hatchery production is fundamental to aquaculture. A shrimp hatchery generally has four units: Maturation section, Unit for micro algae, Unit for Artemia, and Larviculture section.

2.6 Glossary

Alga (plural: algae) - Primitive chlorophyll containing mainly aquatic eukaryotic organisms lacking true stems and roots and leaves.

Artemia - A small crustacean. At certain periods of the year, it produces cysts, metabolically inactive as long as they are kept dry, that float atthe water surface of saline waterbodies; upon immersion in seawater, these cysts hydrate and the embryo resumes its development. The cysts can be easily used as a source of live food for early stages of fish and crustaceans.

Broodstock - Adult animals of both sexes kept for the purpose of controlled reproduction.

Larva (Plural:Larvae) - An organism from the beginning of exogenous feeding to metamorphosis into juvenile. At the larval stage the animal differs greatly in appearance and behaviour from a juvenile or an adult.

Larviculture - The culture of larvae, usually in hatcheries.

Mysis - Pelagic larval stage of a crustacean intermediate between the protozoea (zoea) and postlarva stages.

Nauplius (pl. nauplii) - Earliest larval stage of a crustacean.

Postlarva (pl. postlarvae) - Stage occurring after the larval stage, resembling the juvenile but still lacking certain characteristics. For crustaceans: the stage following metamorphosis from larva (zoea) to juvenile. In penaeid shrimp, this is commonly counted in days after appearance of postlarval features, e.g. PL12 indicates a postlarva that has lived 12 days since its metamorphosis from the zoea stage of development.

Seed - A term used to describe eggs, larvae, post larvae, or juveniles stocked in to the aquaculture production systems.

Spawner - Mature individual of stock responsible for reproduction.

3. Water requirements for shrimp grow-out farms and hatcheries

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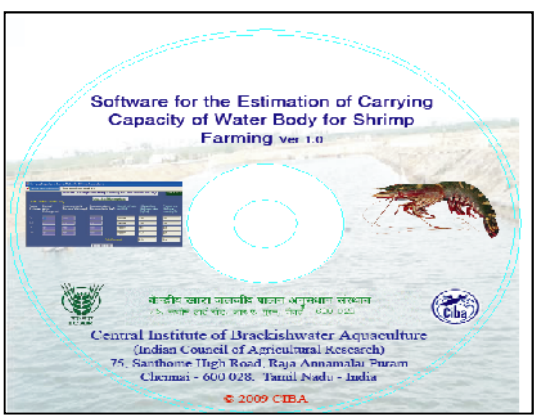
Marine shrimp are traditionally cultured in coastal or estuarine waters. However, inland culture is now being done in many countries. The Pacific white shrimp, *Penaeus vannamei* is found in waters with a wide salinity range (1 to 40 ppt). The high tolerance of *P. vannamei* to low salinity and the year-round availability of healthy post-larvae (PL) make this species an excellent candidate for inland farming.

The water quality variables affecting shrimp survival and growth are determining factors for disease outbreaks. Poor water chemistry leads to deteriorate water quality, which causes stress to the organisms being raised. Disease is an expression of a complex interaction between host (shrimp), pathogen (bacteria/virus) and environment (pond soil and water quality). Severe alterations in the culture environment deviated from the optimum pose stress on the system leading to reduced immune status of the shrimp to fight infections. This problem occurs when shrimp are farmed under poor pond conditions and generally disease will not occur when the culture environment (water and soil parameters) is maintained in optimum condition.

3.1 Suitability of sites for brackishwater aquaculture

The site suitability for shrimp aquaculture is very important aspect for long term sustainability. The Institute has surveyed many areas in different states based on the request by respective Governments or for private entrepreneurs based on the physical verification of sites, soil and water characteristics, hydrodynamic conditions such as tidal amplitude and water availability. Good site selection for shrimp farming operations requires planning for managing entire ecosystem to avoid problems when the numbers of ponds exceed the carrying capacity of the system. Recently carrying capacity estimation has been made mandatory in few countries for the optimisation of aquaculture development. CIBA has developed software for the estimation of carrying capacity of water bodies.



3.2 Shrimp farming under varying source waters

Shrimp species *P.monodon* and *P.vannamei* are being cultured by farmers in sea, brackish and fresh waters. Though high salinity and clear water with less plankton always causes shrimp stunt, but this high salinity water affects shrimp only at juvenile stage when they mainly consume zooplankton. Groundwater may differ significantly in terms of its relative

ionic composition compared to seawater. Most saline groundwater is deficient in potassium although other key ions such as sodium, chloride, calcium and magnesium can also vary considerably depending on the aquifer. The ionic composition of typical seawater, brackish water and freshwater is given in the Table 3.

Table 3. Concentrations of ions in different source water

Ion (ppm)	Sea water	Brackishwater	Freshwater
Chlorides	19000	12090	6
Sodium	10500	7745	8
Sulphate	2700	995	16
Mangnessium	1350	125	11
Calcium	400	308	42
Pottassium	380	75	2
Bicarbonate	142	156	174
Other	86	35	4
Total	34558	21529	263

The optimum range of water parameters is given in Table 4 and the importance of each parameter and effect on shrimp is described below.

Table 4. Optimum water quality parameters for brackishwater aquaculture

Parameters	Optimum range
Temperature (^{o}C)	28 - 32
pH	7.5 - 8.5
Salinity (ppt)	10 – 25
Transparency (cm)	30 – 40
Total suspended solids (ppm)	<100
Dissolved oxygen (ppm)	>3
Chemical oxygen demand (ppm)	<70
Biochemical oxygen demand (ppm)	<10
Total ammonia N (ppm)	< 1
Free ammonia N (ppm)	<0.1
Nitrite N (ppm)	<0.25
H_2S (ppm)	0.002
Nitrate N (ppm)	0.2 - 0.5
Phosphate (ppm)	0.1 - 0.2
Primary productivity (C/lit/day)	1.6-9.14
Plankton (No/lit)	3000-4500

3.3 Salinity

Salinity is the total concentration of all dissolved salts in water. The major ions in seawater (with a practical salinity of 35) are chloride, sodium, magnesium, sulfate, calcium, potassium, bicarbonate and bromine. Seawater has greater sodium and chloride concentration while freshwater usually has a higher bicarbonate ratio.

Total dissolved solids (TDS) combine the sum of all ion particles that are smaller than 2 microns (0.0002 cm). This includes all of the disassociated electrolytes that make up salinity concentrations, as well as other compounds such as dissolved organic matter. In clean water, TDS is approximately equal to salinity. In wastewaters, TDS can include organic solutes (such as hydrocarbons and urea) in addition to the salt ions. Depending on the ionic properties, excessive total dissolved solids can produce toxic effects on fish and fish eggs.

Salinity determines osmotic relationships and also the growth, reproduction and migratory behaviour of the animal as well as its general metabolism. In brackishwater ponds, the salinity of water varies with the salinity of the estuarine water supply. During the wet season, high discharges of fresh water from rivers into estuaries cause salinity values to decline, whereas low discharges of fresh water during the dry season resulting in higher salinities. Salinity range of 10 to 35 ppt with variations not exceeding 5 ppt is considered as optimum level for growth and proper metabolic processes of culture species and also helps in reducing stress on the animal.

At low saline condition, the salinity of shrimp body fluids is higher than the environment and the water in the environment will enter into the shrimp body so that the cell will swell. In this condition, the intake of essential minerals from the feed is low and it leads to the slow growth of shrimp. Molting in extremely high or low salinities may require more time and energy in normalizing hemolymph osmolality. Shrimp under varying salinities between 15 and 20 ppt will stimulate molting and consequently increase growth. Animals at low salinity are more sensitive to metabolites such as ammonia, nitrite and some water contaminants, including heavy metals and pesticides. It also increases shrimp oxygen consumption. As salinity changes, the deviation from the iso-osmotic point in the body fluid of an aquatic animal can cause adverse effects on the animal, such as low resistance to disease and the oxidative stress caused by the salinity enhanced reactive oxygen species (ROS). On the contrary, if the environmental salinity is higher than the salinity of shrimp body fluids, the water in the shrimp body will come out so that the shrimp become thin.

Under salinity stress, aquatic animals are forced to adapt to the changing environment through osmoregulation via the change of various enzymes and transporters, but the physiological

adaptations to these functional changes are highly energy demanding. Therefore, the provision of sufficient nutrients and energy through dietary manipulation is necessary to enhance the ability for shrimp to cope with low-salinity environments. Therefore, shrimp cultured under long-term stress at low salinity without any nutritional modulation in the feed are more susceptible to environmental stress and can cause slow growth and low survival.

3.4 Temperature

Temperature is one factor controlling the speed of biochemical reactions and regulating the activities of cultured animals. The temperature below and above the optimum range (28 to 32^{o}C) is known to weaken the immune status of the shrimp making it more susceptible to diseases due to *Vibrio*. In brackishwater shallow ponds, where regular exchange between the tidal water and the pond water is not maintained during the hot dry months, the temperature of pond water may shoot up beyond the tolerance limit causing mortality of reared shrimps. The variation in temperature is known to lower the levels of total haemocyte counts, phenol oxidase and respiratory burst in addition to reduction in the activity of superoxide dismutase (SOD) responsible for scavenging superoxide anion. It is common to expect outbreak of diseases when environmental temperatures go beyond the optimum range of culture shrimp species. If shrimp are infected, either as PL or older shrimp, they can survive reasonably well as long as the temperature remains above 30^{0} C. However, if the temperature drops below around 27^{0}C, mortality rates increase. Studies show that that the rate of mortality in shrimp infected with some virus diseases such as WSSV and TSV is affected by water temperature and had total crop failures unlike those who stocked later when the temperature was high and stable. Increase in temperature favours high rate of evaporation which increases the water salinity beyond the tolerance level. Similarly, during the winter season, the low temperature will have a chilling effect reducing metabolic and growth rates of cultured shrimps.

3.5 Dissolved Oxygen

Dissolved oxygen (DO) refers to the level of free, non-compound oxygen present in water. Non-compound oxygen or free oxygen (O_2) is oxygen that is not bonded to any other element. The bonded oxygen molecule in water (H_2O) is in a compound and does not count toward dissolved oxygen levels. Oxygen concentration in water is expressed as parts per million (ppm), which is equivalent to mg/L, or as a percent of saturation value for that temperature and pressure. DO is necessary to many forms of life including fish, invertebrates, bacteria and plants. DO in water is utilised by an aquatic organism to hold metabolism and is excreted as carbon dioxide (CO_2). Animals require oxygen for respiration, which physiologists express as mg of oxygen consumed per kilogram of animal per hour (mg O2 /kg/h). The respiratory rate increases with increasing temperature, activity, and following feeding. Bottom feeders, crabs, oysters and worms need minimal amounts of oxygen (1-6 mg/L), while shallow water fish need

higher levels (4-15 mg/L). A dissolved oxygen level that is too high or too low can harm aquatic life and affect water quality.

In ponds, there are three main sources of oxygen: 1) direct diffusion from the atmosphere; 2) wind and wave action; and 3) photosynthesis. Of these, photosynthesis by aquatic plants and phytoplankton is the most important. The availability of dissolved oxygen frequently limits the activities and growth of aquatic animals.

Photosynthesis:

$$6CO_2 + 6H_2O + \text{light energy} \rightarrow C_6H_{12}O_6 + 6O_2$$

Respiration:

$$C_6H_{12}O_6 + 6O_2 \rightarrow 6CO_2 + 6H_2O + \text{heat energy}$$

Oxygen, derived from photosynthesis, is produced during the day when sunlight shines on the plants in the water. Oxygen levels drop at night because of respiration by plants and animals. These predictable changes in DO that occur every 24 hours are called the diurnal oxygen cycle.

Microbes such as bacteria and fungi also require dissolved oxygen. These organisms use DO to decompose organic material at the bottom of a body of water. However, if there is an excess of decaying organic material in a body of water with infrequent or no turnover, the oxygen at lower water levels will get used up quicker.

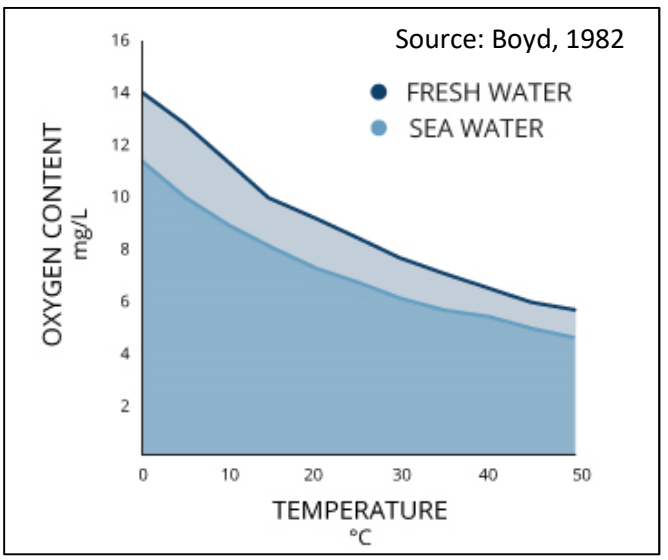
Factors affecting DO concentration

- Two water bodies, both 100% air-saturated do not necessarily have the same concentration of dissolved oxygen. The actual amount of dissolved oxygen (in mg/L) will vary depending on temperature, pressure and salinity.
  - The solubility of oxygen decreases as temperature increases. This means that warmer surface water requires less dissolved oxygen to reach 100% air saturation than deeper and cooler water. For example, water with a temperature of 32°C can hold up to 7.3 mg/L of oxygen, while 7°C water can hold 12.1 mg/L. As water temperature rise, oxygen levels decrease. Higher temperatures also increase the metabolic rate of animal resulting in the need for more oxygen.

    Oxygen depletion usually occurs in the summer months because warmer water holds less oxygen than cooler water. During cloudy weather, the intensity of light reaching surface waters is greatly diminished, resulting in a marked decrease in oxygen production from photosynthesis. Oxygen consumption, however, remains unchanged. This results in a net

depletion of oxygen. Oxygen transfer (from the atmosphere into the water) is minimal because there is little or no wind/wave action. The net result over a period of several days is oxygen depletion.

- Solubility of oxygen decreases with the increase in salinity of the water. Freshwater hold more DO compared to brackishwater and marine water. At the same pressure and temperature, saltwater holds about 20% less dissolved oxygen than freshwater.
- Dissolved oxygen concentrations decrease as pressure decreases (altitude increases). This is true of both atmospheric and hydrostatic pressures. Water at lower altitudes can hold more dissolved oxygen than water at higher altitudes. Gas saturation decreases by 10% per meter increase in depth due to hydrostatic pressure. This means that if the concentration of dissolved oxygen is at 100% air saturation at the surface, it would only be at 70% air saturation three meters below the surface.

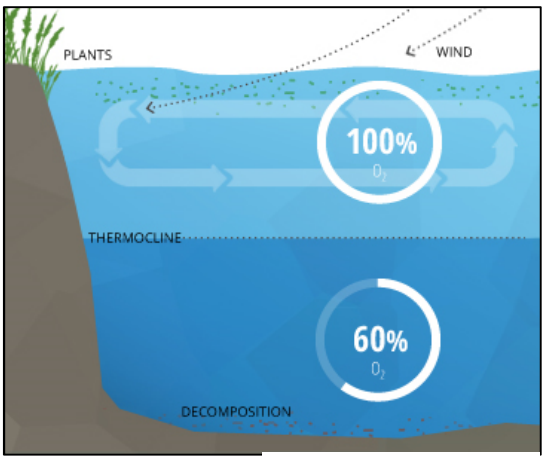


- Oxygen concentration decreases depending on the depth of water. If the pond is shallow in nature oxygen easily diffuse into the water and if depth is more, diffused oxygen will not reach the bottom results in low DO.
- Oxygen production in the pond is considerably limited when a plankton die-off occurs or when there are high nutrient loads, large quantities of feed and faecal wastes are found on the pond bottom. Decomposition of accumulated feed and the animal faeces lead to hypoxic and sometime anoxic conditions particularly at night time.
- Oxygen concentration varies within a day and it is known as diurnal change. The DO levels used to be highest in the afternoon around 2-3 ppm due to maximum photosynthesis under high temperature.

Dissolved Oxygen Saturation

In a stable body of water with no stratification, dissolved oxygen will remain at 100% air saturation. In a pond not all water depths reach 100% air saturation. The water will slowly absorb oxygen and other gasses from the atmosphere until it reaches equilibrium at complete saturation. During hot weather, surface waters warm up more rapidly than deeper waters. As the difference in temperature increases between warm surface water and cool bottom water, a

thermocline develops. When a thermocline is present there is no mixing of surface and deep layers of water. As photosynthesis and oxygen production only occur near the surface, water in the deep layer becomes devoid of oxygen and develops an oxygen demand. In deeper waters, DO remains below 100% due to the respiration of aquatic organisms and microbial decomposition. If there is a significant occurrence of photosynthesis or a rapid temperature change, the water can achieve DO levels over 100% air saturation. At these levels, the dissolved oxygen will dissipate into the surrounding water and air until it levels out at 100%. 100% air saturation is the equilibrium point for gases in water. As oxygen in the atmosphere is about 20.3%, the partial pressure of oxygen at sea level (1 atm) is 0.203 atm. The amount of dissolved oxygen at 100% saturation, sea level and 20° C is 9.03 mg/L.



Source: Boyd, 1982

Effect of low DO

In intensive aquaculture practices, dissolved oxygen (DO) is a major limiting factor especially in the bottom layers of shrimp culture ponds. Changes in the oxidation state of substances from the oxidised to the reduced form can be caused by low levels of dissolved oxygen in the pond environment. The concentration of toxic substances such as un-ionised NH_3, hydrogen sulphide and carbon metabolites (methane) increases when low DO level exists.

If DO concentrations are consistently low, aquatic animals will not eat or grow well and will be susceptible to infectious disease. If concentrations fall to very low levels, the animals may die. DO less than 2.8 mg/l is considered hypoxic condition and it is known to influence growth, survival, feeding, moulting, behaviour, osmoregulatory capacity and immune response of penaeid shrimps. Though lethal DO levels vary from species to species, generally DO of 0.2 to 1.27 mg/l for *P. monodon* while 1 mg/l for *P. vannamei* is considered lethal after one hour of exposure. At the optimum salinity of 15-25 ppt tiger shrimp have better resistance to low DO toxicity than the in the higher or lower salinity ranges. Hence, factors like, body weight, temperature, salinity, pH, and feeding condition have significant effect on ability of shrimp to resist different lethal DO levels.

3.6 Turbidity, total suspended solids and transparency

Turbidity

Turbidity is an optical property of water which describes the cloudiness or muddiness of water.

