CIBA-TM Series – No.8

TRAINING MANUAL

On

RECENT ADVANCES IN SOIL AND WATER MANAGEMENT IN BRACKISHWATER AQUACULTURE

25-30 June, 2018

भा.कृ.अनु.प.- केन्द्रीय खारा जलजीव पालन अनुसंधान संस्थान
भारतीय कृषि अनुसंधान परिषद, कृषि मंत्रालय, भारत सरकार
#75, संथोम हाई रोड़ राजा अण्णामलै पुरम चेन्नई - 600 028, तमिलनाडु, भारत
(ISO 9001:2008 certified)
ICAR - Central Institute of Brackishwater Aquaculture
Indian Council of Agricultural Research, Ministry of Agriculture, Govt. of India
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FOREWORD

Indian shrimp farming sector has kept the upward growth trend which took off in 2010 with the introduction of SPF *Penaeus vannamei*, and recorded spectacular shrimp production of 4,87,470 MT during 2015-16 accounting for 39.53 per cent in quantity and 66.06 per cent of the total earnings in dollar terms. The contribution of Pacific white shrimp, *P. vannamei* was 4,06,018 MT. Though *P.vannamei* seed has SPF status, it is no longer maintains its status, once it is stocked in the pond environment. The productivity of a pond depends upon its soil and water characteristics, where all the abiotic and biotic factors will converge. Generally, fish/shrimp production is low in ponds located in agriculture belt due to degraded soils, while high in those ponds located in fertile soils. The properties of soils and water quality should be considered in selecting a site for aquaculture. The water and soil quality variables affecting shrimp survival and growth are the determining factors for poor growth and disease outbreaks. Disease is an expression of a complex interaction of host, pathogen and environment. Generally disease will not occur when the culture environment i.e., water and soil parameters are maintained at optimum levels, through adoption of better management practices (BMPs).

The issues related to aquaculture and environment belongs to two broad categories - impact of aquaculture on environment and impact of environment on aquaculture. When compared to other industries, pollution from aquaculture sector is negligible. The degradation of aquaculture land due to pesticide residues and heavy metals discharged from agriculture and industries is threatening the aquaculture activity, which are often overlooked, and ignored such adverse impacts of the environment on aquaculture. Aquaculture has both positive and negative impacts; where the advantages comparative outweigh the low adverse effects. There have been substantial socio-economic benefits, which include production of healthy food, employment opportunities, higher income, foreign exchange and nutritional security.

My complements go to Environment Section of Aquatic Animal Health and Environment Division for conducting a training program on "Recent Advances in Soil and Water Management in Brackishwater Aquaculture during 25-30 June, 2018 and to my colleagues in publishing this training manual. I hope this manual covering the aspects related to soil and water management in shell fish and finfish farming systems, environmental impact assessment and carrying capacity assessment of water bodies for optimization of shrimp aquaculture development will be useful to the trainees and other stakeholders.

I wish the training program a grand success.

K.K.VIJAYAN

Chennai
20th June, 2018

CONTENTS

CHAPTER 1

BRACKISHWATER AQUACULTURE IN INDIA: AN OVERVIEW

Vijayan K K

Aquaculture, not internet, represents the most promising investment opportunity of the 21st century –Peter Drucker (management expert)

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 (*Mathieson*, 2014). 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.

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.

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 product 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 (George and Rao, 1963), 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 (Sasidharan et al., 2012), 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.

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 (Chamberlin, 2010).

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. Unfortunately, in India we did not have an SPF programme for any of the candidate species of Indian penaeids, the tiger shrimp or Indian white. 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. Again, the Taiwanese were the first to use SPF *P. vannamei* from US, with an initial success of 13 mt/ha production within 75 days of culture (Wyban, 2002). 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.

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.

Presently, the scenario of Indian brackishwater aquaculture is found to be bright; however, it is certainly not without problems, uncharacterized diseases, problems in the hatchery production, problems due to the pond reared broodstock etc are the challenges of brackishwater aquaculture sector.

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.

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).

Selective breeding and inter-specific diversification

The aquaculture of the native *P.monodon* and *P indicus* in India is at a cross road; technology for the development of post larvae using wild caught broodstock has been standardized, and alleviated the shortage of seed supply in almost all the countries where *P. monodon* farming is carried out. However, the natural progression of *P. monodon* farming from fishery based aquaculture to domestication and selective breeding has not been happened partly due to the constraints related to the induced maturation and reproductive success of captive broodstock. To initiate and take up a selective breeding programme into success, is a long term process, where positive partnership of different stake holders such as industry, research organization, developmental agencies and famers, are imperative. Non availability of a native-SPF, forced the farming countries in Asia to depend on the imported broodstocks of SPF-shrimp, *P. vannamei*, as a short gap technological arrangement. Now it's time to think and plan a selective breeding programme for an Indian penaied, the white shrimp, *P. indicus* can be the ideal choice due to the possession of several aquaculture traits, viz. growth, reproduction and larval rearing, hardiness and stress tolerance, especially to temperature and salinity. Farming trials has showed the suitability of this species for high stocking density culture, in a commercial trial carried out in Tuticorin where a yield of 8 mt per ha in five and half month culture at a stocking density of 70 no/m^2 has been realised (CIBA, 1990). This shrimp is suggested as an alternative species ideal for Indian scenario (Vijayan, et al 2004 and Rajeev et al 2007).

Ecosystem approach to aquaculture

Ecosystem approach to aquaculture (EAA) is needed to provide an unambiguous linkage among aquaculture, environment and society to promote the complimentary role, ensuring environmental sustainability social equity and development. While developing new aquaculture it should ensure that it should be an integral part of community and ecosystem. Further, the diversity of unprocessed aquaculture products, for example, in the case of mud crab there are niche market for gravid females, soft shelled crabs etc, and value added products should be considered. The modern aquaculture, particularly shrimp aquaculture requires high investment in terms of inputs and farm management, often practiced, relatively, by affluent farmers, and not a choice for the landless rural poor at the grass root levels. The aquaculture at the confined ponds near Chilka Lagoon (Reyntjens, 1987 and Balasubramanian et al., 2004) and finfish aquaculture as a livelihood options for poor farmers in Thailand are proven examples. The EAA framework converges, the seemingly mutually exclusive ecological trajectories such as 'aquaculture for rich' and 'aquaculture for poor'. Thus a wide involvement of stake holders occupying different social niches lead to blue revolution. Some components of ecosystem approach are:

a. Non-fed aquaculture

Aquaculture of shell fishes and seaweeds are regarded as non-fed aquaculture forms and they are considered to be the most sustainable form of aquaculture as they feed at the base of the food chain, where no artificial is used, saving money and remain friendly to the aquatic system. They are also highly efficient water filters and directly remove particular matters and reduce the turbidity and directly or indirectly remove the nitrogen and other nutrients, contributing to a healthy environment. The edible oyster, *Crassostraia madrasensis*, is one of the potential species for bivalve farming. Initial experimental work carried out by CMFRI (Devaraj and Appukuttan, 2000), and later by Mohamed et al (2013) demonstrated the potential of the species in brackishwater farming. The diversification of traditional bivalve fishers into shellfish aquaculture as a part time enterprise would act as alternate livelihood, to improve the income of fishing families.

The potential of seaweed farming in brackishwater, using suitable species such as *Gacilaria edulis*, can also be integrated in a multi-trophic aquaculture, and indigenous technology is already available for the culture of seaweed (Devaraj and Appukuttan, 2000)

b. Family farming and rural aquaculture

As mentioned earlier, the potentials of brackishwater aquaculture for improved livelihood and greater house hold food security are poorly addressed. Brackishwater aquaculture can contribute effectively for the improved food security and rural empowerment, exploiting the vast stretches of bracksihwater in the coastal areas and inland saline waters. Initial experiments conducted by CIBA to involve rural women in mud crab aquaculture and sea bass nursery rearing indicate the potential of rural aquaculture. The work was conducted among the self-help group, although the performance was successful, the continuation of the project, once sponsoring authority withdraws the funding in the form of input support, is not found to be encouraging due to the problems in group conflicts and social dynamics. An

alternative or complimentary to the self-help group model is family farming. Family farming is a means of organizing aquaculture production which is managed and operated by a family and predominantly reliant on family labour, including both women's and men's (FAO 2014). A successful model for family farming for aquaculture of *Etroplus suratnesis* in Kerala has been demonstrated (Krishna et al 2013) and this model can further be refined integrating other aquacultured species.

d. Diversification of brackishwater aquaculture

Finfishes: The finfishes such as Asian sea bass (*Lates calcarifer*), grey mullets (*Mugil cephalus*), milkfish (*Chanos chanos*), pearl spot (Etroplus suratensis) are important candidate species for brackishwater aquaculture. The production procedures for these species are under various stages of standardization and transfer of technology. Sea bass is one of the most preferred species and CIBA has successfully bred this species and hatchery production technology has been perfected. Nursery rearing technology for the production of fingerlings have been developed and demonstrated to small farmers. Grey mullets form an important component in the traditional farming systems using wild seeds and technology for poly culture has been demonstrated to farmers.

Mud crabs: Mud crabs, species of *Scylla*, are one of the most traded sea food commodities in India. The technology for seed production, nursery rearing and grow out production have been standardized and under various stages of technology transfer.

Other penaeids: Species such as *F.merguinesis, Marsupenaeus japonicus* and species of genus *Metapenaeus*, are valuable species for aquaculture in India. The hatchery production of these species are standardized and many experimental grow out culture have been conducted. These species have regional importance and have high market values. Strategies have to be developed for the popularization of these species.

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.

In recent years, the sustainability of shrimp farming has been questioned in view of the various environmental and social concerns raised. Shrimp production levels increased mainly due to expansion of farming area and adoption of intensive farming practices. Nutrient enrichment of pond waters and discharging water from ponds are common management practices to ensure adequate water quality for shrimp growth. However, the discharge of such nutrient-rich waters may result in deteriorating water quality in receiving waters, and is the subject of increasing regulation in many countries. Management strategies for sustainable shrimp farming can be done at two levels, one is at the level of the farm which involves proper utilization of resources and inputs and has to be followed by the farmer and the other is at the level of policy makers so as to integrate shrimp farming in the overall development plan of the coastal zone.

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.

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

State	**1990**	**1994**	**1999**	**2016**
West Bengal	33815	34400	42525	51980
Orissa	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
Orissa	8991	28432	5.8
Andra 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**	

Penaeus vannamei farm (courtesy, Dr Saji Chacko)

Penaeus vannamei

CHAPTER 2

SOIL AND WATER SUITABILITY FOR AQUACULTURE

M. Muralidhar, R. Saraswathy and S. Suvana

Aquaculture ponds are normally built on soils. Selection of potential and suitable sites is the first and foremost step for successful aquaculture. The success of shrimp culture depends on essential features namely good bottom soil and better quality of water. Aquaculture ponds are normally built of soils. Properties of soils should be considered in selecting a site, designing earthwork, and specifying construction methods to provide a water-tight pond with stable levels and bottom slopes. The nature of a particular soil type is dependent on its physical properties and nutrient content. Soil quality is an important factor in pond productivity as it controls pond bottom stability, pH and salinity. It also regulates the quality of the overlying water. The bottom soil helps in organic mineralization process, adsorption and release of nutrients to water. It provides shelter and food to bottom biota, influences water quality and hence helps the survival and growth of shrimp. The condition of pond bottoms and the exchange of substances between soil and water strongly influence water quality. A large amount of information is needed for proper planning, design and construction of aquaculture ponds. The lack of knowledge and attention on soil properties will lead to unsuccessful shrimp farming and realize aquaculture ponds to their full potential.

The productivity of a pond depends upon its soil - water characteristics. Generally, fish/shrimp production is low in ponds located in agriculturally poor productive soils and high in those placed in fertile soils. A satisfactory pond soil is the one in which mineralization of organic matter takes place rapidly and nutrients are absorbed, held and released slowly over a long period. Further, in bottom soil, a series of chemical and biochemical reactions take place resulting in either the release of nutrients from soil to water or absorption of nutrients from water by the soil and microbial population. In general, moderately heavy textured soil having moderate organic matter content is desirable for aquaculture. This process governs the growth and population of the micro and macro food organisms in the fish/shrimp ponds. To understand the complete pond ecosystem, it is essential to study the characteristics of pond soil and water to increase the productivity of the ponds in general and thereby augmenting fish and shrimp production.

Soil suitability for aquaculture

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.

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 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:

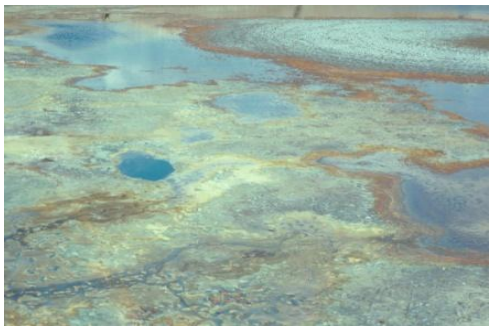
- Supply of sulphate
- Supply of easily decomposable organic matter
- Adequate source of iron
- Anaerobic condition coupled with chemically reducing microbes
- Tidal action

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 of 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 prawn mortality, pH has to be monitored regularly, and necessary lime application is to be done.

Sandy soil



Acid sulphate soil

Mangroves soil

Soils not recommended

Soil quality

The nature of soil affects the shrimp production and hence one should have well acquaintance with the properties of soil.

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.

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.

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.

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%.

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.

Table 1. Soil parameters suitable for brackishwater aquaculture site

Parameter	Optimum range
pH	6.5-7.5
Organic C (%)	1.5-2.0
$CaCO_3$ (%)	>5.0
Av. N (mg/100g)	50-70
Av. P (mg/100g)	4-6
EC (mmhos/ cm)	>4

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.

Soil limitation ratings concept in shrimp aquaculture

A system of limitation ratings and restrictive features for soil properties was offered for use in shrimp aquaculture. Ranges for classes and degree of limitation for each property were based on literature, experience and best judgment.

Soils were placed into three classes according to their limitations for excavated ponds, pond levels, dikes or embankments. The rate class is given in forms of limitations and restrictive features. Only the most restrictive feature should be listed when a limitation class is given. If the rating is slight there is no need for restrictive feature.

Excavated ponds

Interpretation of soil limitations for excavated ponds (Table 2) considered soil properties to a depth of 150 cm.

Pond embankments, dikes and levees

Embankments, dikes and levees are raised structures of soil material constructed to impound water. The soil material is considered as being mixed and compacted to medium density. Soil used for these applications must resist seepage and erosion. The final material should not cause toxic leachate to enter ponds. The ratings given in Table 3 for an in-place soil from the surface to the depth of 100 cm, with the assumption that all soil layers will be mixed in dozing, loading, dumping and spreading. The major properties considered are erosion, stability and permeability.

Definition of limitation ratings

Soils should be rated in-place. Soils are rated to have a slight, moderate or severe limitation for a particular property. A moderate or severe limitation does not mean that a soil cannot be used for aquaculture. Developers can modify soil features, adjust plans and redesign to compensate for many moderate and severe soil limitations. Managers can implement management practices to overcome many severe water limitations. However, the initial cost of pond and dyke construction and cost of maintenance must be considered when on-site soils have a restrictive feature. Limitation ratings are for single properties; consequently, efforts to overcome limitations are different depending on the property and local conditions. The following are limitation-rating definitions essentially used;

(1) Slight - This rating indicates that on-site soils have properties favourable for use. No unusual construction, design, management or maintenance will be required for the designated use.

(2) Moderate - This rating indicates that on-site soils have one or more properties that will require special attention for the designated use. This degree of limitation can be overcome or modified by special planning, design management or maintenance.

(3) Severe - The severe rating is given when one or more properties of on-site soils are unfavorable for the rated use. Major reclamation and modifications in design, management or maintenance will be required for the designated use and sometimes, it may not be economically feasible.

Table 2. Soil limitation ratings for excavated ponds

Property	**Limitation rating**			**Restrictive feature**
	Slight	**Moderate**	**Severe**	
Depth to sulfidic or sulfuric layer (cm)	>100	50-100	<50	Potential acidity or toxicity
Thickness of organic soil material (cm)	<50	50-80	>80	Seepage; hard to compact
Exchangeable acidity (%)	<20	20-35	>35	Exchangeable acidity
Lime requirement (T/ha)	<2	2-10	>10	Mineral acidity
pH of 50-100 cm layer of pond bottom	>5.5	4.5-5.5	<4.5	Too acid
Clay content (%)	>35 Clayey	18-35 Loamy	<18 Sandy/ silty	Too sandy/ silty; excessive seepage
Slope of terrain (%)	<2	2-5	>5	Slope
Depth to water table (cm)	>75	25-75	<25	Hard to drain; dilution
Frequency of flooding	None	Occasional	Frequent	Flooding
Small stones (%)	<50	50-75	>75	Small stones
Large stones (%)	<25	25-50	>50	Large stones
Decomposed OM (%)				
Low clay content soil (<60% clay)	<4	4-12	>12	Excessive humus
High clay content soil (>60% clay)	<8	8-18	>18	Reducing environment
Depth to rock (cm)	>150	100-150	<100	Shallow; seepage

Source : Boyd, C.E. (1994)

Table 3. Limitation ratings for pond embankments, dikes and levees

Property	Limitation rating			Restrictive feature
	Slight	Moderate	Severe	
Clay content (%)	>35 Clayey	18-35 Loamy	<18 Sandy	Too sandy
Depth to sulfidic or sulfuric material (cm)	>100	50-100	<50	Toxicity; potential acidity
Slope (%)	<8	8-15	>15	Slope
Thickness of organic material (cm)	<15	15-50	>50	Subsides; excess humus; difficult to compact
Depth to water table (cm)	>100	50-100	<50	Wetness
Fraction >8 cm diameter (%)	<25	25-50	>50	Large stones
Depth to bedrock (cm)	>100	50-100	<50	Depth to rock
Shrink – swell potential	Low	Medium to high	Very high	Shrink – swell
Erodibility (K)	<0.1	0.1-0.3	>0.3	Erosion

Source : Boyd, C.E. (1994)

In one case study conducted by CIBA at Gopalapuram area of Nellore District, Andhra Pradesh some of the properties of farm area such as low pH, high sand content and low organic carbon comes under moderate rating according to the classification mentioned above i.e., these soils have one or more properties that will require special attention for the designated use. This degree of limitation can be overcome or modified by special planning and management such as liming, organic manuring and additional compaction of soils. The soils may be considered suitable for shrimp farming upon managing these moderate limitation properties.

Water suitability for aquaculture

Water quality and quantity determines the success or failure of an aquaculture operation. The estimation of the quantity of water required in a farm and the ways and means to meet the needs are the essential factors to be considered in the choice of a site. Day-to-day management of ponds requires only an estimation of the topping-up rate of the water supply, to combat evaporation and seepage losses. However, it should be noted that a large supply of water should be on-hand to flush ponds if needed, or refill them after draining. An annual water budget should be calculated for a potential farm site so that the supply is adequate for existing and future needs.

Settleable solids more than 20 ml/l result in rapid silting of the pond and decreasing of water depth. Optimum level of total suspended soilids is < 100 ppm. Excessive TSS led to increased sedimentation of eco-system. Source waters should be analysed for heavy metals and pesticides before use since agricultural fields and industries nearer to water bodies are the sources.

CHAPTER 3

SHRIMP AQUACULTURE IN INDIA: PRESENT STATUS AND WAY FORWARD

C.P. Balasubramanian and K.K. Vijayan

Aquaculture has evolved from a simple but an elegant system, which has deep community and family roots. In 1980s, there was a drive towards the export oriented agriculture crops. Thus, many traditional agriculture has grown from basic food producing system to a market driven complex export oriented enterprise. Shrimp culture in the tropics is the paradigmatic example for this transformation. Tropical shrimp farming is considered to be one of the few success stories of modern aquaculture. Evolution of shrimp aquaculture from a fishery based pond production system of 1970s to a mature industry of 1990s is spectacular. Its early success attracted many farmers, and this industry has become the focal point of export in many tropical developing countries. However, the early success and image of risk-free clean-industry has not lasted for many years due to the frequent disease hits and crop failures. Success of shrimp culture often depends on how successfully disease out-break can be prevented and controlled. Further environmental protection, conservation of biosecurity and social equity are equally important for the long-term sustainability of shrimp farming, although these elements are masked by the short term gains and success.

History of shrimp farming in India is almost similar to the other South East Asian countries. 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 product 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 (George and Rao, 1963), 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 (Sasidharan et al 2012), 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, 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. Again, 2000-2011 witnessed a remarkable upsurge of farmed shrimp, due to the introduction of, the exotic American shrimp, *Penaeus vannamei*, resulting in an increased production of about 1,25,000 tonnes in 2012-13.

Seed production industry in India

The first trials for seed production in India of penaeid shrimps started in 1975 at Narakkal prawn culture laboratory of CMFRI. By the end of 1970s most of the cultivable penaeids were reared under laboratory conditions, and hatchery production of P. indicus was standardized (Silas et al., 1978). A spectacular growth in the shrimp hatchery sector has been found in the 1990s and the number of hatcheries increased from 13 in 1990 to 280 in 2003 with a production of 10 billion seeds (FAO, 2007). However, there has been a great change since 2008, when Pacific white shrimp has been introduced since 2008. The shrimp seed production has been increased exponentially, and crossed 32 billion PL per year, and number of hatcheries also doubled and almost 500 hatcheries are currently under operation (Ramraj, 2015).

Farming systems

Conventionally, shrimp farming systems, applied to most aquacultured species, are categorized into traditional, modified-traditional, extensive, semi intensive and intensive system. By no means, these systems are universal. They are highly dynamic and modifications and refinements are being made constantly. These classifications are made based on the degree of management inputs provided (FAO/NACA 1995)

Traditional farming systems are practiced in West Bengal, Kerala, Karnataka and Goa, also adopted in some areas of Orissa. These farms include large variety of poly culture systems and fully tide fed. These farms are located at the coastal low lying area with tidal effects along estuaries, creeks and canals. These are farms of vast areas ranging from 2-200 ha. Average production is low and ranges from 200 to 500 kg/ha per year (mixed species and size). In improved traditional farms, ponds are stocked with wild seed, and overall yield increase by 100 to 200 kg/ha/ per year

Extensive systems are monoculture systems, usually water taken through pumping from canals, creeks or sea. Farmers use better management practices such as formulated feeds and

water managements. Ponds are prepared with tilling, liming and fertilization, which enables the application of higher stocking densities (up to 10 per square metre) and increases the potential yield to some 1000 kg per hectare per crop. Semi-intensive system are more recent pond system up to 1 ha size, use of good quality SPF seeds with regular water management system such as reservoir ponds, nutritionally well balanced formulate feed, proper aeration, use of other water and pond management inputs such as probiotics and pond bioremediatiors etc. Recently advanced techniques such as recirculation system, zero water exchange systems etc are being used. Since 2010, 90% of the farming is done by introduced SPF white shrimp, *P. vannamei*

Production characteristics

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 65000 ha in 1990, it increased up to 0.16 million ha in 1999, and again it has been reduced to 0.12 ha in 2014. Major reduction in area under farming was recorded in Andhra Pradesh, from 85000 ha in 1999 it reduced to 36000 ha in 2014, whereas West Bengal and Gujarat showed marginal increase. However, Andra Pradesh still contributed more than half of the farmed shrimp production in India. By early 2000 shrimp production also increased up to 100000 mt in 2001. 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, and shrimp farming gradually increased and peaked with a maximum production of about 1.4 lakh tonnes in 2006, but production reduced drastically in 2008. However, the year 2014-15 witnessed a remarkable upsurge of farmed shrimp, due to the introduction of, the exotic American shrimp, P. vannamei, resulting in an increased production of about 426569 tonnes.

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 (Chamberlin 2010).

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, Unfortunately, in India we did not have an SPF programme for any of the candidate species of Indian penaeids, the tiger shrimp or Indian white. 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 *L. vannamei*, although the scale of shrimp farming was only limited in Americas. Again, the Taiwanese were the first to use SPF *L. vannamei* from US, with an initial success of *L. vannamei* production of 13 mt/ha within 75 days of culture (Wyban 2002). Following the success of Taiwan, *P. vannamei* was introduced into several South East Asian countries including India. In India, from 2010, a dramatic growth of farmed shrimp production due to the introduction of *P. vannamei* (Figure 3 and 4)was recorded, with 90,000 MT in 2010 to 1,75,000 MT in 2013-14 (Manimaran, 2014). 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.

Issues in current brackishwater aquaculture

However, the development of *P. vannamei* aquaculture has certainly not been without problems. Currently *P. vannamei* has been facing several problems 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.

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 management,
- expansion of new inland and coastal areas,
- development of non-fed aquaculture (shell fish and sea-weed).

Further, modern aquaculture is profit driven and governed by free market principles, for example, when wild fishery of a species no longer support market demands, the price raises and this makes farming commercially viable (Jolly and Clonts, 1993). If the species is more expensive in the market, it is more attractive for farming and, thus, the concept of aquaculture as a producer of cheap protein is giving way to dynamic industry that target specific market segments (Wickins and Lee 2002).

Better management practices and Biosecurity measures

History of best management practices can be traced back to the history of aquaculture or the history of any production system. It is evolved from the producers' quest to reduce the input and costs, and vast majority of the best management practices are generated by the producers. No single BMP reduces key impact equally, as there is no one-size fit for all. The most effective BMP depends on species cultured, type and magnitude of impact, scale of production, resource available to producers and overall management of the system. As best management practices in aquaculture and biosecurity protocols are intimately linked, in this lecture note, these aspects are treated together.