Turbidity measurements are often used as an indicator of water quality based on clarity and estimated total suspended solids in water. Turbidity of water arises from both biotic and abiotic factors such as plankton, dissolved organic substances, suspended sediment such as silt or clay and solid particles. Turbidity due to plankton is desirable but turbidity arises from soil particles is undesirable. Turbidity reduces the light penetration and thereby affects the photosynthesis and productivity of the ponds. High turbidity may cause temperature and dissolved oxygen stratification in aquaculture pond.

Transparency (water clarity)

It reflects the type and density of plankton. The more intense colour of water signifies the more number of existing plankton. Too high plankton density may affect fluctuations in dissolved oxygen and pH in the pond. Transparency must be maintained at a level of 30-40 cm. Flocculation and turning water milkfish colour with little or no primary productivity and excessive amount of foam are the causes for slow shrimp growth. Water clarity is a physical characteristic defined by how clear or transparent water is. Clarity is determined by the depth that sunlight penetrates in water. Water clarity is directly related to turbidity, as turbidity is a measure of water clarity. The transparency of water is affected by the amount of sunlight available, suspended particles in the water column and dissolved solids such as colored dissolved organic material (CDOM) present in the water. Salinity also affects water clarity. This is due to the effect of salt on the aggregation and settling velocity of suspended particles.

Plankton turbidity: Turbidity arises from plankton population is measured in situ in terms of transparency using Secchi disc. The optimum range of transparency is 25–50 cm. High value of transparency (>60 cm) is indicative of poor plankton density and low value (<20 cm) indicates high density of plankton. High plankton density may affect fluctuations in dissolved oxygen and pH in the pond.

Clay Turbidity: Clay turbidity reduces the magnitude of daily fluctuations in DO concentration, so that it gets neither very high nor very low. However, muddy water tends to have a lower average concentration of dissolved oxygen than water with a green phytoplankton bloom. Clay turbidity which restricts the visibility to less than 30 cm hinders the phytoplankton growth.

Due to the extremely smaller size, clay particles have extremely high surface area relative to the volume of the particle and remain suspended in water. Clay particles are negatively charged and attract positively charged cations. These clouds of cations surround the negatively charged clay particles. Clay particles along with their cloud of ions get repulsed upon coming closer to each other. The cumulative effect of the repulsion between these small particles prevents aggregation in to large molecules and remains in suspension.

Total suspended solids (TSS)

TSS is particles that are larger than 2 microns found in the water column. Anything smaller than 2 microns (average filter size) is considered a dissolved solid. Most suspended solids are made up of inorganic materials, though bacteria and algae can also contribute to the total solids concentration. These solids include anything drifting or floating in the water, from sediment, silt, and sand to plankton and algae. Organic particles from decomposing materials can also contribute to the TSS concentration. As algae, plants and animals decay, the decomposition process allows small organic particles to break away and enter the water column as suspended solids. Even chemical precipitates are considered a form of suspended solids. Total suspended solids are a significant factor in observing water clarity. The more solids present in the water, the less clear the water will be.

Turbidity vs Suspended Solids

Turbidity and total suspended solids refer to particles present in the water column. Turbidity and water clarity are both visual properties of water based on light scattering and attenuation. All three parameters are related to particles in the water column, whether directly or indirectly.

In terms of water quality, high levels of TSS will increase water temperatures and decrease DO levels. This is because suspended particles absorb more heat from solar radiation than water molecules. This heat is then transferred to the surrounding water by conduction. Warmer water cannot hold as much DO as colder water, so DO levels will drop. In addition, when water stratifies, the upper and lower layers do not mix. As decomposition and respiration often occur in the lower layers, they can become too hypoxic (low DO levels) for organisms to survive. Turbidity can also inhibit photosynthesis by blocking sunlight. The higher the turbidity levels, the less light that can reach the lower levels of water and low plant productivity.

3.7 pH

Water pH is one of the most critical chemical parameters for shrimp farming. It is determined by interactions among dissolved CO_2, carbonic acid, bicarbonate, carbonate and carbonate containing minerals. pH levels of the pond water will change depending on the aquatic life within the pond. Carbon dioxide produced by aquatic organisms during respiration has an acidic reaction in the water. The pH decreases at night because of respiration and production of CO_2 by all organisms. The pH in ponds will rise during the day as phytoplankton and other aquatic plants remove CO_2 from the water during photosynthesis. In intensive aquaculture ponds pH fluctuate between 6.6 and 10.2 with the accumulation of residual feed, dead algae and excreta over a 24 hour period with lowest pH occurring near dawn and the highest pH occurring in the afternoon. Waters of moderate alkalinity are more buffered and the degree of pH fluctuation is lower than low alkaline water.

Factors affecting the pH of water in shrimp ponds

- Acid sulfate soil (acidic alum soil, acidic soil)
- Acidic source of water
- Rate of rainfalls in pond areas
- Poorly buffered water
- Stocking density of shrimps
- Feeding & rate of sludge formation in pond bottom.
- Presence of micro/ macro organisms.
- Existence of phytoplankton and rate of carbon dioxide production in pond water
- Quantity of respire by aquatic species in the pond water.

Carbon dioxide is the most common cause of acidity in water. Photosynthesis, respiration and decomposition contribute to pH fluctuations due to their influences on CO_2 levels. The extremity of these changes depends on the alkalinity of the water, but there are often noticeable in diurnal variations. Carbon dioxide exists in water in a dissolved state and can also react with water to form carbonic acid, which can then lose one or both of its hydrogen ions.

$CO_2 + H_2O <=> H_2CO_3$
$H_2CO_3 <=> HCO_3^- + H^+ \ldots. HCO_3^- <=> CO_3^{2-} + H^+$

The released hydrogen ions decrease the pH of water. However, this equation can operate in both directions. At a higher pH, bicarbonate system will shift to the left, and CO_3^{2-} will pick up a free hydrogen ion. This reaction is usually minimal as H_2CO_3 has a low solubility constant. However, with the increase in CO_2 levels d the equation will be carried out from left to right, increases H_2CO_3, which decreases pH.

Impact of changes in pH on shrimp

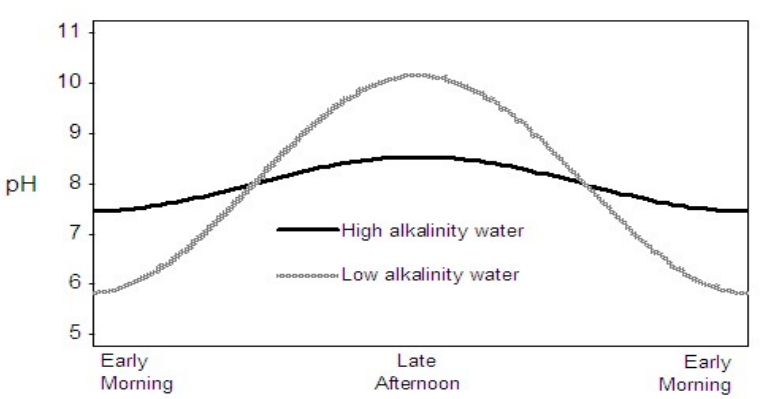
The pH of brackishwater is usually not a direct threat to the health of the aquatic animal, since it is well buffered against pH changes. Calcium is a particularly important modulator of pH toxicity because calcium affects the permeability and stability of biological membranes. The optimum level of pH is between 7.5 and 8.5. For the best water quality, the maximum diurnal pH fluctuation should not exceed 0.5. Below and above this pH range, there will be reduction in total haemocyte counts, granulocyte counts, respiratory burst, SOD activity, phagocytic activities and clearance efficiencies leading to increase susceptibility to infections especially vibriosis. Sub-optimal pH has adverse effects on shrimps. If pH changes significantly, shrimp gets shocked, weakened and stops eating. If high or low pH extends for a long time, shrimp grow slowly, stunting growth and susceptible to diseases. It can cause stress, fewer survivals, low production and leads to poor growth.

The low pH levels will cause the shell of shrimp to become soft. This is due to the calcium carbonate in the shell of the shrimp reacts with acid. Low pH increases nitrite toxicity and also the fraction of H_2S (toxic form). The proportion of total ammonia existing in the toxic, un-ionized form (NH_3) increases as the pH increases. For example, the toxicity of ammonia is ten times more severe at a pH of 8 than it is at pH 7. Whereas in waters with low pH will cause an increase in the fraction of anionic sulphide (H_2S) and the toxicity of nitrite, as well as physiological disorders in shrimp.

3.8 Total alkalinity

Alkalinity is the water's ability to neutralize acid without changing the pH and is a measure of the total concentration of bases (bicarbonates, carbonates, phosphates, hydroxides) in pond water predominantly bicarbonate and carbonate. The alkalinity of pond water is determined by the quality of the water supply and nature of pond bottom soils. It is the capacity of water to buffer against wide swings in pH and enhanced natural fertility of water. Waters with high alkalinities generally have a greater complement of most ions than water of low alkalinity; it also increases pH that favours rapid decomposition of organic matter by organisms. In addition to that phosphorus availability will be increased by increasing total alkalinity by the addition of lime, which helps for phytoplankton growth. Ponds with a total alkalinity of 20-150 ppm have sufficient supply of CO_2 for phytoplankton growth and it may improve productivity. In addition it decreases the potential for metal toxicity.

Though alkalinity and pH are closely related, there are distinct differences. The alkalinity of water or a solution is the quantitative capacity of that solution to buffer or neutralize an acid. In other words, alkalinity is a measurement of water's ability to resist changes in pH. This term is used interchangeably with acid-neutralizing capacity. Due to the presence of carbonates, alkalinity is more closely related to hardness than to pH, though there are still distinct differences. However, changes in pH can also affect alkalinity levels, as pH lowers, the buffering capacity of water lowers as well. pH and alkalinity are directly related when water is at 100% air saturation.



Changes in pH during a 24-hour period in waters of high and low total alkalinities (Wurts and Durborow, 1992)

Alkalinity between 75 to 200 mg L^{-1}, but not less than 20 mg L^{-1} is ideal in an aquaculture pond. But it may vary based on the animal size. Newly released shrimp requires 100-120 ppm; 45 to 90 days shrimp requires 120-150 ppm and 90 days to older shrimp requires 150-200 ppm. More than 300 ppm alkalinity is undesirable due to non- availability of CO_2. If the alkalinity is less than 20 mg L^{-1} pond water pH can swing widely during the day, measuring from 6 to 10.

During the culture, alkalinity is naturally decreased over time through bacterial action which produces acidic compounds that combine with and reduce the alkalinity components. Especially in lined ponds, when ammoniacal nitrogen in the pond water is oxidized by nitrifying bacteria creating acidity, alkalinity declines because there is no input of replacement of alkalinity in incoming water or from the dissolution of carbonate minerals in pond bottoms. Hence supplements should be given to maintaining an acceptable level.

3.9 Hardness

Total hardness is a measure of the concentration of all metal cations with the exception of alkali metals. Water hardness is important in aquaculture and is a commonly reported aspect of water quality. Much of the concern about hardness in water treatment is with all the ions involved, in aquaculture the concern is mostly with calcium and magnesium concentration. There are many different divalent salts; however, calcium and magnesium are the most common sources of water hardness. Another important aspect of hardness is its effect on pH- soft water has low pH and hard water has high pH.

A desirable range would be between 75 to 200 mg/L $CaCO_3$. A low level of hardness (10-20 mg/L $CaCO_3$) can cause stress to animals. A high level of hardness is not desirable as it increases the pH and reduces the availability of other nutrients. Euryhaline species have high tolerance limit to hardness.

An important impact of hardness on aquatic life is the effect this has on the presence of metals as cadmium, lead, chromium and zinc. The toxicity of these metals decreases with hardness as they form insoluble precipitates and settle at the bottom and become unavailable to the aquatic organisms. A low $CaCO_3$ hardness value is a reliable indication that the calcium concentration is low. However, high hardness does not necessarily reflect a high calcium concentration. A high hardness reading could result from high magnesium concentrations with little or no calcium present.

3.10 Minerals

Minerals are important for the growth and metabolism of animals. Calcium and magnesium are essential in the biological processes of fish-bone and scale formation, blood clotting and other

metabolic reactions. Calcium is important in the moulting process of shrimp and other crustaceans and plays a major role in hardening of the shell. Crustaceans absorb calcium from the water when moulting, and if the water is too soft their exoskeletons begin to soften and they may cease to moult. In addition, bone deformities and reduced growth rates may result if water is too soft. Calcium reduces the toxicity of metals, ammonia, and the hydrogen ion. Major ion deficiencies can have serious physiological consequences ranging from stunted or poor growth through to asphyxiation, oedema and death. Potassium has an essential role in regulating sodium and therefore fluid balance within the haemolymph. Hence there is a need to supplement potassium as and when required.

Aquatic organisms can absorb calcium and magnesium directly from the water or from food. The presence of free calcium at relatively high concentrations in culture water helps reduce the loss of other salts (e.g. sodium and potassium) from body fluids. Sodium and potassium are the most important salts in hemolymph and are critical for normal heart, nerve and muscle function. In low calcium water, fish can lose substantial quantities of these salts into the water. The ratio of Na to K and Ca to Mg in the water are highly important for survival, growth and production rather than salinity. The ratio of minerals should be maintained similar to the ratio of sea water.

3.11 Iron

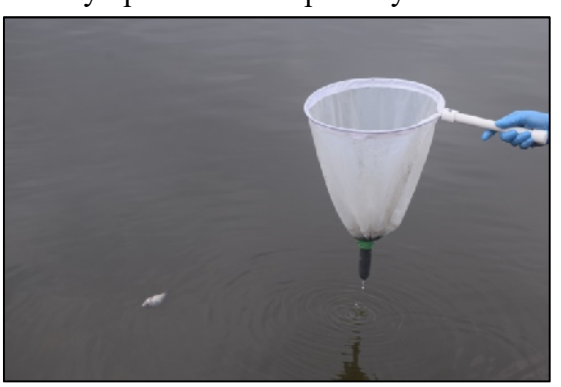
Iron is an essential element for bacteria, plants, and animals. Many enzymes important in energy transformations contain iron. Iron forms the centre of the hemoglobin molecule, important in oxygen transport in the blood of vertebrate and some invertebrate animals. Iron exists in two forms, soluble ferrous iron and insoluble ferric particulate iron. In most aquaculture systems there will be a high oxygen concentration, and all iron present in the water will be in the form of insoluble ferric Fe^{3+}. Ferric iron as a chemical though nontoxic, may exert a pathological response. The solubility of iron in water is governed mainly by pH. The concentration of ferric iron will seldom exceed 2 mg/L unless the pH is below 4. Nevertheless, freshwaters may contain up to 1 mg/L or more of dissolved iron, because iron forms soluble hydroxides and ion pairs, and it also forms soluble complexes with dissolved organic matter (chelated iron). Circumstances where reversible oxidation and reduction prevails, as in shrimp ponds constructed on acid sulphate soils, hydrated iron oxide, is expected to be the dominant form of iron and soluble manganese is presumed to be the most stable form of manganese.

In many aquaculture systems, the presence of iron at concentrations above 0.1mg/l, will damage the gills of the fish. The gills of the fish are in effect acting as a mechanical filter, and small particles of iron with dimensions of a few microns are becoming trapped in the gill

lamella. A brown or black discoloration of the gills is the most common symptom, which in severe condition can lead to death, due to asphyxiation. The presence of the small iron particles causes irritation of the gill tissues leading to gill damage and secondary bacterial and fungal infections. Iron encrustation on the cuticle of shrimp has been reported in the acidic culture condition. Iron acts like a catalysts in water, and will promote the dissociation of oxygen molecules in water to form free radicals. The free radicals are extremely reactive and short lived. If the levels are above 0.1mg/l (especially in very clean water with low organic concentrations) then there will usually be gill damage.

3.12 Phytoplankton and primary productivity

Phytoplankton is made up of single-celled algae and cyanobacteria. As algae can be single-celled, filamentous or plant-like, they are often difficult to classify. Most organizations group algae by their primary color (green, red, or brown), though this creates more problems. The various species of algae are vastly different from each other, not only in pigmentation, but in cellular structure, complexity, and chosen environment. Phytoplanktons are photosynthetic and thus have the ability to use sunlight to convert carbon dioxide and water into energy. Phytoplankton can be divided into two classes, algae and cyanobacteria. These two classes have the common ability of photosynthesis, but have different physical structures. Regardless of their taxonomy, all phytoplankton contain at least one form of chlorophyll (chlorophyll A) and thus can conduct photosynthesis for energy.



Phytoplankton, both algae and cyanobacteria, can be found in fresh or saltwater. As they need light to photosynthesize, phytoplankton in any environment will float near the top of the water, where sunlight reaches. Most freshwater phytoplankton is made up of green algae and cyanobacteria, also known as blue-green algae. Marine phytoplanktons are mainly comprised of microalgae known as dinoflagellates and diatoms, though other algae and cyanobacteria can be present. Light appears to be limiting phytoplankton growth and it is suggested that a shallower pond depth and/or higher turbidity would increase algal productivity. Changes in nitrogen-to-phosphorus ratios and ammonia concentrations coincided with changes in phytoplankton community structure.

Primary productivity is directly related to the temperature and the available nutrients in water and soil. Among the different type of nutrients, nitrogen, phosphorus and potassium are the essential pre-requisite for productivity of any aquatic system. Nutrient limitation of phyto

plankton communities may change depending on salinity levels. The availability of different forms of nitrogen and their relative rates of utilization are important factors contributing to the relative success and productivity of different phytoplankton. Typically, fast-growing diatoms have been found to be highly correlated with large and/or frequent additions of NO_3^-. By contrast, microflagellates (including dinoflagellates) have been correlated with low nitrate concentrations and high rates of NH^{4+} or dissolved organic nitrogen (DON) supply. Phosphorus regulates the phytoplankton production in the presence of nitrogen.

3.13 Metabolites

Unfortunately a single metabolite may not be responsible for retarded growth or mortality of shrimp in ponds. It is essential to study at what level of toxicity shrimp can tolerate under combinations of two or more metabolites (ammonia, nitrite, sulphide). Another major consequence of aquaculture production is a high degree of variability in the concentration of dissolved nitrates, nitrites and ammonia. High feeding rates observed in prawn farms lead to eutrophic conditions characterised by substantial phytoplankton blooms, which ultimately senesce and cause rapid increasing in ammonia levels in the ponds. The environmental conditions that create high ammonia concentrations may also cause increases in nitrite concentration. Both ammonia and nitrite can be directly toxic to culture organisms or can induce to sub lethal stress in culture populations that results in lowered resistance to diseases.