Biosecurity

The entire stake holders of aquaculture concerned about biosecurity: Consumers need to ensure the seafood that they eat are safe, the processors have to follow HACCP guidelines to provide safe seafood, investors should protect their investment from the preventable losses. The Biosecurity workshop for aquaculture defined biosecurity as: "an essential group of tools for the prevention, control, and eradication of infectious disease and the preservation of human, animal, and environmental health" The principles of bio-security are not only to keep away the pathogen from the farming environment but also from the country. The success of poultry industry world-wide is the successful implementation of biosecurity, It has been prompted the use of similar protocol in shrimp farming. In Poultry biosecurity is defined as: "cumulative steps taken to keep disease from a farm and to prevent the transmission of disease within an infected farm to neighboring farms." It is a team effort, shared responsibility and an ever-time process. Basic philosophy behind biosecurity is to prevent the entry of pathogen, ensure the best living condition to the animals and to provide a clean product to the customer. The principles of biosecurity in the poultry can be applied to the aquaculture

Site selection: Poorly located sites are often found to be failed and provide negative ecological impacts. Potential problems should be identified and measures should be taken to avoid maximum problems. Mangrove sites and other coastal wet lands should be avoided, as these habitats are inherently important for ecological well-being.

Farm design: Modular seawater system with reservoir ponds before use in culture is found to be effective. All these farm design directly depends on the characteristic site and level of intensity. All inlet and outlet system should be free from leakage, and to avoid carriers such as crabs and birds, preventing nets should be installed. Additionally, the management

measures to improve soil quality and other preventive measures should be taken as per the following Table

Strategies	Benefits
Sludge removal and disposal away from the pond sites	Increase the carrying capacity of the pond, and improve the pond general conditions
Adoption of minimal water exchange	Increase the stability of culture environment, minimize the entry of influent pathogens
Water filtration using twin bag filters of 300 µm filters	Prevent the entry of disease carrying vectors,
Water treatment using approved chemicals such as chlorine, and aging the water	Eradication of pathogens
Maintaining the water depth at least 80 cm at shallow part of depth	Prevent the formation of benthic algae

Broodstock and post larvae

During the early days of shrimp farming, farmers used wild seed stock entering along with the tidal inflow or captured wild broodstock. This practice was replaced later with the use of hatchery produced seeds obtained from the wild caught broodstock. This wild caught broodstock are often carriers of pathogens. Thus, it is understood that dependence of wild broodstock are important source of pathogen entry, and without de linking the wild fishery and aquaculture, the disease management cannot be attained effectively. Thus, use of captive reared and specific pathogen free broodstock are found to be crucial. The process of development of Specific pathogen free broodstock are given below

Development of Specific Pathogen Free stock

Whatever the methods have been incorporated to eradicate the occurrence and out -break of disease in aquaculture ponds, none could provide enough protection, if we use seed stocks derived from the wild brooders. Therefore, the most important principle of biosecurity is the use of domesticated stocks, which have been cultured under controlled conditions and that have been under active disease surveillance program. The development and use of specific pathogen free (SPF) stock is, perhaps, the best management strategy for stock control in farms or regions or countries. Although in market place, these stocks are called as "disease free" in reality they are free of specific disease causing agents. It should be understood that no living being is completely free of diseases. SPF means the stock of interest has at least 2 years of documented historical freedom of pathogens listed on the working list. These pathogen should have the following criteria: 1) the pathogens must be excludable, 2) adequate diagnostic methods should available and 3) pathogen should poses significant threat to industry.

The process of SPF development begins with identification of wild or cultured shrimp stocks. The samples of this stock then will be tested for specific pathogens using appropriate diagnostic procedures. If these stocks are free from specific pathogens they are designated as

founder population or F_0, and they will be reared in a primary quarantine facility. During the primary quarantine F_0 stock will be monitored periodically for the specific pathogens. If this stock is detected for any of the specific pathogens, the stock will be destroyed. The stock will be moved to secondary quarantine, if they are free of specific pathogens. At this facility these stocks will be matured, selected and produce F_1 generation. These F_1 stocks will be maintained in quarantine further to ensure that they are free from specific pathogens. These SPF stocks will be supplied to hatcheries and breeding centers.

Use of stress test

Exposure to weak concentration of formalin or with change in salinity can be used to determine whether post larvae are strong enough to survive stocking into ponds.

Feeds and feed management

Manufactured feeds account for 60 -70% of total operating cost in shrimp aquaculture. Feeds are one of the important concerns for environmental group because it depends marine capture fishery for fish meal and fish oil. Further, 20 to 40% of feed becomes remained unused by shrimp become pollutant to the pond. Use high quality feed, and feed should not contain more Nitrogen and Phosphorus than shrimp needed. Feed management practices should be carefully monitored. It should be assured that shrimp consume as much as feed shrimp consume, to avoid the wastage of feed. Check tray should be used to avoid over feeding and under feeding. Feeding should be practiced four to five times per day, and it should be broadcasted widespread. Feed should be adjusted with biomass and appetite of shrimp. Natural productivity has an important role in the nutrition of farmed shrimp. The larvae at early stages cannot consume the pelletted feed as efficiently as larger shrimps, and therefore, natural biota of the culture pond plays an important role in the nutrition during the early phase of culture. Therefore, production and maintainence of natural productivity has important role in the sustainable shrimp farming. Do not use fresh feed or other material for feeding the farmed shrimp.

Health management.

Regular monitoring of shrimp for the health status should be carried out, the sick and moribund shrimp should be removed regularly. In the case of disease out-break of disease strict quarantine protocol should be followed to prevent the spread of disease. As many stressors reduce the innate immunity of many cultured shrimp, the measures should be taken to minimize the stress such as maintenance of high oxygen content in the water, maintaining stable pH, temperature and salinity of rearing water, minimize the use of feed, water exchange etc. The eradication of disease at the beginning is easier and do not use antibiotic. Use probiotics judiciously and only when the efficacy of the product is proved.

Selective breeding and inter-specific diversification

The aquaculture of the native *P. monodon* and *P. indicus* in India is at a cross road; technology for the development of post larvae using wild caught broodstock has been standardized, and alleviated the shortage of seed supply in all most all the countries where *P. monodon* farming is carried out. However, the natural progression of *P. monodon* farming

from fishery based aquaculture to domestication and selective breeding has not been happened partly due to the constraints related to the induced maturation and reproductive success of captive broodstock. To initiate and take up a selective breeding programme into success, is a long term process, where positive partnership of different stake holders such as industry, research organization, developmental agencies and famers, are imperative. Non availability of a native-SPF, forced the farming countries in Asia to depend on the imported broodstocks of SPF-shrimp, *L vannamei*, as a short gap technological arrangement. Now its time to think and plan a selective breeding programme for an Indian penaied, the white shrimp, *Penaeus indicus* can be the ideal choice due to the possession of several aquaculture traits, viz. growth, reproduction and larval rearing, hardiness and stress tolerance, especially to temperature and salinity. Farming trials has showed the suitability of this species for high stocking density culture, in a commercial trial carried out in Tuticorin where a yield of 8 mt per ha for a five and half month culture at a stocking density of 70 no/m^2 has been realised (CIBA, 1990), the shrimp is suggested as an alternative species ideal for Indian scenario (Vijayan, et al 2004 and Rajeev et al 2007).

CHAPTER 4

BRACKISHWATER FINFISH FARMING SYSTEMS

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Introduction

Fish is one of the best animal protein sources for nutritional security. The world fish production is in the order of 148 million tons (FAO, 2010), more or less equal contribution by capture and culture fisheries. The increasing demand for fish by the growing population worldwide (expected to surpass 9 billion by 2050) to cater the need, fish production through capture fisheries alone may not meet the requirement and necessarily aquaculture has to contribute great extent. Aquaculture has grown many folds with annual growth rate of 8.8 % in the last 3-4 decades represents more than 90% of global aquaculture output by volume. The growth of the industry has largely been a result of two major factors; intensification through technological advances and increased use of inputs like feed. Fish farming is an age old practice in many countries carried out by the coastal community with their indigenous traditional knowledge. However, in the recent years fish culture has become full time profession taken up on large scale by the farmers, self-help groups, corporate and entrepreneurs in a much more improved manner. The phenomenal growth during the past decades was tremendous and guided significant increase on the employment opportunity, food security and economic upliftment of the people engaged in this business. Brackishwater aquaculture was synonymous with farming of shrimps. However, this monoculture practice of shrimp has resulted to disease problems and the sector has suffered with serious setbacks. Diversification of farming practices to other groups such as finfishes is suggested as one of the remedial measures. Culture of fish is mostly carried out from ponds, pens and cages with supplementary feeding, water quality and disease management.

Culture systems and utilization of brackishwater bodies

Traditional culture systems

In India, fish culture is being practiced traditionally in the ponds and pens. The bheri fish culture in West Bengal, the fish and shrimp culture in Pokkali paddy fields of Kerala, Ghery fish farming in Chilka lagoon of Orissa and Khazans of Goa are some of the practices. In the traditional method, fish culture was done by extensive method without much of inputs like stocking of seed, feed, water exchange etc., Fish seeds are allowed to enter in to pond though sluice gate (auto stocking) during high tide. Seed stocking is done by this method during the season when fish seeds are available from the wild. Without any supplementary feeding with low stocking density fish production is achieved from 500 to 700 kg/ha in a season (maximum of six months).

Improved culture systems

In the improved culture system, selective stocking of seed is done either from wild collection or from hatchery production. Stocking is done @ 6000- 8000ns/ha. After pond preparation, feeding is done with the artificial diet and proper water quality management is carryout.

Sampling is done at regular intervals to assess the growth and enhancement feeding or health assessments are done. Water exchange is also carried out according to the requirement. Aerators are provided in the fish pond in order to improve the dissolved oxygen levels. All the activities are conducted based on the schedules to achieve the maximum production. In the improved pond culture method, fish production can be achieved from 4000 to 6000 tonnes / ha.

Mono species culture

Mono species culture of finfishes is generally practiced for the high valued species. However, other low value species also can be taken up by extensive culture method. Carnivore fish species such as seabass *Lates calcarifer*, grouper *Epinephelus spp*, Snapper *Lutjanus spp* and omnivore species like Cobia *Rachycentron canadam* can be taken up either in ponds or in cages. Since these fishes are highly carnivores, it can predate other species of smaller size fishes and hence they are suitable for monoculture practice.

Polyculture

Culture of many fish species in a single pond is called polyculture. Polyculture method is practiced by the farmers in order to utilize the available phytoplankton, zooplankton, and benthic organisms in the pond as feed for the fishes. By this method, the production can be achieved by introducing fish species which can be the surface water feeders, column feeder and bottom feeder. Ponds that have been enriched through chemical fertilization, manuring or feeding practices, contain abundant natural fish food organisms living at different depths and locations in the water column. Fish species for the polyculture have to selected based on their feeding habits. Poly culture is being done since the fish farming was started by traditional way.

Fishes having the feeding habits of planktonicvore, herbivore and bottom feeders have to be selected for the polyculture. In the polyculture ponds, organic and inorganic fertilizers can be added in order to enhance the productivity particularly the plankton production. These phytoplankton and zooplankton can serve as food for the fishes stocked in the ponds. Herbivore fishes can feed the micro algae, filamentous algae and other weed plants present in the pond. Species like milkfish *Chanos chanos* can consume decaying organic substances present in the bottom of the pond. Shrimp can feed on the benthic organisms such as polychaetes, molluscan etc., from the sediment. Predatory fish like seabass is also cultured along with the Tilapia *Oreochromis spp*. In this system, small tilapias were consumed by the seabass and small tilapia will be available continuously in the pond, since Tilapia can breed throughout the year.

In the brackishwater culture system, fish species such as Milk fish *Chanos chanos*, grey mullet *Mugil cephalus*, Pearl spot *Etroplus suratensis* and Tilapia *Oreochromis spp* can be taken up together with the varying stocking densities. Along with the finfishes, shrimp species such as *Penaeus monodon, P.indicus, F. japonicus* and *F.merguensis* also can be cultured. In the polyculture ponds, manures and fertilizers are added heavily. This can cause over blooming of algae and there by resulting oxygen depletion, which is stress to the fish. To

overcome this problem, the bloom has to be monitored and controlled. Proper stocking densities of each species have to be maintained in order to utilize the pond productivity at different depths of pond water effectively

Economically important cultivable Brackishwater finfishes

Seabass (Lates calcarifer)

Asian seabass *Lates calcarifer* is highly priced fish in India, which is distributed in Indo-Pacific region and in Australia. It can withstand the salinity from 0-40 ppt. The fish can be cultured in freshwater, brackishwater and marine condition in earthen ponds and cages. Under pond condition, the fish can attain the growth of 800-1000gm in 6-8 months period either with trash fishes or artificial diet. With the stocking density of 4500-5000 nos/ha, seabass production can be achieved between 4.0 to 4.5 tonn/ha. The fish is being widely cultured in Australia, Thailand, Singapore, Malaysia, Philippines, Indonesia and India. In the recent days, seabass is also cultured in Europe. In India, the seed production technology of seabass has been standardized by Central Institute of Brackishwater Aquaculture, Chennai. In India, seabass farming is practiced in many states by obtaining either hatchery produced seed or wild source seed. Seabass can be farmed in the sea cages and also in the shallow backwater net cages.






Milk fish* (*Chanos chanos)

The milkfish *Chanos chanos* is an important food in South East Asia. They occur in Indo-Pacific region. In Philippines, milkfish seed are collected from the backwater, low lying region areas and raised for grow out culture either in ponds or in cages. Milkfish is an ideal fish for pen culture and can be cultured even in fresh water. Milkfish can grow to 500gm in 5-6 months. It is a plankton feeder and the diet of the milkfish either supplied wholly by natural productivity or is fed wet particulate diets. Milkfish sold fresh, frozen, canned, or smoked. The milk fish is a national symbol of the Philippines. The fish can withstand maximum salinity up to 35 ppt. In India milkfish seed is available in short period of three months in Andhra Pradesh coast and near Rameshwaram in Tamil Nadu. Milkfish can be cultured along with other fishes and shrimps in ponds. In monoculture pond, milkfish can be stocked @ 7000-8000 fingerlings/ha and formulated feed can be provided @ 3-5% body weight daily. After 6 months culture, milkfish can attain the mean body weight of 500g with the productivity of 3.5-4.0 tonnes/ha.

Grey mullet (Mugil cephalus)

Grey mullets are the preferred food fishes in India due to its white tender meat. Mugil cephalus inhabits estuarine, freshwater, coastal and marine water bodies. It occurs in lagoon, with juvenile fishes most common in impounded areas, around mangroves, in seagrass beds, and offshore. Mullets are herbivore fish and it consumes the decaying organic matter. It is one of the ideal candidate species for brackishwater culture and can withstand salinity from 0 to 35 ppt. The fish can grow 500gm in six months period under pond conditions with low cost feed. Among the mullets, *M.cephalus* grows faster than other species. *M.cephalus* is distributed in the Indo-pacific region, Hawaii, Israel, Iran and Egypt. In India mullet is being cultured by traditional method. In India mullet is being cultured in Kerala, West Bengal and certain parts of Tamil Nadu as polyculture mode along with other finfish and shrimp species. In monoculture, mullet can be stocked @ 7000-8000 fingerlings/ha to achieve a production of 3.5-4.0 tonn/ha.

Culture of finfishes in the Cages

Cage culture of fish is a method of raising fish in containers enclosed on all sides and bottom by materials that hold the fish inside while permitting water exchange and waste removal into the surrounding water. Cage culture of finfishes has been practiced for years in many southeast Asian countries and Norway.. It allows the opportunity of holding fish of varied sizes and utilizing deep waters that are difficult to seine because of stumps or other obstructions. Because of the high stocking rates, crowded conditions, and the cage's fixed, special attention to water quality and feeding management is required for successful production. Some of the highly valued species mostly on higher trophic levels are farmed in cages.

Species selection

- The fish should have faster growth rate
- Amenable to crowded conditions
- Ability to withstand environmental changes
- High market demand (Possibilities for live fish market or processed market)
- Size of the fish preferred for the market
- Resistance to disease

Potential fish species suitable for cage farming

	Common name	Scientific name	Suitability
1	Seabass, Giant perch, Cock up, Barramundi	*Lates calcarifer*	It can grow up to 1.0 kg in 6-8 months culture period. Tolerate wide range salinity from 0-35 ppt. Fetches Rs.200-350/kg depending upon the size
2	Cobia	*Rachycentron canadum*	It has fast growth rate attaining the size from 4-6 kg in 12 months under sea cages. Ideal salinity range 27-35 ppt. Accepts pellet feed and market price ranged from Rs.200-300/kg
3	Estuarine grouper, Greasy grouper, Brown spotted grouper	*Epinepheleus tauvina*	Groupers can grow from up to 1 kg in 12-18 months under sea cage condition depending upon the management protocols followed. But international market available for small size live groupers also. *E.tauvina* can be cultured in brackishwater saline cage also and other species are purely marine in nature
4	Humpback grouper	*Cromileptes altivelis*	
5	Tiger Grouper	*Epinepheleus fuscoguttatus*	
6	Mangrove snapper	*Lutjanus griseus*	Snappers are the ideal species suitable for cage culture either in sea based or brackishwater lagoon based conditions. It can grow up to one kg in 10-12 months period and accepts artificial conditions
7	Mangrove red snapper	*Lutjanus argentimaculatus*	
8	Spotted rose snapper	*Lutjanus guttatus*	

Advantages of cage culture

- for cage culture man-made or natural bodies of water e.g., ponds, reservoirs, lakes and streams, canals can be potentially used
- Cage system provides natural environmental to the fishes.
- Can be cultured in high density
- Can be operated to any scale
- Close monitoring in terms of feeding, sampling, observation and harvesting is possible

Disadvantages of cage culture

- Vulnerability of crowded and confined fish to incidence of diseases and parasites
- Rapid spread of diseases
- Localized poor water quality, e.g., dissolved oxygen in and around cages
- Caged fishes need a nutritionally complete, fresh feed
- Cages area attractive to predators, vandals and poachers

Site selection

Net cages should be set up in calm water bodies like sheltered lagoons, Bayd behind an island or a river mouth. This is to avoid damage due to strong waves and current. The criteria for selecting a suitable site for cage culture are

- Tide and water depth. Water depth should be more than 4 meters.
- Current and waves. An ideal area would be a protected bay, sheltered cover or island sea
- Water quality. Pollution free
- Water circulation to improve the poor water quality
- Must be accessible and preferably secured from vandals and poachers

Design of cages

Cages are designed usually rectangular, square or circular in shapes. Size of the cages varied according to the purpose, species to be farmed and depth of the water bodies in which cage has to be fixed. The size can be 5×3×1M, 5×3×2M, 5×5×1M and 5×5×2M. The cages have to be designed in such a way so that a series of cages can be fixed as row in one place and platform has to be fixed to connect from the road or main land or to get down from the boat. All the cages have to be connected together.

Materials for cage preparation

The following materials are required for fabricating the cages

- Nylon net materials with the mesh size of 1-2 inches
  (Nylon material is highly preferred because it has high breaking strength, high melting point, and high extensibility)
- HDPE twines
  (The high density poly ethylene (HDPE) is highly suitable for fabrication of net cages because of high breaking strength (110%) in water, less shrinkage in water (5-8%), non-absorbance of moisture, easy for handling and cleaning, rigid nature enables free water exchange)
- Wooden or galvanized iron or PVC pipes frame
- Plastic drums for floating
- Anchor & wooden platform/walkways

Types of cages

Cages can be set up according to the water depth and three types of cages can be used for the culture purpose. They are floating cages, submergible cages and stationary cage.

Floating cage

Floating cages are set up usually in the deeper water bodies. In this cage, floats can be fixed at the bottom of the frames, which enables the cage to float. The top, four corners of the net are tied in all the four corners of the cage frames and the bottom, four corners of net have to be tied separately with the separate anchor and allowed to hang so that the cage is positioned its shape. In the top frame, wooden planks can be fixed tightly and can be used as walkway.

Stationary cage

Stationary cages can be fixed in shallow water bodies. Wooden poles can be used for fixing the cage. Bottom of the net has to be tied with the poles. All the four sides of the cage have to be tied with series of poles at one meter intervals.

Stocking of fish juveniles

Size of the fish to be stocked in the cages can vary from 5 to 10 cm and larger than the mesh size of the net. The stocking density up to marketable size varies from 10 to 100 fish per m^3. Before stocking in the cages, fishes have to be acclimatized to the temperature and salinity of the water in the cages.

Feeding in cages

Seabass, grouper, and snapper are carnivorous and voracious in feeding habit feed on live fish and crustaceans. It is advisable that these finishes must be weaned to feed on trash fish/formulated feed which to be fed in the cages. In the initial period of culture, feeding rate can be fixed at 5-7% body weight and later it can be gradually reduced to 3% body weight.

Cage management

Cage management is an important component to be considered seriously. Periodical cleaning has to be carried out to remove the debris and other adhering materials from the cages which would be brought by the tidal water movements. There after the water circulation within the cage will be improved. Damage of the cages by other possibilities like crabs is common and this has to checked and repaired. At the time of flooding, lot of debris may be brought in to the sea by the terrestrial run off, which may cause clogging and these materials have to be removed. Poaching is a major problem everywhere, and therefore, a security has to be arranged to take care the cage as well as the stocked fish.

CHAPTER 5

SOIL QUALITY MANAGEMENT IN BRACKISHWATER AQUACULTURE

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Good bottom soil and water quality are vital ingredient for any successful aquaculture practices. Although such problems are related to site characteristics bottom soils have undesirable properties viz acid sulphate, high organic and excessive porosity etc. On the other hand, even if the site is good, problems may still crop up by the large quantity of inputs like feed and fertilizers, 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 such as pond preparation, liming, moderate stocking etc.

Pond preparation

Pond preparation is meant to prepare the pond to have favourable environment to the shrimp post larvae to live and grow in steady manner so as to attain marketable size within crop duration of 100 - 120 days. Pond preparation is generally dealt in two categories viz., newly constructed ponds and existing culture ponds. The main objectives of pond preparation are to produce the shrimp with a clean pond base and appropriate stable water quality by ensuring the following

(i) Removal of predatory and unwanted animals from the pond

(ii) Removal of poisonous gases - obnoxious gases such as H_2S, NH_3, etc.

(iii) Generation of natural productivity in the culture ponds.

Newly constructed ponds

In newly dug out ponds, the characteristics of the soil has to be understood first before adopting the various measures to prepare the pond. Soil samples taken from different locations of the pond are thoroughly mixed together and a representative portion is taken for analysis. Understanding of the soil parameters helps to decide the management strategies to be followed in terms of liming, manuring, fertilization, water management etc.

Pond preparation after harvest

Before initiating a second crop in a pond, the pond has to be prepared for stocking the shrimp post larvae. In this case, pond preparation is entirely different from that of a newly dug-out pond except for liming, fertilization and raising the water level.

Cleaning

During production cycle, considerable quantity of waste accumulates in the ponds depending upon the culture practices. This waste must be removed to ensure sustained production in the pond. 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.

Draining of ponds

The first step in pond preparation is draining the pond after harvest of the previous crop. 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. The coastal waters are heavily laden with silt and this gets accumulated in the pond bottom. After draining, pond should be desilted. Often black mud is noticed on the pond bottom. This black colour is caused by an accumulation of iron when the mud is depleted of oxygen. When the mud is oxidised (contains oxygen), the ferrous iron changes to ferric iron and the mud will no longer be black in colour. This accumulated black material can be removed either by wash away the waste before it dries off or to allow the pond to dry out and then remove the waste.

Wet method

In this 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. It is quick and more efficient process than the dry method, reducing the period between production cycles. 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.

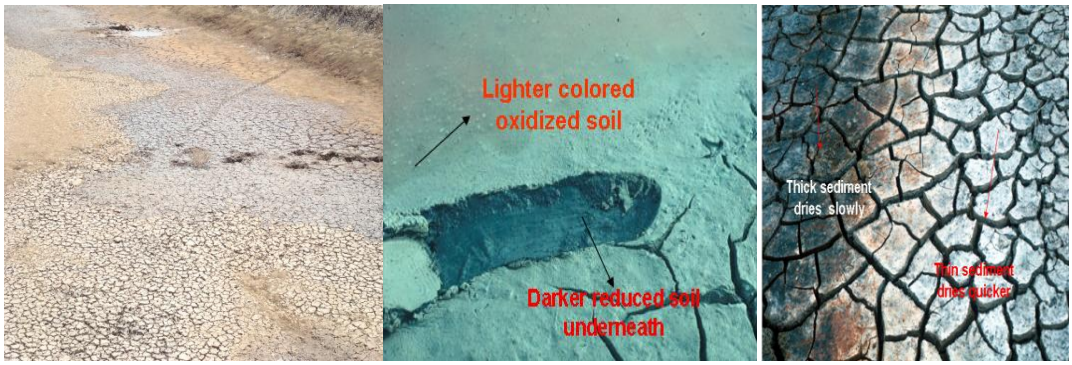
Pond mud drying and sediment removal

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. This method has some advantages, for example, the solid waste can be easily handled and transported away from the ponds. It has also the beneficial effect of making pond bottom harder and it may reduce the levels of some pathogens in the pond. However it needs site for dumping of the removed waste.

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. When ponds are drained and bottom soils exposed to air, which contains 21% oxygen compared to 0.0007% in water, oxygen supply for organic matter decomposition is greatly enhanced. Soil respiration measured in a pond bottom increased drastically during first 3 days after drying. 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. Pond drying certainly enhances the mineralisation of organic phosphorous but mineralised phosphorus is subjected to available for water column as well as to pond mud. It is always

better to allow the mud saturated with mineralised inorganic P rather than existing in organic forms under reducing bottom environment.

Properly dried pond bottom



Unevenly dried pond bottom
(Source: Boyd, et al., .2002)

Even though ponds left for drying for considerable time some portion i.e low lying area may remain wet with black soil. These microsites can be treated by nitrate salts which improve the microbial growth by acting as electron source and accelerate the organic matter degradation. Appropriate amount and type of salt has to be selected for this purpose**.**

Wet black pond bottom **Nitrate salt treated pond bottom**

Nitrate treatment on pond bottom improvement
(Source: Chainark, S and Boyd, C.E.2008)

Sludge removal obviously took organic material out of the pond before it could mineralise and release inorganic nutrients back to the water column. Both ammonia and reactive ortho phosphate were lower in the sludge removal process. Disposal of sludge on high ground reduces the impact of drain harvest effluent on the receiving stream and in certain situations, may improve high ground soil quality.

Shrimp pond soils consist primarily of mineral soil (95 - 98%) and contain only a little organic carbon (2-5%). In our study in Nellore District of Andhra Pradesh and Tuticorin District of Tamil Nadu, it was observed that the organic carbon content of the soil never increased beyond 1.5% during culture harvest. Hence removal of sediments from pond bottom may not be necessary. In the modern shrimp farming with central drainage system it is easy to remove the organic sludge.

Pond maintenance

The pond dike is strengthened with soil wherever it has become weak and the inner slope of the dike is consolidated with soil. Tunnels and holes caused by burrowing organisms are to be closed/plugged. Reconditioning of the bottom trench levelling of pond bottom, repairs of sluice structures and sluice screens are also to be attended.

Tilling

Tilling or ploughing or raking the bottom soil improves soil quality by exposing subsoil to the atmosphere thereby speeding up oxidation process and release of nutrients that are locked in the soil.

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. Pests are species that generally do not have direct harmful effects on the cultured stock. Some pests like crabs burrow into the dikes. This can damage the dikes and cause leakage, which may allow entry of undesirable species into the pond or the escape of cultured species. Competitors are species that compete for space, food, oxygen etc. with the stocked species. Predators are the species that prey on the culture stock. 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. Research carried out by CIBA has indicated that white spot disease can be transmitted by WSSV contaminated inadequately dried pond sediment, resulting in mass mortality of shrimps. Under field conditions WSSV was found to be infective even 26 days after harvest with sun drying. 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 such as Mahua oil cake (100-150 ppm) and tea seed cake (15-20 ppm) 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.

Plant extracted pesticides are recommended since they are biodegradable and also in most cases contribute to the fertility of the soil. The commonly used pesticides are:

1. Mahua oil cake (Bassia lafifolia): 100 - 150 ppm.
2. 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.
3. 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).

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

Identification of ponds needing lime

Generally waters softer than 10 mg/l of total hardness usually need lime application for inorganic fertilisation to be effective, while ponds with water of 20 mg/l or more total hardness seldom responded to liming. Actually total alkalinity is a more reliable indicator of the need for liming than total hardness because some ponds may have a low total hardness and a high alkalinity or vice-versa. But total hardness and total alkalinity rarely should be of concern in brackishwater ponds. Many times the need for lime is first suggested when inorganic fertilisation fails to produce an adequate plankton bottom. In brackishwater soils, where water exchange is not used and soils are acidic or even where water exchange is used but where soils are extremely acidic (acid-sulfate soils), liming may assume more importance.

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 CaC03 with the PECC value of that particular lime material with the formulae given below.

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

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

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 to 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

Fertilization

The usual way of increasing the carrying capacity of the shrimp pond is to improve its natural fertility through the addition of organic and inorganic fertilizers. Pond fertilization is an important and necessary step in extensive and semi-intensive methods of farming operations.

Prawns being bottom dwellers, benthic organisms constitute their main food items. Hence fertilization of soil instead of water is more effective. Fertilization of pond should be done after 20-25 days of liming. It should be broadcast/spread all over the pond bottom and mixed thoroughly. Due to the intensification of shrimp farming, off-late farmers are not applying fertilizers and manures.

Organic fertilizers or manures are animal wastes or agricultural by-products which when applied to ponds, decompose slowly to release nutrients. Application of organic fertilizers especially in newly developed ponds is advisable because it serves as soil conditioner. The rate of application of organic manure in shrimp ponds ranges from 500 to 2000 kg/ha as a basal dose.

Enhancement of nutrients using inorganic fertilisers is required in ponds to increase the phytoplankton production. Inorganic fertilizers are synthetic fertilizers that generally contain an amount of at least one of the major plant nutrients like nitrogen, phosphorus and potassium. The rate of application of inorganic fertilizers ranges from 25 - 100 kg/ha as a basal dose during pond preparation with minimum water depth of 10 - 15 cm. During the shrimp culture, depending upon the phytoplankton density as exemplified by turbidity of the pond water, the required quantity of the fertilizers may be applied in split doses at short intervals for sustained plankton production. The main nutrient limiting phytoplankton production in brackishwater ponds is phosphorus. Hence both phosphorus and nitrogen should be applied in the ratio of 1:1.

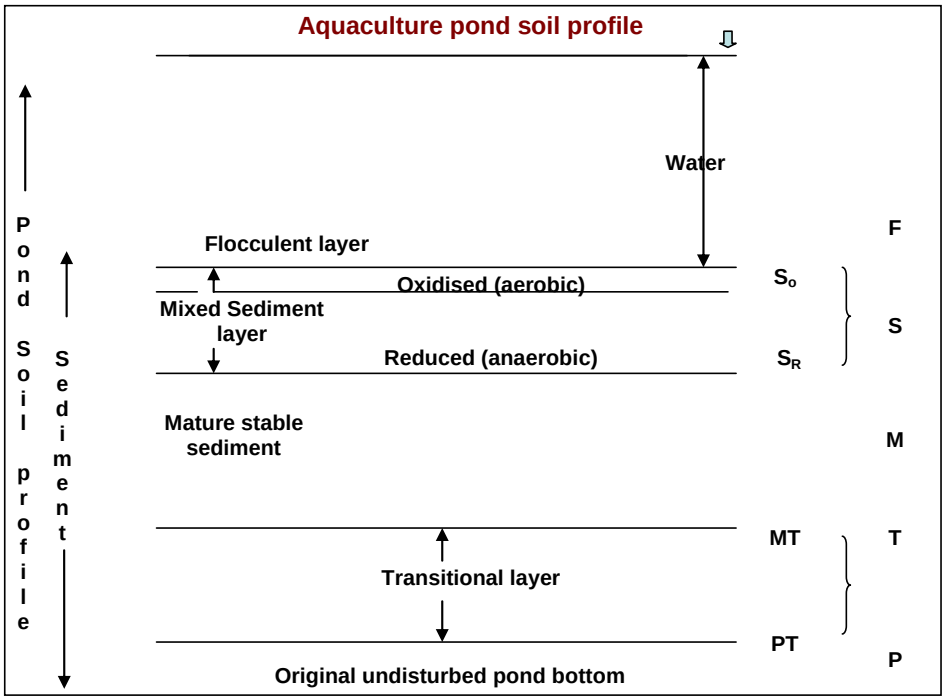
Management of pond bottom during culture

All aquaculture pond bottoms become covered with sediment, and this sediment can be considered as aquaculture pond soil. In describing various physical, chemical and biological processes occurring in the pond bottom, it is convenient to refer to bottom deposit as sediment. A core taken through the sediment and extending into the original bottom soil is called a profile. Layers in the profile are known as horizons. For practical purposes, the F and S horizons are most important in aquaculture because they exchange substances with overlaying water to influence water quality.

Oxidized Layer

The oxidized layer at the sediment surface is highly beneficial and should be maintained throughout the shrimp culture. Metabolic products of aerobic decomposition are carbon dioxide, water, ammonia, and other nutrients. In anaerobic sediment, some microorganisms decompose organic matter by fermentation reactions that produce alcohols, ketones, aldehydes, and other organic compounds as metabolites. Other anaerobic microorganisms are able to use oxygen from nitrate, nitrite, iron and manganese oxides, sulfate, and carbon dioxide to decompose organic matter, but they release nitrogen gas, ammonia, ferrous iron, manganous manganese, hydrogen sulphide, and methane as metabolites. Some of these metabolites, and especially hydrogen sulfide, nitrite, and certain organic compounds, can enter the water and be potentially toxic to shrimp. Methane and nitrogen gas pass through the

layer and diffuse from the pond water to the atmosphere. These two gases do not cause toxicity to aquatic organisms under normal circumstances.



Pond soil profile showing different horizons

Soil Horizons
F - Water with high concentrations of mineral and organic solids, aerobic
S - Sediment with high water content and low dry bulk density, abundant organic matter, well stirred by physical and biological agents,
S_O - Thin aerobic surface (Oxidised)
S_R - Anaerobic below (Reduced)
M - Sediment with medium water content and intermediate dry bulk density, abundant organic matter, not stirred, anaerobic
T - Transition between M (MT) and P (PT) horizons with characteristics intermediate between M and P horizons, not stirred, anaerobic

The oxidized layer at the sediment surface prevents diffusion of most toxic metabolites into pond water because they are oxidized to non-toxic forms by chemical and biological activity while passing through the aerobic surface layer. Nitrite will be oxidized to nitrate, ferrous iron converted to ferric iron, and hydrogen sulfide will be transformed to sulfate. Thus, it is extremely important to maintain the oxidized layer at the sediment surface in shrimp culture ponds.Loss of the oxidized layer can result when soils accumulate large amounts of organic matter and dissolved oxygen is used up within the flocculent layer (F horizon) before it can penetrate the soil surface. F and S horizons are most important that influences overlying water quality. Even in ponds without high concentrations of organic matter in sediment, high rates of organic matter deposition resulting from large nutrient inputs and heavy plankton blooms can lead to oxygen depletion in the F horizon. Ponds should be managed to prevent large accumulations of fresh organic matter in the F horizon at the soil surface,

or in the upper few millimeters of soil. Toxic metabolites entering well-oxygenated pond water will be quickly oxidized. However, if the rate of release of toxic metabolites into water exceeds the rate that metabolites that are oxidized, equilibrium levels of metabolites in the water may be high enough to have detrimental effects on culture animals.

Nutrient exchange between soil and water

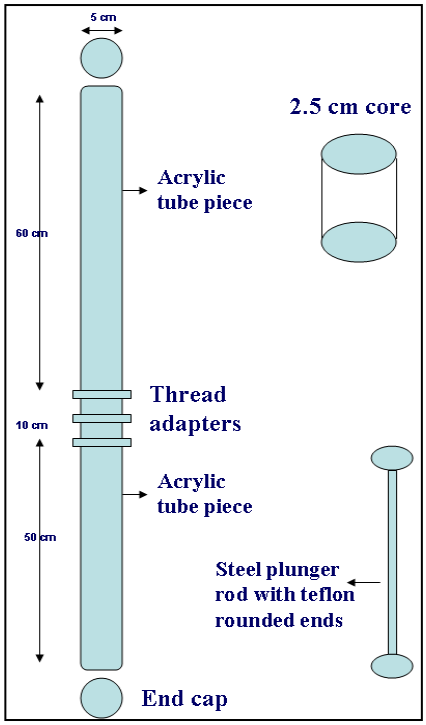
The two most important nutrients in pond aquaculture are nitrogen and phosphorus because these two nutrients often are present in short supply and limit phytoplankton growth. These two nutrients are added to ponds in fertilizers, manures, and feeds. Fertilizer nitrogen usually is in the form of urea or ammonium, and urea quickly hydrolyzes to ammonium in pond water. Ammonium may be absorbed by phytoplankton, converted to organic nitrogen, and eventually transformed into nitrogen of shrimp protein via the food web. Ammonium may be oxidized to nitrate by nitrifying bacteria, and nitrate may be used by phytoplankton or denitrified by anaerobic microorganisms in the sediment. Nitrogen gas formed by denitrification diffuses from sediment to pond water to the atmosphere. Ammonium is in equilibrium with ammonia, and ammonia also can diffuse from pond waters to the atmosphere. A small amount of ammonium may be adsorbed on cation exchange sites in pond bottom soils. Organic nitrogen in plankton and in aquatic animal feces may settle to the bottom to become soil organic nitrogen. Nitrogen in soil organic matter may be mineralized to ammonia and recycled to the pond water, but the rate is slow.

Monitoring of soil parameters during culture period

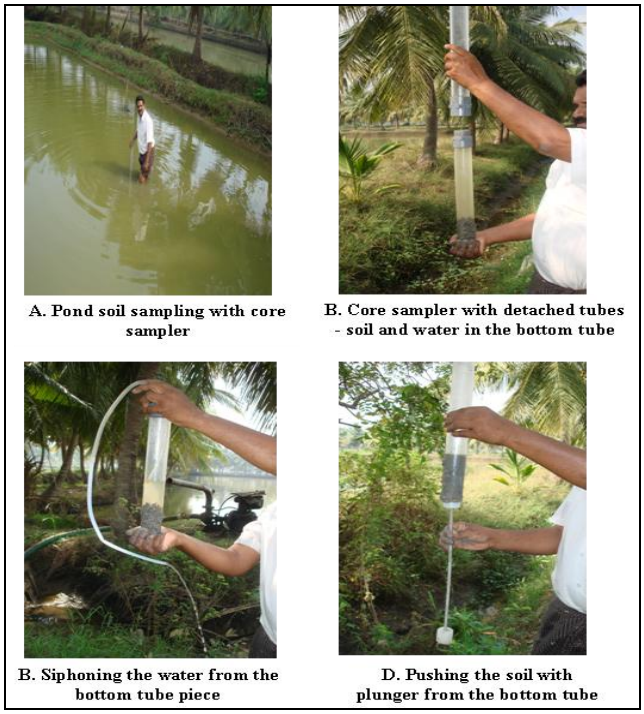
Monitoring of soil quality condition can be valuable in shrimp culture pond management. During culture the carbonaceous matter, suspended solids, faecal matter and dead plankton etc. also settle at the pond bottom. Major concerns in pond bottom soil management are low soil pH, high soil organic matter, loss of the oxidized layer, and accumulation of soft sediment. Pond managers should still strive to prevent severe soil quality problems from developing. In older ponds with impaired soil quality, problems should be corrected and prevented from recurring. These materials have combined effect on the environment of the pond bottom. In order to characterize the soils based on soil type, a pond core sampler (Fig.2) fabricated by the Environment Group of CIBA can be used for the depth-wise collection of cores. The soil type name broadly depicts the physico-chemical characteristics of the soil. The type of soil will help in formulating the location specific BMPs. The BMPs adopted in one type of soil can be extrapolated to the other areas having similar soil types. To understand the condition of the pond bottom, the following parameters are to be monitored regularly.

pH of soil

This is one of the most important soil quality parameters since it affects the pond condition. Generally, soil pH ranging between 6.5 and 7.5 is the best suited where availability of nitrogen, phosphorus, potassium, calcium and magnesium is maximum. The low pH of bottom sediment indicates unhygienic condition and needs regular check-up.



Pond soil core sampler



Pond soil sampling method

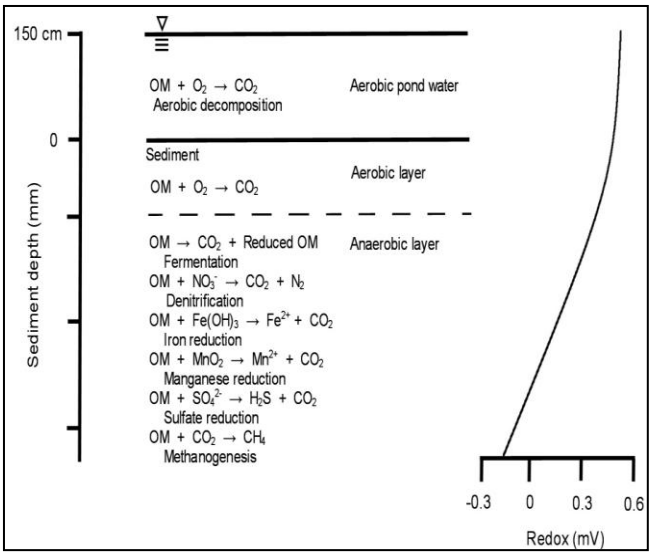
Organic matter

Unutilized feed, carbonaceous matter, dissolved solids, faecal matter, dead plankton etc. settle at the pond bottom and results in the accumulation of organic loads. The change in the bottom in terms of increasing organic load should be recorded regularly for the management of the pond bottom.

Redox-potential

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.

Anaerobic condition can be developed in pond, when input of organic matter exceeds the supply of oxygen needed for decomposition of organic matter. This reducing condition can be measured as the redox potential (Eh). Eh indicates whether the water or soil is in reduced (Eh with `-ve' value) or oxidized (Eh with `+' ve value) condition. In anaerobic sediment, some microorganisms decompose organic matter by fermentation reactions that produce alcohols, ketones, aldehydes, and other organic compounds as metabolites. Other anaerobic microorganisms are able to use oxygen from nitrate, nitrite, iron and manganese oxides, sulfate, and carbon dioxide to decompose organic matter, but they release nitrogen gas, ammonia, ferrous iron, manganous manganese, hydrogen sulphide, and methane as metabolites (Fig.6). Some of these metabolites hydrogen sulfide, ammonia and nitrite can enter the water and be potentially toxic to shrimp. The redox potential (Eh) of mud should not exceed -200 mV.



Reactions at the pond bottom soil during aerobic and anaerobic conditions

On-farm measurement of redox potential

ORP can be measured at soil water interface (SWI) near sluice gate and away from the aerators by portable multi parameter analyser with ORP probe. If probes are not available, the sediment sample at 10-cm depth is to be collected in a polythene bag under air tight condition near sluice gate and away from the aerators. Once the sample is brought out of the pond, immediately ORP has to be measured under air tight condition by using a portable/ bench top redox meter. In order to minimise the errors of ORP variability, minimum of three sampling places have to be fixed near sluice gate and repeated measurements are to be taken at each sampling place (SWI or 10-cm depth soil in polythene bag) and the average value can be taken as final value.

Chain dragging once in fortnight improves the pond bottom condition by marinating the bottom in oxidised condition. It has to be started from the initial period of culture. Central drainage system removes the accumulated sludge and improves the pond bottom condition. 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. Central drainage canal in the pond may also help in the removal of organic waste periodically. The redox potential (Eh) of mud should not exceed -200 mV.

Application of pond conditioners and chemicals to improve soil quality

A variety of chemicals both indigenous and imported are available in the market with high claims of efficiency. But the efficiency of most of them has not been scientifically proved. For example, bacterial and enzyme preparations are used to enhance nutrient removal, organic matter oxidation and removal of ammonia. Such bacteria and enzymes are present in the ponds and further addition is unnecessary. Similarly, Zeolite is used for the absorption of ammonia but for the efficient absorption the quantity required will be very high. Application

of 350 - 500 kg / ha health stone after fertilization of the pond is recommended for optimum production of phytoplankton due to mineral ions present in it.

In shrimp ponds bottom pollution is a major problem caused by the unconsumed feed, dead plant and animal matter, exuviae of shrimps, faecal matter of shrimps etc. The very slow rate of microbial degradation naturally occurring in the pond bottom is not enough to cope up with the rate and bulk of waste accumulation. The pond bottom, thereby, becomes unhygienic and leads to a host of problems like formation of NH_3, H_2S, nitrites, nitrates, acidity, and depletion of DO. External fouling is usually associated with deterioration in the pond bottom or the water quality. The shrimps are under tremendous stress and diseases set in causing heavy mortality. The first priority, therefore, should be to ensure a clean environment for the shrimp. Chemical treatment should be resorted only if the environment has been improved but the shrimp have not moulted.

The most commonly used compound for this purpose is formalin (37 to 40% formaldehyde). The dose of formalin used to treat external fouling in shrimp a pond is much lower than that used for finfish or shrimp hatcheries. The recommended dose of formalin is 25 to 30 ppm. The diluted formalin should be applied widely over the surface of the pond. Five to six hours after adding formalin the pond should be filled up to its normal level. Formalin is a reducing agent, which removes dissolved oxygen from the water; therefore if it is applied at night the DO levels must be very carefully monitored.

CHAPTER 6

WATER QUALITY MANAGEMENT IN BRACKISHWATER AQUACULTURE

R. Saraswathy, N. Lalitha, Satheesha Avunje, R. Aravind and P. Mahalakshmi

Introduction

Successful aquaculture largely depends on providing animals with a satisfactory environment in which to grow. The most important principle regarding soil and water is that a pond has a finite capacity to assimilate nutrients and organic matter. When the capacity is exceeded, water and soil quality will deteriorate resulting in growth inhibition, vulnerability to diseases and ultimately mortality. Soil and water quality can be maintained within the optimal range by giving due importance right from site selection, suitable pond preparation, good culture practices and post-harvest pond management for next crop. Maintaining a good pond environment through use of proper management practices will reduce the stress, risk of disease, increase production, improve productivity and livelihood.

Critical water quality parameters

All living organisms have tolerable limits of water quality parameters in which they perform optimally. A sharp drop or an increase within these limits has adverse effects on their body functions. Water quality variables such as salinity and temperature are important when assessing the suitability of a site for a culture of particular species. Other properties such as alkalinity, turbidity, compounds of phosphorus and nitrogen are important because they affect plant productivity, which in turn, may influence aquaculture production. Dissolved oxygen, carbon dioxide, ammonia and other factors come to play during grow out period, because they are potential stressors for the animal in culture.

Water quality management

Maintenance of good water quality is essential for both survival and optimum growth of animal.

Source 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. Chlorination should be done in reservoir pond to sterilize the water by applying enough chlorine (approximately 30 ppm) to overcome the chlorine demand of organic matter and other substances in the water. Chlorine dose varies with pH, concentrations of organic matter and ammonia. 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 thio sulfate 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.

Culture period

During culture, the parameters that should be monitored routinely are water temperature, salinity, pH, dissolved oxygen, total alkalinity, minerals, nutrients and metabolites.

Physical characteristics

Water temperature

Temperature of water is obviously very vital. All metabolic and physiological activities and life processes such as feeding, reproduction, movement and distribution of aquatic organisms are greatly influenced by water temperature. Temperature also affects the speed of chemical changes in soil and water, and the contents of dissolved gases. On account of unequal distribution of temperature with higher temperature near the surface layer and decreasing temperature with depth, thermal stratification can occur resulting in formation of methane, hydrogen sulphide and ammonia causing degradation of water quality. Optimum level of pond water temperature is 25-30^{0}C. Operation of aerators during warm and calm afternoons helps to break thermal stratification by mixing warm surface water with cool sub surface water.

Salinity

Salinity refers to the total concentration of ions in water (Calcium, magnesium, sodium, potassium, bicarbonate, chloride and sulphate). It determines osmotic relationships and also affects the growth, reproduction and migratory behaviour of the animal as well as its general metabolism. 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.

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 result in higher salinities. Maintenance of salinity of 18 to 35 ppt with variations not exceeding 5 ppt will help in reducing stress on the animal. The stress response associated with the sudden

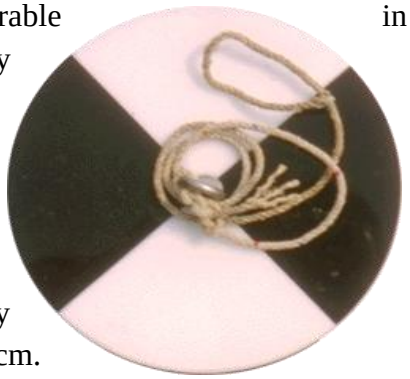
decrease in salinity is reduced when the calcium concentration of the low salinity is increased from 84 to 150 ppm.

pH

pH of most pond water is determined by interactions among dissolved CO_2, carbonic acid, bicarbonate, carbonate and carbonate containing minerals. pH can fluctuate between 7.5 and 9.5 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. Unusually high afternoon pH values typically occur in waters of moderate to high total alkalinity (50-200 ppm as $CaCO_3$) and low total hardness (< 25 ppm as $CaCO_3$). The proportion of total ammonia existing in the toxic, un-ionized form (NH_3) increases as the pH increases whereas low pH increases nitrite toxicity and also the fraction of H_2S (toxic form). However, 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. Optimum level of pH is between 7.5 and 8.5.

Turbidity

Turbidity refers to an optical property of water that causes light to be scattered or absorbed rather than transmitted through the water in a straight line. Turbidity can be measured in terms of transparency using secchi disc. Turbidity caused by plankton is desirable whereas turbidity resulting from suspended particles of clay is undesirable in aquaculture ponds. It will restrict light penetration, adversely affecting plant growth and destroy benthic organisms. In case of very high turbidity fish die due to gill clogging. High value of transparency (>60 cm) is indicative of poor plankton density and the water should be fertilized with right kind of fertilizers. Low value (<20cm) indicates high density of plankton and hence fertilization rate and frequency should be reduced. Optimum range of transparency is 25-35 cm. Alum (Aluminium sulfate) is an excellent coagulant and is used widely in water-treatment plants to clarify the water. Calcium sulfate, calcium hydroxide calcium ferric chloride, organic matter, certain synthetic polymers and chemical fertilizers are used in removing suspended solids from ponds.



Total solids

Organic and inorganic, settleable, suspended and dissolved matter is termed as total solids. Portion of organic and inorganic solid that settles in 1 hr in an Imhoff cone is known as

settleable solids and dissolved solids are portion of organic and inorganic solids which is not filterable. Settleable solids more than 20 ppm result in rapid silting of the pond and decreasing of water depth. Portion of inorganic and organic solids that are not dissolved are suspended solids (TSS). Deforestation, poor soil management practices and erosion in drainage basins of rivers are major causes for heavy load of silt and clay in intake water. Optimum level of TSS for most of the shrimp is < 100 ppm. Excessive TSS lead to increased sedimentation of eco-system.

Chemical characteristics

Dissolved oxygen

Dissolved oxygen is the most important and critical water quality parameter because of its direct effect on the feed consumption, stress-mediated immunosuppression, metabolism of animal, growth, survival, behaviour and physiology of aquatic organisms as well as indirect influence on the water quality. The concentration of toxic substances such as unionized NH_3, Hydrogen sulphide and carbon metabolites (methane) increases when low DO level exists. However, in the presence of optimum level of oxygen, the toxic substances are converted into their oxidized and less harmful forms. Optimum DO concentration for aquatic animal growth is 3-10 ppm.

Aeration is the best option to maintain DO concentration. Use of aerators result in mixing of water at surface and bottom and breaks down DO stratification and can also eliminate black mud formed at soil-water interface. Paddle wheel aerators are commonly used and the latest ones such as the long arm aerators and spiral aerators can circulate oxygen to the pond bottom more effectively. Management of DO in pond waters is very closely related to the amount and type of phytoplankton, animal biomass, organic matter in the pond and bacterial activity. Water exchange is the best solution to prevent low DO problem in the pond where aeration is not practiced, but it comes with the inherent risk of disease outbreak.