TAN (Total Ammonia Nitrogen)

The concentration of total ammonia nitrogen (TAN) in intensive grow-out ponds increases as culture progress and levels of more than 1.0 ppm are toxic. Ammonia is present in water in two forms, a toxic un-ionized ammonia (NH_4^+) form and a non-toxic ionized ammonia (NH_3) form, The relative amounts of these are dependent on the pH of water and to a lesser extent on water temperature. The percentage of the toxic form increases as pH and temperature rise during the day and can reach critical levels. Hence, it is necessary to know the pH of the pond water and use conversion tables to estimate the level of un-ionised ammonia in the pond. In addition to immune response, elevated concentration of TAN affects the growth, moulting, oxygen consumption and ammonia excretion. Continuous exposure of shrimp to ammonia leads to reduced phenol oxidase activity without affecting the number of circulating haemocytes. Increased concentration of TAN decreases the activity of superoxide dismutase responsible for the scavenging of reactive oxygen species (ROS) leading to increase in superoxide anion. Reduced phagocytic activity and clearance efficiency leads to increased susceptibility to vibrio bacterial infections. Shrimp growth and survival can be reduced with long-term exposure to un-ionised ammonia at 0.1ppm and short term exposure to as low as 0.4 ppm. Level of ammonia excretion by shrimp is altered by environmental factors like temperature, salinity and dissolved oxygen.

Nitrite

Nitrite (NO_2) is the intermediate product of bacteria mediated conversion of ammonia to nitrate. Imbalance in levels of denitrifying and nitrifying bacteria leads to accumulation of nitrite. Among the metabolic toxicants nitrite is considered most dangerous as it can accumulate in haemolymph up to 10 fold higher than in water via active chloride uptake mechanism and passive entry. Increased concentration of nitrite in haemolymph leads to reduced levels of oxyhaemocyanin and increased deoxyhemocyanin. In addition to extracellular fluids, nitrite accumulates in gill, liver, brain and muscle tissue. The higher concentration of the nitrite is known to decrease the levels of total haemocyte counts to the reduced Prophenoloxidase and phagocytositic activities. Further, there is a reduction in superoxide dismutase activity consequently increasing the levels of cytotoxic superoxide anions. Shrimps when exposed to higher concentration of nitrite, increase oxygen consumption and ammonia excretion indicating increase of energy and protein catabolism and ultimately has adverse impact on growth and moulting. Nitrite is more toxic in low saline conditions compared to brackish and seawater based culture ponds.

Hydrogen sulphide

Under anaerobic condition, certain heterotrophic bacteria can use sulphate and other oxidized sulphur compounds as terminal electron acceptors in metabolism and excrete sulphide. Sulphide is an ionization product of hydrogen sulphide and pH regulates the distribution of total sulphide among its forms (H_2S, HS- and S^{2-}). Un-ionized hydrogen sulphide is toxic to aquatic organisms. Concentration of 0.01 to 0.05 mg/l of H_2S may be lethal to aquatic organisms and any detectable concentration is undesirable. Presence of sulphide affects the immune parameters like total haemocyte count, hyaline cells, phenol oxidase activity, phagocytic activity and clearance efficiency thereby making the shrimp more susceptible to pathogenic infections like, vibriosis.

3.14 Water requirements for shrimp hatchery

One of the most important aspects with respect to both location and functionality of shrimp hatchery is the quality of water. Good quality water indicates the water capable of supporting the desired species. A thorough knowledge on the water quality requirement (Table 5) of the candidate species as well as the water quality management techniques is the essential tool for the successful hatchery operation. The most important criterion for selection of site for a penaeid hatchery is the availability of clean, clear and pristine quality seawater. The objective is to reproduce the near constant condition found in the deeper ocean where shrimp breeds and completes the larval phase of the life cycle.

Table 5. Suggested water quality criteria for penaeid shrimp hatchery

Parameter	Nauplii	Protozoea	Mysis	Post larvae
Ammonia(NH_3-N) (µg/l or ppb)	10	17	48	100
Nitrite(NO_2-N) (mg/l)	0.11	0.29	0.45	1.36
Nitrate(NO_3–N) (mg/l)	-	-	-	<200
Dissolved Oxygen (%)	----------	----------	>95	----------
H_2S (µg/l)	----------	----------	<2	----------
Chlorine residue (µg/l)	----------	----------	<10	----------
pH	----------	----------	7.9-8.2	----------
Temperature (°C)	----------	----------	28-32	----------
Salinity ppt	----------	----------	28-34	----------
Metals				
Cadmium(µg/l)	----------	----------	<5.0	----------
Chromium(µg/l)	----------	----------	<25	----------
Copper(µg/l)	----------	----------	<3	----------
Iron(µg/l)	----------	----------	<300	----------
Mercury(µg/l)	----------	----------	<0.1	----------
Manganese(µg/l)	----------	----------	<50	----------
Nickel(µg/l)	----------	----------	<50	----------
Lead (µg/l)	----------	----------	<50	----------
Zinc(µg/l)	----------	----------	<50	----------
Pesticides				
Aldrin/Dieldrin(µg/l)	----------	----------	<0.003	----------
BHC(µg/l)	----------	----------	<4	----------
DDT(µg/l)	----------	----------	<0.001	----------
Endrin(µg/l)	----------	----------	<0.004	----------

4. Soil requirements for shrimp aquaculture

P. Kumararaja, M. Muralidhar, R. Saraswathy and S. Suvana

The nature of soil affects the shrimp production and hence one should have well acquaintance with the properties of soil. In India, aquaculture ponds are located under different agro-climatic conditions. The soils are classified mainly under eight major heads: alluvial, black, red, laterite, forests, desert, saline and alkaline and peat in India. The brackish water aquaculture is done on salt affected soils or coastal soils. Generally acidic soil and acid sulphate can cause low pH and high sulphide production respectively, unless proper management practices is followed, these soils are not suitable for aquaculture.

4.1 Soil type

The brackish water aquaculture is done on salt affected soils or coastal soils.

Saline soil

Saline soils are also called "white alkali" soils Saline soils are classified as saline if the EC exceeds 4 or more mmhos/cm at 25^0 C, exchangeable sodium < 15% and pH < 8.5. These soils usually have white crust upon drying.

Alkali soil

Alkali soils are often called "black alkali" soils. These soils have high sodium content causing dispersion of organic matter. The solution extracted from the saturation paste have an EC > 4 mmhos/cm at 25^0 C, exchangeable sodium >15 % and pH between 8.5 and 10.0.

The brackishwater aquaculture is practiced enormously where the above mentioned soils are located. The soluble salts, measured as electrical conductivity (EC) consists of cations like Ca^{2+}, Mg^{2+}, Na^{+}, K^{+}, and anions like CO_3^{2-}, HCO_3^{-}, Cl^{-} and SO_4^{2-}. The total salt affected area in India is about 8 million ha, of which 0.5 million ha is mangrove area and 3.1 million ha coastal.

Acid sulphate soil (ASS)

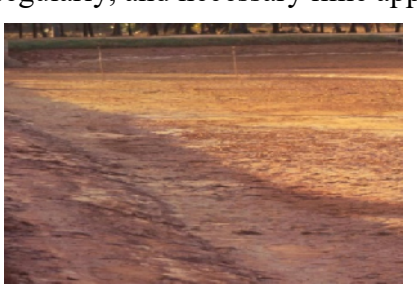
Acid sulphate soils are found extensively on the coastal plains of tropics. India has more than 2 million ha of acid sulphate soils, which poses a potential threat for the long term production if excavated. Acid sulphate soils contain oxidisable or already oxidised sulphides. The principal form of sulphides is iron pyrites, along with other forms like monosulphides in smaller concentrations. When sea level rises and inundates ponds, sulphate in sea water mixes with the land sediments containing iron oxides and organic matter. The resulting chemical reactions

produce large quantities of iron sulphides in waterlogged sediments. The conditions necessary for the formation of sulphides in coastal sediments are:

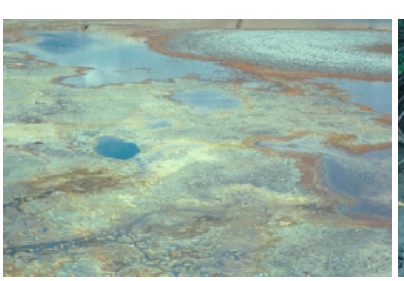
- Supply of sulphate
- Supply of easily decomposable organic matter
- Adequate source of iron
- Anaerobic condition coupled with chemically reducing microbes
- Tidal action

A potential acid sulphate soil (PASS) is one which remains in reduced condition and no oxidation of sulphide occurs, whereas, actual ASS (AASS) or sulphuric soil (pH 3.8) is one which get exposed to air and undergoes oxidation. Oxidising conditions frequently overlie reducing conditions in the same profile, so AASS and PASS occur in different parts of the same profile. The formation of ASS can be avoided by following correct pond preparations. Excessive turning over of pond bottom should be avoided, as this will expose sites of PASS to become AASS. However, rapid reclamation of ASS can be done as follows:

- In the early part of dry season, dry the ponds, harrow them thoroughly and fill with brackishwater. Measure the pH of water as the pH will drop below 4 initially. Once the pH stabilizes, drain the pond and repeat until the pH stabilizes above 5. Usually, three or more drying and filling cycles will be required.
- At the same time, when the pond is being reclaimed, acid must be removed from the surrounding levees. To achieve this, level the levee tops and build small bunds along each side of levee tops to produce shallow basins. Fill the basin with backwater. When the pond is drained for drying, also drain the small basins on the levee tops for drying. Repeat the process if required. Finally remove the bunds and broadcast lime over the tops and sides of levees at 0.5 and 1.0 kg/ m^2.once the last drying and refilling cycle is over, broadcast $CaCO_3$ over the pond bottom at 500 kg/ha. To prevent shrimp mortality, pH has to be monitored regularly, and necessary lime application is to be done.



Sandy soil



Acid sulphate soil

Mangrove soil

Soils not recommended

4.2 Soil texture

Soil texture refers to the relative percentage of sand, silt and clay in the soil and has direct bearing on the productivity of the ponds. In brackishwater ponds, benthic production is more important. The clayey soils rich in organic matter promote growth of benthic blue algae, which along with other micro-organisms constitute the main food of brackishwater animals. Clayey soils are best suited for building ponds as they have good water retention capacities. Sandy soils are porous and are not recommendable for bund preparation. Moderately heavy textured soils are suitable for pond preparation. Hence, some of the textures suitable for aquaculture are- sandy clay, sandy clay loam, clay loam.

4.3 Soil pH

The pH indicates whether the soil is acidic or alkaline and is an important parameter which affects pond condition. Slightly acidic to slightly alkaline soil pH is suitable for higher production. The nutrient availability, mineralization rate, bacterial activities and phosphorus fixation are influenced by pH. The pH range from 6.5 to 7.5 is best suited for brackishwater environment as the availability of nutrients like nitrogen, phosphorus, potassium, sulfur, calcium and magnesium is highest under this range. The availability of micronutrients like iron, manganese, boron, copper, chlorine and zinc is higher under acidic pH than under neutral or alkaline. Since the requirement of micronutrients is less, it is sufficient to maintain the pH at 6.5 to 7.5.

4.4 Organic matter

The most important index of soil fertility is soil organic matter. The presence of organic matter increases aeration, nutrient supply, reduces seepage loss, turbidity and acts as antioxidant. The microbial activity mainly depends on the organic matter content. In brackishwater aquaculture, soils with high organic matter are desirable.

4.5 Calcium carbonate

This parameter gives an indication of the amount of free $CaCO_3$ present, the absence of which shows acidic reaction. The harmful effects of sulphides and acids can be reduced by application of lime which is calcium carbonate. The soils with high calcium carbonate content promote biological activity and hence accelerates breakdown of organic matter. This creates more oxygen and C reserves in the soil. The $CaCO_3$ precipitates suspended or soluble organic materials, decreases BOD and increases nitrification due to requirement of Ca by nitrifying microbes. A productive soil should have $CaCO_3$ more than 5%.

4.6 Soil salinity

Saline soils are potentially productive soils. The excess of Na ions in these soils exerts

antagonistic effects on Ca and Mg absorption. These soils commonly occur in arid and semi-arid regions nearer to the sea and the salinity increases with the increase in salinity of water. The transformation of N, native or fertilizer added, is greatly influenced by the soil salinity. The available N content in water increases with salinity. The amount of nitrogen held in soil complex is higher at higher salinities and hence reduce nitrification. The rate of decomposition is also affected under different salinity and is comparatively lower at low salinity.

In general, the soil requirements for shrimp aquaculture are given in Table 6. Other parameters to be taken care of while selecting a site is the slope (2-5 %) and water table (25-75 cm) for excavated ponds. Embankments, dikes and levees are raised structures of soil material constructed to impound water. The major properties considered are erosion, stability and permeability. The clay content should be in the range of 18-35 %, slope 8-15 %, depth of water table 50-100 cm, medium to high shrink-swell potential, erodibility factor of 0.1-0.3 for proper pond embankments, dikes and levees.

Table 6. Soil requirements for brackishwater aquaculture

Parameter	**Optimum Range**
pH	6.5-7.5
Organic carbon (%)	1.5-2.0
Available nitrogen (mg/100g)	50-70
Available phosphorus (mg/100g)	4-6
Calcium carbonate (%)	>5.0
Electricl conductivity (dS/m)	>4
Exchangeable acidity (%)	20-35
Depth to sulfidic or sulfuric layer (cm)	50-100
Clay content (%)	18-35
Textural class	Sandy clay, sandy clay loam and clay loam

5. Pond management and treatment applications for maintenance of water and soil parameters in optimum range

M. Muralidhar, P. Kumararaja, R. Saraswathy and S. Suvana

Good bottom soil and water quality are vital ingredient for any successful aquaculture practices. The maintenance of good water quality is essential for both survival and optimum growth of culture organisms. In view of the observed effects of environmental stress on immune system of cultured shrimp, the management strategies should include, maintaining optimum condition of pond environmental parameters. Good pond management is critical as the water quality can deteriorate quickly due to the accumulation of organic matter from uneaten feed, faeces, dead shrimp and algal bloom crashes. Better control of water quality within the ponds became vital when farms reported incidences of shrimp coming up to the surface and problems of shrimp mortality. Regular monitoring of water and bottom soil in culture ponds for pH, DO, ammonia, nitrite and H_2S is the key in protecting the losses due to diseases Water management for the production of *P. vannamei* is to focus attention on measures to maintain colour changes (plankton density) and increase DO concentration, use of chemical and biological technologies to improve water quality and sediment.

5.1 Intake water treatment

Water treatment is necessary during pond preparation for the maintenance of good water quality at later stages. Water from the source should be filtered through 60µ filters to prevent the entry of parasites and crustaceans that are carriers of diseases. Reservoir has to be integral component and should be attached to grow-out ponds for sedimentation to settle organic loads and silt and chlorination treatment (approximately 10 ppm). Water has to be pumped in the grow out pond after 12 days of treatment, at which time, the permissible levels of chlorine residuals should be less than 0.001 ppm. Intense aeration, addition of 1 mg/lit of sodium thiosulfate for every mg/L of chlorine and exposure to sunlight are some of the management practices. Inorganic turbidity should be removed by providing sedimentation in the reservoir pond before water is taken into production ponds.

Grow out pond should be filled with water from reservoir pond. The water level is maintained to 30 - 40 cm and allowed to remain for few days. By this time, the colour of water may tum dark

green with algal bloom and a layer of benthic algae along with associated food organisms will form at the bottom. Subsequently small doses of organic and inorganic fertilizers are applied based on the observations (transparency with secchi disc 30 - 40 cm) of algal production. The water level is then raised to 100-125 cm. Once the pond is filled, nutrients will release into water column resulting higher nutrient concentrations. High dissolved inorganic nitrogen (DIN) concentration might contain considerable amount of ammonia and nitrite which may harm cultured animals and thus it is always safer not to stock fish/ shrimp right after filling pond. Perhaps one week time may be given to decrease DIN concentration allowing plankton to absorb. Adding treated water from reservoir (approximately 30%) throughout the crop is essential to prevent excess salinity which may gradually increase through evaporation.

5.2 Water exchange

Traditionally the management of water quality is through water exchange to reduce organic and to flush excess nutrients and plankton (cyanobacteria) out of the pond. Periodic partial removal of cyanobacteria and algal blooms by flushing or scooping out the scum facilitates optimum density and prevents sudden die-off of the bloom. However, due to increasing farm density, deteriorating intake water quality and rise in viral diseases, the use of water exchange as a method of pond water quality management is questionable. This practice increases the operating costs due to high water and energy consumption, and the lower retention time of nutrients within the culture systems, which would otherwise be available for biogeochemical recycling by bacteria and phytoplankton, thereby increasing the availability of natural food. Minimisation of water exchange will prevent viruses and carriers/bacterial pathogens from entering the ponds and reduce the possibility of disease transmission into shrimp ponds. This also led to the reduction of wastewater discharges and only the wastewater during harvest needs to be treated. But the reduction of water exchange requires closer control of water quality parameters such as pH and ammonia, effective sediment management, careful control of feeding and reduction of stocking density. However, improperly managed closed system increases the risk of stressful rearing conditions, bad water quality and diseases in ponds. Hence, the best water management option available to farmers is limited water exchange from treated reservoir, which enables good water quality conditions in ponds, while reducing the potential of disease introduction to the farms through intake water. The potential of zero water exchange system will be greater if the nutrients generated within the system and further accumulated in the sediment could be removed.

5.3 Aeration

In a typical black tiger shrimp pond, low rpm (revolution per minute) aerators may suffice but those with high rpm are required for *P. vannamei* culture. Paddle wheel aerators are commonly used and the newer ones such as the long arm aerators and spiral aerators can circulate oxygen

to the pond bottom and apply more efficient aeration. In general, aeration to achieve more than 4 ppm of DO is related to production targets, stocking density, feed usage and salinity. Manage the concentration of DO in pond waters are very closely related to the amount and type of phytoplankton, the number and condition of the existing aerator, shrimp biomass, total organic matter content in the pond, and bacterial activity. Generally, one horsepower is suggested for 500 kg production and 50 PL/m. The placement of aerators is important to prevent localized deposition of sludge. Maintaining sufficient level of DO facilitates oxidation of ammonia to harmless nitrate by nitrifying bacteria.

5.4 Feed management

The practice of providing food for the shrimp is trade-off between food source and water quality in the pond. It has been estimated that as much as 0.4 ppm ammonia can be added to the system for each 100 kg of feed used. Overfeeding, even in one feed can lead to sudden increases in ammonia, sometimes called ammonia spikes, a few hours later. These spikes can often be missed during daily or weekly sampling of water for ammonia levels. Thus, it is a prudent management strategy to reduce ammonia in ponds, even at lower pH. Feeding quantity should be strictly controlled, according to the weather, water quality, containing shrimp density and the actual flexibility to adjust food intake and other factors, so that smaller meals and scientific feeding.

5.5 Management of water parameters during culture period

Use of chemicals, disinfectants and probiotics

Various chemical products and probiotics have been recommended for reducing the load of harmful bacteria in the pon and to improve water and soil parameters in optimum range. There is very little evidence for the efficiency of these compounds. Most of the recommended substances are broad-spectrum disinfectants including quaternary ammonium compounds (Benzalkonium chloride), buffered iodophores and calcium hypochlorite. External fouling is usually associated with deterioration in the pond bottom or the water quality. Chemical treatment should be resorted only if the environment has been improved but the shrimp have not moulted. Probiotics generally includes bacteria, cyanobacteria, micro algae fungi, etc. which can improve the water quality of aquaculture, and (or) inhibit the pathogens in water there by increasing production. Probiotics in aquaculture water to regulate the micro flora of aquaculture water, control pathogenic microorganisms, to enhance decomposition of the undesirable organic substances in aquaculture water, and improve ecological environment of aquaculture. Effective use of scientifically proven products helps in maintaining the optimum pond environment.

Salinity

Many hatchery operators are requested to provide low salinity acclimatized post larvae. As the salinity tolerance of shrimp varies with strain, each hatchery operators has to modify the protocol accordingly. Acclimation can be conducted in a series of 100-500 l tanks. Fill the tanks with filtered sea water, and stock the post larvae. Normal aeration and feeding procedures should be followed. These tanks should be supplied with filtered fresh water from a separate water tank. Salinity reduction rate is adjusted by changing the flow restriction and reduction of water volume in the rearing tank. More than 3 ppt of salinity should not be reduced daily. As the salinity tolerance capacity is directly related to the age of the PL, during the initial phase of larvae, the reduction should be carried out carefully.

pH

To maintain optimum pH level, periodical application (twice weekly) of dolomite at 80 kg/ha at night after 20 days of culture and upto the end of the crop gives good result. Fermented juice of the rice bran and molasses @30 kg/ha are also being applied to reduce the pH lvels.

Dissolved oxygen

- Water exchange - Change the water with other good quality water from reservoir pond.
- Reduce the feed.
- Maintain optimum stocking density.
- Periodic monitoring of plankton growth and removal of excess algae.
- Providing aeration through aerators.
- Application of oxygen releasing compounds such as calcium peroxide (10 litre/acre) sodium percarbonate, sodium perborate and. (1-2.5 kg/ ha) based on the efficiency of the products.
  H_2O_2: $2H_2O_2 \rightarrow 2H_2O + O_2$

Turbidity management

i) Physical treatment - Involves sedimentation and filtration. If the source water is turbid, sedimentation should be carried out to remove the large suspended particles. Water is retained in the sedimentation tank at least for a minimum of 10-12 hours, so that larger particles settle down. The top clean water is used for further treatment. Finer suspended particles are then removed by passing the water, either by gravity or under pressure through sand filter. Water exchange is the most suitable method for removing the excess plankton growth.

ii) Chemical treatment - If the plankton density is too low as indicated by high transparency, the pond should be fertilized to improve the plankton growth. Turbidity arises by suspended clay particles in the aquaculture pond can be managed by adding substances which form

bridge between clay particles and make small particles called 'flocs' i.e. flocculation. Depending on the pH of the water, metal salts could be used as flocculants. Hydrolysed metals salts create flocs by adsorbed onto the surface of clay particles and form bridges between particles. As these particles begin to settle, they ensnare other particles, become progressively heavier, and settle much more readily from suspension. The hydrolysed metal compounds destabilize the colloidal particles by shrinking the layer of positively charged ions around clay particles and leads to coagulation. As the charge of the metal, increases the effectiveness of the coagulants increases. As sodium is monovalent ion sodium chloride is ineffective as coagulant. Divalent and trivalent metal salts like gypsum ($CaSO_4$), lime ($CaCO_3$) Epson salt ($MgSO_4$), Alum, Poly aluminium chloride, ferric chloride and ferric sulphate are more effective because of their higher charge. Synthetic "polyelectrolytes," which are large, long-chained molecules with even more charge than the metal salt coagulants. The effectiveness of coagulants depends on pond water chemistry, availability of product and application capacity.

Gypsum

Gypsum can be used to control turbidity under low saline aquaculture ponds without the loss of alkalinity. Calcium sulphate (gypsum) dissolves in water to increase calcium and sulphate concentrations. Calcium and sulphate may be absorbed in small amounts by plants and animals to become normal biological constituents. Gypsum has to be added to achieve a concentration of 100 to 300 mg/L for effective turbidity control. For most ponds, gypsum application rates will range from about 1,000 to 2,000 kg per hectare pond. Brackishwater pond water is highly saturated with calcium and magnesium and gypsum may be ineffective. In that situation, alum will be the only effective coagulant.

$$\text{Soil colloids - (Clay)} + CaSO_4 \rightarrow \text{Clay-Ca} + SO_4$$

Limestone (Calcium carbonate)

Agricultural limestone is a material commonly used to remove the suspended clay from the water. Similar to gypsum it is effective in low saline aquaculture ponds. Application rates of 500–1,000 kg per surface hectare are typically used. The mechanism of limestone is similar to gypsum.

Alum (Aluminium sulphate)

Alum is the most effective material for clearing clay turbidity from a pond, often within a few hours. A dose of 15 to 25 mg/L (150 to 250 kg/ha) should be sufficient to remove the turbidity from most waters. The dose varies with the turbidity level; low concentration for moderate turbidity (< 30 cm visibility) and higher dose for highly turbid water (< 15 cm visibility).

$$\text{Soil colloids - (Clay)} + (Al_2 (SO_4)_3\ 14H_2O \rightarrow \text{Clay-Al} + SO_4$$

Dissolve the gypsum/aluminium sulphate in clean water and spray over the surface on a calm day. Add 1/3–1/2 of the required amount, wait a day, and then determine if additional amount is required to increase transparency to about 45 cm. Late evening is often an ideal time to make the application as most nights are wind-free. Water movement from the wind prevents the suspended clay from quickly settling out, reducing the effectiveness of amendment. In ponds equipped with aerators, releasing slurry of amendment and water in front of the aerator will distribute it quickly. Both aluminium sulphate and ground limestone are best applied in slurries across as large surface area of the pond as possible. This extremely large surface area requirement is to maximize treatment to turbid water contact and in the case of aluminium sulphate, minimize the localized water pH depression. Alum treatment produces small amounts of sulfuric acid which can decrease pH significantly to levels harmful to aquatic life. Therefore, alkalinity and pH should be tested prior to application. Alkalinity should exceed 100 mg/l and pH should be greater than 7.0. If not, hydrated lime needs to be added simultaneously to buffer the effects of the acid produced by the alum addition. All the coagulants mentioned can remove phosphorus from water. As phosphorus is an essential plant nutrient, it may be necessary to fertilize the pond after treating it for turbidity.

Alkalinity

To improve alkalinity and stabilize pond water quality, dolomite, shell lime, calcium carbonate, egg shells and zeolite could be added depending upon soil pH and buffering capacity. If alkalinity is low, lime or dolomite should be applied @ 30-50kg/1600 m^2 one time at night every 2-3 days interval until the pH reaches the required levels. Agricultural limestone will not increase pH beyond a maximum of 8.3. The use of hydrated lime [$Ca(OH)_2$] or quick lime (CaO) is not recommended because either of these compounds can cause the pH to rise very rapidly to levels that are harmful to aquatic life. Sodium bicarbonate is an alternative as it dissolves quickly and the limited solubility of traditional liming materials. To reduce the alkalinity level, EDTA @ 20-30 kg/ha can be applied at night.

One ppm of Al^{3+} reduces 5.6 ppm alkalinity and 1 ppm of Fe^{3+} reduces 2.7 ppm alkalinity. In ponds with low alkalinity (< 20 mg/L) it can reduce water pH to levels that may affect animal growth and survival. In low alkalinity ponds, add half part $Ca(OH)_2$ for every part of alum applied in order to maintain proper pH. Al^{3+} and Fe^{3+} ions from aluminium sulphate and ferric chloride applications quickly precipitate as Al and Fe oxides. Nevertheless, these two compounds have a strongly acidic reaction in water because of the hydrolysis of Fe and Al.

Hardness

Agricultural limestone, dolomite or gypsum can be used to increase hardness in areas with low pH. When the pH increases due to the hardness, if we add any products to decrease the pH,

the minerals in the hard water buffers the water and hinders the reduction in pH. Hence, to reduce the hardness of water, the minerals present in the water has to be removed. This can be done by using water conditioners in the field level and reverse osmosis or any chemical which forms a complex and binds the mineral strongly like zeolites can be used in the small scale (hatcheries).

Minerals application

In order to calculate the desired mineral levels at different water salinities, the water salinity (in ppt) is to be multiplied by the factors shown for each mineral (Table 7).

Minerals	**Salinity**		
	1 ppt	5 ppt	10 ppt
Calcium (ppm)	11.6	58.0	116.0
Magnesium (ppm)	39.1	195.5	391.0
Potassium (ppm)	10.7	53.5	107.0
Sodium (ppm)	304.5	1522.5	3045.0

Generally the deficiency of mineral is seldom observed in brackish water, whereas after introduction of *P.vannamei* in low saline, farmers are very keen on mineral application. If pond water is deficient with the above said mineral, it has to be corrected by the addition of following salts.

Salt	**Formula**	**General name**	**Mineral (%)**
Calcium sulphate	$Ca_2SO_42H_2O$	Gypsum	Ca: 22 %, SO_4: 55%
Potassium chloride	KCl	Murate of potash	K: 50%, Cl: 45%
Potassium magnesium sulphate	$K_2SO_42MgSO_4$	K-Mag	K: 17.8%, Mg: 10.5% SO_4: 63.6%
Potassium sulphate	K_2SO_4	-	K: 41.5%, SO_4: 50.9%
Hydrated magnesium sulphate	$MgSO_47H_2O$	Epsom	Mg: 10%, SO_4: 39%

Amount of salt to be added in the pond will be calculated based on the desired mineral level and the selected salt.

- Amount of salt to be added = Concentration of minerals required in the pond (in ppm) / % of mineral ions in the selected salt.
- For example, to get the potassium content of 200 ppm, the amount of murate of potash to be applied = 200 / (50% / 100) = 400 mg / l

Metabolites

Zeolites, although widely used, have been shown in several studies to be ineffective in reducing ammonia at salinities above 1 ppt due to competition with other ions in salt water such as sodium, potassium, magnesium and calcium. Application of gas adsorbents or probiotics to adsorb or reduce ammonia and H_2S are being practiced. However, application of probiotics can give inconsistent results due to wide differences between bacteria counts and strains, differences in the environmental conditions in which they are used, and the slow growth of many probiotic bacteria strains in ponds.

5.6 Pond bottom management

Pond bottom management is very important because most of the shrimp activities performed in the pond bottom. Pond bottom is a feeding area which is also where the accumulation of dirt as a result of the culture process. Keeping the pond bottom clean will indirectly protect water quality and shrimp health.

Pond preparation after harvest

Before initiating a second crop in a pond, the pond has to be prepared for stocking the shrimp post larvae.

Draining of ponds

The first step in pond preparation is draining the pond after harvest of the previous crop. Removal of waste by draining and drying of the pond bottom after the production cycle are some of the steps to be followed for keeping pond environment clean. This could be done either by pumping or draining through sluice. For effective and complete drain, the pond should be designed in such a way that the bottom must have a gradual slope from the inlet gate to drain gate. The effective slope is 1:500. After draining, pond should be desilted. In wet method, after the final drain harvest, the accumulated black material on the pond bottom is flushed in the form of thin slurry using a high pressure pump. The advantage of this method is that waste is removed in suspension. This method needs a settling pond where waste is removed from the water and treated repeatedly to avoid polluting the local environment.

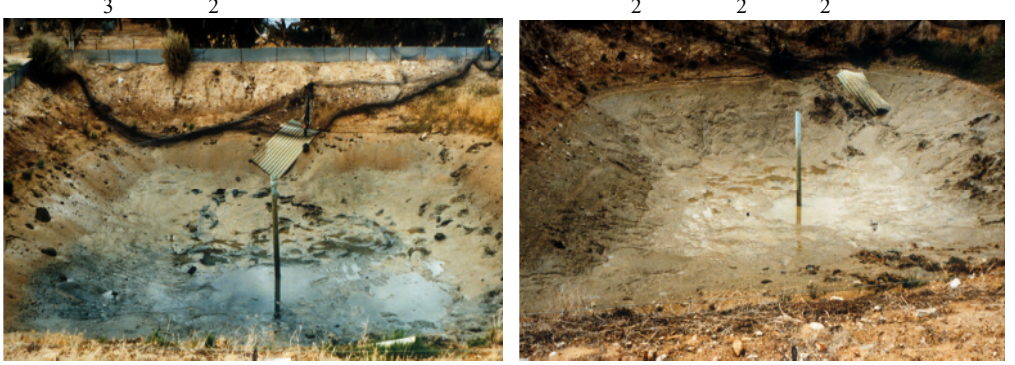
Pond mud drying and sediment removal

In this method after the final drain harvest, the pond bottom is allowed to dry and crack, primarily to oxidize the organic components left after the previous culture. The pond bottom is sun dried for at least 7-10 days or until it can support a man's weight without subsiding and the soil should crack to a depth of *25* - 50 mm. After drying, the waste can either be removed manually or with machines. Pond drying between crops is a common practice for releasing nutrients to the pond water in brackishwater aquaculture ponds. In aerobic decomposition, the

organic matter is oxidized to inorganic substances such as carbon dioxide, water, ammonia, sulphate, phosphate etc. Drying and cracking of pond bottom enhances aeration and favours microbial decomposition of soil organic matter. The moisture level of pond muds affects the rate and amount of decomposition. The optimum moisture content for drying is 20%, but it might vary among soils from different ponds. Excessive drying of water-saturated soil may have adverse effect on microbial activity resulting counterproductive without any benefit. The effect of drying period on the viability of white spot syndrome virus (WSSV) in pond bottom soil after harvest indicated that 19 days drying make the WSSV unviable and hence recommended a minimum of three weeks drying between successive crops to avoid WSSV infection.

Generally after the crop harvest, water draining is not uniform throughout the pond bottom in most of the farms and it takes more time for draining compared to the other portions on the pond bottom. To enhance the oxidation of such wet patches nitrate salts at 20-40 g/ m^2 could be applied. The nitrate salts enhances the organic matter degradation by acting as nitrogen for microbes.

$$4NO_3 + 5CH_2O \text{ (Organic Matter)} \rightarrow 4H^+ + 2N_2 + 5CO_2 + 7H_2O$$



Wet reduced soil **Nitrate treated soil**

(Source: Boyd, 1995)

Eradication of predators and unwanted species

After the crop is harvested, undesirable species like pests, competitors and predators remain in the ponds, which should be removed. These species include finfishes, crustaceans and molluscs. Elimination and control of undesirable species from shrimp culture pond is very important to get good yield. There are two methods to control the undesirable species.

Physical method

The most effective method is drying the ponds. Unwanted organisms are removed from the pond by drying of the pond bottom. Direct sunlight helps to disinfect the light sensitive

pathogenic microorganisms (bacteria, fungus, virus) and to desiccate egg, larval and adult stages of predators. It also helps in elimination of undesirable algal mats of filamentous algae. Other methods include installation of appropriate screens in the outlet/inlet gates to prevent entrance of undesirable species, proper maintenance of dikes and water gates to prevent leakage and to eradicate boring organisms like crabs and eel. During culture, selective harvesting or the use of cast net can be resorted to minimize the impact of undesirable species.

Chemical method

In cases, where complete drying is not possible, organic, biodegradable, piscicides can be used. Eradication of undesirable species is very effective, easy, efficient and fast when chemicals are used. This is because chemicals act as contact or systemic poison. After the application of the organic piscicide at least a minimum period of 10 days should be given for its toxic effect to be degraded.

1. Mahua oil cake (Bassia lafifolia) @ 100 - 150 ppm and tea seed cake @ 15-20 ppm.
2. Saponin: The recommended doses are 12 and 20 g/m^3 for salinities above and below 1.5 ppt, respectively.
3. Croton tialium seed: - The seed of Croton tigliurn @ 3 - 4 g/m3 water is applied.
4. Calcium carbide: It is used to kill crabs. After applying calcium carbide into the crab holes, water is poured to activate it, which kills the crabs.
5. Ammonium sulphate: This chemical compound, which is also a fertilizer (21 - 0 - 0), is an effective eradicant when used in combination with lime. Ammonia is released from the reaction of ammonium sulphate with lime. It is applied in pond at a dosage of 1 part of ammonium sulphate to 5 parts of lime. Lime must preferably applied first to raise the pH since the rapid release of ammonia from ammonium sulphate is dependent on high pH (above 8.0).

<u>Pond treatments to enhance nutrient use efficiency</u>

Fertilization of ponds should be delayed for 1-2 week after liming to avoid precipitation of fertilizer phosphorus as calcium phosphate. Alum is sometimes applied to fertilized ponds to remove suspended soil particles and reduce turbidity and also for reducing phosphorus concentrations and phytoplankton abundance. Phosphate fertilizer should be applied 3-4 d after alum treatment to encourage phytoplankton growth and reduce the likelihood of underwater weed infestations developing in the clear water. Elevation of Ca^{2+} concentrations by gypsum treatment can drastically lower phosphate concentrations and retard phytoplankton growth in ponds with naturally low Ca^{2+} concentrations. Applications of agricultural limestone or lime [$Ca(OH)_2$ or CaO] might also be effective in precipitating phosphate as calcium phosphate from some ponds. Manual raking of the pond bottom on alternate days accelerated the bacterial activity, improving sediment-water interactions and nutrient availability suggesting that

provision of suitable environmental conditions is as important a requisite as the substrate availability for optimum manure utilization.