Minerals

Minerals are important for the growth and metabolism of animals. Among the minerals, 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. In general, water is suitable for aquaculture if levels of minerals are similar to the levels in seawater diluted to same salinity. 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.

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.

Minerals	Formula	General name	%
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

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

Total alkalinity

Alkalinity is the water's ability to resist changes in pH and is a measure of the total concentration of bases in pond water predominantly bicarbonate and carbonate. 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. Ponds with a total alkalinity of 20-150 ppm have sufficient supply of CO_2 for phytoplankton growth and it may improve productivity. According to alkalinity between 75 to 200 mg L^{-1}, but not less than 20 mg L^{-1} is ideal in an aquaculture pond.

In addition, increasing alkalinity may improve productivity because waters of higher total alkalinity generally have a pH that favours rapid decomposition of organic matter by microorganisms. It decreases potential of metal toxicity. It also decreases potential of metal toxicity. Very high alkalinity (200-250 ppm) coupled with low hardness (less than 20 ppm) results in rise in afternoon pH beyond 11 and cause death of animal. Bhatnagar and co-workers suggested that <20ppm indicates poor status of water body, 20-50 ppm shows low to medium, 80-200 ppm is desirable for fish/prawn and >300 ppm is undesirable due to non-availability of CO_2. Dolomite, Shell lime, calcium carbonate, egg shells and Zeolite depending upon soil pH and buffering capacity to improve alkalinity and stabilize pond water quality.

Nutrients

Nitrogen and phosphorus along with carbon and other trace elements serve as nutrients thus accelerating the growth of phytoplankton, which is the base of the food web in culture system. Nitrate is harmless and is produced by the autotrophic *Nitrobacter* bacteria combining oxygen and nitrite. Maximum concentration of nitrate-N was recorded during monsoon, while minimum during pre-monsoon. Abrupt increase in nitrate-N recorded after heavy shower or continuous rainfall. Almost all of the phosphorus (P) present in water is in the form of phosphate (PO_4) and in surface water mainly present as bound to living or dead particulate matter and in the soil is found as insoluble $Ca_3(P0_4)_2$ and adsorbed phosphates on colloids except under highly acid conditions. Fertilizers are applied to increase nitrate and phosphate level (N:P=15:30) and stimulate phytoplankton growth and to prevent reducing conditions that lead to sulphide production in pond bottom soil.

Primary productivity

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^{-1}. 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.

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.

Metabolites

Ammonia

Ammonia in the pond water is a by-product of metabolism by animals and bacterial decomposition of organic matter such as wasted food, faeces, dead planktons etc. As ammonia in water increases, ammonia excretion by aquatic organism diminishes, and levels of ammonia in blood and other tissue increases. In water, ammonia nitrogen occurs in two forms, un-ionized ammonia and ammonium ion (NH_4). Un-ionized ammonia is determined by total ammonia concentration, pH, and water temperature and to a lesser extent on salinity. It is considered more toxic form of ammonia due to its ability to diffuse readily across cell membrane, hence should be less than 0.1 ppm. A given concentration of un-ionized ammonia is more toxic when dissolved oxygen concentrations are low. Ammonia toxicity reduces when salinities are near the optimum levels and when high concentrations of calcium are present. Toxic effect of ammonia may be minimized by maintaining sufficient level of dissolved oxygen, periodic partial removal of algal blooms, water exchange and addition of liming agents such as hydrated lime or quick lime. Addition of liming agent is effective only in ponds with low alkalinity

Nitrite

Nitrite is an intermediate product in the bacterial nitrification of ammonia to nitrate. Nitrite is highly toxic to fish as it oxidizes hemoglobin to form methemoglobin, which is incapable of transporting oxygen. Nitrite toxicity is affected by water pH and the presence of chloride and calcium ions. Toxicity increases with increasing pH and decreases with increasing calcium and chloride concentrations. Optimum level of nitrite is less than 0.25 mg/L. Optimum level can be maintained by effective removal of organic waste, adequate aeration, correct application of fertilizer & feed and use of biofertilizer to accelerate nitrification.

Hydrogen Sulfide

Under anaerobic condition, certain heterotrophic bacteria can use sulphate and other oxidized sulphur compounds as terminal electron acceptors in metabolism and excrete sulphide. pH regulates the distribution of total sulphide among its forms (H_2S, HS^- & S^{2-}). Un-ionized H_2S is toxic and it decreases rapidly with increasing pH. H_2S builds up mostly in sediment which is highly reduced (redox potential < 100 mv), within a pH range of 6.5 – 8.5 and low in iron. Sulfide can be reduced by aeration, water exchange and circulation of water to minimize anaerobic zones in the pond bottom. Application of lime or potassium permanganate or iron oxide will reduce the hydrogen sulfide. Iron reacts with H_2S and forms insoluble iron sulphide. Periodic pond draining and drying of bottom muds will result in oxidation of sulfide and enhance the decomposition of organic matter. Concentration of H_2S more than 0.01 mg l^{-1} may be lethal to aquatic organisms.

Optimum water quality parameters for brackishwater aquaculture

Parameters	Optimum range
Temperature (^oC)	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

Conclusion

Of the many factors that affect the production and productivity of aquaculture, soil and water quality play a pivotal role. Deterioration of pond environment leads to stress to animals and increases susceptibility to diseases. Key to successful aquaculture is intervention at the right time through appropriate management practices. Hence, it is imperative to constantly monitor and maintain soil and water quality parameters within the optimum range. Water dissolved oxygen, should be measured at dawn and late afternoon, which will normally provide information on the daily extremes. pH and metabolites should be measured at weekly intervals as a minimum. Apart from the monitoring of the pond conditions, observing animal behavior along with accurate record keeping helps the farmer to recognize and prevent deleterious environmental conditions through better management practices at early stages and thereby maximize the production.

CHAPTER 7

ROLE OF MICROORGANISMS AND BIOREMEDIATION APPROACHES FOR SUSTAINABLE AQUACULTURE

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Introduction

Microbiology includes studies on diverse organisms including bacteria, virus and fungi. Although microbes were observed over three hundred years ago, the field of Aquatic microbiology is in its infancy relative to other biological disciplines. Microorganisms are of major importance in aquaculture, as they are present in the water, sediments and as well as in and on the aquatic animals like fish and shrimps. Microorganisms have special impact, especially on the aquatic zone of ecosystem where light cannot approach. Some microbes are decomposers which have ability to recycle the nutrients. They also play a positive role in the elimination of toxic wastes in the aquatic environment like ammonia, nitrite and hydrogen sulphide. Hence, they are also used for microbial bioremediation of aquatic environments.
Successful aquaculture depends on health status of aquatic animals which depends on their inherit resistance to microbial invasion. Biological equilibrium between beneficial and detrimental microorganisms in the aquatic environment has direct impact on the health status of aquatic animals. These and other functions make micro-organisms key players in the health and sustainability of aquaculture.

Maintaining good water quality during culture is vital for better growth and production. However, intensification of aquaculture practices leads to accumulation of uneaten feed, excreta at the pond bottom, resulting in building up of toxic nitrogenous metabolites (ammonia and nitrite) concentration in water. Bioremediation is one of the technologies to reduce the metabolites concentration, thereby improving the water quality and increasing the productivity. Bioremediation involves organic matter mineralization to carbon dioxide, nitrification, denitrification and maximizing productivity to stimulate production of aquatic animals. This process eliminates excess nitrogen from ponds, maintain microbial diversity and establishes the desirable microbial species.

Micro-organisms and their importance

Maintaining an environment of diverse microbes in the aquatic ponds is very important. Microbes contribute significantly to the food web in all systems of aquaculture such as extensive, semi-intensive and some intensive aquaculture systems. They may be eaten directly by the cultured species or by small animals on which the cultured species feed. Diverse microbes must be in a proportion that beneficial microbial population predominates to maintain the health status of aquatic animals. The activities of microbes play an essential role in supplying the oxygen content to the aquatic animals in the aquatic ponds during the day. Water quality factors, such as pH, and the content of ammonia affected by both aerobic and anaerobic microbial processes. Beneficial microbes improve the water quality by speeding up decomposition of organic matter as well as reducing Chemical Oxygen Demand, NH_3–N and NO_2–N in water as well as sediments. Microbes stimulate the primary productivity of the aquatic animals through the activity of the heterotrophic decomposers,

which recycle nitrogen and phosphorus. These microbes produce clean culture models with no toxin, no side effects, no residue, and no resistance which paves way for sustainable aquaculture by improving environment, immunity of aquatic animals, health, maintaining eco equilibrium as well as reduce diseases. These microorganisms play an important role in suppressing the water-borne pathogens and diseases of aquatic animals. Beneficial microbes produce and integrate bioactive compounds like B type vitamins, antibiotic substances, hormones and enzymes.

In concise microbes play major role in aquatic pond culture by performing various functions, including regulation of microdysbiosis, maintenance of microeubiosis, improving the health level of hosts. Further, they are involved in decomposing the organic matter, nutrient cycling, maintain dynamic balance among organisms in water and sediments and to create a favourable environment for fish and shrimp growth.

Diversity of microbes in aquatic environment

Microorganisms can be grouped into two types; Reviving and Disintegrating

***Reviving types*:** These are beneficial synergistic microbes which can enhance the biological, chemical and physical properties of water, soil and sediments. They can decompose the organic matter to meet the growth requirements of cultured aquatic animals and reduce pollution. They can inhibit the proliferation of harmful organisms and disintegrate harmful chemical substances in ecological environment and maintain the eco equilibrium.

The beneficial organisms are

a. Fresh water: *Aeromonas, Pseudomonas* and *Achromobacter* etc.

b. Seawater : *Pseudomonas, Vibrio spp., Achromobacter, Flavobacterium and Micrococcus* etc.

Other bacteria such as Photo Synthetic bacteria (*Ectothiorhodospira*), *Bdellovibrio*, *Bacillus* (*Bacillus cereus, B. megatherium var. phosphaticum*), *Lactobacillus* and Desulphurobacterium are present in nature.

Apart from bacteria, there are yeast, *Aspergillus niger*, *Actinomycetes*, Bacteriophage, cyanobacteria and micro algae (*Pyrrophyta, Cyanophyta and Cryptophyta*) present in the aquaculture environment.

***a.* Organic matter decomposition:** Aquaculture ponds are deliberately fertilized with organic manures, chemical fertilizers or agricultural wastes, resulting in accumulation of organic matter. Yield increases as primary productivity increases. More feed input, metabolites produced by the aquatic animals and more feed spills results in more organic matter accumulation in aquatic environment.

Organic matter is decomposed by microorganisms using aerobic or anaerobic pathways depending on the conditions that favour the one or the other. Organic carbon is mineralized by both aerobic and anaerobic mechanisms. Anaerobic carbon mineralization includes denitrification, sulphate reduction and methanogenesis Nitrification is performed in two steps: oxidation of ammonia to nitrite and oxidation of nitrite to nitrate.

The aerobic process yield compounds which are either beneficial, non-toxic or have low toxicity levels in aquaculture ponds. Whereas, uncontrolled anaerobic microbial processes produce compounds that are highly toxic to aquatic animals.

b. **Nitrogen fixation:** Nitrogen input in aquaculture ponds comes from feed, manure and fertilizers which are used to enhance productivity. Nitrogen fixation by blue green algae (*Anabaena sp* and *Aphanizomenon sp*) can also be a source of nitrogen in aquaculture ponds.

c. **Biotic control:** It is the biocontrol of microbial pathogens in aquaculture system by the use of antagonistic micro-organisms, especially the co-existing bacteria. This minimises the negative impacts of antibiotics. The biological control organisms aquaculture belong to the Lactic Acid Bacteria (*Lactobacillus, Carnobacterium* etc.), *Vibrio* (*Vibrio alginolyticus*), *Bacillus* and *Pseudomonas.*

Eg.1.Penaeid shrimp larvae associated bacterium, *Alteromonas*, species suppresses the activity of *Vibrio harveyi* and improves the survival of *Penaeus indicus* larvae *in-vivo.*

2. Non-pathogenic isolates of *Vibrio alginolyticus* suppresses the pathogenic vibrios like *Vibrio harveyi, Vibrio parahaemolyticus* and *Vibrio splendens* and reduces the invasion of these pathogens in shrimps.

d. **Bioremediation:** Bioremediation is the application of microbes to ponds for improving water quality and maintain health and stability of aquaculture systems. It involves organic matter mineralization to carbon dioxide, nitrification, denitrification and maximising primary productivity to stimulate production of aquatic animals.

This process eliminates excess nitrogen from ponds, maintain microbial diversity, eliminate pathogens from the system and and as well as establishes the desirable species. Eg. *Bacillus* sp, *Pseudomonas, Aeromonas, Lactobacillus sp Nitrosomonas, Nitrosovibrio, Nitrosococcus, Nitrolobus, Nitrospira, Nitrobacter* and *Nitrococcus.*

Disintegrating types: The disintegrating types of microorganism are pathogenic which cause diseases in cultured aquatic animals. Eg. Systemic vibriosis disease in shrimp can be caused by pathogenic species of *Vibrio* such as *Vibrio alginolyticus, V. parahaemolyticus, V. anguillarum, V. vulnificus, V. damsella, V. fluvialis and V. mimicus.*

Bioremediation for sustainable aquaculture The different processes, approaches and technologies available for bioremediation and enhanced production in aquaculture are as follows.

Physical, chemical and biological process

a. Physical and chemical process

Physical process involves sedimentation for the settleable solids whereas mechanical filtration for suspended and fine solids. The chemical process ozonisation is used in combination with physical process. But the chemical disinfection has the toxic by-products so the UV irradiation is considered as the alternative to chemical disinfection.

b. Biological process

Nitrification is the major biological process. It has two types (i) emerged fixed film filters (rotating biological contractors, trickling filters) and (ii) submerged fixed film filters (fluidized bed filters, bead filters). Rotating biological contactor technology is based on the rotation of a submerged substrate, which is made of high-density polystyrene or polyvinyl chloride, attached to a shaft. Nitrifying bacteria grow on the media and because of the rotation they alternately contacting nitrogen rich water and air. Trickling filters consist of a fixed medium bed through which aquaculture wastewater flows downwards over a thin aerobic biofilm. The organic material present in the wastewater is adsorbed on the biological slime layer and degraded by aerobic microorganisms. Downflow microbead filters are combinations of trickling filters and granular type biological filters. As the recirculating water passes through the packed bed, suspended solids are captured and biofiltration processes are active. Fluidized sand biofilters have been widely adopted in recirculating systems. Rotating biological contactors have the highest total ammonia nitrogen removal rate, followed by bead biofilters and trickling filters, and fluidized sand biofilters. Fluidized sand biofilters and bead biofilters are the least expensive options for water treatment when the cost per kg of aquatic animal produced per year is considered.

Microbial approach

a. Biological nitrification

The process that involves two groups of bacteria aerobic ammonia-oxidizing bacteria oxidize ammonia to nitrite via hydroxylamine and then the nitrite-oxidizing bacteria oxidize nitrite to nitrate ($NH3–NO_2^-–NO_3^-$) under aerobic condition which consumes a abundant of oxygen resulting in the lower dissolved oxygen in the area. These groups combined with ammonia-oxidizing archaea, known collectively as the ammonia-oxidizing microorganisms. Autotrophic bacteria in these groups use the reducing power of the nitrogenous substrates to fix CO_2 via the Calvin–Benson cycle as their source of carbon. The ammonia oxidizers belong predominantly to the β-subclasses and γ-subclasses of the Proteobacteria, whereas the nitrite oxidizers belong to the α-subclasses and g-subclasses of the Proteobacteria and the phylum Nitrospirae.

b. Biological denitrification

Denitrification is the main mechanism for converting fixed nitrogen to N_2 gas, which returns to the atmosphere. It occurs under low oxygen conditions, in energy-generating reactions where oxides of nitrogen, including nitrate, nitrite, and nitric and nitrous oxides, are used as electron acceptors in place of oxygen, finally becoming reduced to N_2 gas as the end product. A broad spectrum of microorganisms is capable of denitrification reactions, including various bacteria, Archaea and Eukarya.

c. Anammox process

Anaerobic ammonium oxidation is another route to denitrification. Planctomycetales capable of oxidizing ammonium using nitrite as electron acceptor. Anammox activity would be a major contributor to denitrification of anoxic aquaculture-derived sediments.

d. Microbes for bioremediation of organic detritus

Dissolved and suspended organic matter mainly carbon chains, carbonaceous wastes, leached or excess feed, feces, non-organic particulate form. Bacteria will mobilize C, N, P. Bacteria used for the bioremediation of organic detritus were *Bacillus subtilis, Bacillus licheniformis, Bacillus cereus* and *Bacillus coagulans.*

e. Microbes for bioremediation nitrogenous compounds

Excessive nitrogen applications in pond than its assimilatory capacity result in deterioration of water quality through the build-up of nitrogenous compounds (e.g., ammonia and nitrite) which were toxic to fish and shrimp. Fish excretion and sediment flux derived from the mineralization of organic matter and molecular diffusion from reduced sediment were the sources of ammonia.

Nitrification

$NH_4^+ + 1\ 1/2\ O_2 \rightarrow NO_2^- + 2H^+ + H_2O$

$NO_2^- + 11/2\ O_2 \rightarrow NO_3^-$

Bacteriological nitrification is achieved by biofilter is the practical method for the removal of ammonia in closed aquaculture systems. Bacteria that oxidize ammonia are *Nitrosomonas, Nitrosovibrio, Nitrosococcus,* and *Nitrospira,* whereas the nitrite oxidizing bacteria are *Nitrobacter, Nitrococcus* and *Nitrospira.* There are also some heterotrophic nitrifiers. Denitrifying filters helps to convert nitrate to nitrogen. It creates an anaerobic region where anaerobic bacteria can grow and reduce nitrate to nitrogen gas.

$NO_3 \rightarrow NO_2 \rightarrow NO \rightarrow N_2O \rightarrow N_2$

Nitrate reducing bacteria are *Pseudomonas, Bacillus* and *Alkaligenes*

f. Microbes for bioremediation hydrogen sulphide

Organic loading can stimulate H_2S production which is soluble in water and causes gill damage and other ailments in fish. Bacteria involved in bioremediation of hydrogen sulphide - Photosynthetic bacteria of importance in aquaculture are *Rhodospirillaceae (Rhodospirillum, Rhodopseudomonas, Rhodomicrobium), Chromotiaceae (Chromatium, Thiocystis, Thiosarcina, Thiospirillum, Thiocapsa, Lamprocystis, Thiodictyon, Thiopedia, Ameobobacter, Ectothiorhodospira), Chlorobiaceae (Chlorobium, Prosthecochloris, chloropseudomonas, Pelodictyon, clathrochloris).*

Technological approaches for bioremediation

a) Probiotics

Now, researchers are trying to use probiotic bacteria in aquaculture to improve water quality by balancing bacterial population in water and reducing pathogenic bacterial load. Researchers are increasingly paying more attention to this new approach (ecological aquaculture), and have made considerable headway. Probiotics generally includes bacteria, cyanobacteria, micro algae fungi, etc. In English literature, probiotic bacteria are generally called the bacteria, which can improve the water quality of aquaculture, and (or) inhibit the pathogens in water there by increasing production. "Probiotics", "Probiont", "Probiotic bacteria" or "Beneficial bacteria" are the terms synonymously used for probiotic bacteria.

Recently, the bio-controlling theory has been applied to aquaculture. Many researchers attempt to use some kind of 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. In addition, the use of probiotics can increase the population of food organisms, improve the nutrition level of aquaculture animals and improve immunity of cultured animals to pathogenic microorganisms. In addition, the use of antibiotics and chemicals can be reduced and frequent outbreaks of diseases can be prevented.

b. Green water technology

Green water technology is the bioremediation approach which incorporates the fishes viz., mullet, milkfish, pearl spot and tilapia. This was carried out in the shrimp reservoir ponds where zerowater exchange and recirculatory aquaculture system existed. In these technology integrated fish is a bioaugmentor for green water with abundance of reviving microbes which a vital role in microbial bioremediation which in turn have a competitive exclusion of pathogens replaced by reviving microbes as well as decreased vibrio load, improvement of water quality and enhanced immunity of cultured aquatic animal . The fishes included in these are euryhaline nature and they feed on uneaten feed and algae. These fishes secrete slime which have the antagonistic property against the luminous bacteria and also clean up the water. Enhanced shrimp production was observed. The herbivores fish which feed on wide range of diet and water quality is suitable for green water technology. Characterization of green water by molecular techniques for heterotrophic bacteria and metagenomics for unculturable bacteria revealed the diversity of bacteria which can be used as bioaugmentors. Development of simple cost effective bioremediation technology in zero exchange and water reuse systems is the need of the hour. This technology in Philippines was evinced as biocontrol agent in shrimp grows out culture system.

Procedure

- Identification of aerated zero water exchange ponds
- Analysis of water quality parameters
- Salinity adjustment of finfish seeds
- Selection of pen size (10m x 10m x 2m or 9m x 9m x 2m or 8m x 8m x 2m or 7m x 7m x 2m) and mesh size depending on the DOC and pond size
- Green water area – minimum area of 5-10% of the shrimp pond
- Transportation of finfish seeds and pen to the site
- Installation of pen in the centre / corners of ponds
- Adjustment of finfish seeds (0.75 – 1.7g size each) in the pen

c. Biofloc technology

Suspended growth in ponds consists of phytoplankton, bacteria, aggregates of living and dead particulate organic matter, and grazers of the bacteria. Biofloc technology is a technique of enhancing water quality in aquaculture through balancing carbon and nitrogen in the system. The technology has recently gained attention as a sustainable method to control water quality. By adding carbohydrates to the pond, heterotrophic bacterial growth is stimulated and

nitrogen uptake through the production of microbial proteins takes place. The added carbon source, together with the waste nitrogen, is converted into microbial bio-flocs, which in turn can be eaten by the cultured organism. This technique provides an inexpensive protein source with a higher efficiency of nutrient conversion of feed. The C:N ratio in an aquaculture system can be increased by adding different locally available cheap carbon sources and a reduction of protein content in feed.

Conclusion

As demand for fish, crustaceans and other aquatic organisms increases, and natural fisheries reach their maximum degree of exploitation, aquaculture will be looked to for extra supplies. The present status of the utilization of beneficial microorganisms cannot meet the demand of cultured aquatic animals and water environment requirements. When conditions are favourable to the growth of harmful microorganisms, they can rapidly increase their populations and become dominant with devastating effects on cultured aquatic animals. Uses of medications are only transitional measures, leaving safety problems in aquatic food products for humans. Hence, it is essential to adopt biological and ecological measures to deal with these problems. Research on microorganisms will help for accurate quantification of the roles of bacteria and other microorganisms in food chains, health, and disease and to understand the microorganisms in ponds to optimise aquaculture production.

Bioremediation application is the applied research which is vital for the successful aquaculture. But has to be validated through scientific investigation will be a boon for the aquaculture industry. The application of beneficial bacteria, probiotics and biodegrading microorganisms, to the pond water and soil is vital for sustainable aquaculture production. Enormous technologies, principles and process are discussed here but the package of practices combining process, approaches and technologies has to be developed for the sustainable aquaculture production.

CHAPTER 8

MICROBIAL PRODUCTS FOR ENHANCED SURVIVAL AND PRODUCTION IN AQUACULTURE

Satheesha Avunje, N. Lalitha, P.K. Patil, M. Muralidhar and S.V. Alavandi

Introduction

Association of macro-animals with micro-organism is part of the evolutionary development, and they play a significant role in the survival of animals. Under general presumption, two functions of microbes were believed, in nutrient cycling and disease development. With modern research tools, a third and multiple roles of microbes in the evolutionary development of animals/plant were recognised, opening a new chapter in microbiology. Animal-microbial interaction is not mere occurrence but part of the evolutionarily developed association. Microbial interaction in the gastrointestinal tract of the animals may influence in nutrition and food digestion, disease resistance by inhibiting colonisation of pathogenic microbs, immunomodulation, colouration *etc.* Besides, the metabolites produced by bacteria may act as nutraceuticals, anti-allergen, neuro compounds, anti-inflammatory *etc.* Thus bacterial population play a pivotal role in the healthiness of animals that can be taped for better productivity in aquaculture.

Aquaculture is an activity that acts a prominent role in livelihood as well as corporate business. However, diseases are the single most concern in hindering the expansion of the farming. With vertical expansion in the farming system, degradation of the pond system and natural environment is evident, attracting more health issues in the culture animals. For mitigating the problem, application of disinfectants, drugs have become unavoidable part of the farm inputs. Lack of scientific knowledge, awareness and guidelines, on application of the drugs and other farm inputs, there has been reports on detrimental impact on the eco-system as well as the host. Alternatives to drug that are efficient and safe are the need of the day, probiotics can be one of such alternative to drug that not only considered as safe to environment and also have capacity to remove the pollutants. Probiotics being very effective in improving the farm productivity, its use as farm input has been ever-growing in aquaculture industry. In such a scenario, guidelines for manufacturing and application of the probiotics are present day requirement. Probiotics can be used for various purposes that include bioremediation agent, as discussed earlier; as gut probiotics and immunostimulant, through feed.

Probiotics

Probiotics word was coined from Greek words, ‘pro’ and ‘bios’ refereeing to the beneficial microbes. Though the microbes in living forms were believed to be effective as probiotics, recent studies confirmed immunostimulatory role of inactivated bacteria in fish. Thus probiotics can be considered for not only living bacterial cells but also metabolites, peptides derived from bacteria or dead cells that ultimately impart beneficial effect on the host animal. Based on the probiotic components, they may be grouped into different sub-groups or categories:

(i) Postbiotics are those microbial products that provide health benefit even in the absence of the viable bacteria. This category includes bacterial metabolites such as bacteriocins, organic acids, ethanol, diacetyl, acetaldehydes, hydrogen peroxide or even killed bacterial cell. Postbiotics are known to have anti-bacterial property; hence they can be used as alternative to antibiotics. In addition, postbiotics are healthy to the host, non-toxic or non-pathogenic and resistant to digestive enzymes.