Liming

Liming of the pond bottom is one of the most important items in pond preparation to keep the pond environment hygienic for sustainable shrimp production. Liming is an agricultural practice that has been adopted by fish/shrimp culturists and lime materials used in aquaculture are the same that is applied in agriculture. As a practice lime materials such as agricultural limestone ($CaCO_3$, quick lime or unslaked lime (CaO), and hydrated lime or slaked lime [$Ca(OH)_2$] are commonly used in agriculture. Besides above lime materials other materials such as dolomite, calcite, seashell and hydrated granules gained importance recently in shrimp culture. Most of the shrimp/fish farmers use these materials depending on local availability. Application of lime is not for fertilisation but is a remedial procedure necessary in acidic ponds to accomplish one or more of the following tasks:

1. Neutralising acidity
2. Increasing pH of bottom soil and thereby enhancing the availability of phosphorus added through fertiliser
3. Accelerating the microbial activity and thereby diminishing the accumulation of organic matter in pond bottoms and favouring recycling of nutrients
4. Maintaining the alkalinity and other physico-chemical characteristics of soil which in-turn helps in enhancing fish/shrimp production
5. Improve the hygiene of the pond bottom
6. Permit normal reproduction and growth
7. Improve survival of aquaculture species
8. Greater availability of carbon dioxide.
9. Enhances the nitrification due to the requirement of calcium by nitrifying organisms

Quality evaluation of lime materials

The commercially available lime materials from market have to be collected and analysed for their neutralisation value. The term "neutralising value" refers to the relative ability of lime materials to neutralise acidity. Pure calcium carbonate is assigned a neutralisation value (NV) of 100 per cent and is the standard against which various lime materials are compared. Thus, the neutralising power is nothing but a statement of its strength with reference to calcium carbonate or its calcium carbonate equivalent (CCE). The lime materials were sieved through 60-mesh sieve (0.25 mm) and analysed for their CCE values. The finer the lime material, quicker is the reaction with the soil. Different lime materials available in the market vary considerably in their particle size. Hence, a fineness guarantee is desirable. A mechanical

analysis is made by the use of different mesh sieves to calculate the fineness factor or efficiency rating (ER).

The calculation of fineness factor rating for a sample of agricultural limestone that was subjected to sieve analysis is as follows. The particles of lime passing through 60 mesh sieve are rated 100 per cent efficient, those passing through the 8 mesh sieve are rated 50 per cent efficient and those retained on 8 mesh sieve are rated 20 percent efficient. Finally, the percent effective calcium carbonate (PECC) value was obtained by multiplying the estimated CCE with fineness factor values.

Calculation of lime requirement for ponds:

The lime requirement of a soil can be defined as the amount of lime material that must be added to raise the soil pH to 7.0. First, the amount of lime needed as pure calcium carbonate is calculated based on the actual pH of pond soil and the extent of the area to be applied. Values of liming rate as pure $CaC0_3$ (tons/ha) with an efficiency of 100 percent are calculated from the formulae given below.

$$Lime\ needed = \left[\left(\frac{\text{Desired pH} - \text{Actual pH}}{0.1}\ X\ 0.5\right) /\ Efficiency\ of\ lime\right] X\ area$$

Then, the recommended dose for various lime materials was calculated by dividing the value of lime needed as pure $CaCO_3$ with the PECC value of that particular lime material with the formulae given below.

Recommended rate of application of lime material (tons/ha):

$$\frac{Liming\ rate\ as\ pure\ \text{CaC03}\ \left(\frac{tons}{ha}\right)}{(PECC)/(100)}$$

where, PECC = Percent effective calcium carbonate or efficiency percent.

Methods of liming

Liming can be done in two ways.

- By broadcast over dried pond which includes the dike inner walls and
- By mixing with water and spraying over the pond bottom

In using the above methods, the lime should be spread as uniformly as possible over the complete surface of the pond and should be ploughed upto 10-15 cm depth for thorough mixing. This should be done at least 20 -25 days before fertiliser application in minimum water

column. This is important because liming materials will precipitate phosphorus if applied at or near the same time in the form of fertiliser. Depending upon the soil pH, the lime is evenly spread over the whole pond bottom and upto the top of the dike and left for 10 - 15 days. During this time, lime will react with mud and will result in greater availability of phosphorus at later stage when phosphatic fertilizers are applied. Ploughing and tilling is recommended only if pond is deeply contaminated. Effective plough depth is 15 cm. A large proportion of the lime should be spread on the feeding areas and any part of the pond that has remained wet. During the crop, lime in smaller dose may be applied to maintain the pH of the pond between 7 and 8. The recommended levels of lime application during pond preparation are given below.

Amount of lime (tons/ha) to raise the soil pH to 7.0

Soil pH	Quantity of lime material (tons/ha)		
	Dolomite	Agricultural	Quick lime
6 to 6.5	5.7 to 2.8	5.5 to 2.8	4.6 to 2.3
5.5 to 6.0	8.5 to 5.7	8.3 to 5.5	6.9 to 4.6
5.0 to 5.5	11.3 to 8.5	11.1 to 8.3	9.2 to 6.9
4.5 to 5.0	14.2 to 11.3	13.9 to 11.1	1 1.5 to 9.2
4.0 to 4.5	17.0 to 14.2	16.6 to 13.9	13.8 to 11.5

5.7 Management of pond bottom during culture

During culture, the feed not eaten by the shrimps and carbonaceous matter, suspended solids, faecal matter and dead plankton etc. settle at the pond bottom. To understand the condition of the pond bottom, the following parameters are to be monitored regularly; pH, organic carbon content and redox potential are the indicators of pond bottom quality. Reduced or anaerobic sediments may occur at the pond bottom of heavily stocked pond with heavy organic load and poor water circulation. Under anaerobic condition of the pond bottom, reduced substances such as H_2S, NH_3, CH_4 etc. are formed which are toxic to benthic organisms. Among the pond bottom quality indicators redox potential can be measured in situ by using portable redox meter or probe. The redox potential (Eh) of mud should not exceed -200 mV. The following management practices are recommended to improve the pond bottom quality.

- Central drainage canal in the pond may also help in the removal of organic waste periodically. Management of pond bottom:

- Water circulation by water exchange, wind or aeration helps to move water across mud surface and prevent the development of reduced condition. Bottom should be smoothened and sloped to facilitate draining of organic waste and toxic substances.
- Bottom Raking - The oxygenated water and surface should be always in contact for the purpose of maintaining the oxidized layer. Stirring the bottom layer by manual raking and chain dragging are the common methods to improve the contact with oxygenated water maintain the oxidized layer.

5.8 Wastewater management

Coastal Aquaculture Authority has made wastewater (effluent) treatment system as mandatory for *L.vannamei* farming irrespective of the size of the farm. Shrimp farm wastewater after harvesting has to be treated and disinfected by chlorine before discharge to open water sources. The wastewater from the pond may be allowed into a settlement pond before letting it into the environment so that suspended solids may settle at the bottom and the sludge has to be removed periodically. Shrimp farm wastewater is rich in nutrients such as nitrogen and phosphorus and can be utilised by integration with other aquaculture production systems. Culture of finfish, molluscs and seaweeds in the wastewater from shrimp ponds can remove nutrients and particulate organic matter. To reuse the water, reservoir is required to ensure that water treated along the treatment system is within the standards acceptable for culture.

5.9 Conclusion

Sustainability of aquaculture depends on the maintenance of a good environment. The two-pronged approach of combining pond management and health monitoring is the key for successful shrimp production. It is important to know how much shrimp can be supported by the pond environment (carrying capacity of pond). Although the ideal carrying capacity can be low, higher production volumes can be achieved by partial harvesting more than once. The promotion of growth of natural planktonic or benthic microbial and microalgae communities (bioflocs and periphyton, respectively) present in the pond environment helps in the utilization of nutrients through autotrophic and heterotrophic processes accelerating the removal of organic and inorganic wastes, thus improving water quality. Regular monitoring of environmental parameters and timely mitigation is the key to protect potential losses due stress and opportunistic bacterial infections. The understanding on ecological process occurring in shrimp culture ponds through regular monitoring will help to solve some of the disease issues faced by shrimp farms.

6. Use of disinfectants as biosecurity measure in aquaculture systems

N.Lalitha and Satheesha Avunje

Disinfection is one of the most important biosecurity measure practiced in aquaculture to control entry or exit of pathogens or spread of diseases/infection. Disinfection is carried out for eradication of pathogens carried through water, culture animals and their eggs, equipment and accessories used, hand wash, foot dips etc. Disinfection is a structured process that uses physical and chemical procedures to remove organic material and destroy or inactivate pathogenic agents. Disinfection efficiency is highly dependent on the cleanliness and nature of the targeted surface, disinfectant concentration and contact period, temperature and pH, organic and inorganic load in the water, level of microbial contamination, biofilm formation etc. Disinfection process depends on the objective of disinfection, either eradication, control or disease prevention.

6.1 Disinfection process and efficacy

A disinfection process should include three stages, (i) Cleaning/washing, (ii) application of disinfectant, and (iii) removal of inactivation of the disinfectant. Disinfection efficacy depends on the selection of appropriate disinfecting agent and they may be chosen based on: (i) pathogen targeted, (ii) materials to be disinfected, (iii) safety of the disinfectant, (iv) Environmental impact, (v) stability, solubility, wetness/penetration and corrosiveness, and (vi) availability, handling and cost. Nature of the pathogen is equally important to achieve better disinfection efficacy. Viruses, bacteria and protozoan parasites can be classified based on their resistance to different disinfection processes. Viruses are classified into three categories, (i) Category A: lipid enveloped, easy to disinfect using soaps or detergents (*e.g:* WSSV); (ii) Category B: non-enveloped virus with strong protein coat. These are resistant to mild disinfectant and high dose or stronger disinfection protocol to be followed (*e.g:* betanodavirus or NNV); (iii) Category C: intermediate group (*e.g:* Iridoviruses). Bacterial pathogens may be classified as (i) Gram-positive vegetative isolates are highly susceptible to disinfectants; (ii) Gram negative bacilli are more resistant than cocci; (iii) Mycobacteria that are classified as intermediate of the two groups; (iv) Spores of bacteria, highly resistant. Before neutralising parasites, understanding their life cycle is important as they may have dormant stage, intermediate hosts or non-aquatic forms. With detailed life cycle, most susceptible stage of the parasite can be targeted for disinfection.

6.2 Types of disinfectants

Disinfection could be achieved either by physical or chemical means.

6.2.1 Physical process of disinfection

Desiccation, light (act on the fish pathogens on earthen bottoms), dry heat (acts fish pathogens on concrete, stone, iron, plastic surfaces), damp heat (acts on fish pathogens on transport vehicle tanks) and ultraviolet rays (acts on bacteria and viruses in water).

6.2.2 Chemical process of disinfection

Based on the principle, mode of action or chemical groups, disinfectants can be classified into several groups.

i) Oxidising agents

They are highly reactive and effective disinfectants to control microbes. However, presence of organic matter significantly reduces their effective doses. Hence removal of organic load will reduce quantum of application and increase effectiveness.

a. Chlorine liberating compounds

Chlorination is one of the most common disinfection method followed in shrimp hatcheries and farms. Its high efficacy and cost effectiveness are the advantages. Three forms of chlorine are available, gaseous chlorine, diluted sodium hypochlorite (NaOCl) and granular calcium hypochlorite ($Ca(OCl)_2$). The first one is in pure form and highly toxic to the users; hence not practiced in aquaculture. Second form is highly diluted and less effective in aquaculture. The third form, $Ca(OCl)_2$ contains about 65% of active ingredient, chlorine, and widely used in aquaculture systems. The active ingredient in chlorination is chlorine gas, hypochlorous acid (HOCl) and hypochlorite ions (OCl^-). Negatively charged chlorine oxidises peptide links and denatures proteins to kill microorganisms. The process of chlorination is highly pH dependent, at pH below 2 chlorine gas dominates, between 2 and 6 dominated by hypochlorous acid, at alkaline pH hypochlorite ions are dominant. Demerit of chlorination other than health hazard is its reactivity to organic matter. Chlorine reacts with ammonia to form chloramine that has less disinfecting property. They react with organic matter to oxidise them and lose efficacy. Hence it is essential to compensate chlorine demand of the system where chlorination is required to achieve efficient chlorination. Dose for disinfecting effect thus depends on the organic load in the water and generally ranges from 10 to 50 ppm of active chlorine. It is essential to dechlorinate the system before used for farming, and can be achieved by keeping the treated water for few days or treat with sodium thiosulphate ($Na_2S_2O_3$). Application of 7 ppm of $Na_2S_2O_3$ will remove 1 ppm of free residual chlorine.

The procedure for chlorination is as follows

- Pump seawater in to a reservoir via sand filter
- Determine and weigh the amount of calcium hypo chlorite needed for disinfection using the formula:

Weight of hypo chlorite=10 X Volume of water to be added in tone/Percentage of hypo chlorite in the product

- Dissolve hypochlorite in a pail of clean water. Aerate vigorously.
- Add hypochlorite solution to the water, which is to be treated. Aerate vigorously for homogeneous mixing
- Neutralize12-24 hours after disinfection with hypochlorite

b. Iodine compounds

Iodophore is an iodine containing disinfectant, commonly used in medical facilities to control infection. The chemical is admixed with surfactants such as povidone (thus povidone-iodine) to make it more effective disinfectant. Iodine interacts with electron transport chain of bacteria, thus blocking respiration and killing. It also interferes with functioning of cytoplasmic membrane protein (Maris, 1995). Reactivity of iodine to organic molecule is three fold lower than chlorine, thus it is stable and effective even in the presence of organic matter. Nevertheless, iodophore are affected by water hardness and alkalinity.

In shrimp aquaculture, iodophore used for brief wash (1 min) eggs or treat nauplii to control microbial infection. It is used as disinfectant to control bacteria, viruses, protozoa, fungi, pathogens and parasites. Also it is used for treating farm/hatchery utilities such as smooth surfaces, nets etc. or even for hand wash. Free iodine molecule concentration is increased 10 fold when 10% povidone-iodine is diluted 100 fold. Best disinfection effect will be achieved by using 0.1% of povidone-iodine. Majority of the oxidising agents are highly corrosive except iodophore. However, older aqueous or alcohol solutions of iodine are highly corrosive and toxic, and are not advised for application as disinfectant.

In addition to povidone-iodine, acidified iodine can be used for disinfection of equipment or pipelines. Due to lower pH, their corrosiveness will be higher; however, the bactericidal effect is increased by bringing down the pH. Brown colour of iodophore is due to available free iodine. The colour can be used as indicator of iodine concentration since consumption of iodine reduces brown colour of iodophore.

c. Per oxygen agents

Disinfectants such as hydrogen peroxide, peracid solution and monosulphate of sodium or potassium. These agents are not affected by organic load, but may cause corrosion to steel, aluminium or alloys. Per-acetic acid, acetic acid and hydrogen peroxide are mixed to form peracid solution, which is strong biocide with low toxic residues. Peracetic acid is affective against different types of microorganisms including spores.

Peracetic acid (PAA) is a relatively new compound suggested for use to treat pathogens in aquaculture. Peracetic acid is an antimicrobial disinfectant registered by the US Environmental Protection Agency for use in agriculture, food processing and medical facilities. In Europe, PAA is also approved for use in veterinary medicine and is one of the very few compounds approved for use in aquaculture as a disinfectant. It is the peroxide of acetic acid and is commercially available in an equilibrium mixture with acetic acid, hydrogen peroxide and water. The mixture is represented by the following equilibrium:
Acetic acid + hydrogen peroxide= peracetic acid + water

Peracetic acids' direct oxidation and destruction of cell wall of microbial pathogen allows for its prime candidacy as a disinfectant in waste water treatment. The pH is less than 2 with a specific gravity of 1.10 to 1.11 depending upon the temperature. The PAA product is a clear, colourless liquid with a pungent odour due to the presence of acetic acid. Commercially, this product is available in concentration from 2- 15 % (wt/wt). Solutions exceeding 15% exhibit explosive nature, instability and reactivity. The active disinfecting agent within this equilibrium, PAA, is highly active in very low concentrations, across a wide range of microbes. The germicidal property is found to be bactericidal @ 0.001%, fungicidal @ 0.003% and sporicidal @ 0.3%. The disinfection efficiency can be ranked as bacteria> virus> bacterial spores> protozoan cysts. The bacterial effectiveness depends upon the organisms. The PAA @ 10 ppm was found effective against heterotrophic bacteria and luminescent *Vibrio* sp.

There are three reactions in which PAA is consumed in aqueous solutions: spontaneous decomposition, hydrolysis and transition-metal catalysed reaction. The pH range of 5.5 to 8.2 favours spontaneous decomposition of PAA to acetic acid and oxygen. The residues of PAA reduced to negligible amount after 48 h of application when applied @ 10 ppm dose. Although hydrogen peroxide is also a disinfecting agent, PAA is more potent antimicrobial agent than hydrogen peroxide, rapidly active against a wide range of microbes at low concentrations. The disinfectant activity is based on the release of active oxygen. The sulfhydryl and sulfur bonds in proteins, enzymes and other metabolites are oxidised and the double bonds are oxidised. It acts as a protein denaturant.

Disinfection efficiency of PAA is affected by pH, which decreases at higher pH. The biocidal form of PAA is the undissociated acid. It has a pKa of 8.2, i.e at pH above 9; the predominant species is the dissociated form which has lower disinfection efficiency. The disinfection efficiency of PAA decreases with increasing total suspended solids (TSS) and biological oxygen demand (BOD). The degradation of PAA depends upon the amount of organic matter in the system; it degrades faster in a system with higher organic load. The efficiency of PAA was found to be less in turbid systems (>40 NTU). The composition of ions also has an impact on the PAA degradation; low Mg and Ca and higher Na and K ratio accelerate its decay.

Compared to other disinfectants like, bleaching powder and sodium hypochlorite, the residual effect of PAA is less and harmful and less. It takes utmost 4 days for the residual chlorine to reduce to zero when applied at 10 ppm, which is the effective dosage, whereas in the case of PAA only 2 days. The disinfection efficiency was also high compared to these disinfectants. The main drawback in the usage of PAA is its high cost compared to the other disinfectants in the market.

Hydrogen peroxide (H_2O_2) causes significant cellular damage by oxidising internal cellular components causing apoptotic and necrotic cell death. H_2O_2 is used widely in washing of finfish eggs (@ 500-1000 mg/L) to control fungal and bacterial infection and treatment can be continued once a day until eggs hatch. H_2O_2 is one of the safe disinfectants with no residual effect or withdrawal period. In addition to disinfectant role, H_2O_2 can be used for removing biological oxygen demand of organic matter in the water. During low oxygen period, it can be used for increasing dissolved oxygen level in pond water. H_2O_2 with 35% active ingredient is approved by USFDA for application for disinfection in aquaculture, while the domestic use H_2O_2 is having 3% active ingredient.

d. *Ozone:*

Action on fish pathogens, water and sterilization and was used at the rate of 1mg/L for 1 minute.

ii. Detergents

Quaternary ammonium compounds (QACs)

These detergents are generally effective against vegetative form of bacteria and not on spores. More specifically, they kill majority of Gram-Positive bacteria, while some Gram Negative bacteria show resistant. The QACs (Benzalkonium chloride derivatives) are noncorrosive and have wetting property, hence have high contact efficiency. Removal of QACs is important as they can be toxic to the animals. They are effective at high pH range, stable at higher temperature and generally do not affected by organic load. A dose of 1 mg/L will kill bacteria and viruses, and the dose is good for hand disinfection. 2 mg/L for a period of 15 min is used for disinfection of surfaces. BKC is effective disinfectant in treating culture tanks, utilities used like net, plastic wares etc.

iii. Alcohol

Alcohol chemical compounds viz., ethyl alcohol and isopropyl alcohol are water soluble with bactericidal property 60-90% (v/v) concentration. It causes the cells dry, dislocate membranes and denaturation of protein. Ethyl alcohol is virucidal at 60-80% concentration. Eg. Ethyl alcohol, isopropyl alcohol and methyl alcohol.

iv. Reducing agents (Aldehyde compounds)

Formaldehyde is available as 37% formaldehyde by weight, formalin, water-based solution. It has the property of bacteriocide, fungicide, virucide and sporicide. In aquaculture it was used as disinfectant in the hatcheries and in culture system applied in the pond water and this has the property of natural degradation so food safety hazard for the use of the formaldehyde in the ponds. They act on the protein by denaturation.

$O = CH_2$ O Protein-NH-CH_2-OH => Protein -NH-CH_2-NH- Protein

Acts on nucleic acids by alkylation

The reaction is irreversible at the level of nucleic acids:

$O = CH_2$ => Protein-NH-CH_2-NH-DNA.

Glutaraldehyde is the saturated dialdehyde used as disinfectant. It acts on the microbes similar to formaldehyde at the neutral or alkaline pH.

Formalin was used in the shrimp hatcheries. Nauplii and fertilized eggs were washed with formalin to prevent the viral infection.

Collection of nauplii using plankton net	=>	Running sea water for 1-2 minutes	=>	Formalin 400 ppm for 30 seconds to 1 minute
Collection of fertilised eggs	=>	Running seawater for 1-2 minutes	=>	Formalin 100 ppm for 1 minute

v. Acidic and alkaline compounds

Acidic agents efficacy is associated with concentration of hydrogen (H^+) ions which act on nucleic acid resulting in the destruction of amino acid bond, alteration of the pH of cytoplasm and thereby precipitate the proteins. Eg. Nitrous acid. Similarly, alkaline agents efficacy is associated with concentration of hydroxyl (OH^-) ions which act on the membrane envelope by lipid saponification. Eg: NaOH and KOH. Sodium hydroxide acts on the resistant surfaces with cracks and on dried earthen ponds. Mixture of Sodium hydroxide 100g, teepol 1g, calcium hydroxide 500g, water 10L can be used by spraying on the surfaces 0.1 /m^2 and leave for 48 h and in earthen ponds 2L/ m^2 and leave for at least two weeks.

vi. Phenolic compounds

Phenol byproducts are orthophenylphenol, ortho-benzyl-para-chlorophenol with bactericidal, fungicidal and virucidal obtained when hydrogen atoms on the aromatic ring were replaced by a functional group viz., alkyl, halogen, phenyl, benzyl. Essential intracytoplasmic enzymes and metabolites in the system and the cell wall respectively were inactivated resulting in the death

of the bacteria. At high concentration causes bacterial protein denaturation and cell membrane lysis, whereas at low concentration cell elements are released in the outside media.

vii. Metal salts

Metal salts are bacteriostatic and mode of action on protein it causes precipitation and oxidation of sulfydryl groups Eg. Silver nitrate, copper sulphate,

viii. Other disinfectants

Ethylene oxide, sulphur dioxide (gaseous form), sulphides, anilides, esters, betapropiolactone, bronopols, parabens, hexachlorphene are the other disinfectants.

ix. Biguanides

Chlorhexidine belong to the family of polyhexamethylene biguanide (PHMB). At low concentration, membrane permeability of the bacterial cell was altered causes break of membrane and releases of elements of cells, whereas at high concentration causes cytoplasm coagulation therefore resulting quicker bactericidal effect.

x. **Dyes**

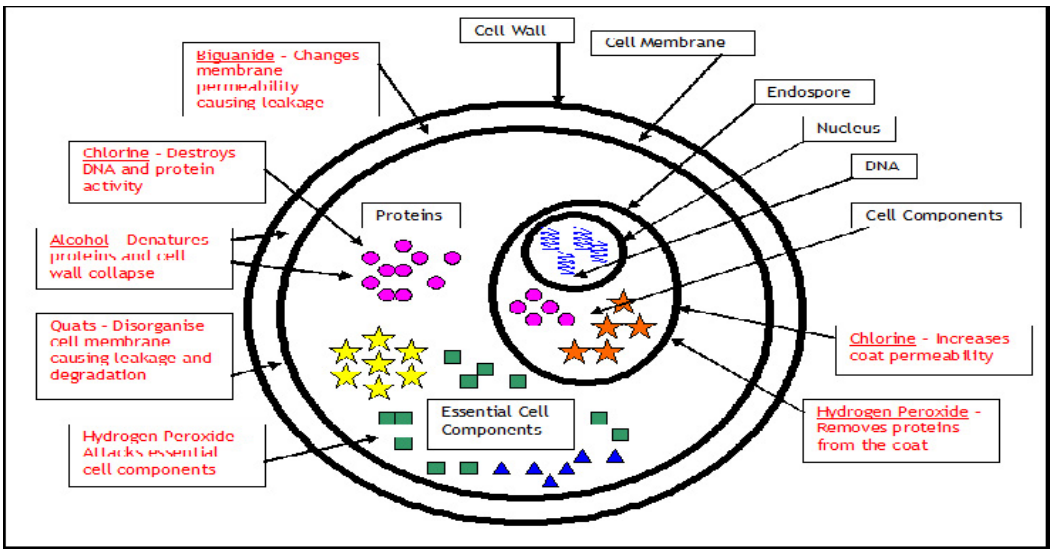
Acridine dyes act on the bacterial nucleic acid thus exhibiting antibacterial action. Acridine dyes such as acriflavin and aminacrine have antimicrobial properties. Ethidium bromide acts against gram positive than gram negative bacteria. Base pairs in DNA were intercalated.

xi. **Plant based disinfectants**

Garlic in whole form used to treat the helminth and sea lice parasites in marine salmonids at all life stages. Onion in whole form was used to treat Crustaceans parasites and sea lice infestation in Salmonids at all life stages. Papain was used to reduce the disease incidence and improve the hatchability of the eggs at a concentration of 0.2% solution.

6.3 Action of disinfectants on bacteria

Bacteria were the prokaryotic microorganisms which lack nuclei and other organized cell structures. External membrane play a role in survival of the bacteria since it protects from the environment. They were made of phospholipids, lipopolysaccharides with divalent cations, Ca and Mg. There are two stages of the disinfection action on the bacteria primary based on the mode of action and secondary on the consequence of action. When the bacteria comes in contact with disinfectant, it enter the lipid phase by dissolving non-polar molecules, membrane organization was disturbed by other molecules. Disinfectants can act on the cytoplasmic membrane where the nutrients enter the cytoplasmic membrane by active diffusion and passive diffusion, energy metabolism of ATP and, on the cytoplasm and nucleus. Bacterial spore have the dipicolinic acid which is impermeable where the highly oxidizing products may be used.



Mechanism of disinfectant action

6.4 Disinfectants at the outbreak of the disease

- Disinfection of the earthen ponds can be achieved by destructing the solid particles by removal of the top 10-15cm sludge and decontaminating it. CaO can be used @ 1kg/m^2 when the ponds were dried completely. 1% NaOH + 0.1% teepol can be used as the disinfectant. Application rate of 2L/m^2 and the ponds should be maintained in the dried condition.
- Disinfection of the concrete tanks was done using water with low pressure and an alkaline detergent. Spray the disinfectant on the top and then towards the bottom and rinse with clean water. After disinfection unpainted concrete can be painted using latex or acrylic paint. Disinfections of installations in seawater can be done by mechanical brushing with aldehyde or phenol based disinfectants, sodium hypochlorite.
- Disinfections of the pipelines with 15% of 2l sodium hypochlorite solution to 1000l of water @ 300mg/L concentration.
- Disinfections of the outdoor areas - 0.4L /m^2 of NaOH, KOH 1% or Na_2Co_3 5%; on dry soil $Ca(OH)_2$ can also be used.
- Disinfections of the rooms in the buildings by formaldehyde at the rate of 1L (30% formaldehyde) and 300g $KMnO_4$ per 20m^3 volume. Aldehyde gas disinfects the room in 24 hours. Paraldeyhde can be used at the rate of 1kg / 100 m^3.
- Disinfections of the pipelines using 0.3L commercial grade formaldehyde in 10L of water for 24hrs and then the pipes were aerated.
- Disinfection of the transport vehicles done using chemical disinfectants or 100◦C damp heat.

7. Therapeutic use of chemicals and biosafety of chemical products in aquaculture

Satheesha Avunje and N.Lalitha

7.1 Therapeutic agents

Chemicals are also employed for therapeutic purpose to treat fish/shrimp when they are diagnosed with infection. Based on the pathogen, disease condition and host, a suitable therapeutic agent need to be selected and applied. Common therapeutic agents used in aquaculture may be as follows:

a. Acriflavin: It is an antiseptic dye used for treating fish or their eggs from bacterial or fungal infection. Dose: dip treatment with 25 ppm for 3-5 sec for seabass, 5 ppm for walking catfish. For aquarium fishes, bath treatment of 24 h with 5-10 ppm concentration.
b. Copper compounds: Age-old chemical and widely used as disinfectant and therapeutic agent. Bath treatment with 40-50 ppm of copper sulphate for 20-30 min for controlling protozoan parasites. 0.5 ppm of copper ethanolamine complexes is used to treat filamentous bacteria in shrimp. Repeated use of copper compounds should be avoided in shrimp farms, as they will be dangerous to the shrimp.
c. Trichlorfon: used in fish ponds to control parasites of crustacean, monogeneans and protozoan groups. Repeated treatment of 3 day interval for 2-3 times with 0.25-0.30 ppm concentration.
d. Formalin: One of the few chemicals approved by USFDA for treatment in aquaculture. 25-30 ppm of formalin is used to control parasitic infestation in fishes while in shrimp ponds, used to control phytoplankton bloom @ 25-40 ppm.
e. Malachite Green: It is banned compound in USA and EU. It is very effective to control ecto-parasitic infestation along with formalin (formalin: malachite green ratio of 25:0.1).
f. Potassium Permanganate ($KMnO_4$): Used to treat aquarium fishes with ectoparasites and external bacterial infection @ 5ppm for bath treatment. For dip treatment, 500 ppm of $KMnO_4$ can be used for min.
g. Benzalkonium Chloride (BKC): It is well-known bactericide and fungicide used in shrimp hatcheries at 1.0-1.25 ppm for long term treatment or bath treatment with 200 ppm for 30 min.
h. Trifuralin: It is generally recommended for treatming fungal infection in shrimp hatcheries @ 0.01-0.05 ppm daily
i. Sodium chloride: Common salt can be used for detachment of ecto-parasites from fishes by dipping the fish in salt water (25-30g/L) for 30 -60 min.

j. Antibiotics: Florfenicol, Oxytetracycline (OTC) and sulfadimethoxine/Ormetoprim are antibiotics or anti-microbials that are approved for use in aquaculture. Though these antimicrobials are approved for use as therapeutants in aquaculture, the residual withdrawal period has to be followed before harvesting the fish/ shrimp. Florfenicol is used @ 10-15 mg/ kg fish biomass for consecutive 10 days period. OTC is used @ 80 mg/kg biomass for fish and 4.5g/kg feed for shrimp with a feeding period of 10 and 14 days respectively. Sulfadimethoxine/Ormetoprim is fed to the fish @ 50 mg/kg body weight for a period of 5 days. It is important to note that no antibiotics should be administered simultaneously and the second antibiotics if required can be used after withdrawal period of the previous one.

7.2 Biosafety of chemical products used in Aquaculture

Disinfectant is an agent that destroys pathogenic and other kinds of microorganisms by chemical or physical means. A disinfectant destroys most recognized pathogenic microorganisms, but not necessarily all microbial forms, such as bacterial spores. Disinfectants used in general are highly reactive in nature and may cause ill-effect to the handling person, animals under contact and environment. In such a scenario, it is important to understand the biosafety of the chemicals used for disinfection and their handling. Before application of chemicals of hazardous nature, it is important to understand the environmental safety protocols, residual effect, withdrawal period and handling protocols to be followed.

Oxidising agents are highly reactive and caution to be taken while applying for disinfection. Chlorination is one of the most common disinfection process followed in aquaculture. The reactive chlorine may cause health hazard to the users, if not handled cautiously. The chlorine gas released from the disinfectants may cause serious damage to lungs and eye while handling and utmost care to be taken. Application of calcium hypochlorite to highly acidic water may release free chlorine gas that may cause serious health hazard to the personnel. Chlorination kills plankton and benthos, hence no natural food will be left out after chlorination. Development of primary productivity is essential before fish larvae are released into the culture ponds. Chlorine residual compounds remain in the water 48-72 h post treatment depending on water temperature and pH. Residual chlorine reacts with organic matter in the water body to form toxic chemicals, like reaction between free chlorine and ammonia develops chloramine, toxic to fishes or other aquatic animals. Chlorine residues can cause serious damage to the fish/shrimp gills causing mortality. The discharge of the treated water should be free of chemical residues to avoid damage to the natural aquatic fauna and flora.

Formalin, a reducing agent used to control fungal and parasitic infection, is a toxic chemical to fishes. It has a residual period of about 36h and the fish should be released after residues are

cleared. Glutaraldehydes are commonly used as anti-bacterial agents are known to cause skin and respiratory irritation to the users, and are toxic to the fishes. However, no bioaccumulation is observed. Thus chemical disinfectants should be used with precaution so that neither the user nor the culture animal should be adversely affected.

Generally heavy metals in the pond water are well below the toxic levels and their concentration may exceed the permissible limit due to excessive addition, and one such example is copper. Excess growth of blue-green algae releases geosmin in low saline ponds. Shrimp cultured in such waters will have unpleasant flavour. Farmers often apply excess amount of copper sulphate due to lack of information to eradicate the filamentous and blue-green algae. Dose of copper sulphate application varies from 0.1 to 0.2 ppm and it depends mainly on the total alkalinity of pond water. Copper based disinfectants are known to inhibit growth of plankton and induce shrimp moulting, may cause harmful impact if proper dosage is not used or residues are leftover. Environmental deterioration due to Cu accumulation in the pond sediments poses serious concern to shrimp health and growth. Excess copper concentration is known to decrease the haemocyte count, phenol oxidase activity, phagocytic activity and respiratory burst in cultured shrimp. Exposure of copper sulphate, as low as 5 mg/l for 24h leads to cytotoxic levels of superoxide anion. This immune suppression is correlated with the increased susceptibility to *Vibrio* challenge. In addition to immune suppression, Cu-exposure causes oxidative stress leading to structural damages in the gills and hepatopancreas. Cu^{2+} disturbs the cell calcium homeostasis leading to altered mechanism of apoptosis. Mechanism of cupper toxicity has been attributed to generation of reactive oxygen species, over production of these cause oxidative damage to tissue macromolecules including DNA, proteins and lipids.

8. Policies and guidelines for sustainable shrimp aquaculture in India

M.Jayanthi and M.Muralidhar

Shrimp farming has grown rapidly in recent years in many tropical and subtropical countries, but there have been setbacks resulting from diseases and the growing awareness of the environmental and social impacts of shrimp farming. At the global level, rapid expansion of coastal aquaculture has resulted in large-scale removal of valuable coastal wetlands and subsequent loss of goods and services generated by natural resource systems. In India, aquaculture has transformed from a traditional to a commercial activity in the last two and half decades and the area under shrimp culture has increased manifolds. The rapid development of shrimp aquaculture in the coastal areas of the country also raised some environmental issues, and the need for regulatory mechanism to control the indiscriminate growth of aquaculture was realized.

8.1 Guidelines for aquaculture as per CAA act 2005.

The Aquaculture Authority has brought out guidelines for the development of sustainable aquaculture. Coastal Aquaculture Authority Act was enacted in 2005 and a new Coastal Aquaculture Authority was instituted as per the Gazette Notification No. 1336 dated 22nd December 2005. The Aquaculture Authority constituted under the directives of the Supreme Court laid down certain conditions, related to the nature and conversion of the land used for shrimp farming, banning intensive and semi-intensive farming systems, requirement of Effluent Treatment Ponds and EIA etc., for issuing approval (license) for the shrimp farms. State level and District level committees were constituted by the State Governments for screening the applications on the basis of the above guidelines for recommendation to the Aquaculture Authority for issue of license.

- Under this Act coastal area for aquaculture includes the land within a distance of two kilometers from the High Tide Line of seas, rivers, creeks and backwaters.
- The delineating boundaries for coastal aquaculture along rivers, creeks and backwaters shall be governed by the distance unto which the tidal effects are experienced and where salinity concentration is not less than 5 ppt. In the case of ecologically fragile areas, such as Chilka Lake and Pulicat Lake the distance would be up to 2 km from the boundary of the lakes.
- No license for aquaculture should be granted allowing aquaculture within 200 m of the high tide line or any area within the coastal regulation zone. However, this is subject to the provision that it does not apply to any aquaculture farm in existence at the time of the establishment of the Aquaculture Authority. Noncommercial and experimental aquaculture farms operated by any research institute of the Government or by the Government
- Mangroves, agricultural lands, saltpan lands, ecologically sensitive areas like sanctuaries, marine parks, etc., should not be used for shrimp farming.

- Shrimp farms should be located at least 100 m away from any human settlement in a village / hamlet of less than 500 population and beyond 300 m from any village / hamlet of over 500 population. For major towns and heritage areas it should be around 2 km.
- All shrimp farms should maintain 100 m distance from the nearest drinking water sources.
- The shrimp farms should not be located across natural drainage canals / flood drain.
- While using common property resources like creeks, canals, sea, etc., care should be taken that the farming activity does not interfere with any other traditional activity such as fishing, etc.
- Spacing between adjacent shrimp farms may be location specific. In smaller farms, at least 20 m distance between two adjacent farms should be maintained, particularly for allowing easy public access to the fish landing centers and other common facilities. Depending upon the size of the farms, a maximum of 100 - 150 m between two farms could be fixed. In case of better soil texture, the buffer zone for the estuarine based farms could be 20 -25 m. A gap having a width of 20 m for every 500 m distance in the case of sea based farms and a gap of 5 m width for every 300 m distance in the case of estuarine based farms could be provided for easy access.
- Larger farms should be set up in clusters with free access provided in between clusters.
- A minimum distance of 50-100 m shall be maintained between the nearest agricultural land (depending upon the soil condition), canal or any other water discharge / drainage source and the shrimp farm.
- Water spread area of a farm shall not exceed 60 per cent of the total area of the land. The rest 40 per cent could be used appropriately for other purposes. Plantation could be done wherever possible.
- Areas where already a large number of shrimp farms are located should be avoided. Fresh farms in such areas can be permitted only after studying the carrying / assimilation capacity of the receiving water body.