(ii) Prebiotics are certain nutrients that are non-digestable but propagate beneficial gut microflora. Sugar compounds like oligofructose, galactose/ xylose containing oligosaccharides are known to benefit growth on *Bifidobacterium* in the gut. *Bifidobacterium* ferment the prebiotics in the intestine to produce organic acids that gives health benefits. Commonly available vegetables, fruits and grains are not only energy source but also supplement prebiotics. Prebiotics play important role in nutrient uptake in intestine, increase mineral availability for assimilation. More research in this line will significantly support good health of culture animals by use of natural products.

(iii) Synbiotics: can be considered for those which come with composition of prebiotics with probiotics that synergistically enhances gut colonisation and beneficial effect of probiotics in the gut of animal fed. Since combination of postbiotics, prebiotics and synbiotics are more efficient than unitary use, development of different combination will drastically change microbial application in aquaculture in improvement in health status and nutritional uptake.

Source of probiotics

Origin/ source of the probiotic microbe may be from host or non-host but the bacteria should have beneficial role in improving health/ growth of the culture animal, however sourced from host is ideal probiotics, which have diverse biochemical features. Thus in general, bacteria isolated from intestine of aquatic/ terrestrial animals are developed as probiotic bacteria. Generally, Gram positive spore forming bacteria are used as probiotic strains, due to the benefit of better viability and shelf life though some Gram negative bacteria have good beneficial effect.

Mode of action

Probiotics play beneficial effect by various mechanisms. In mammalian system, they are known to take part in several activities such as anti-pathogenicity, anti-diabetic, anti-obesity, anti-inflammatory, anti-cancer, anti-allergic, angiogenic activities, neurostimulatory activities *etc*. However, understanding on their function in the beneficial role in aquatic animals is not well known, but research has confirmed few prominent features.

(i) Anti-pathogenic activity: Probiotics are more known for anti-pathogenic activity than any other activity and are used for disease control. The bacteria have evolved several mechanisms for their survival and compete from other bacteria, thus provide the health benefit to the host. The mechanism can be subclassified as:

(a) Competition for space: generally pathogenic bacteria need to attach to the mucosal layer of the gut lumen to infect the host. Probiotic bacteria colonise on

the gut mucosal layer and exclude attachment of pathogenic bacteria, thus competing with pathogenic strains and avoid their colonisation. Probiotic bacteria such as *Lactobacilli* get itself colonised over epithelial cells of intestinal villi and deny space for the pathogenic bacteria.

(b) Production of antibacterial compounds: bactericidal compounds that are produced by probiotic bacteria inhibit replication and establishment of pathogenic bacteria strain in the gastrointestinal tract of the host. Hydrogen peroxide, siderophores, lysozymes, proteases, organic acids are major bacteriocins produced by the probiotic bacteria. Bacteria are also known to exhibit antiviral and antifungal activity, though the mechanism has not been well understood.

(c) Competition for energy source: Efficiency of beneficial bacteria to grow in an environment where nutrients are deficient makes it a good probiotic candidate. Such microbe sequesters the essential mineral/nutritional component making unavailable for the pathogenic bacteria. Bacteria that produce siderophores are known to chelate iron molecules when they are deficient, thus pathogenic bacteria will be excluded from establishing in the host gut.

(ii) Growth promotor: Microbes produce several metabolites and assist in the digestion of complex macromolecules thus improving food digestion and nutrient uptake and reduce food conversion ratio. Bacteria are known to produce proteases, lipases and growth promotors supporting nutritional health of the host. In addition, some bacteria secrete vital vitamins, essential amino acids and fatty acids in the gastrointestinal tract. Such bacteria play an important role in larval development, by acting external source of enzymes and growth promoters.

(iii) Inhibition of quorum sensing: Quorum sensing is one of the major cause of disease occurrence and transfer of genetic material between bacterial strains, thus acquiring toxic genes from one bacterium to the other. Pathogenic bacteria such as *Vibrio* are known to exhibit quorum sensing. Probiotics like *Lactobacillus, Bifidobacterium, Bacillus* etc. are known to degrade quorum sensing signals produced by pathogenic strains.

(iv) Immunomodulation: Bacterial compounds are known to stimulate host innate immunity and activate signal transduction for production of anti-bacteria secretions by the host. In fishes, it is established that bacterial metabolites/secretions stimulating the production of cytokines, anti-microbial peptide, interferons, growth factors etc. In shrimps, they enhance production of cellular compounds like phagocytosis, encapsulation, formation of nodules, humoral components such as agglutinins, anticoagulants, phenol oxidase enzyme etc.

(v) Stress mitigation: Stress I sine of the major concern in aquaculture system and is the main cause of disease outbreak. The reason may be due to physicochemical agents such as temperature, salinity, photoperiod, metabolic toxins or biological agents such as higher density. These will cause multiply in negating growth and survival of culture animals. Thermal, nutritional, anoxia/ hypoxia, chemicals, toxins etc. are common stressors in aquaculture. Enhancement of production of reactive

oxygen species (ROS) causes oxidative stress interferes with cellular metabolism. A probiotic bacterium like *Lactobacillus delbrueckii* is known to mitigate temperature stress in fish. *Bacillus* sp is known to reduce transportation stress in fish larvae. Probiotics may bring down cortisol level in fish or catalase and superoxide dismutase in shrimp to mitigate stress. With the ever-increasing environmental and biological stresses, microbes may play important role in stress mitigation; however detailed research in this regard is essential.

Table: Different applications of probiotics in aquaculture (Cruz et al, 2012)

Application	**Probiotic bacteria**
Growth promoter	*Bacillus sp., Carnobacterium divergens, Alteromonas* sp., *Lactobacillus helveticus, Lactobacillus lactis, Streptococcus thermophiles, Streptomyces* sp., *L. casei, Vibrio* sp., *Bacillus coagulans*
Pathogen inhibition	*Bacillus sp., Enterococcus faecium, L. rhamnosus, Micrococcus luteus, Pseudomonas fluorescens, P. fluorescens, Pseudomonas sp., Roseobacter sp., Saccharomyces cerevisiae, S. exiguous, Phaffia rhodozyma, Vibrio alginolyticus, V. fluvialis, Tetraselmis suecica, Carnobacterium* sp., *Lactobacillus acidophilus, Bacillus* spp., *Enterococcus* sp., *Lactococcus lactis*
Nutrient digestibility	*L. helveticus, Bacillus* sp., *Vibrio* sp., *Carnobacterium* sp., *Lactobacillus acidophilus,* Shewa*nella putrefaciens*
Water qual i ty	*Bacillus* sp., *Vibrio* sp., *Lactobacillus acidophilus, B. coagulans, Saccharomyces* sp.
Stress tolerance	*Lactobacillus delbrueckii, Alteromonas* sp., *B. subtilis, L. acidophilus, S. cerevisiae, L. casei, Pediococcus acidilactici, Shewanella putrefaciens*
Reproduction improvement	*Bacillus subtilis, L. rhamnosus, L. acidophilus, L. casei,, Enterococcus faecium, Bifidobacterium thermophilum*

Issues in the better management of probiotics in aquaculture

Probiotics are considered as one of the farm inputs that could be decisive in future of aquaculture. However, it is essential to use the technology judicially and scientifically. Generally, probiotics in human use are considered safe and no emphasis was given in policy or guideline development. The bacterial strains like *Lactobacilli*, *Bifidobacterium* are used as probiotics for human are studied for several years and deemed to be safe to even immunocompromised hosts. However with ever increasing use of bacterial strains as probiotic strains, side effects *viz.* systemic infections, undesired metabolic activities, excessive immune stimulation, and horizontal gene transfer between bacteria strains should be seriously considered. Some strains of Gram-negative bacteria like *Enterococcus* are known to have the capacity to transfer plasmid genes horizontally, may exchange genes responsible for antibiotic resistance or infectivity. Considering seriousness of deleterious effect, FAO with WHO has developed set of guidelines in the development of human probiotics.

In line with the international standards, Indian Council of Medical Research (ICMR) constituted a committee in drafting policy guideline in development of probiotics for human

use (Ganguly et al, 2011). The guideline sets a different set of experimental procedures to prove the probiotics is safe to use as food probiotics. Requirements of probiotics as per the guideline should include

(i) Strain identification:
(ii) *In-vitro* tests to screen potential probiotic strains:
(iii)In vivo safety and efficacy studies in an animal model
(iv) Evaluation of safety and efficacy studies for human use
(v) An effective dosage of probiotic strain
(vi) Labelling
(vii) Manufacturing and handling procedures

However, there are limited or no policy guidelines exist for aquaculture probiotics. With present-day farming practice, where minimum water exchange is practised, probiotics for improving animal health along with environmental health has been ever increasing. The surge in demand will cause propping of new probiotic products with poor performance. It is advisable to use verified probiotic compounds for better performance and increased production.

CHAPTER 9

FINFISH AND SHELLFISH FEEDS AND FEED MANAGEMENT FOR BETTER POND HEALTH

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Introduction

Feed management is a critical factor affecting soil water quality and production economics in aquaculture. Feed is not only a initial source of physiological wastes, but it accounts for 50% to 60% of the operation costs in intensive systems and around 40% in semi-intensive systems. Survival and growth have the direct impact on the economic performance of aquaculture production, and optimal feeding is essential for both. Feed management strategies should therefore be aimed at optimizing feed inputs, reducing feed conversion ratios and reducing the potential impact on the culture and effluent water. In recent years, the intensity of shrimp production systems has increased, resulting in higher stocking densities and greater feed inputs, which in turn commonly result in a high FCR. Quite often, failures are blamed on PL quality, feed, water quality and /or disease; although in most cases the origin of the problem is poor feed management.

Influence of Feed quality on pond Soil and water quality

What is meant by Feed quality?

The quality of the feed is the single most factors which has got major influence on the successful operation of the aquaculture enterprise. A good definition of quality is difficult to explain and in the common parlance quality is about meeting the needs and expectations of customers. If we are able to assess the quality of the feed before it is fed to aquatic animals it will save us lots of trouble and money. Hence, attempt has to be made to assess the feed quality as quickly as possible. There are different types quality which can be done easily and quickly.

Physical quality

This is the important quality normally used by farmer for evaluating the feed. The nutritionist/ farmer or the mill manager should train himself to use all his five senses to identify the changes in the nature of finished feed. The appearance of the feed will reveal its quality and the colour will depend on the type of the ingredient used. However in certain conditions change in the colour of the feed is an indication of the storage condition, presence of toxins etc. The size of the pellet should be uniform and it should be free from other contaminants with powder percentage to the acceptable level. Smell is the important indicator in shrimp and carnivorous fish feed. A good fishy smell indicates that it contains considerable level of marine protein sources and hence the feed will be highly attractable and palatable for the shrimp and fish. Water stability of dry shrimp/fish feed pellets is the crucial physical quality which is determined by the loss in weight of pellets kept in water for a specified time interval. The loss in weight of pellets indicates the stability, higher the loss poorer the stability. Normally the water stability for shrimp feed is not less than 2 hours and 1 hour for fish feed.

Chemical quality

Even though physical quality of the feed will indicate the worthiness of the feed the actual nutritional quality can be assessed only by subjecting it for chemical/laboratory evaluation. The feed can be analyzed for proximate and chemical composition and ensured that the nutrient contents are in the desirable level for the candidate species.

Biological quality

The physical and chemical quality n will not be able to reveal everything about the ingredient sometimes when all the tests prove ideal nature of an ingredient but it may cause problems to the fish and shrimp, hence this particular feed may be subjected for biological evaluation to know its suitability to the candidate species.

What is feed management?

Feed management means control and use of feed for aquaculture operation in such a manner that the utilization of feed is optimum with minimum wastage, negligible impact on environment, achieving best feed conversion ratio (FCR) and maximum growth of fish and shrimp and production. Such feed management practice if adopted, aquaculture production will be not only economical and profitable but also sustainable and eco-friendly. A best can produce poor results if the feed management is poor. On the other hand a moderate feed can produce best results under good feed management.

Most of the feed suppliers provide feeding charts for feeding fish and shrimp during the period of culture operation. These tables may be prepared based either some experiences or based on theoretical models. Since most of the feeding charts are based on size of fish and biomass in the culture pond still errors occur because accurate estimation of biomass in a pond is very often not possible correctly. In many farms excess feeding may occur due to this error. In some cases farmers may be over enthusiastic in achieving faster growth may over feed the stock leading to poor feed management.

Rate of feeding

Even though there are some investigations on the quantities requirements of feed in relation size and stage of the growing fish/shrimp still research on these aspects is needed for making the feeding tables more accurate. Generally the method of calculating the daily ration is based on the body weight of fish. The quantity of ration varies from 100% of body weight for larvae and fry and gradually reduced to 50%, 20%, 10%, 5% and 2-3% as the fish/shrimp grow marketable size. Suppose if W grams is the average weight of the stoked animal and if there are A number of animals in the pond then the total biomass in the pond is W x A grams which is equal to W x A/1000 kg. If feed is to be given at 10% of body weight then the quantity feed required per day is

$$\frac{W \times A}{1000} \times \frac{10}{100} \text{ kg}$$

In pond to estimate the biomass accurately is not possible. Generally periodically (once a week or 10 days) using a suitable net, sampling of the fish/shrimp and the average weight of the animal is calculated. Total biomass is calculated by multiplying the average weight by the number of animals surviving at that time. This is mainly by done by counting the number of animals caught per each netting and estimating the total number of animals taking into accounts the area covered by each netting and the total area of the pond. Some times the number of animals surviving in the pond is approximately estimated by giving a margin of 5 –10% mortality per month on the total number of animals initially stocked.

The alternative method of feeding is not by calculating the daily ration but by leaving the fish on self-demand feeding conditions. When the fish is hungry it will approach the demand feeder for its food requirements. It was observed that fish quickly learn how to obtain feed. The growth of fish also is good with best FCR and minimum wastage of feed. This method works best with finfish farming. Mechanical demand feeders and feed bags suspended at different places in pond are used in this method feeding.

Floating pellet feeds for finfish have the advantage in controlled feeding. Since the feed floats on the surface of water, the active feeding by fish can be directly observed and the consumption of feed can be monitored. Based on the observations the quantity of feed to be broadcast can be regulated.

Schedule and frequency of feeding

The total quantity of feed required in a day should not be fed at time. Scheduling and frequency of feeding greatly help in successful feed management. Time schedules for feeding the fish may be fixed such that larger ration may be given when the fish is expected to be most hungry. If night feeding is limited the morning feeding should have larger ration. There should be a minimum of three time schedules of feeding in a day – morning, noon and evening. Some species are more active during night and should receive comparatively larger portion of the ration. Observations and experiences show that frequent feeding of small portions of the ration seems to help in better utilization of the feed and there by lead to efficient FCR. The daily ration can be offered at every 2- 4-hour interval in divided doses. There must also a mechanism in each case to monitor the feed consumption and offering of the next scheduled dose should be regulated according to the consumption from the previous feed offered. Regular observations and experience help in mastering the management of feeding in a culture farm.

Feeding shrimp in grow-out ponds

The quantity of feed required in a day for feeding shrimp is estimated based on biomass in the culture pond. To start with feed is offered at 15 – 20% of body weight. As the shrimps grow, it is gradually reduced and brought down to 2-3% towards the end of the culture period. A model chart for feeding is given in Table 1. The entire quantity of feed required for a day in a pond should not be put at one time. The shrimps should be offered feed at every 3 - 4 hours in small doses. This helps in better utilization of feed and reduces wastage. Shrimps are active feeders during night, hence large doses may be offered in the evening and

during night. Keeping the feed in bamboo or velon screen trays kept inside the pond at different locations is a good practice. These are known as check trays. Periodically these check trays can be lifted up to check the feed consumption. A part of the feed may also be broadcasted for proper distribution. Instructions of the feed supplier with regard to feeding may be followed. Excess feeding leads to uneaten feed at the pond bottom. This will cause pollution of pond water and stimulates algal blooms, which may cause stress to shrimp. Under these conditions mass mortality of shrimp may occur. Feeding a little less does not do any harm, but feeding a little excess may be harmful and can cause heavy loss. Feed management needs experience and skill to obtain best results. Water quality in culture pond is also linked to feed management. If the water quality (such as dissolved oxygen, ammonia, nitrite, nitrate, hydrogen sulphide) in the pond is poor, even the best feed may give poor performance.

Shrimp feeds should be stored properly. Absorption of moisture during storage leads to mould growth and lowers the quality. Certain kinds of fungi (*Aspergillus* sp) produce aflatoxin, which is very toxic to shrimps. Feedstocks required for use of one month may be purchased at a time and stored in a cool and well-ventilated place. For longer shelf-life, the feed may be stored at lower temperature of 10^0 C.

Farmers should look for feeds that are as fresh as possible. Fresh feeds generally give good fishy smell. Stale smell indicates that the feed is not fresh. Water stability of feed also affects the performance of the feed. It will not disintegrate fast but also causes water pollution leading to economic loss. The feed should be stable under water at least for 2 hours. Feed should not be too hard also as it not properly assimilated the animal. Feed with poor water stability leads to poor FCR and higher cost of production.

CHAPTER 10

DISEASES IN BRACKISHWATER AQUACULTURE AND ENVIRONMENT PERSPECTIVE OF HEALTH MANAGEMENT

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In 2009, the Coastal Aquaculture Authority of India (CAA), after the risk analysis conducted by ICAR- CIBA, permitted the entrepreneurs to introduce a new species, *P. vannamei* (Pacific white leg shrimp) in India with prescribed guidelines. Because of its SPF status, fast growth rate and culture feasibility in wide salinity range, this got readily accepted by the farmers and subsequently became the dominant cultured species. Indian aquaculture sector has achieved remarkable growth during the past decade, especially with respect to shrimp production through aquaculture after the introduction of Pacific white shrimp. During 2016-17, seafood exports crossed11,34,948 MT tons valued an all-time high of Rs 37, 870.90 crore (US$ 5.78 billion) and frozen shrimp being the top item of export, accounting for 38.28 per cent in quantity and 64.50 per cent of the total earnings. However, the increasing trend in intensification and commercialization has exacerbated the epidemics of diseases in culture systems and mortality problems continue to confront shrimp aquaculture sector including emergence of new disease problems. WSD alone is estimated to cause losses of over US$6 billion since its emergence in 1992. Since 2009, the newly emerging diseases early mortality syndrome (EMS), specifically known as Acute Hepatopancreatic Necrosis Disease (AHPND),caused economic loss excess of US$ 1 billion per year. Considering the limited therapeutic options available for the control of viral diseases, timely disease detection using novel diagnostic tools for disease surveillance followed by active response to adopt and practise proper health management approaches would ameliorate the magnitude of the problem. In this context, it is essential to understand aspects of disease management in aquaculture systems and explore possibility of available avenues for disease prevention and control.

Economically Important Diseases in shrimp culture

Disease can be described as an expression of complex interaction of host, pathogen and environment. A decline in host's immunity is the main cause of disease. The pre-disposing that will impair shrimp health and most important factors leading to diseases in shrimp culture are: Adverse environment, high stocking density ,nutritional deficiency/poor nourishment, accumulation of unused feed, inadequate aeration, sub-optimal or heavy algal blooms in the pond, physical injury and presence of virulent pathogens in high count etc.

OIE listed diseases

WHITE SPOT SYNDROME (WSD)

White spot syndrome (WSD) is the most devastating diseases of penaeid shrimp and virus that has wide spread presence throughout the world. WSD caused by white spot syndrome

virus (WSSV) which is a rapidly replicating and highly virulent shrimp virus. The typical clinical symptoms of WSSV infection are the formation of circular white spots on the carapace often with reddish discolouration. Outbreak of this disease wipes out the entire shrimp populations in the ponds within 7-10 days. Rapid and specific diagnosis of the WSD can be accomplished using nested or quantitative polymerase chain reaction. WSSV is transmitted vertically from infected broodstock to larvae and horizontally by ingestion of infected organisms. Other than the penaeid shrimps which serve as host for this virus, a large number of other crustaceans serve as carrier and therefore it has been impossible to eradicate this virus from the culture system. While WSD transmission vertically is being prevented by screening out infected broodstock, its horizontal transmission in grow out farms is a serious challenge. At present there is no treatment available to prevent the unrestrained occurrence and spread of the disease. Better management practices (BMPs) have helped alleviate this problem to a great extent, by minimising risks of its transmission through carrier organisms such as mud crabs, *Artemia*, rotifer eggs, molluscs, polychaete worms, insect larvae and seabirds etc. However, concerns of WSD transmission through contaminated water and pond sediment remain unaddressed. Pond preparation practices have proved to be useful in eliminating the virus from the pond and reducing the risk of disease outbreaks.

White spots on carapace seen in shrimp infected with WSD

YELLOW HEAD DISEASE (YHD)

Yellow head disease is a major viral disease that caused extensive losses to shrimp farms in Thailand during 1990-91. Outbreaks of YHD with heavy mortalities have been reported in farmed black tiger shrimp and pacific white shrimp. YHD is caused by Infectious type I yellow head virus (YHV). YHV principally affects pond reared juvenile stages of 5-15 g. Affected shrimp typically feed voraciously for two to three days and then stop feeding abruptly and are seen swimming near the periphery of the pond. YHV infections can cause

swollen and yellow discolouration of hepatopancreas in infected shrimps. The primary mechanism of spread of YHV in pond culture appears to be through water and mechanical means. Infected broodstock can pass on the virus to larvae in the maturation/hatchery facilities if thorough disinfection protocols are not strictly adhered to. Methods of YHV eradication in ponds are much the same as for other viruses and involve BMPs that include pond preparation by disinfection and elimination of carriers and production of virus free broodstock and PL for pond stocking.

INFECTIOUS HYPODERMAL AND HAEMATOPOIETIC NECROSIS (IHHN)

IHHN was first reported in *P. Stylirostris* from America and the disease is caused by a small (20-22 nm) single-stranded DNA Brevidenso virus.. However, it was thought to have been introduced along with live *P. monodon* from Asia.Large-scale epizootics were responsible for multi-million dollar losses in *P. vannamei* culture in the Americas during the 1990s. Gross signs of disease are not specific to IHHN, but may include reduced feeding, elevated morbidity and mortality rates, fouling by epicommensals and bluish body coloration. Larvae, PL and broodstock rarely show symptoms. In *P.vannamei*, IHHNV can cause runt deformity syndrome (RDS), which typically results in cuticular deformities (particularly bent rostrums), slow growth, poor FCR and a greater size variation at harvest, contributing substantially to reduction in profits. Transmission of IHHN is known to occur rapidly by cannibalism shrimp. It can also be transmitted through waterborne route and cohabitation. Vertical transmission from broodstock to larvae is common. Strict hatchery biosecurity, use of SPF broodstock, washing and disinfecting of eggs is essential in combating this disease.

Stunted shrimp with size variation

TAURA SYNDROME (TS)

Taura Syndrome was first identified from farms around the Taura River in Ecuador in 1992 and the disease spread rapidly to other regions, probably through the regional and international transfer of live PL and broodstock of *P.vannamei*. Taura syndrome virus (TSV) is responsible for the TS.TSV infections occur in juvenile shrimp (0.1-1.5 g body weight) within two to four weeks of stocking ponds and occur largely within the period of a single moult cycle. In the acute phase of the disease, during pre-moult stage, the shrimp are weak, soft-shelled, have empty gut and diffuse expanded chromatophores that appear red, particularly in the tail (hence the common name - red tail disease). Such animals will usually die during moulting (5-95%). Adult shrimp are known to be more resistant than juveniles. Shrimp that survive infection show signs of recovery and enter the chronic phase of the

disease. Such shrimp show multiple, randomly distributed, irregular, pitted, melanised lesions of the cuticle. These gross lesions will persist, but may be lost during moulting, and the shrimp thereafter appear normal. TS can be diagnosed using standard histological and molecular methods of detection.The mechanism of transmission of TSV can be through contaminated PL and broodstock. Recently it has been shown that mechanical transfer through insect. The disease can be prevented by avoidance of reintroduction of the virus from wild shrimp and carriers and stocking with TSV-free PL produced from TSV-free broodstock.

INFECTIOUS MYONECROSIS (IMN)

Infectious myonecrosis is an emerging *P. vannamei* disease, and has been detected in Indonesia and Brazil. The principal host species is *P.vannamei* in which IMNV known to cause significant disease outbreaks and mortalities. IMN is caused by putative totivirus. IMN disease causes significant mortality in grow-out ponds and is characterized by acute onset of gross signs including focal to extensive whitish necrotic areas in the striated muscle, especially of the distal abdominal segments and the tail fan, which may become necrotic and reddened similar to the colour of cooked shrimp. Severely affected shrimp become moribund and mortalities can be instantaneously high and continue for several days. Mortalities from IMN range from 40 to 70% in cultivated *P.vannamei*, and food conversion ratios (FCR) of infected populations increase from normal values of ~ 1.5 to 4.0 or higher. Juveniles and sub-adults of *P.vannamei*, farmed in marine or brackish water, appear to be the most severely affected by IMN disease. IMNV has been demonstrated to be transmitted through cannibalism. Transmission via water and vertical transmission from broodstock (trans-ovarian or by contamination of the spawn eggs) to progeny is also likely to occur. IMNV may also be transmitted among farms by faeces of seabirds or shrimp carcasses. Outbreaks of IMN with sudden high mortalities may follow stressful events such as capture by cast-net, feeding, sudden changes in salinity or temperature, etc., in early juvenile, juvenile, or adult *P. vannamei* in regions where IMNV is enzootic. The disease can be prevented by stocking with virus free PL produced from IMNV-free broodstock.

Shrimp infected with IMNV displaying whitish necrotic areas in the distal abdominal muscle segments

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Important bacterial diseases

VIBRIOSIS

Vibriosis is ubiquitous and all marine crustaceans, including shrimp, are susceptible. Epizootics occur in all life stages, but are more common in hatcheries. *Vibrio* species are part of the natural microflora of wild and cultured shrimp and become opportunistic pathogens when natural defence mechanisms are suppressed. They are usually associated with multiple

aetiological agents. However, some *Vibrio* species, or strains of certain species, have been identified as primary pathogens. Pathogenic strains of *V. harveyi, V. parahaemolyticus* and *V. Vulnificus* have caused massive epidemics. Mortalities due to vibriosis occur when shrimp are stressed by factors such as poor water quality, crowding, high water temperature and low water exchange. High mortalities usually occur in post-larvae and juvenile shrimp. Shrimp suffering vibriosis may display localised lesions of the cuticle typical of bacterial shell disease, localised infections from puncture wounds, loss of limbs, cloudy musculature, localised infection of the gut or hepatopancreas and/or general septicaemia.