8.1.1 Shrimp farm registration and renewal

All persons carrying out aquaculture in the coastal areas shall register their farm with the CAA. Such registration made for a period of five years with facility for further renewal. Aquaculture will not be permitted within 200m from HTL and also in creeks, rivers, and backwaters with in the CRZ. However it is not applicable to the existing farms set up before CAA act 2005. Every application for the registration of a coastal aquaculture farm shall be made to the District Level Committee as set up by the Authority, obtainable from the office of the District Level Committee or the office of the Authority or be downloaded from the website of the Authority. On receipt of an application, the District Level Committee shall verify the particulars given in the application in respect of all coastal aquaculture farms irrespective of their size; and

- In the case of coastal aquaculture farms up to 2.0 ha water spread area, the District Level Committee upon satisfaction of the information furnished therein shall recommend the application directly to the Authority for consideration of registration under intimation to the State Level Committee.
- In the case of coastal aquaculture farms above 2.0 ha water spread area, the District Level Committee shall inspect the concerned farm to ensure that the farm meets the norms specified in the guidelines with specific reference to the siting of coastal aquaculture farms and recommend such applications to the State Level Committee, which upon satisfaction shall further recommend the application to the Authority for consideration of registration.

The time frame of four weeks to the DLC for the detailed inspection and dispatching to SLC and two weeks for the SLC to give the recommendations are prescribed.

8.1.2 Regulations for SPF *P. vannamei* farms

- Aquaculture farmers who are registered with CAA will be required to submit a separate application for permission for farming *P. vannamei*. In case of so far unregistered farms, the application for registration must clearly spell out the intention to culture *P. vannamei*. Decision on such applications will be taken in accordance with these guidelines.
- Inspection team authorized by Coastal Aquaculture Authority shall inspect the farm and based on its recommendation regarding the suitability of the facility for farming of *P.vannamei*, applications shall be processed by the Member Secretary for consideration of the Coastal Aquaculture Authority for issuing permission to farms for farming of *P.vannamei.*
- Farms must establish adequate bio-security measures including fencing, reservoirs, bird-scare, separate implements for each of the ponds etc. The farms should be managed by the personnel who are trained and/or experienced in management of bio-security measures.
- *P.vannamei* shrimp is tolerant to low salinities but the rearing water should have a salinity of more than 0.5 ppt. The Govt. of India has notified that farmers who desired to culture vannamei outside the jurisdiction of CAA having the water salinity of above 0.5 ppt shall get registered with the Department of Fisheries (DoF) of the state government concerned. The farms should possess all the required infrastructure and biosecurity. The DoF may constitute a separate district level committee to inspect and give registration to the farms within a reasonable time frame of 60 days and other guidelines are same as that of brackishwater area.
- Farms irrespective of their size should have an Effluent Treatment System (ETS). Since loading of the environment with suspended solids is very high during the harvest, the ETS should be able to handle the waste water let off during harvest. Harvesting should be sequential depending on the size of the ETS. The quality of the waste water should conform to the Standards prescribed under the Guidelines issued by Coastal Aquaculture Authority.

8.2 Guidelines for culture of *P.vannamei* in fresh water / inland farms

Government of India have communicated approved guidelines that the farmers who desire to culture the exotic species, *P.vannamei* in fresh water/inland farms located outside the jurisdiction of the Coastal Aquaculture Authority(CAA), having a water salinity of 0.5ppt shall be required to register their farms with the State Fisheries Department. However, the farms located within the jurisdiction of the CAA shall register with CAA only.

The following guidelines and instructions for culture of *P.vannamei* in fresh water / inland farms located outside the jurisdiction of the CAA having a water salinity of 0.5ppt:

- No person shall carry on the culture of *P.vannamei* in fresh water / inland waters without permission in accordance with this Order.
- The District Level Committee (DLC) constituted in the GO (2nd) read above shall be the Competent Authority to permit the culture of *P.vannamei* in fresh water / inland farms located outside the jurisdiction of CAA.
- Permission for taking up culture of *P.vannamei* shall be accorded only to farms which have been already registered with the Fisheries Department and which have complied with the guidelines.

8.2.1 Guidelines for granting permission for culture of *P.vannamei* in fresh water /inland waters:

- The DLC shall consider only the farms which are outside the jurisdiction of the CAA and water salinity in the farm is above 0.5 ppt.
- Permission shall be accorded within 60 days based on the recommendations of the inspections conducted by the DLC regarding the suitability of the farm for farming of *P.vannamei*.
- Stocking density should not exceed 60 number / sq. m.
- The farm should maintain a detailed record of the name and address of the hatchery from where the seed is procured, quantity of seed procured, water quality parameters and daily feeding data during the culture period in the prescribed format.
- Banned drugs and antibiotics should not be used (list is given in the CAA website)
- The farm must establish adequate bio-security measures including crab fencing, bird scare and separate implements for each of the ponds.
- If the farm is not connected to the outside water sources (rivers, canals, lakes etc.) the reservoirs need not be insisted for disinfection.
- The farms with connections to open fresh water sources like rivers or canals or lakes etc. which are geographically adjoining to brackish water areas, irrespective of their size should have an Effluent Treatment system (ETS). The quality of treated water should conform to the standards of the standards prescribed by the A.P. Pollution Control Board.

- In case of any outbreak of disease, the farmer shall report immediately to District Fisheries Officer. Distress harvesting is permitted through netting only and the discharge water should be chlorinated and dechlorinated before release into drainage systems.
- Farms approved for *P.vannamei* culture shall not be permitted for farming of any other crustacean species simultaneously.
- Tested and certified seed should be procured only from the hatcheries approved by the CAA for *P.vannamei* seed production.
- For ponds not connected with open water sources, the accumulated organic wastes should be removed and disposed of safely.
- Farms located within the jurisdiction of CAA shall register with CAA invariably.

8.2.2 Advisories for sustainable culture of SPF *P.vannamei* in fresh water/ inland farms.

- It is advisable not to culture in fresh water with 0 ppt salinity since it could lead to poor growth, poor survival and poor quality
- Lower stocking rate is advised to reduce the operation cost and improve sustainability.
- Gradual acclimatization of the post larvae to the existing salinity conditions is very important for ensuring good survival.
- Younger stages of larvae below 15 days age old will not be able to tolerate lower salinities, hence PL15 and above should be used. In case of inland saline water culture, the ionic composition of pond water should be assessed continuously with respect to Potassium, Magnesium and Calcium for making necessary amendments.
- Feed with proper fortification of minerals as required should be followed for ensuring better survival rate and growth.
- Only probiotics suitable to the culture environment should be used.

8.3 Conclusion

Sustainable coastal aquaculture hinges on environmental protection and social responsibility. The guidelines are framed to ensure environment friendly, socially acceptable and sustainable aquaculture which should not disturb the other production systems and end users of natural resources. Self-discipline is the secret of sustainability. Therefore, the shrimp farmers and other stakeholders need to follow the regulatory guidelines and should integrate themselves with the Coastal Zone Development programmes so that shrimp farming can be sustained and continue to help in improving the socio-economic capabilities of the coastal population.

9. Sampling protocols and analysis of essential water and soil parameters

S. Suvana, A. Nagavel, P. Kumararaja, R. Saraswathy and M. Muralidhar

9.1 Collection of water samples

Since it is not possible to analyse the whole of a water body, samples, which are considered representative of whole of water mass are taken for different analyses. Sampling method depends largely on the parameter to be measured. Use only sample bottles with glass or plastic stoppers. Unbreakable polyethylene and polypropylene bottles are much more convenient.

9.2 Salinity

Salinity is not measured directly, but is instead derived from the conductivity measurement. This is known as practical salinity. The units used to measure salinity fluctuate based on application and reporting procedure. Parts per thousand or grams/kilogram (1 ppt = 1 g/kg) used to be the standard. Now salinity values are reported based on the unit less Practical Salinity Scale (psu). Absolute salinity is reported in g/kg and is denoted by the symbol S_A. The units psu, ppt and S_A g/kg are nearly equivalent and often interchanged.

Principle: The salinity of seawater can be determined by titrating the precipitable halides (Cl, Br^- and I^-) with silver nitrate solution as silver chloride using a chromate end point, the mohr titration. (Rapid low precision method):

Reagents:

(a) Silver nitrate solution: Dissolve 6.82 g of pure $AgNO_3$ in 250 ml of distilled water and store in a dark bottle. Standardize the solution by titrating against standard sodium chloride solution using potassium chromate indicator solution.

(b) Standard sodium chloride solution: Dissolve 2.06 g analytical NaCl in 250 ml of distilled water. Each ml of this NaCl contains 5 mg of $C1^-$.

(c) Indicator diluent solution: Dissolve 5 g potassium chromate in 80 ml of distilled water and dilute to 100 ml.

Procedure: To 5 ml of sample, add a few drops of indicator. Titrate with standard silver nitrate solution, with constant agitation of flask, until the colour just changes permanently from yellow to brown red and will not return to yellow with further shaking. Salinometer (Refractometer): Low precision salinity measurements in the field can also be made using Salinometer.

Calculation
Chlorinity (ppt) = volume of $AgNO_3$ used for titration
Salinity (ppt) = 0.03 + 1.80655 x Chlorinity (ppt)

9.3 pH

Principle: pH can be measured more accurately and conveniently with a pH meter and combination glass electrode

Procedure (Potentiometric): Take the water sample in a clean beaker and dip the electrode of the pH meter or portable pH probe into it. The indicator of the pH meter shows the pH readings directly. The meter should be calibrated routinely at pH 7.0 using appropriate buffer solution and then accuracy verified by testing a pH 9.2 buffer.

9.4 Alkalinity

Principle: It can be measured by titrating the water sample with a standard acid using methyl orange.

Reagents:
(a) **0.02 N** Sulphuric Acid: Dilute 30 ml of concentrated H_2SO_4 to 1 litre with distilled water to get approximately 1N stock solution. To make 0.02N H_2SO_4, take 20 ml of this stock solution and dilute to 1 litre with distilled water. Standardise this solution against 0.02N sodium carbonate using methyl orange as in indicator.
(b) **0.02 N** Sodium carbonate: Dissolve 5.3 g anhydrous sodium carbonate in 1 litre distilled water. Dilute 50 ml of this solution to 250 ml to get 0.02 N sodium carbonate.
(c) Methyl orange indicator: Dissolve 0.05 g reagent in 100 ml of distilled water.

Procedure: Add 2 drops of methyl orange indicator to 50 ml of water sample. If the sample remains colourless, no alkalinity is there. If it is yellow, titrate with 0.02N H_2SO_4 till the colour turns taint orange.

Calculation

Total alkalinity (ppm of $CaCO_3$) = volume of 0.02 N H_2SO_4 required for titration x 20

9.5 Hardness

Principle: Calcium and magnesium ions are titrated with the complexing agent ethylene diamine tetra acetic acid disodium salt (EDTA] to form the stable complexes. The end point of the titration is signaled with an indicator called Erichrom black-T.

Reagents:

(a) **Buffer solution:** Disolve 67.5 g of ammonium chloride in 570 ml of conc. Ammonium hydroxide. Dilute to 1000 ml with distilled water.

(b) **Erichrome black-T:** Dissolve 4.5 g of hydroxyl amine hydrochloride and 0.5 g of Erichrome black-T in 100 ml of 70 % ethanol.

(c) **Standard calcium solution:** Transfer 1.0 g of anhydrous calcium carbonate to a 1 litre beaker. Add 1: 1 HCl slowly to dissolve the calcium carbonate and dilute to about 200 ml with distilled water. Boil for ***5*** to 10 minutes to expel carbon dioxide, cool and adjust to pH 7.0 as determined with a pH meter, with 3N NH_4OH. Transfer to a 1000 ml volumetric flask and dilute to volume with distilled water.

(d) **Standard EDTA solution:** Dissolve 4.0 g EDTA disodium salt and 100 mg of $MgCl_2$.6H20 in distilled water and dilute to 1 litre. The solution must be standardized against the standard calcium solution. Pipette 10 ml of the standard calcium solution into a 250 ml beaker and add 90 **ml** of distilled water. Titrate the calcium solution with EDTA solution according to the procedure given below. Compare the molarity of the EDTA solution with the equation: **NV = N'V'**

Procedure: Measure a 100 ml of water sample into a 250 ml Erlenmeyer flask. Add 2 ml of the buffer solution and mix. Add 8 drops of Erichrome black-T indicator and titrate with the EDTA solution. At the end point, the solution will change from wine red to pure blue.

Calculation:

$$\textbf{Total hardness (mg/l as } CaCO_3\textbf{)} = \frac{\textbf{T. M. 10000}}{\textbf{S}}$$

Where, T = Volume in ml of EDTA solution

M = Molarity of EDTA solution

S = Volume in ml of sample

9.6 Turbidity

Turbidity is most often measured with a turbidity meter. Turbidity is reported in units called a Nephelometric Turbidity Unit (NTU), or a Jackson Turbidity Unit (JTU). The JTU was the original turbidity unit based on the visibility of candlelight in a tube (Jackson Candle Turbidimeter). However, this method is considered out of date and inaccurate in comparison to newer methods. NTU is more precise and has a wider range, whereas JTU cannot measure above 25 JTU. In addition NTU is the standard unit of many turbidity meters with wavelength of 400-680 nm. Nephelometric refers to the measurement of technology used, where the photodetector in the meter is placed at a 90 degree angle from the illumination source.

Principle (Nephelometric method): Turbidity can be caused either by planktonic organisms or by suspended soil particles. Turbidity due to suspended soil particles is measured by Nephelo-turbidity meter, which is based on the scattering of light beam, produced by tungsten filament lamp by particulate material. The quantity of light scattered is taken as a measure of turbidity in NTU. Higher the intensity of scattered light, the higher the turbidity.

Reagents:

(a) Turbidity free water

(b) Standard turbidity suspension

Solution-I: Dissolve 1g hydrazine sulphate in distilled water and dilute to 100 ml in a volumetric flask

Solution-II: Dissolve 10g hexamethylene tetramine in distilled water and dilute to 100 ml.

Mix 5 ml each of Solutions I and 11. Let stand 24 hours at 25OC. Dilute to mark and mix. The turbidity of this suspension is 400 NTU. Dilute 10 ml of this stock suspension to 100 ml with turbidity free water. Prepare daily. The turbidity of this suspension is 40 NTU.

Procedure: Calibrate the instrument using standard turbidity suspension. Shake the sample thoroughly. Wait until air bubbles disappear and pour sample into turbidimeter tubes. Place the tube in instrument and read turbidity in NTU directly from instrument scale.

9.7 Transparency

A standard Secchi disc is a circular metal plate having 10 cm radius. The upper surface of the disc is divided into four quadrants, painted in black and white colours. The disc is gradually lowered into the water and the depth (cm) at which the upper surface just disappears is noted (dl). Now the disc is slowly lifted upward and the depth at which the disc reappears is noted (d2). The value (dl+d2)/2 in cm gives a measure of transparency.

9.8 Total settleable solids

Principle: This is a portion of organic and inorganic solids that settles in 1 h in an Imhoff cone and is measured in terms of ml/l.

Procedure: Shake the water sample vigorously and pour 1 litre water into Imhoff cone graduated at the lower end and leave it for 1 h. Measure the quantity of settleable solids in ml/l.

9.9 Total suspended solids (TSS) and Total dissolved solids (TDS)

Principle: A well-mixed sample is filtered through a weighed standard glass fibre filter disc or Gooch crucible made of porcelain and the residues retained on the filter is dried to constant weight at 103^0C to 105^0C. The increase in weight of filter represents the total suspended solids. For total dissolved solids, the filtrate is evaporated to dryness in a weighed dish and dried to constant weight. The increase in dish weight represents the total dissolved solids.

Procedure: Wash filter disc with three successive 20 ml volumes of distilled water using vacuum. Continue suction to remove all traces of water. Filter a measured volume of well-mixed sample through the glass fibre filter disc or Gooch crucible. Wash with three successive 10 ml volumes of distilled water allowing complete drainage between washings and continue suction for about 3 minutes after filtration is complete. Transfer filtrate to a weighed evaporating dish for measurement of total dissolved solids.

TSS:

Dry filter disc/crucible containing residues for at least 1h at 103^0C to 105^0C in an oven. Cool in a desiccator and weigh. Repeat the cycle of drying, cooling, desiccating and weighing until a constant is obtained.

$$\text{TSS (mg/l)} = \frac{(A-B) \times 1000}{\text{Sample volume (ml)}}$$

A = Weight of filter or crucible + dried residue (mg)

B= Weight of filter or crucible (mg)

TDS:

Evaporate the filtrate in dish to dryness on a steam bath. Dry for at least 1h in an oven at 180°C, cool in a desiccator and weigh. Repeat drying, cooling, desiccating and weighing until a constant weight is obtained.

$$\text{Total dissolved solids (mg/l)} = \frac{(A-B) \times 1000}{\text{Sample volume (ml)}}$$

A =Weight of dried residues + dish (mg)
B = Weight of dish (mg)

9.10 Dissolved oxygen

Principle: DO can be determined by Winkler's method. In this method divalent manganese solution, followed by strong alkali, is added to the sample. Any dissolved oxygen rapidly oxidises an equivalent amount of divalent manganese to basic hydroxides of higher valency states. When the solution is acidified in presence of iodide ions, the oxidised manganese ions again revert to divalent state and iodine, equivalent to the original dissolved oxygen content of the water, is liberated. This iodine is titrated with standardised thiosulphate solution.

Reagents:
(a) Winkler **A** solution (Manganous sulphate) : dissolve 480 g MnS04.4H20 or 400 g of $MnSO_4.2H_2O$ or 365 g of $MnSO_4$. H_2O in distilled water and make up the volume to 1 litre.
(b) Winkler B solution (alkaline iodide) : Dissolve 500 g of sodium hydroxide and 300 g of potassium iodide in 900 ml of distilled water and make up the volume to 1 litre.
(c) Standard thiosulphate solution (0.025 N): To prepare 0.1 N stock solution of sodium thiosulphate, dissolve 24.82 g of crystalline $Na_2S_2O_3$.5H20 and 4.0 g of borax as a preservative in 700 ml of distilled water and make up the volume to 1 litre. Standardise the strength of this solution to exactly 0.1 N by titrating against 0.1 N potassium dichromate. To make 0.025 N thio solution, dilute 125 ml of this standardised stock solution (0.1 N) to 500 ml
(d) Concentrated sulphuric acid.
(e) 0.1 N potassium dichromate: Dissolved 4.904 g of dried and crystalline K~CI-207in
1 liter of distilled water.
(f) Starch solution **(0.2%)** : Add 2.0 g starch and 30 ml20% NaOH solution in 350 ml of distilled water. Stir until a thick, almost clear solution is obtained. Neutralise the alkali with HCl and acidify with 1 ml of glacial acetic acid.-Finally dilute the solution to 1 litre with distilled water.

Procedure: Collect the water sample in stoppered BOD bottle and add immediately 1 ml of manganous sulphate reagent with a pipette followed at once by 1.0 ml of alkaline iodide solution. Restopper the bottle immediately and mix the contents thoroughly by shaking to develop a flocculent precipitate. No air bubble should be trapped in the bottle. Add concentrated sulphuric acid (about 1 ml) to dissolve the precipitate. Transfer 50 ml of dissolved

solution into a conical flask. Titrate at once with 0.025 N standard thiosulphate solution until a very pale straw colour remains. Add starch (about 5 ml) indicator and continue the titration until the blue colour is just discharged. Solution should remain colourless for at least 20 seconds at the end point

Calculation:
DO (ppm) = 8000 N **x** V_1 / V_2
V_1 =volume (in ml) of $Na_2S_2O_3$ of normality N required for titration
V_2 = volume of water sample titrated.
If N = 0.025N and V_2 = 50 ml then **DO** (ppm) = $\mathbf{V_1}$ x **4**

Measurement of DO by DO meter :
DO can also be measured by **DO** meter (YSI, USA) in field.

9.11 Ammonia-N

Principle: Water sample is treated in an alkaline citrate medium with sodium hypochlorite and phenol in the presence of sodium nitroprusside, which acts as a catalyser. The blue indophenol colour formed with ammonia is measured spectrophotometrically.

Reagents
(a) De-ionised water
@) Phenol solution: Dissolve 20 g of analytical grade phenol in 200 ml of 95%v/v ethyl alcohol.
(c) Sodium nitroprusside solution: Dissolve 1.0 g of sodium nitroprusside, $Na_2Fe(CN)_5NO.2H_2O$, in 200 ml of de-ionised water. Store in a dark glass bottle. The solution is stable for at least a month.
(d) Alkaline reagent: Dissolve 100 g of sodium citrate and 5 g of sodium hydroxide in 500 ml of de-ionised water. The solution is stable indefinitely
(e) Sodium hypochlorite solution
(f) Oxidising solution: Mix 100 ml of reagent (d) and 25 ml of reagent (e). Prepare fresh every day.
Standard curve: Dissolve 0.9433 g of analytical reagent quality ammonium sulphate in 950 ml of distilled water. Add 1 ml of chloroform and make up the volume to 1 litre. Store in refrigerator, sheltered from strong light. This solution contains 100 ppm and is stable for many months if well stoppered. Prepare a series of standard solutions from this stock solution and carry out the method exactly as described above. After colour development, measure absorbance at 640 nm and prepare a calibration curve from the absorbance of a series of standards.

Procedure: Add 50 ml of seawater to an Erlenmeyer flask from 50 ml measuring cylinder. Add 2 ml of phenol solution, swirl to mix and then add in sequence 2 ml of nitroprusside 5 mi of oxidizing solution. Mix after each addition by swirling the flasks. Cover the flasks with aluminum foil to lessen the contamination by atmospheric ammonia and allow the flasks to stand at room temperature for 1 hr in dark. The colour is stable for about 24 hr after the reaction period. Read the absorbance at 640 nm in a spectrophotometer against blank or distilled water using 10 cm cell. Carry out the method exactly as described above for blank also using 50 ml of de-ionized water.

Calculation: Calculate the ammonia concentration by using calibration curve.

9.12 Nitrite-N

Principle: The nitrite in water is allowed to react with sulfanilamide in an acid solution. The resulting diazo compound is reacted with NED and forms a highly coloured azo dye.

Reagents:
(a) Sulfanilamide solution: Dissolve 5.0 g of sulfanilamide in a mixture of 50 ml of conc. HCl and about 300 ml of distilled water. Dilute to 500 ml with distilled water. The solution is stable for many months.
(b) NED (N-(1-naphthy1)- ethylene diamine dihydrochloroide solution) Dissolve 0.5 g of the dihydrochloride in 500 ml of distilled water. Store the solution in a dark bottle. The solution should be renewed once a month or directly a strong brown colouration develops.
(c) Standard nitrite: Dissolve 1.064 g anhydrous, analytical grade potassium nitrite, KNO_2, (dried at 105^0C for 1 hr) in distilled water. Add 1 ml 5 N NaOH and dilute to 250 ml. This solution contains 700 mg/l nitrite-N and should be stored in a dark bottle with 1 ml of chloroform as a preservative in refrigerator. The solution is stable for several months.

Procedure: Add 1.0 ml of sulphanilamide solution from a pipette to each 50 ml sample, mix and allow the reagent to react for more than 2 minute but less than 10 min. to assure a complete reaction. Add 1 ml of NED reagent and mix immediately. Leave for 10 minutes and then measure the absorbance (OD) of the samples and standards against a reagent blank at 540 nm. The colour is stable for **2** h. Calculate the nitrite concentration by using calibration curve.

9.13 Hydrogen sulphide

Reagents
(a) Hydrochloric acid, HCL, 6N.

(b) Standard iodine solution, 0.0250N**:** Dissolve *20* to 25 g KI in a little water and add **3.2** g iodine. After iodine has dissolved, dilute to 1000 ml and standardize against 0.0250 N $Na_2S_2O_3$, using starch solution as indicator.
(c) Standard sodium thiosulfate solution, 0.0250N: Dissolve 6.205 g $Na_2S_2O_3.5H_2O$in distilled water. Add 1.5 ml 6N NaOH or 0.4 g solid NaOH and dilute to 100 ml
(d) Starch Solution: Dissolve 2 g starch + 0.2 g salicylic acid as a preservative in 100 ml hot distilled water.

Procedure: Measure from a burette into a 500-ml flask an amount of iodine solution estimated to be an excess over the amount of sulphide present. Add distilled water, if necessary, to bring volume to about 20 ml. Add 2 ml 6N HCl. Pipette 200 ml sample into flask, discharging sample under solution surface. If iodine color disappears, add more iodine so that color remains. Back titrate with Na2S203 solution as end point is approached, and continuing until blue color disappears.

Calculaltion
ml of 0.0250N iodine solution reacts with 0.4 mg S^{--}

$$mg\ S^{--}/L = \frac{[(A \times B)-(C \times D)] \times 16000}{\text{ml sample}}$$

Where: A = ml iodine solution, B = normality of iodine solution, C = ml $Na_2S_2O_3$ solution, and D = normality of $Na_2S_2O_3$ solution.

9.14 Residual chlorine (Free, Combined and Total)

Principle: N, N-diethyl-p-phenylenediamine (DPD) is used as an indicator in the titrimetric procedure with ferrous ammonium sulfate (FAS). Where complete differentiation of chlorine species is not required, the procedure may be simplified to give only free and combined chlorine or total chlorine. In the absence of iodide ions, free chlorine reacts instantly with DPD indicator to produce a red colour. Subsequent addition of iodide ions acts catalytically to cause chloramines (mono & di) to produce colour.

Reagents
(a) Phosphate buffer solution: Dissolve 24 g anhydrous Na2HP04 and 46 g anhydrous KH_2PO_4 in distilled water. Combine with 100 ml distilled water in which 800 mg disodium ethylenediamine tetra acetate dihydrate (EDTA) have been dissolved. Dilute to 1 L with distilled water and add 20 mg $HgCl_2$ to prevent mold growth and interference in the free chlorine test caused by any trace amounts of iodide in the reagents. (CAUTION: *$HgCl_2$* is *toxic, hence take care to avoid ingestion).*

(b) *N,* **N-Diethyl-phenylenediamine** (DPD) indicator solution: Dissolve 1 g DPD oxalate, * or 1.5 g DPD sulfate pentahydate, or 1.1 g anhydrous DPD sulfate in chlorine-free distilled water containing 8 ml (l + 3)HzsO~an d 200 mg disodium EDTA. Make up to 1 L, store in a brown glass-stoppered bottle in the dark, and discard when discolored. Periodically check solution blank for absorbance and discard when absorbance at 515 nm exceeds 0.002/cm. CAUTION: *The oxalate is toxic - take care to avoid ingestion.*

(c) **Standard ferrous ammonium sulfate** (FAS) titrant: Dissolve 1.1 06 g $Fe(NH_4)2(SO_4)_2$. $6H_2O$ in distilled water containing 1 ml 1 + 3 H_2SO_4 and make up to 1 litre with freshly boiled and cooled distilled water. This standard may be used for 1 month, and the titer checked by potassium dichromate. For this purpose add 10 ml 1 + 5 H_2SO_4, 5 ml conc. H_3PO_4, and 2 ml 0.1% barium diphenylamine sulfonate indicator to a 100-ml sample of FAS and titrate with 0.100 N primary standard potassium dichromate to a violet end point that persists for 30 **s.** The FAS titrant is equivalent to 100 ug Cl as Cl_2/ 100 ml.

Procedure : Mix 5 ml each of buffer reagent and DPD indicator in a conical flask. Then, add 100 ml of sample (upto 5 ppm, if > 5 ppm use diluted sample) and mix and titrate rapidly with standard ferrous ammonium sulphate titrant until red colour is discharged (titre value A). For combined chlorine, add about 1 gm of KI, mix and then continue titrating until red colour is discharged again.(titre value B).

Calculation

For a 100-ml sample, 1 ml standard FAS titrant = 1 .00 mg Cl as Cl_2/ L

A = Free chlorine

B = Combined chlorine (mono-chloramines and di-chloramines)

C = (A+B) = Total chlorine

9.15 Collection of soil samples

Collection of representative soil sample for different analyses merits greater attention since, error at the time of sampling cannot be corrected at a later stage. Soil tests and their interpretations are based on the soil samples sent in for analysis. It is therefore important that soil samples should be properly collected and be representative of the area to be tested. Methods of sampling depend largely on the purpose for which the sample is drawn.

Materials required

Spade, Auger, Tins, Polythene bags, Khurpi

Procedure

- The area from which the soil samples are collected should be divided in to different sampling units. The size of plot or the farm area that could be represented by one 'composite sample' depends on the spatial variability in the fields. Sampling units should not be more than 10 per hectare. Thus for each acre (approximately 4000 m^2) field one composite sample may be sufficient. For this purpose after scraping the surface litter a thin 112" to 314" slice of soil from 8-10 spots, scattered uniformly over the area (preferably a zigzag pattern) should be collected.
- Proper sampling tools should be used. Any of the tools such as tube auger, screw type auger, posthole auger or a spade can be used for digging the soil. Spade or tube auger is satisfactory for moist and soft soil. Screw type auger is convenient for hard and dry soil, while posthole auger is useful for wet soil. For samples up to 30 cm depth, a cut in the soil can be made with a spade and a thin slice of soil taken at a desired depth (0-15 and/ or 15-30 cm) with the help of khupra. If samples from deeper soil layers have to be taken an auger should be used. For collecting depth-profile core samples (0-30 cm, 30-60 cm, 60-90 cm and 90-120 cm), soil core sampler can be used.
- After collecting the sub samples, they should be combined together and mixed thoroughly. All the lumps should be broken and mixed well in the container or on a clean cloth. The size of the composite sample should be reduced by successive quartering to about half a kilogram.
- The sample has to be dried in the shade, till it dried ground to fine powder with the help of wooden hammer, passed initially through a 2 mm sieve and finally through a 80 mesh sieve and packed in an air tight polythene or ordinary cloth bag for subsequent analyses, with sufficient information.

9.16 Soil reaction

The soil reaction (pH) is meant to express the acidity or alkalinity of soil.

Measurement of pH by Potentiometric method

Potentiometric method with electrically or battery operated pH meter with the help of suitable electrodes is used for determination of soil pH values for greater accuracy.

Instrumentation

PH meter with glass and calomel electrodes

Materials and reagents

1. Glass beakers 50 ml
2. Glass rods

3. Buffer solutions
(a) 0.05 M Potassium hydrogen phthalate has a pH value of 4.001 at 20°C and 4.02 at 35'C. Dissolve 10.21g of potassium hydrogen phthalate in distilled water and dilute to 1 litre.
(b) 0.01 M Borax solution has a pH value of 9.22 at 22OC: Dissolve 3.81 g of borax in distilled water and dilute to 1 litre.
(c) Standard buffer tablets1 solutions.

Procedure
Take exactly 10 g of prepared soil sample in a clean beaker and add 25 ml of distilled water. Shake it occasionally by stirring with glass rod and keep it for about half-an-hour. Then dip the electrodes of pH meter into soil solution that has already been checked with standard buffers of known pH. The indicator of the pH meter shows the pH readings directly. The pH meter should be calibrated routinely at pH 7.0 and then accuracy verified by testing a pH 9.2 buffer.

Observations:
Soil pH (1 :2.5 Soil-water ratio) :

9.17 Determination of electrical conductivity
Electrical conductivity (E.C) is commonly used for indicating the total concentration of the ionized constituents of solutions.

Principle
When water is added to the soil, the soluble salts get dissolved. Solutions offer resistance to the passage of electric current through them depending upon the concentration and type of ions present. Higher the salt content, less the resistance to the flow of current. The resistance (R) by Ohms' law is defined as the ratio of electric potential (E) in volts and strength of current (I) in amperes. Electrical conductivity (E.C) is the reverse of the resistance and is expressed in reciprocal of Ohms or as mhos per cm. As the values of E.C obtained for soil solutions are very small, it is therefore, convenient to express them in milli mhos per centimetre.

Instrumentation
Conductivity meter

Materials and Reagents
1. Glass beaker
2. Glass rod
3. 0.02M potassium chloride - Dissolve 1.4912 g of Kc1 in distilled water and dilute to one litre. The specific conductance of this solution at 25OC is 2.268 mmhos/cm.

Procedure

Same soil-water (1: 2.5) suspension for pH estimation may be used for electrical conductivity determination also. Meanwhile the instrument is put on by connecting the conductivity cell to the proper electrodes and calibrated with 0.02 M Kc1 solution. Rinse the conductivity cell with distilled water and then twice with soil water suspension. Dip the electrodes in the soil-water suspension and the multiplier is brought to the suitable range and the compensation knob is brought to the temperature of the solution and read directly the specific conductance of the solution.

Observations and calculations

E.C m mhos/cm (L) = Dial reading X Cell constant X multiplier range
Milli equivalents of salts1 litre of soil solution = L m mhos/cm X 10 (approximately)
ppm of salts in soil solution = 640 L m mhos/cm
Osmotic pressure of soil solution = 0.36 L m mhos/cm

9.18 Estimation of organic matter

Organic matter in a mineral soil is regarded as an index of its fertility status. The organic matter content of soils can be obtained by organic carbon estimation. The rapid titration method of Walkley and Black is being universally used as it has an advantage that it excludes the less active elementary carbon and includes those parts of organic carbon of soil, which play an important role in nutrient availability. This method is widely used for estimating the organic carbon content of freshwater pond soils and with some modifications may be used for brackishwater fish pond soils also.

Principle

A known quantity of soil is digested with known excess of chromic acid using the heat of dilution of sulphuric acid. The excess chromic acid, which is not utilized for the oxidation of organic carbon, is back titrated against standard ferrous ammonium sulphate solution using diphenylamine indicator till the bright blue colour changes to light green colour.

Reagents

(a) 1N Potassium dichromate solution: Dissolve 49.048 of solid $K_2Cr_2O_7$ in distilled water and make the volume to 1 litre.
(b) Sulphuric acid with silver sulphate: Dissolve 5 g of AgS04 in 100 ml of conc. H_2SO_4.
(c) 85% Orthophosphoric acid: Commercially available
(d) Diphenylamine indicator: Dissolve 0.5 g of reagent grade diphenyl amine in 20 ml water and 100 ml conc. H_2SO_4

(e) IN Ferrous ammonium sulphate: Dissolve 392.2 g of ferrous ammonium sulphate in 800 ml distilled water containing 20 ml conc. H_2SO_4 and dilute to 1 litre with distilled water.
(f) Sodium fluoride salt: Commercially available.

Procedure
Take 1 g of soil sample in a 500 ml conical flask and moisten with few ml of distilled water. After about 10 minutes add exactly 10 ml of 1N $K_2Cr_2O_7$ and 20 ml of $AgSO_4$ mixed H_2SO_4. The contents of the flask are stirred slowly for 5 minutes and then flask is placed on asbestos plate and allowed for digestion of contents for 30 minutes with intermittent shaking. After digestion about 100 ml of distilled water is added followed by 5-10 ml of orthophosphoric acid. About 1g of NaF and 10-20 drops of diphenylamine indicator should be added. The contents are thoroughly shaken and titrated against 1N Ferrous ammonium sulphate solution. The colour is dull green at the beginning, which turns to a turbid blue as the titration proceeds and at the end point sharply changes to a brilliant green. A blank titration is also conducted without soil sample.

Observations and Calculations
Organic carbon (%) = (Blank titration value - sample titration value) X 0.3
% organic matter in soil = Organic carbon X 1.724

9.19 On-farm monitoring of water parameters with test kits
The water quality parameters can be regularly monitored at the farm site itself with the help of commercially available kits, which works based on the principle of colour change and further calculation either directly by colour comparison or by a factor multiplied with the no. of drops. Kits have been developed by ICAR-CIBA for detection of pH, DO, ammonia, nitrite, calcium, magnesium, total hardness and total alkalinity in water samples. Kits have been tested for variety of samples including freshwater, brackishwater and coastal waters. Kits for detection of ammonia and nitrite have been commercialized to private entrepreneurs Chennai based Shrimpex Biotech Services and pH and DO kits to Itarasi based FisherMan's.

Benefits
- Higher accuracy and sensitivity; less requirement of water samples; increased shelf life of the reagents; cost effectiveness; wider range of detection; user-friendliness and easy to use in the laboratories and fields.
- Since all the tests are mainly based on the color chart comparison and number of drops, no equipment is required.

These kits are useful for aqua-farmers and hatchery operators for regular monitoring of these critical parameters in aquaculture, hatchery waters and related aquatic environment for maintaining optimum water quality parameters in the system.

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