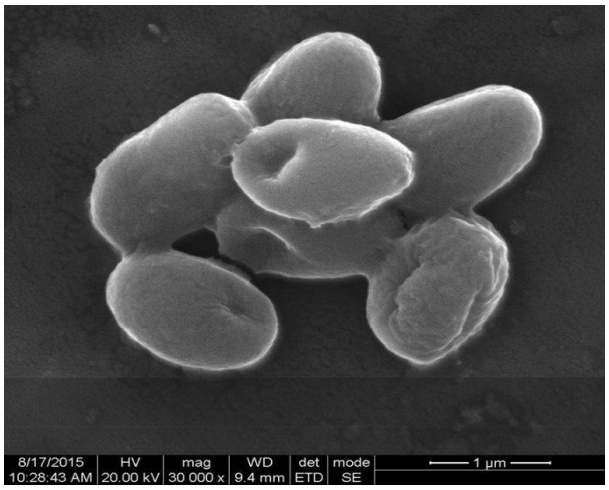
NECROTIZING HEPATOPANCREATITIS (NHP)

NHP has been reported as an important disease since its first diagnosis in 1985. It has been reported to cause mass mortalities to the tune of 20-90 percent of *P. vannamei* in highly saline commercial grow-out ponds nearly every year since then. NHP has not yet been reported in Asia, but could cause significant damage were it to be transferred here with untested shrimp introduction. Necrotizing hepatopancreatitis is caused by obligate intracellular Rickettsia-like bacterium, a member of the order α-Proteobacteria (Gram-negative, pleomorphic, rod-shaped or helical-shaped bacterium). Affected shrimp become lethargic, anorexic with empty gut and show epibiotic fouling. Exoskeleton becomes soft and show abdominal muscle atrophy. Affected ponds have increased FCR and growth of affected shrimp is retarded. The hepatopancreas becomes watery with white or black streaks. Mortality rates reach up to 90% within 30 days of the appearance of clinical signs. NHP could be transmitted horizontally with infected PLs. Maintaining optimal environmental parameters using BMPs will be useful in preventing NHP.

Emerging diseases of concern

HEPATOPANCREATIC MICROSPORIDIOSIS (HPM)

Hepatopancreatic microsporidiosis (HPM) is caused by *Enterocytozoon hepatopenaei* (EHP) and is currently known to infect both *P. monodon* and *P. vannamei*. It has been found that EHP can be transmitted directly to shrimp by cannibalism and cohabitation. EHP shows no gross signs of disease except retarded growth. More recently, samples of frozen A*rtemia* mass has been reported to test positive for EHP by PCR, but again, it is not known whether *Artemia* is susceptible to EHP infection or just a mechanical carrier. Although EHP does not appear to cause mortality in *P. monodon* and *P. vannamei*; information from shrimp farmers indicates that it is associated with severe growth retardation in *P. vannamei*. The best approach for maturation and hatchery facilities to



EHP spores observed under SEM

avoid EHP is not to use wild, captured, live animals (e.g., live polychaetes, clams, oysters, etc.) as feeds for broodstock. Alternatively, polychaetes could be selected and tested for freedom from shrimp pathogens and then reared as broodstock feed in biosecure settings designed to maintain their freedom from shrimp pathogens (i.e. SPF polychaetes).

ACUTE HEPATOPANCREATIC NECROSIS DISEASE (AHPND)

Since 2009, an emerging threat, popularly known as early mortality syndrome (EMS) and recently termed as acute hepatopancreatic necrosis disease (AHPND) severely affected shrimp farming in many countries in the Southeast Asian region. The disease is reported to affect mainly Pacific white shrimps (*P. vannamei*), tiger shrimp (*P.monodon*) and Chinese shrimp (*P.chinenesis*) and is characterized by mass mortalities (reaching up to 100% in many cases) during the first 20-35 days of culture (post-stocking in grow-out ponds). The disease has caused severe economic losses in affected region. Some of the farm level observations include onset of clinical signs such as soft shells, significantly emaciated pale to whitish hepatopancreas (HP) and partially full to empty guts and mortality starting as early as 10 days post stocking. Recently detection of the causative agent of AHPND by molecular methods such as PCR has been developed. AHPND/EMS is caused by an infectious agent, particularly by a specific strain of a *V.parahaemolyticus* (VPAHPND) bacterium that is commonly found in the shrimp culture environment. Adoption of biosecurity measures to prevent this pathogen entering the culture system. The potential risk factors for EMS / AHPND include factors such as high stocking densities, older farms closer to the sea using higher salinity water, farms not employing reservoirs, farms overusing chemicals, inadequate aeration, and presence of toxic levels of H_2S etc.

PENAEUS VANNAMEI NODAVIRUS (PvNV) INFECTION

The virus is reported to cause muscle necrosis. This was first reported in 2004 from P. *vannamei* cultured in Belize. Infection with this virus resulted up to 50% reduction in production in the affected farms. The gross signs were whitened abdominal muscles, coagulative muscle necrosis that is similar to the signs of IMNV. Effect (s) on farmed shrimp has not been fully evaluated and is not clear at present. RT-PCR method needed to detect *Pv*NV and differentiate it from IMNV.

COVERT MORALITY DISEASE (CMD)

The disease first reported from China. In China since 2009, continuous mortality of shrimp especially peaked at 60-80 days with cumulative losses reaching up to 80%.Thenew disease was named as Covert mortality disease (CMD) because affected shrimp died at the bottom of the pond and farmers would initially be unaware of the mortality. The causative agent was identified and named as covert mortality nodavirus (CMNV). The virus cause gross signs of muscle whitening, similar to that caused by infectious myonecrosis virus (IMNV) and *P.* vannamei nodavirus (PvNV). Experimental studies conducted at Thailand showed injection of extracts derived from tissue homogenates of RT-PCR positive shrimp into naïve shrimp at 32^oC resulted in conversion of the injected shrimp to CMNV-positive status, but was not associated with any gross signs of disease or mortality.

DISEASES OF UNKNOWN ETIOLOGY

BLACK GILL DISEASE

Black gill disease is highly prevalent in the shrimp farms. More plankton in water, high stocking density, insufficient aeration and more mud in the pond bottom are the predisposing factors. The gill becomes black in colour and is generally colonized with different bacteria (*Flavobacterium*, *Cytophaga*, etc.) and parasite (e.g. *Zoothamnium*sp.). Increase in duration of aeration, water exchange and addition of lime according to pH may be the corrective measures. In poor management/culture conditions the increase in population of the fouling organisms can cause impairment of physiological functions of the host. Usually, heterogenous mixture of filamentous and non-filamentous bacteria (*L.mucor*, *Vibrio* sp., *Thiothrix* sp., *Flavobacterium* sp., *Flexibacter* sp., and *Cytophaga* sp.,), blue green algae and protozoa (*Zoothamnium*) causes fouling and black gill.

Black gill condition in shrimp

WHITE FAECES SYNDROME (WFS)

White faeces syndrome reported since last decade, has recently been noted as serious problem for *P.vannamei*. WFS usually occur after 60 days of culture (DOC) and may be associated with high mortalities. Ponds affected with WFS show white faecal strings floating on the pond surface while the shrimps show white/golden brown intestine, reduced feed consumption and growth retardation and often associated with loose shell. The disease can cause moderate to severe economic loss by reducing the shrimp survival by 20–30 percent when compared to normal ponds. The cause of white faeces syndrome and treatment is uncertain. However while investigating the aetiology of WFS; this disease has been associated with vibriosis, EHP, blue-green algae and loose

Faecal strands floating on Pond surface affected with WFS

shell syndrome. Six species of fungi (*Aspergillus flavus, A. ochraceus, A. japonicus, Penicillium* spp., *Fusarium* spp., and *Cladosporium cladosporioides*) were found associated with from shrimp naturally infected with white faeces syndrome. Reduced stocking density, proper water exchange together with better management practices will be helpful in evading WFS.

WHITE MUSCLE SYNDROME (WMS)

In recent years, shrimp farmers have been suffering from several cases of white muscle with muscle necrosis in the *P. vannamei* grow-out cultures associated with low mortalities. The white muscle syndrome affected shrimps show focal to extensive necrotic areas in striated muscle tissues, displaying a white, opaque appearance. Similar lesions have been described with Infectious myonecrosis (IMN), penaeid white tail disease (PWTD) and non-infectious aetiology with sudden changes in water quality parameters such astemperature, salinity and dissolved oxygen.

White, opaque appearance of shrimp with WMS

White muscle in shrimp can also be caused by the advanced infection of microsporidians belonging to the genera Ameson and Agmasoma, or dietary deficiency of selenium.

RUNNING MORTALITY SYNDROME (RMS)

Since 2011, a new syndrome has affected the shrimp industry and causing substantial mortality. The affected ponds show different mortality patterns with unusual symptoms, no relation to any known diseases and a slow mortality rate, but the cumulative loss over the culture period will be high. Some farmers have lost up to four crops, with mortality percentage reaching 70% in most of the cases. Generally mortalities start after a month or 40 DOC, but few shrimp continue to survive and can grow to fully harvestable size. The causative agent is unknown. Investigations conducted in ICAR-CIBA revealed no association of RMS with known shrimp pathogens except affected shrimp indicated predominance of *Vibrio* spp., such as *V.parahaemolyticus* and *V.azureus* .

Shrimp mortalities seen in RMS condition

WHITE GUT DISEASE (WGS)

This disease of *P. vannamei* is mostly caused by different species of *Vibrio* and is very much prevalent in Andhra Pradesh and Tamilnadu. Vibrios are normally present in water bodies. But sometimes stressed environmental factors such as sudden change of environment and salinity, low DO, mechanical injury, higher stocking density, etc. caused rapid multiplication of this organism in the gut and hepatopancreas. Vibriosis causes red discolouration and melanization of appendages (red disease), necrosis of tail, broken antennae, etc. Six species of *Vibrio* viz. *V. harveyi*, *V. parahaemolyticus*, *V. alginolyticus*, *V. anguillarum*, *V.vulnificus* and *V. splendidus* are generally associated with the diseased shrimp. The diagnosis of the disease can be done by isolation of organism by plating haemolymph on the TCBS agar followed by identification of the species of *Vibrio* by biochemical tests or 16S rRNA gene sequencing.

ABDOMINAL SEGMENT DEFORMITY DISEASE (ASDD)

Abdominal segment deformity disease described in *P. vannamei* from Malaysia and Thailand. The disease is characterized by gross signs of deformed abdominal segments that were enlarged or twisted laterally and/or dorsoventrally and in few with opaque muscles. Due to unpleasant appearance it caused economic loss of approximately 10% in affected specimens. Despite the distortion, shrimp growth and survival were unaffected. The disease is not caused due to known shrimp pathogens. it is recommended that control measures for ASDD include avoidance of long-term broodstock use

MUSCLE CRAMP SYNDROME (MCS)

This disease is mainly caused by environmental stresses, mainly low DO and sudden rise of environmental temperature and is especially common when the stocking density is high. The body of the shrimp bends and stiffness of the muscle observed. The rate of mortalities varies. The increase in duration of aeration circumvents the problem to some extent.

Interactions of pond environment in shrimp culture

Disease prevalence in populations and ecosystems is influenced by numerous environmental factors, and their interactions. The diseases of cultured penaeid shrimp include syndromes with infectious viral, rickettsial, bacterial, fungal, etiologies, as well as noninfectious diseases, caused by environmental extremes, nutritional imbalances, toxicants and other factors.The two significant components of the pond environment are the pond water and sediments which interact continuously to influence the culture environment. Pond management activities which influence the culture environment include feeding, use of aerators, water exchange and liming. The production system evolved from extensive toward intensive with increasing inputs of high quality feed and water supply. Consequently, waste loads from culture ponds as uneaten feed and metabolic wastes also increase. A major concern shrimp farming industry is the discharge of nutrients from shrimp farms, with the potential to contribute to increased algal blooms, oxygen depletion of bottom waters and reduced biodiversity. Most of the nutrients discharged from intensive shrimp farms originate

from the formulated feed. Therefore, efforts to improve feeding strategies must focus on both optimizing production and minimizing waste. Feeding strategies also influence water quality and shrimp health. The time of feeding was very important to ensure rapid consumption of the feed by shrimp, thereby minimizing the loss of nutrients and resulting in an improvement in growth rate. The organic component of the accumulated sediment is a mixture of pond soil organic content and detrital material. This detrital material is composed of sedimented organic material from plankton, shrimp faeces and uneaten feed. The character of the accumulated sediment is therefore dependent upon culture intensity, pond soil organic content, and water exchange practices. Problems associated with the pond bottom and accumulated sediment occur when excessive organic material builds up causing release of ammonia, organic sulphur compounds. As accumulated sediment is known to be undesirable, need to be removed. Frequent water exchanges are advocated. The development of acid sulfate soils, and associated release of toxic levels of aluminum, precipitation of iron, and alterations of water chemistry e.g. calcium and magnesium, may indirectly cause production failure by increasing physiological stresses and lowering the immune response. Inadequate sediment removal would cause water quality problems in the subsequent crop. In areas with high pond density, the emitted chemical and biological pollutants are recirculated among farms, and consequently, the degree of self-pollution increases. In order to reduce disease risk, the grow-out period in shrimp farming is often shortened, resulting in harvesting of smaller shrimp. Sometimes, cultivation continues until first signs of disease appear when the crop is immediately harvested and can still be marketed, but at lower quality.

The role of pond environmental factors in disease outbreak

Viral and bacterial diseases, together with poor soil and water quality and deficient environmental management of shrimp farms are the main causes of shrimp mortality. Chemical and biological pollution by farms includes disposal of pond effluents and sludge in coastal waters, salinization of soil and water, misuse of chemicals, including antibiotics and pesticides and under some conditions, the host and its pathogen may be co-existing with little or no adverse effect. In penaeid shrimp, there are examples of normally innocuous epicommensal organisms on shrimp gills causing disease when host populations are crowded and environmental conditions are stressful such as high BOD combined with low dissolved oxygen conditions. Instances are common in which bacteria that may be part of the shrimp's normal microflora, and viruses such as WSSV, YHV are found to cause disease in stressed shrimp. Common problems in the open water exchange system include phytoplankton crashes, deteriorated pond bottoms and bacterial diseases. A phytoplankton crash causes a significant increase in ammonia in the water, a decrease in dissolved oxygen and a rise in organic material. This stressful situation, together with increased bacterial concentrations, often leads to outbreaks of vibriosis, *Zoothamnion* infections, and luminescent *Vibrio* in the ponds. Fluctuations in normal environmental conditions e.g. oxygen, temperature, salinity have a significant effect on the virulence of *Vibrio harveyi*, with salinity being more lethal to shrimp than temperature. Low oxygen levels, which are a common problem in ponds with high shrimp stocking density, increase sensitivity to vibriosis in penaeid shrimp.

The disease occurrence in shrimp ponds in Hainan, China was closely associated with excessive stocking and poor water quality. In the Philippines, IHHNV prevalence in various wild populations of *Penaeus monodon* has been correlated with shrimp culture intensification and mangrove status. White Spot virus disease seems to be triggered or aggravated by changes in sea water quality including, hardness, temperature and dissolved oxygen. Sudden change in pH or low dissolved oxygen levels can precipitate an outbreak of YHV disease, and pollution from outside, such as insecticide residues that have a very high direct toxicity on shrimp may be important at sublethal levels as predisposing factors for disease. Other studies have shown that salinity reductions cause physiological stress in crustaceans and lower their tolerance to pollutants, indicating that toxicants in combination with environmental factors may act synergistically. Clearly, physiological stress seems to be one of the most important factors triggering the disease outbreak.The method for ameliorating this problem is high levels of water exchange. Water quality management was achieved by a combination of flushing the pond with clean seawater and management of the phytoplankton bloom by assessment of pond colour. There appears to be a clear linkage between environmental conditions and disease, although the precise nature of the relationship is complex and has to be established.

Environment management strategies for disease prevention and control in shrimp farming

An understanding about the environment, biota and biology of the target species along with the in depth knowledge of the disease, pathogen, disease development, diagnostics, epidemiology and control measures are essential factors in management of a disease problem. Hence, Shrimp health management requires a holistic approach, addressing all aspects that contribute to the development of disease. Disease outbreak is an end result of negative interaction between pathogen, host and the environment. Hence, management of disease problems must be aimed towards broader ecosystem management with a view to control farm-level environmental deterioration and to take preventative measures against the introduction of pathogens into the aquaculture system. The emphasis should be on better management for prevention, which is likely to be more cost effective than treatment, involving both on-farm management and the management of the environment. Steps must include reducing the use of chemicals and drugs. Regulations with respect to land and water usage, environmental protective measures, inputs that go into the aquaculture systems, farm-

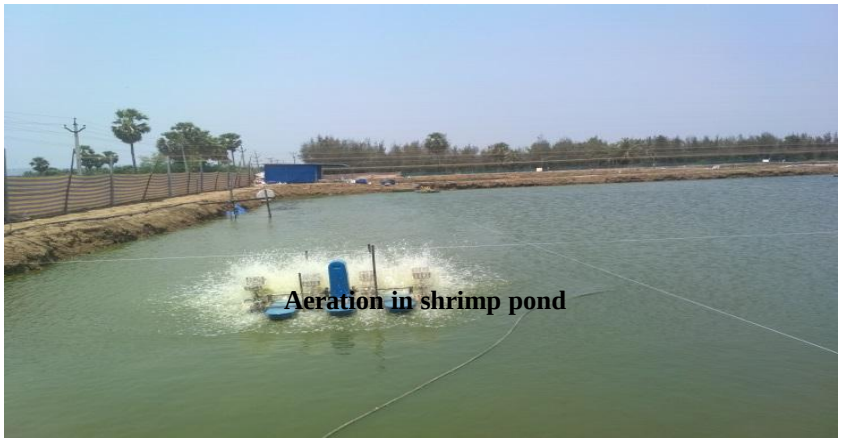
Optimal water quality

wise and region-wise must be put in place by the Government for disease management of aquatic animals and sustainable development of aquaculture at large. In addition, research and development, training programs, extension, and information exchange would help achieve the objective of disease prevention and control in aquaculture effective. The ultimate goal of most aquaculture operations is to produce maximum possible biomass per culture unit area in a sustainable manner, regardless of the type of operation and the species cultured. However, the production depends upon a number of factors including environmental conditions, availability of good quality water, nutrition and disease and mortality of cultured stock. Incidence and severity of infectious disease outbreaks very often depend on the quality of environment. Hence the foremost important step in aquaculture health management is to provide the best quality environment within the culture unit.

Approaches to reduce disease problems in shrimp farming

Several factors may be involved in the occurrence of epizootics of cultured stock and are often complex and difficult to pinpoint. Therefore, disease management must be viewed with a holistic angle, considering the host, pathogen and environment and their inter-relationships. Hence, treatment of disease should not consider the pathogen alone. Management of disease problems must comprise of broader ecosystem management with a view to control farm-level environmental deterioration and to take preventative measures against the introduction of pathogens to aquatic animals. Overall, health management procedure includes crop-planning, pond preparation, post-larvae/fry selection and stocking process, management ofwater quality, pond bottom, feed , health monitoring, farm record keeping, biosecurity measures, dealing with disease outbreaks, if any, and appropriate use of chemicals, if necessary. However, even the stringent health management procedure may not be able to eliminate the risk of diseases or mortality completely. The emphasis should be on better management for prevention, which is likely to be more cost effective than treatment, involving both on-farm management and the management of the environment . An effective health management programme comprises steps and control measures that are carried out on a daily-basis. Main preventive measures at pond / farm level include proper pond preparation to eliminate pathogens and their carriers, treatment of water in reservoirs to inactivate free viruses and kill virus carriers, water filtration using fine filters to keep carriers out, closed systems to avoid contamination from source water, reduced water exchange to minimize the entry from source water and even changing the water source. Transmission of virus can be prevented by providing crab fencing, fencing, foot baths, wheel baths, and disinfection protocols. Improved husbandry practices have been successfully employed for the control of diseases. Shrimp ponds with a history of disease outbreaks have a greater likelihood of future disease outbreaks, and hence, special attention is required during pond preparation in such farms. Pond preparation is essential to reduce the risks of shrimp disease outbreaks. Removal of bottom sludge, especially in ponds with high stocking densities, ploughing of soil when wet, use of lime during pond preparation will help in minimizing disease risks. Farms with poor bottom soil quality such as presence of a black soil layer, will suffer crop failures. Hence, the sludge must be removed and disposed away from the pond site. Extra precaution should be taken while disposing sludge from farms affected by disease outbreak during the

last crop. Sludge removal should pay attention to areas of the pond where there is a high accumulation of organic matter from pervious crop, such as feeding areas. Ponds must be ploughed to expose the black soil layer underneath bottom soil to sunlight and atmospheric oxygen. By this process, the organic waste (sludge) will be oxidized. Ploughing on wet soil is particularly recommended for ponds if the sludge cannot be removed properly by manual or mechanical methods. After ploughing, ponds must be dried for 2-3 weeks and even more when pond had a history of WSD outbreak since WSSV can be viable for three weeks despite sun-drying. In case a heavy tractor is used for ploughing, then plough the dry soil and then fill the pond with water to wet the soil and then again dry. Ploughed pond bottom leads to turbid Water conditions during culture period. Therefore, compaction of the bottom using heavy rollers after the whole process of pond preparation, i.e., before water intake, helps avoid the turbid water condition. Liming during pond preparation optimizes pH and alkalinity conditions of soil and water. The type and amount of lime to be added depends mainly on the soil pH and also on pond water pH. The other general approaches include lower intensity and pond densities, aeration, treat and re-circulate pond water, integrate systems for effluent treatment and resource management, Keep farming within carrying capacity of local environment, judicial use of antibiotics and medicines.



Aeration in shrimp pond

Water quality deteriorates during culture mainly due to the accumulation of metabolic wastes of living organisms, decomposition of unutilized feed, and decay of biotic materials. Changes in water quality can influence survival of organisms as they become vulnerable to disease. But an addition of beneficial bacterium as probiotics helps maintain water quality, thereby improving survival and growth. Dissolved oxygen in the culture medium is an important factor not only for the respiration of aquatic organisms but also to maintain a favorable chemical and hygienic environment in the water body. The pH of the culture medium plays an important role on the organisms. It changes with the accumulation of residual feed, dead shells, and excreta. The toxicity of ammonia is pH-linked. Ammonia is the main end product of protein catabolism in aerobic conditions. Nitrate is reduced to ammonia in anaerobic conditions. Microorganisms can convert ammonia into nitrate by nitrification through the intermediary product nitrite. Steps must be initiated towards reducing the use of chemicals and drugs. The water probiotics contain multiple strains of bacteria like *Bacillus acidophilus, B. subtilis, B. licheniformis, Nitrobacter sp*., *Aerobacter sp*., and *Saccharomyces cerevisiae* while feed probiotics contain *Lactobacillus sp., Bacillus sp*. or Saccharomyces *cerevisiae* .Probiotic supplementation of live microorganisms in aquaculture aids in preventing disease, thereby increasing production and decreasing economic loss. Probiotics applied through feed beneficially act upon shrimp growth, ultimately increasing production. Water quality plays an important role in aquaculture production.

SUMMARY

Aquaculture is now integral to the economies of many countries. Growing demand for seafood and limitations on production from capture fisheries will inevitably lead to the increased intensification in commercialization of shrimp aquaculture. Aquaculture sector has undergone spectacular transformation through expansion, intensification and diversification. This in turn increases the number of diseases and leads to emergence of new diseases. However, as a consequence of disease problems and the resultant production losses have been the major limiting factor in aquaculture. The emergence and spread of infectious disease is usually the result of a series of linked events involving the interactions between the host, the environment and the presence of pathogens. Focusing efforts on producing high quality seed, better pond manage to reduce stress and risk of infection, following routine farm biosecurity, responsible trade practices, response to disease outbreak, and improved better management practices shall aid in preventing the epidemics of diseases. Further health management is a shared responsibility, and each stakeholder's contribution is essential to the health management process.

CHAPTER 11

BIOSECURITY AND QUARANTINE MEASURES FOR BRACKISHWATER AQUACULTURE

S.K.Otta, M. Muralidhar, P.K.Patil, S.V.Alavandi and K.K.Vijayan

Shrimp aquaculture industry has been continuously progressing at a rapid pace contributing to the demand of protein need and increasing the overall economy of many of the countries throughout the world. India has also witnessed similar progress and particularly after the introduction of the Pacific white shrimp, there is a steady increase in the production. During 2016-17, India's seafood export was all time high touching the value of $ 5.78 billion. Frozen shrimps accounted about 38.28% in terms of quantity and about 65% in value indicating a major share and again a majority of this has been contributed by aquaculture. India has a huge potential for brackishwater aquaculture and still a major chunk of it remains unexploited. With years to come, it is highly expected that there will be increase in effort to explore and utilise this unexploited treasure land. At the same time, there should be proper planning and protocols in hand to counteract all the associated risk factors.

Disease always comes as a major hurdle on the way to a successful aquaculture practice. During the initial period, tiger shrimp (*Penaeus monodon*) was the major and successful cultured species in India till it was severely threatened by white spot syndrome virus (WSSV) during the late 90's. Subsequently, it was totally replaced by the Pacific white shrimp (*Penaeus vannamei*). However, this species has also been facing similar threat at present due to outbreak of existing and emerging diseases. Species replacement is not at all a best alternative to coup with the disease prevalence. Rather, aquaculture sustainability will be a possibility more with the placement of required corrective measures like biosecurity and quarantine.

Biosecurity consists of set of well-designed practices that minimizes the introduction and spreading of infectious diseases. In this process, animals can thus avoid the unnecessary burden of several stress factors and mortality and thereby avoid huge economic loss. It is highly essential to accurately determine the specific points of production system that favours the introduction and spread of pathogens. Once the specific points are determined, it will then help to develop precautionary measures to avoid untimely losses.

Quarantine is a standard protocol to isolate individual/group of organisms, conduct all the necessary tests and ensure existing/new pathogen entry to the point of release. Like biosecurity, this is also an important step in aquaculture practice to avoid disease outbreak and prevent loss. Sustainability of aquaculture practice is very much dependent upon these two aspects.

Biosecurity measures for Aquaculture

Determining the point of entry of pathogens and their spreading possibilities are the major steps in designing the biosecurity protocol. This depends on several aspects.

1. **Cultured animal** – It depends on particular animal species or strain and its susceptibility status, life stage at stocking, health and immune status at the time of stocking
2. **Environment** – This involves source water and its pathogen load, water quality and maintenances and the type of culture practice
3. **Pathogen characteristics**: It includes the very basic biology and life cycle of the pathogen and survival strategy of the pathogen (free living state, ability to form spore, ability to survive on inanimate objects, adopting a carrier etc.)
4. **People involved** – This entirely depends on the nature and education status of all the peoples such as management staff, workers and visitors (how much they understand the principle and how they follow it)

Based on the above, maintenance of biosecurity in aquaculture mainly aims at managing and maintaining the followings steps;

Animal Management

Obtaining pathogen free and healthy stock is prime criteria. Established stress test should be carried out on a sample to determine the health status. Visual and microscopic observation should also be carried out to determine the health status. Wherever possible, it should be tried to get specific pathogen free (SPF) or specific pathogen resistant (SPR) stocks. All the larvae should be tested for the presence of pathogens through a well-established diagnostic protocol. If SPF/SPR stocks are imported, these should pass through proper quarantine for the detection of all possible pathogens. Care should be taken to avoid importing larvae/broodstock from areas known to be affected with specific diseases. For stocking, the concept of "all-in-all-out" should be followed. This means a single batch or group of animals should be stocked till harvest and any additional stoking during the culture period should be avoided.

Environment management

This starts with pond preparation. Details regarding soil test and pond construction can be found somewhere else in this manual. Preparation of ponds during infection to eliminate the pathogens and preparation between the culture periods to further carryover of pathogens is also very important. When chlorine is used to treat the pond water, effective concentration should be used. Based on the research work carried out in CIBA, it was observed that effective concentration to kill WSSV only with water base is 5 ppm for 2 day, if the water contains infected animals (dead) it is 10 ppm 2 days, in soil based system, with planktonic WSSV, it is 15 ppm for 2 day and in soil based system, with dead infected animals it is 20 ppm for 2 days, Soil based system, WSSV (filtrate)added, water drained, exposed to sunlight for 2 days and then chlorine treatment, it is 10 ppm 2 days. Chlorine concentration also depends on the organic load and therefore should be determined based on the water condition. Accurate chlorine concentration will help to eliminate the pathogen, avoid unnecessary expenditure and will also help to maintain soil health condition. It is necessary to

provide sufficient time gap between the culture practices. Again with respect to WSSV, the work carried out at CIBA indicates, the pathogen can remain viable for at least 35 days in non-drainable ponds and in drainable ponds it is 19 days. In addition to this other treatments such as lime should also be carried out.

Obtaining good water is an important aspect of aquaculture practice. Water can be a primary source for the entry of pathogens. Wherever possible, it is advisable to go for recirculatory systems and thereby any pathogen entry can be avoided. Otherwise, it is necessary to go for adequate amounts of reservoir ponds where water can be stored initially, treated and finally matured before taking into culture ponds. Throughout the culture period, it is required to maintain good water quality and thereby avoid stress. Along with feed and other management practices, the aquatic environment should always be maintained healthy. Animals should be supplied with good quality diets and preferably supplemented with immunostimulants to maintain good immunity during the culture period and thereby avoid infection by opportunistic pathogens.

Some of the commercial feed and live feeds can also serve as a source for pathogens. These feed should be properly tested before use. If necessary proper steps such as pasteurization should be adopted. All the animals should periodically be monitored for their health status.

Pathogen management

Pathogen management mainly involves preventing the entry of pathogen. However, if due to some reason, the pathogens enter the system, and then take necessary steps either to eliminate it completely or reduce the number substantially to prevent mortality.

It is necessary to know the nature and virulence status of each pathogen and act accordingly. Many of the pathogens have reservoirs either as living organisms, water or inanimate objects. Therefore, one should be thorough with the nature of the pathogen and accordingly steps should be taken. Suitable environment and conditions for pathogen multiplication should be avoided. Ponds should have proper fencing system to avoid reservoirs or passive carriers for disease spread.

It is always better to have preventive methods such as vaccination or use of immunostimulants than treatment. Similarly biological controls such as phage therapy should be preferred. However, during inevitable infection period, appropriate sanitizers should be used to reduce the pathogen load. Active ingredients and the mode of action of each chemical should be known properly and accurate dosages should be used. Indiscriminate use of antimicrobials should be avoided to prevent stress on the animal and on the environment.

It is necessary to maintain good diversity of planktons and bacteria in the pond. This will avoid the multiplication of pathogens. Biodiversity can be maintained through the use of good quality probiotics bacteria. Similarly, it is necessary to maintain proper plankton density in the pond and avoid bloom conditions.

People management

All the necessary biosecurity protocols can be put in place. However, this will be successful only when the people involved understand it clearly and practice it effectively. This involves management staff, workers and visitors those get a direct access to the aquaculture facility. Effective measures are required to prevent the entry of pathogens or spread through these people. Sensitive areas should be designated only for the authorized personnel through strict security arrangement.

Visitors from another farm are considered as serious risk factors and should be allowed after thorough sanitizing protocols. Disinfectant foot baths, hand washing stations or spray bottles, net disinfection station, vehicle disinfection station and showers should be in proper places to avoid pathogen entry. Continuous awareness programmes should be arranged for all the employees to make them understand the basic principles and importance of biosecurity.

Aquaculture Quarantine Measures

The principles of quarantine apply to a cultured species coming to one area from other area (within the country or another country). This is an important animal management and biosecurity measure. This is a procedure by which individual animals or population can be isolated and acclimatized to the new place for a specific period of time, during this period, these are observed for any abnormality or disease appearance. Even if these animals look healthy, these are tested for all possible pathogens. If necessary, these are treated to make disease free. Once it is made sure that animals are free from pathogens, these are then either released to culture facility or live market. However, for exotic pathogens or presence of viral pathogens wherever treatment is not possible, the stock should be destroyed carefully making sure that the pathogen does not spread.

The quarantine facility should be well designed and located in an appropriate isolated place or ensure that the facility is physically separated from farms/hatcheries. The facility should have easy access for transportation of animals, get sufficient quality water and the discharge water should be handled properly. The facility should have well established protocols and well trained staffs. This should have competent and readily available diagnostic support.

When in quarantine facility, animals should be sampled at the beginning, at the end and at point of disease appearance. Usually random sampling is done to determine the health status. In case of valuable stocks like shrimp brooders, non-lethal sampling is preferred.

Conclusion

Both biosecurity and quarantine measures are essential parts of a sustainable aquaculture practice. With the adoption of new culture practices involving high stoking density and with the import of several new species for aquaculture, it is expected to have more disease prevalence and culture loss. Therefore, these two systems are very much important and should be well established to avoid mortality, unwanted situations due to introduction of exotic pathogens or appearance of emerging diseases.

CHAPTER 12

DISCHARGE WATER MANAGEMENT FOR ENVIRONMENTALLY SUSTAINABLE BRACKISHWATER AQUACULTURE

M. Muralidhar, R. Saraswathy and S. Krishna

Introduction

Nutrient enrichment of pond waters and discharging water from ponds are common management practices to ensure adequate water quality for shrimp/fish growth. However, the discharge of such nutrient-rich waters may result in deteriorating water quality in receiving waters, and is the subject of increasing regulation in many countries. When the concentration of wastes builds up to undesirable levels in pond water, some water is discharged and ponds are topped up with water to maintain better water quality. The disposal of sludge from the ponds into the drainage channels and creeks causes sedimentation of mudflats, wetland and mangrove swamps, greatly reducing their primary productivity potential and often leading to irreparable damage. There is a need for maintaining water quality for increased production and ecosystem impact.

Characteristics of discharge water from aqua farms and culture ponds

The general term pond waste is used for the mixture of liquid, semi-solid and solid forms unless it is clearly stated as discharge water or solid waste. In general, the nutrient levels and suspended solids in the discharge water practicing improved traditional and extensive methods are within the accepted norms and much less when compared with the effluents generated from the industries.

Comparison of discharge water (DW) from aquaculture farms with effluent water quality from few industries

Parameter *	DW from aquaculture farms (CIBA data)	Effluent water quality from Industries in India				
		Tannery[1]	Distilled[2]	Sugar[3]	Paper[4]	Rubber[5]
pH	7.0-8.2	7.8	5.2	7.9	8.6	7.7
DO	2.1-5.0	Nil	Nil	Nil	1.9	2.3
TSS	20.0-96	1688	1810	1666	-	134
Alkalinity	97-140	-	3959	1650	764	218
BOD_5	9.0-48.0	1335	8420	735	115	-
COD	16.4-73.6	5125	-	-	171	326
NH_3-N	0.009-0.226	-	2.0	2.5	-	14.6
TN	0.80-1.9	-	5.4	3.5	-	-

*All Parameters are in ppm, except pH

Superscript 1- Mariappan, 1994; 2&3- David & Ray, 1996; 4 - Ray *et al*, 1979; 5- Ghosh *et al*, 1979.

Higher nutrient concentrations appeared related to higher stocking densities and feeding rates. Discharge water from aquaculture ponds contain living and dead particulate organic matter,

dissolved organic matter, ammonia, nitrite, nitrate, phosphate, suspended soil particles, and other substances that can be considered potential pollutants. Total N and P, dissolved oxygen and oxygen demand increased and water visibility decreased in intensive ponds throughout the grow-out period.

Aqua farms discharge is characterized being high in volume, turbid with suspended solids and rich in particulate and dissolved organic matter and nutrients. Although this waste is biodegradable, the intensity of the discharges from a pond/farm depends on the technology and on the quantity of fertilizers, un-consumed food and metabolism of nutrients, etc. The shrimp farm/pond water quality tends to deteriorate through the grow-out period, as feeding rate increases with fish size and biomass. Periodic discharge of pond water contains high concentrations of suspended solids and nutrients, particularly nitrogen relative to intake levels and the highest quantity and poorest quality of discharge water was found just before harvest time, when fish biomass is at the maximum. Discharge water during harvest, especially the last 5 cm drainage is usually the most important contributor to overall waste loading, comprising over 75% of the total load. Discharge water during regular flushing and at harvest can account for 45% of nitrogen and 22% of organic matter output in intensive ponds. In areas with significant concentrations of aqua farms and with poor flushing capacities, the most commonly reported impacts are the increased sedimentation of suspended solids, eutrophication of receiving waters, resulting in increased primary productivity, low dissolved oxygen and higher demand for biological oxygen and chemical oxygen in the receiving water bodies. The solid waste have higher value of organic matter, total nitrogen and phosphorous than normal soil.

Legal obligation for maintaining water quality

In many countries, overcrowding of farms in certain areas and limited carrying capacity of the creeks/ estuaries serving such farms has been a matter of concern. As of now for *Penaeus monodon* culture, stocking densities of upto 6-10 no/sq. m. is permitted under the Coastal Aquaculture Authority (CAA) registration and the discharge water treatment system (DWTS) is mandatory for farms of 5 ha and above. But with the introduction of *Penaeus vannamei*, stocking densities of upto 60 no./m2 is permitted and the DWTS has become mandatory for all the farms culturing L. vannamei irrespective of their size. Post culture discharge water management is not well practiced in most farms simply because it is not seen to directly affect production and also due to additional cost. Presently, most of the farms lack treatment system for treating the discharge water before it is released into the open waters. The farms which do have such facility also do not conform to the scientific requirements. Government regulations are important component of management in supporting aquaculture development, maintaining environmental quality, reducing negative environmental impacts, allocating natural resources between competing users and integration of aquaculture into coastal zone management.

Aqua farms are located near the brackishwater sources (canal, creek, back water, agricultural drain) that are common property and used as sink for discharge of wastes. Post culture discharge water management is not well practiced in most farms simply because it is not seen

to directly affect production and also due to additional cost. Presently, most of the farms lack discharge water treatment system (DWTS) before the water is released into the open waters. The Ministry of Agriculture in its Guidelines for Sustainable Development and Management of brackishwater aquaculture has prescribed standards for the water discharged from the coastal aquaculture farms. Establishment of cost effective DWTS is necessary to bring the discharge water within the prescribed standards and mitigate any adverse impact on the ecology of the open waters.

Guidelines/ standards for discharge water from coastal aquaculture farms in India

S.No	Parameter	Final Discharge Point	
		Coastal Marine Waters	Creeks/estuaries-when the same inland water course are used as water source and disposal point
1.	pH	6.0-8.5	6.0-8.5
2.	Suspended Solids mg/I	100	100
3.	Dissolved Oxygen mg/I	Not less than 3.0	0.5
4.	Free Ammonia (as NH_3 – N) mg/l	1.0	0.5
5.	Bio-chemical Oxygen Demand (BOD) (5 days at 20°C) mg/l	50	20
6.	Chemical Oxygen Demand (COD) mg/l	100	75
7.	Dissolved Phosphate (as P) mg/l	0.4	0.2
8.	Total Nitrogen (as N) mg/l	2.0	2.0

Source: CAA (2001)

Technologies for managing solid waste within the pond

In a production period farmers employ different techniques to manage the solid waste depending upon culture situation, pond and environmental condition and resources availability. Management techniques are different from one farm to another depending upon personal preference, affordability, suitability and pond management techniques. Three of the most useful approaches to solid waste management are remain, re-suspend and remove. The 'remain' management technique refers to accumulation of solid waste within the pond where it may produce least negative effects to animal population. Some solid waste is resuspended through bottom aeration techniques and bioturbation using hanging substrata to allow additional growth of microorganisms that degrade re-suspended pond waste aerobically, reducing waste volume and improving water quality. The 'remove' management technique implies removal of waste from grow out ponds during the culture period. This aims to create more clean space for the cultured animals in order to avoid the unfavourable conditions of higher oxygen demand at the mud-water interface and production of hydrogen sulfide gas in pond environment.

Discharge water treatment management

By minimizing and even avoiding altogether the discharges of organic matter and nutrients from the grow-out ponds into the natural waters, the adverse environmental impacts often associated with intensive farming is completely avoided. This can be achieved only through a closed culture system where discharge water is treated and released into grow out ponds.

Discharge water treatment system (DWTS)

CAA has issued guidelines for the DWTS and has suggested a suitable design. The treatment system should consist of a common discharge channel (CDC), settlement pond (SP), bio-pond (BP) with aeration facility and sludge disposal facility. In the case of small holdings ranging from 0.2 to 2 ha, individual settlement ponds would not be practicable. From each culture pond, water is discharged into the primary drainage canal leading to main drainage canal. Water from such drainage canals have to be discharged into a 'common discharge channel' to hold and transport the discharge water from the entire farm. The drainage canals have to be designed in such a way that they are wide enough to slow down the flow of water from ponds, so as to allow the settlement of the suspended solids. Water from the CDC will flow gravitationally through a stream into sedimentation pond. Each sedimentation pond will have baffle walls to facilitate the discharge water to move slowly for a longer distance, enabling the settlement of solid waste materials. The supernatant water from the sedimentation ponds will be discharged into the biopond/aeration pond through sluices. The biopond is used for secondary aquaculture by stocking various fishes, mollusks, oysters and aquatic plants that actively feed on, and thereby remove particulates from discharge water. Methods of economically important sea weeds species such as *Gracilaria* and *Kappaphyccus* should be standardized as secondary aquaculture species. This integrated approach would also offer scope for improving the discharge water quality, reducing the organic and nutrient loss and producing an additional cash crop. The sludge accumulated in the sedimentation ponds will be removed after dewatering and drying and subsequently disposed at a safer place or can be used for other purposes such as manure for agricultural crops.

Common discharge water treatment system (CDWTS)

More than 90 per cent of the farmers own less than 2.0-hectare water area. In the case of small holdings ranging from 0.2 to 2 ha, individual settlement ponds would not be practicable. As number of farms is coming up in many areas, there is every chance of one farm's discharge becoming another farm's water source. Even though this is not a healthy practice, the situation is unavoidable, taking into account the land holdings of small farms, which constitutes a majority of the total area. For maintaining the sustainability of aquaculture, it is necessary to establish a common discharge water treatment system (CDWTS) for the cluster of farms.

A CDWT system consisting of a common discharge channel from cluster of farms, sump with pumps, settlement ponds (SP) with adequate baffles, bio-ponds (BP) with aeration facility is proposed for the cluster of farms. Since each pond would have an individual discharge point, all these could be connected to common discharge channel, leading to a

settlement pond or bio-pond for treatment of effluents. These may be set up at the tail end of the discharge channel or still far away from the culture ponds to be discharged to the mouth of the creek/sea. Such discharge points should be sufficiently away from the supply point of water to the farms, to reduce the possibility of the discharged water getting mixed with waste at the supply point even after removal of the effluents. By incorporation of the CDWTS facility, the farm wastewater is expected to be as good in quality as that of intake water.

Use of discharge waters for secondary aquaculture of seaweed Kappaphycus alvarezii and green mussel Perna viridis

The finfish hatchery of ICAR-CIBA has a well-designed 800 m^2 discharge pond to receive the discharge water from the broodstock holding tanks and other hatchery facilities as a mandatory requirement prior to being released into Muttukadu estuarine waters. The discharge pond of the fish hatchery acts as a zero feed extractive aquaculture system.

The discharge pond has three chambers:-

i) Primary chamber- receives the initial discharge water from the holding tanks and the hatchery. This is designed with two baffles so that the water gently flows through a longer stretch before it reaches the secondary chamber separated from the primary chamber by a sluice gate. The stretch of flow and the presence of baffle slow down the water current and settle suspended matter. This chamber is stocked with omnivorous fish like tilapia and pearlspot to feed on the organic particles.

ii) Secondary chamber- This is the largest chamber of the discharge pond and the water flows from the primary chamber to the secondary chamber. It is stocked with filter feeding bivlaves, the green mussels *Perna viridis* to feed on the suspended particles and seaweed *Kappaphycus alvarezii* to assimilate other dissolved nutrients. The chamber is also stocked with herbivorpus/omnivorous species like the milkfish *Chanos chanos* and bottom feeding species like the grey mullet *Mugil cephalus* and *Liza sp* which feed on the algae and the benthic diatoms in the pond. Green mussels were tested for culture in pond system and the satisfactory specific growth rate of 2.6 % day^{-} was recorded in a 30 day trial, the culture is being continued for extracting organic particles. For seaweed three models were tested, - PVC raft with horizontal and vertical culture and cage culture. The best growth was recorded in the cage experiment with a daily growth rate of 3.19 %. The product quality of carrageenan extracted from the seaweed was found to be at par with its marine counterpart.

iii) Tertiary chamber - Water in the tertiary chamber is further clarified by seaweeds and herbivorous fish species *Scatophagus argus* and then discharged into the open waters.

Harvested seaweed

Rope culture of *P. viridis*

Pond based water recirculation system

It is clear that water resources will be under increasing pressure for human use and future demands for aquaculture based food supply will increase water demands from the sector. By minimizing and even avoiding altogether the discharge of organic matter and nutrients from the grow-out into the natural waters, the adverse environmental impacts often associated with intensive farming is completely avoided. This can be achieved only through a closed culture system where discharge water is treated and returned to the growing ponds. During culture period, even under emergency situations, many farmers are unable to exchange water due to non-availability of good quality water from the source. Under these circumstances, treatment of aquaculture water for its reuse purposes is a sensible mean to support the further growth of aquaculture industry without excessive water demands that are environmentally unsustainable. Hence, the development of simple, cost effective, water reuse systems using quite basic techniques without much technical sophistication may offer advantages of water and area savings, reduced risk of contamination and better environmental control. Quality-wise, the treated discharge water would also be suitable and ideal for recirculation within the farm, making the farming practice conform to the zero discharge norms. However, such a recirculation system would need the establishment of a reservoir pond of suitable size. In areas where the intake water quality is below the desired standards, a recirculation system can be resorted to using a storage reservoir in combination with the discharge water treatment system. Closed water recirculation system not only conserve the water but also prevents entry of pathogens and thus control disease outbreaks.

Post-culture solid waste management

In recent years, farmers have started paying attention to post culture management of solid waste for different reasons. Unsustainable practices such as removing solid waste by pressurized hose after the harvest particularly in crowded farming areas may raise conflicts with other users of the water resource. Proper post culture solid waste management procedure can be divided into four phases; control, treatment, disposal and reuse/utilization. The four management phases carried out after harvest are in sequential order and its level of management, in terms of environmental sustainability, increases with the phase.

a) Control

The control phase refers to preventing solid waste effects on cultured species itself, and minimizing the discharge of untreated waste into open environments.

b) Treatment

Treatment phase aims to reduce the volume and toxicity of solid waste and make it useful for other purposes. This phase is beyond the reach of most farmers at present. Simple primary treatment such as dewatering of solid waste by sun drying or sand bedding are within the reach of farm operators' capacity.

c) Disposal

Disposal implies proper planning and the provision of area for discharging solid waste in an environmentally friendly and safe manner. Implementation of this phase greatly improves environmental quality and reduces health risks. The disposed solid waste is usually settled or sun-dried naturally and its salinity thoroughly reduced by rain.

d) Utilisation

The ultimate goal of solid waste management is 'utilization' including recycling waste products and increasing productivity of other production sectors.

The guidelines for solid waste management are:

- Pond waste should not be discharged to outside environment.
- There should be proper and sufficient disposal area for pond waste on farm.
- Primary treatment such as sedimentation and sun drying should be performed before the waste is disposed of.
- A certain degree of treatment should be applied to solid waste before the disposal based on solid waste condition: quality, volume and especially if the pond had received some probiotic and antibiotic treatment or if the pond had disease problems.
- Avoid disposing any form of solid waste either dried or wet into freshwater aquatic environments.
- Solid waste disposal areas should not be near freshwater sources that are shared by other resource users.
- Solid waste should be recycled to use in pond where possible.

Conclusion

Aqua farmers now aim not only for disease free animals with high growth rates and high yield, but also on resource conservation. Farmers should be encouraged to undertake environmentally sound and non-polluting ways of dealing with pond solid waste. The development and dissemination of better management practices for solid waste disposal should be considered a priority by governments. Development of a regulatory and legislative management framework with enough flexibility is necessary to implement discharge water treatment systems. The integration of water reuse system, based on better management practices in the farms will therefore assist the farmers to improve discharge water quality and make their farming practices more sustainable. The efficiency of the suggested designs of DWTS in decreasing the suspended solids and removing the nutrient load from the discharge water has not been tested under field conditions. It is essential that the awareness is to be created among the farmers about the necessity of the DWTS. Research on management systems to reduce sediment loads is also important. Research should also be directed towards formulating pond water and discharge water quality standards that can be used to establish better management practices without conflict with other users.

CHAPTER 13

ENVIRONMENTAL IMPACT ASSESSMENT, MONITORING AND CARRYING CAPACITY OF SOURCE WATERS FOR SUSTAINABLE AQUACULTURE DEVELOPMENT

M. Muralidhar, M. Jayanthi and P. Kumararaja

A need to determine the environmental limits for sustainability of aquaculture is felt by the people concerned with the industry. Environmental management studies and the remedial measures to counteract any environmental problem due to shrimp culture will have to be made in this perspective. The full socio-economic benefits of coastal aquaculture development can only be achieved by adopting the principles of sustainable development, which is defined by FAO as

"Sustainable development is the management and conservation of the natural resource base and the orientation of technological and institutional change is such a manner so as to ensure the attainment and continued satisfaction of human needs for present and future generations. Such sustainable development conserves land, water, plant and animal genetic resources are environmentally non-degrading, technically appropriate, economically viable and socially acceptable".

Quantitative evaluation of the impacts of aquaculture on the environment has only recently been seriously attempted, and most of the bio-physical relationships involved have yet to be firmly established. Sustainable aquaculture needs adequate interaction among the social, economic and ecological changes, which accompany development. This can be achieved through an integrated approach to planning and management of coastal aquaculture. The biggest initiative for sustainable aquaculture has to come from the farmers and entrepreneurs.

Environmental impact assessment (EIA)

EIA should encompass the evaluation of social, economic and ecological impact of a proposed development as well as the identification of impact mitigation measures and alternative development options. EIAs have frequently consisted of collections of largely descriptive data with a little priori consideration of the specified changes to be expected from the proposed development. Data and model predictions from the EIA are needed to design efficient monitoring programmes.

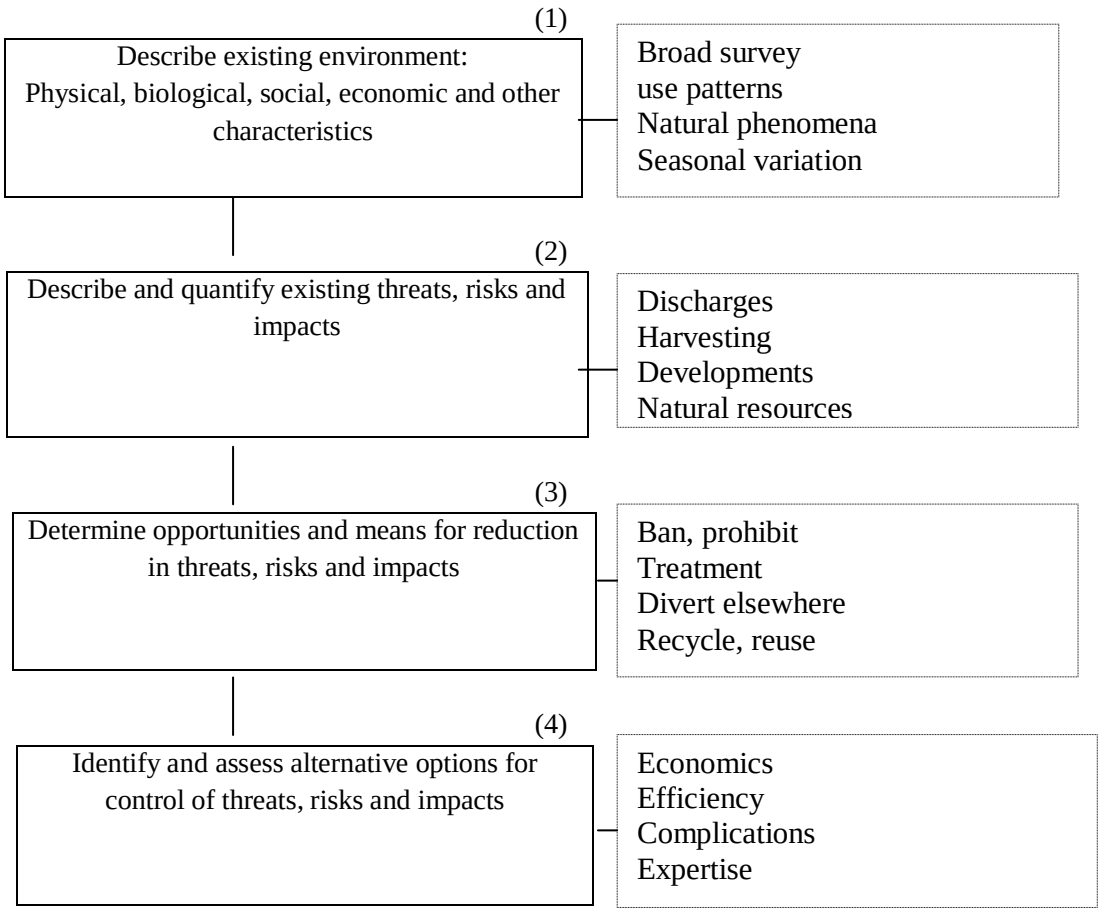
Environmental Quality Objectives (EQOs) and Environmental Quality Standards (EQSs)

To satisfactorily manage the scale of enrichment and ensure that ecological change does not exceed pre-determined and accepted levels, a management framework should be adopted prior to development. Such a framework should include the establishment of environmental quality objectives (EQOs) and environmental quality standards (EQSs) and must include scope for environmental impact assessment (EIA) and a monitoring programme. EQOs define the conditions to protect a particular use. EQSs are levels of particular variable associated with that use which may be imposed to ensure that the objectives are not compromised. An

example of the EQO/EQS approach related to protection of the natural environment is given below.

EQO	Criterion and standard or approach to standards	
	Criterion	**Standard**
Beyond the immediate farm area, the chemical quality of the receiving environment will be indistinguishable from that of the adjacent marine or brackish water environment.	E_h (redox potential) and sediment carbon content	E_h and sediment carbon content shall not be significantly different from that of selected control sites.
	Dissolved oxygen level in water column	Should not fall below prescribed standard (except in cases where de-oxygenation is due to other causes.)

In many occasions, monitoring has been imposed as a result of public pressure because of the perceived ecological damage caused by aquaculture wastes. The result has often been the measurement of a wide range of ecological variables, many of which are inappropriate, failure to analyse and interpret data and implement feedback mechanisms to modify farm practice production and the monitoring programme itself. The key elements of EIA process framework are given.



Key elements of environmental impact assessment (EIA) frame work.

Environmental monitoring - General Principles

- Environmental capacity
- Environmental quality standards
- Baseline studies
- Reference stations
- Delegation of monitoring responsibility
- Mixing zones
- Detecting ecological change
- Feed back
- Flexibility in monitoring intensity
- Monitoring effluents versus receiving waters
- Developing aquaculture specific guidelines for ecological monitoring

Use of models in EIA

The use of EIA in the management of coastal aquaculture development requires the application of ecological knowledge. Models can be an important tool in management both, for predicting impacts and as an aid in the design of monitoring programs. For example, with respect to wastes from shrimp culture operations, there are three processes to model, the quantity of material generated, dispersion after release of discharge and biological consequences. Models may be empirical or mechanistic. The former is based on a statistical relationship between variables derived by observation, and does not necessarily require any understanding of underlying principles. Mechanistic models describe the relationship between cause and effect with the exception that all variables have significance within the natural system. Complex ecosystem models are typically mechanistic and have generally been developed in relationship to large scale or multiple developments. In most instances however, given to low risk of large scale ecological change from shrimp culture wastes there is little justification for the use of complex ecosystem models except in areas where there is likely to be large scale development. For the reasons, it is suggested that complex ecosystem models are more appropriate for research rather than use as management tools.

A sequential approach should therefore be adapted. That is, to use simple models as a first stage and if the results of the investigation indicate that such models is inadequate, the second stage would be to evaluate the conceptual framework, and if necessary, increase the complexity of the model. Simple models may therefore provide an efficient means of screening for potential ecological impacts from shrimp culture and other developments in coastal environments. Data are required to initialise the model and validate predictions.

The rapid development of tropical coastal aquaculture (particularly shrimp farming) requires the urgent adaptation and validation of existing models. A first step in this direction would be to test the applicability of simple empirical models to tropical systems to save time and resources in ecological assessment.

Example: Eutrophication models

Eutrophication, the biological consequence of nutrient enrichment, may be manifest as changes in the biomass and community structure of phytoplankton or macrophytes. The main components associated with developing such models include.

- Defining the basin or water body influenced by waste inputs.
- Estimating dilution rate
- Calculating the level of nutrient enrichment
- Relating nutrient enrichment to the response by phytoplankton

Environmental management plans

Formulation of coastal aquaculture management plan

The Department of Environment and Forests, Government of India has issued a notification declared coastal stretches as coastal regulation zone (CRZ) and regulating activities in the CRZ. The notification has directed the coastal states and union territory administrations to prepare coastal zone management plans (CZMP) identifying and classifying the CRZ areas within their respective territories is accordance with the guidelines given and obtain approval of the central Government in the ministry of Environment and Forests. Through the thematic maps prepared by space application center using satellite data, conflicts of aquaculture with other land uses can be avoided. Buffer zones wherever required may be planned and use of such zones for mutual benefits may be considered.

Preparation of master plans for all suitable sites

Haphazard and unplanned development of shrimp farms will not be in the interest of aquaculture itself, besides coming into conflict with other uses. Technical suitability, economic viability and socio-cultural acceptability of the areas proposed for shrimp farm and their impacts should be accessed and accordingly master plan should be prepared for allocation of sites.

Sustainable aquaculture plans

Analyzing the fundamental causes of the collapse of shrimp industry it can be concluded that most of the factors that led to the mass mortality of the tiger shrimp caused by the virulent diseases were manmade and could be averted. The carrying capacity of the environment was far exceeded. The lesson thereof is to fix levels of sustainable production with reference to the carrying capacity of the environment and manage aquaculture at that level for long term benefits.

Improvement of management operations

No matter how carefully the plans are made, finally it is the farmer who has to ensure proper implementation of plans and management of the farm. Farmer /operator has to choose the appropriate technologies and management practices which would reduce environmental impact. The stocking density, biomass, feed type, rations and schedules, water quality and exchange, algal blooms, aeration and effluent management are the important factors the farmer should bestow attention.

Regulations

There is need for framing and implementing certain regulations to safeguard aquaculture and the environment. It should include effluent treatment and permissible levels of chosen water quality parameters such as suspended solids, nutrients, organic matter, BOD and COD. For tropical brackishwater shrimp culture reliable data are meagre and necessary database should be created through special effort. There should also be regulations on the use drugs and chemicals. Monitoring and implementing agencies at different levels should be identified and responsibilities entrusted with laws and mechanisms for enforcement.

Carrying capacity assessment of water sources

The World Commission on the Environment and Development defines sustainable development as meeting the needs of current generations without compromising the ability of future generations to meet their own needs. Sustainable development requires pragmatic management of natural resources through positive and realistic planning that balances human expectations with the ecosystems carrying capacity. The concept of sustainable development is closely linked to the carrying capacity of ecosystems. Ecosystem carrying capacity (ECC) provides the physical limits to economic development and may be defined as the maximum rate of resource consumption and waste discharge that can be sustained indefinitely in a defined planning region without progressively impairing bio-productivity and ecological integrity. It aims not only at environmental harmony, but also at long term sustainability of the natural resource base. A key of sustainable development of aquaculture is to stay within the "carrying capacity" of the environment. The indicators of carrying capacity designed on sustainable development criteria are quality of life, environmental status degradation, resource balance and, ecological loading ratios. It is difficult to manage one particular coastal natural resource or activity in isolation as it has impact on others or affected by others. Hence different coastal activities viz., fishing and aquaculture, navigation, settlement, ports and harbours, recreation and tourism, industries, waste disposal, exploitation of minerals, oil and natural gas etc. need to be managed together to sustain them. Ideal way to sustain any resource or activity is adoption of concept of integrated management.

The country has witnessed a faster development of shrimp aquaculture since 1990. The unplanned and uncontrolled expansion of shrimp aquaculture can lead to exceeding the carrying capacity of the source water bodies. This would result in negative impacts of poor productivity and occurrence of diseases. The present day failure rate in shrimp farming experienced in the country is at least partly related to the very high concentration of farms in certain areas and declining water quality. Because of the proximity of the shrimp farms to source water and the large volumes of water exchange there is a great concern that accumulation of waste byproducts from the shrimp culture facilities will impose a limit to the number of ponds that can be operated.

Importance and necessity of carrying capacity

Most environmental assessment guidelines require analysis of the relationship between new developments or development programs and ECC. The Honduras government had stopped

further development of shrimp farms until an objective determination of carrying capacity has been achieved and guidelines provided for considering further increase in area under shrimp farming for the various estuaries in Gulf of Fonseca, a large estuarine embayment on the Pacific coast of Central America. NACA study on the preliminary assessment of carrying capacity of Kandaleru Creek in Nellore District, Andhra Pradesh has recommended that Government should limit any development (intensification and or horizontal expansion). Codes of conduct and codes of practice refer to carrying capacity either explicitly or implicitly. According to FAO Code of Conduct for Responsible Fisheries, under Aquaculture development "States should produce and regularly update aquaculture development strategies and plans, as required, to ensure that aquaculture development is ecologically sustainable and to allow the rational use of resources shared by aquaculture and other activities" (Article 9.1.3). In many countries there is a continued need for aquaculture and planning authorities to produce and regularly update comprehensive plans for promoting, regulating and reporting on the aquaculture sector. Given the possible contributions of aquaculture to enhanced food supply and rural development, it may be very useful to design aquaculture development plans with due consideration of existing plans and efforts aiming at food security, sustainable agriculture and rural development. Bangkok FAO Technical Consultation on Policies for Sustainable Shrimp Culture held in December, 1997 recommended that appropriate research should be undertaken to determine carrying capacity of coastal ecosystems for shrimp culture with an emphasis on application of this knowledge to local areas. International Principles for Responsible Shrimp Farming mentioned that do not locate new shrimp farms in areas that have already reached carrying capacity for aquaculture. Coastal Aquaculture Authority, Government of India in the report submitted to Supreme Court, suggested that the type of culture system and the magnitude of intensification permitted should be clearly defined for each zone based on the carrying capacity of the zone to prevent nutrient loading in the ecosystem.

The operational framework for internalization of the concept of capacity in decisions related to environmentally compatible developmental planning process involves estimation of supportive capacity, estimation of assimilative capacity and optimal allocation of resources. The carrying capacity based developmental planning process involves generation of alternative socio-economic developmental scenarios by incorporating aspirations and preferences of people, assessment of decision-makers choices and expert opinion within the assimilative and supportive capacities in the region. The supportive capacity of a region is the capacity of the ecosystem to provide resources for various anthropogenic activities in the defined planning region without impairing bio-productivity and ecological integrity. The formulation of models using water quality and estuarine dynamics data for predicting carrying capacity of water bodies will be of immense benefit to the shrimp aquaculture sector for environmentally compatible development planning.

Carrying capacity of water source

Carrying Capacity (CC) is the number of organisms, or number of enterprises, or total production, which can be supported by a defined area, ecosystem or coastline. Environmental capacity is sometimes confused with carrying capacity and has been subject to a range of

interpretations and definitions. Environmental capacity is a property of the environment and its ability to accommodate a particular level of activity with acceptable levels of impact i.e., the rate at which nitrogen can be assimilated. While carrying capacity determination depends on both environmental capacity and the rate of waste output from aquaculture. In shrimp aquaculture CC of a water body can be used to estimate the maximum area under shrimp farming that can be accommodated without excessive water quality degradation. The water sources (brackishwater canals, estuaries, creeks, agricultural drains) are a common property and withdrawal of water from and discharge of wastes by the farms into the same water source leads to potential eutrophication and hence there is a need to study the carrying capacity of such water source. In relation to the water body receiving the discharge water from shrimp farming CC can be defined in terms of the maximum nutrient loading which can be assimilated by the water body without exceeding the permissible levels. This self-limiting density *i.e*., the number of ponds that can be operated sustainably must be quantified as a basic management parameter and its estimation requires detailed field studies and modeling. As the water quality deteriorates, carrying capacity actually shrinks, leaving the water body no longer able to support even the number of ponds existing. Carrying capacity of the water bodies is likely to become a significant issue as levels of shrimp culture activity increase.

Conceptual basis

The conceptual model for carrying capacity based planning process uses various modelling and analytical techniques to estimate changes in carrying capacity indicators. The development of shrimp culture requires an evaluation of water quality in the regions of existing and proposed shrimp farm operation, especially how the water quality is influenced by the anticipated waste loads from the shrimp farms and from other wastewater discharge sources located in the region. If the combined effect of effluent loads is to reduce water quality below an acceptable value, then it can be said that the carrying capacity of the system has been exceeded. The interaction between individual farms, in which the effluent from one farm is drawn into the intake of another, may necessitate the detailed field studies and development of suitable mathematical models in a simple and cost-effective way to determine the concentration of important parameters that result from a given level of waste loading.

Carrying capacity depends largely on the rate at which the water body (creek) can dilute the effluent. The dilution rate is of great importance in predicting nutrient enrichment in the creek. Creek bathymetry and hydrology (flushing rates, volumes at high and low tide), morphology of the creek (influence water movement, mixing and stratification) along with impact from other land uses and freshwater runoff from the catchment area decides the final level of nutrients. The flushing time can be estimated in relation to the dilution rate, which is the inverse of the flushing time. A greater density of farms can be developed in areas with higher flushing rates. Current flows and water depths are important in calculating dilution rate. Phosphorous is the limiting factor for fresh water, whereas nitrogen is the limiting factor for coastal and marine water. Since both, fresh water and saline water environments persist in the brackishwater bodies it is necessary to apply fresh water as well as coastal water model. Mass balance equation with a dilution term can be used to predict the nutrient loading.

The calculated carrying capacity is very sensitive to the driving parameters, which may be site dependent and also vary with time. They are contingent on technology, preferences, and the ever-changing state of interactions between the physical and biotic environment. It is a function of position i.e., based on well mixing areas and dead zones. The distribution of critical regions in a water body (well circulated areas or poorly circulated areas) and the resultant carrying capacity will vary with the hydrodynamic conditions.

Methodology for the estimation of carrying capacity

The carrying capacity of different tropical aquatic systems is not well established and is likely to vary significantly according to local physical, chemical and ecological conditions. A variety of mass balance, steady state and dynamic dilution and dispersal models have been developed for the estimation of environmental capacity in respect of organic matter and nutrient loads, based mainly on temperate conditions and semi-closed water bodies. Some approaches go further, and seek to model the impact on phytoplankton dynamics, with a view to predicting more subtle impacts associated with nutrient loads, or the impact of shellfish farming on plankton density and composition. Majority of the works related to carrying capacity of water bodies were centered on bivalve culture and there is very limited studies available pertaining to shrimp culture.

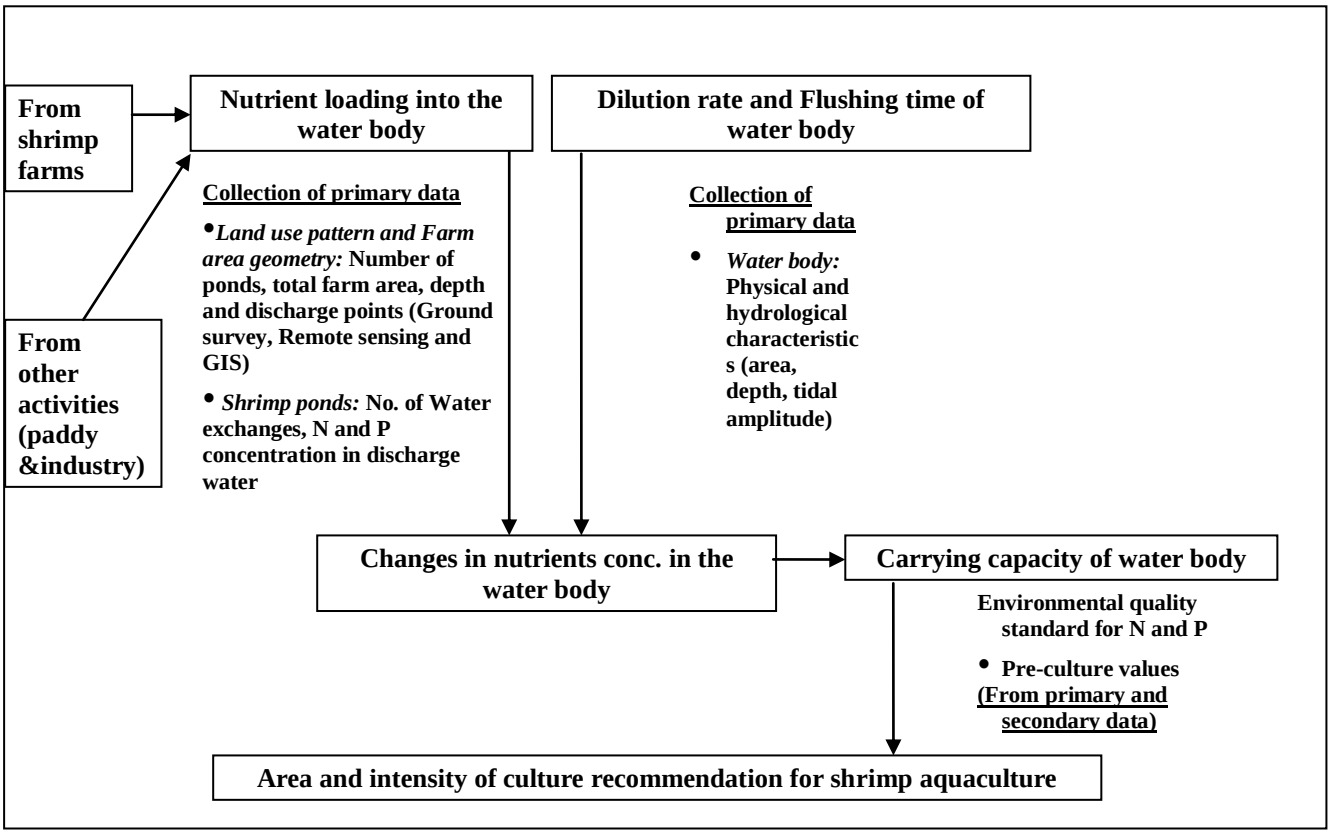
Simple mass balance models were applied to Kung Krabaen Bay lagoon in Eastern Thailand for the estimation of carrying capacity. These models may tend to be inaccurate when applied to complex lagoon systems, and are difficult to apply to estuarine and delta systems. Furthermore, the rate processes and environmental quality standards required to estimate environmental capacity are poorly specified for tropical aquatic systems, as are biological indicators. The Norwegian LENKA project used mixed theoretical-empirical modeling to assess environmental capacity of different coastal systems in relation to aquaculture. Some of the studies were conducted at Rio Chouleteca Delta on the Gulf of Fonseca, Southern Honduras based on water quality, farm chemical budgets and estuarine fluid dynamics.

Central Institute of Brackishwater Aquaculture has developed the methodology for the assessment of carrying capacity of source water bodies in relation to shrimp farming with the below mentioned step-wise activities.

1. Data collection on land use pattern and existing shrimp farm area, culture system and management practices, average shrimp production.
2. Establishment of environmental quality parameters and standards
3. Quantification of the amount of nutrient load (N and P) released into the water body from shrimp farms and other activities. The nutrient overload could be from aquaculture and also from other activities on the land.
4. Use of numeric models to predict the total nutrient load in the water body and the resulting level of nutrients.
5. Estimation of carrying capacity by relating the predictions to the pre-culture values.

Software package for carrying capacity

Decision support software has been developed in Visual Basic to estimate the maximum allowable shrimp farming area, for a particular creek or drainage canal. The flow chart of process for the estimation of carrying capacity is depicted.



Flowchart of steps in CC estimation software

Case studies on carrying capacity of source waters

Case studies were conducted to validate the computer model in Andhra Pradesh and Tamil Nadu, where discharge water was only from shrimp farms or from both shrimp farms and paddy fields and the area recommendations for shrimp aquaculture were made by taking into account of rules of Coastal Regulation Zone and Coastal Aquaculture Authority and supportive capacity of the ecosystem. Based on the studies carried out, the tool can be customized and applied to determine the carrying capacity of other water bodies.

Case study 1: Water bodies that are source and sink for only one activity, shrimp farming

Polekuru Island in East Godavari District, Andhra Pradesh is surrounded by 4 major source water bodies viz., Bandha Creek, Sarrihaddu Kaluva, Gaderu River and Vadalanali Creek. Based on the monthly estimates of nutrient loading from the shrimp farms and assimilation capacity for one year, area that can be taken up for shrimp culture was calculated. The nutrients loading into Bandha Creek and Sarihaddu Kaluva will exceed the assimilation capacity at harvest time, if the whole area is under culture, whereas the nutrients loading into Gaderu Creek and Vadalanali Creek are within the assimilation capacity. Though Gaderu and Vadalanali Creeks are having good dilution and flushing rate, there is no scope for further development on Gaderu Creek as there was no land area and on Vadalanali Creek,

unauthorised development has taken place. Out of 2000 ha developed, a total area of 1300 ha was recommended for culture.

Case study 2: Water source for shrimp farms and sinks for discharge water from both shrimp farms and paddy fields

Mogalthur drain in West Godavari District, Andhra Pradesh was divided into three zones for collection of discharge water samples from the shrimp farms and paddy fields. Based on the nutrient loading into the drain from shrimp farms and paddy fields and carrying capacity of the drain allowable maximum area was recommended for shrimp farming in each zone.

Utility of software

1. The tool will help state governments and other regulatory organizations to regulate the level of shrimp farming activity for each receiving water body.
2. It will also results in awareness among shrimp farmers of the impact of shrimp farming on the environment and encourages them to pursue sustainable production methods.
3. The software permits a reliable estimation of the combined impacts of the shrimp farms and other land use impacts in a region under various scenarios of increased development
4. The carrying capacity models will provide information necessary for the formulation of strategies (preferred scenario) to integrate shrimp farming into coastal zone management.
5. The water quality data generated through field sampling and analysis will serve as the baseline to monitor the long-term trends in quality of water bodies.
6. It will help in framing future guidelines and policies for sustainable development of shrimp farming.
7. The tool increases the capacity of fishery and planning professionals to develop management systems that will reduce the likelihood of aquaculture development having deleterious impact on the environment.
8. For private entrepreneurs who would like to develop large areas for shrimp farming, this software can be used as a planning tool so as not to exceed the carrying capacity of the receiving water body.

Conclusion

The carrying capacity based developmental planning aims at the delineation of guidelines for decision-making related to overall regional development within the region and can be used to sensitize stake holders of the issues involved in and guide the agencies that are interested in environmentally sustainable shrimp development. The carrying capacity of all the coastal water bodies should be assessed and guidelines to be provided for considering further increase in area for aquaculture in the coastal region. Almost all of the discussions on carrying capacity focus on its estimation rather than on the issue of how to ensure that it is not exceeded or how to manage or allocate it once it has been estimated. Furthermore, approaches must take account of other resource users, both in terms of their contribution to the problem (e.g nutrient and organic wastes) or their susceptibility to it. Allocation depends critically on the nature of ownership and/or access rights as well as other broader legal, institutional, and socio-political circumstances. Allocation issues are therefore best addressed through a case study approach, covering a range of socio-political systems.

CHAPTER 14

PRECISION AND ACCURACY IN ANALYSIS

S. Suvana, A. Nagavel and M. Muralidhar

Precision refers to agreement of two or more replicate determinations of a given value. Accuracy refers to the closeness with which a measured value approaches the true value. To illustrate precision and accuracy, consider the determinations of salinity by four students. The instructor determined that the sample had a salinity of 25.2 ppt (considered to be the true value). The results are given in Table 1.

Table 1. Illustration of precision and accuracy in salinity measurement

Student	Replicate					Standard deviation
	a	b	c	d	Mean	
1	25.1	25.2	24.9	25.2	25.1	0.14
2	23.1	23.2	23.0	23.1	23.1	0.08
3	22.1	20.1	23.2	19.1	21.1	1.86
4	22.2	23.2	28.7	25.1	24.8	2.86

Student 1 obtained both high precision (low standard deviation) and accuracy. While Student 2 achieved good precision, accuracy was poor. Student 3 obtained low accuracy and low precision. Student 4 obtained good accuracy in spite of low precision. Obviously, the most desirable results were those of Student 1.

Relative accuracy may be expressed as:

$$\text{Percent relative error} = \frac{\text{True value} - \text{measured value}}{\text{True value}} \times 100.$$

Precision and Accuracy Checks

Once an analyst has accepted a certain method of analysis, obtained the necessary reagents and equipment, and learned to perform the analysis, precision of the measurements should be estimated. Precision can be determined on standard solutions of the substance to be measured, but a better procedure is to obtain real water samples and make the precision estimates on them. An acceptable procedure is to obtain three water samples: one low, one intermediate, and one high in concentration of the substance to be measured. The analyst then makes a number of repetitive measurements on each sample and calculates the mean and standard deviation or confidence interval for individual measurements. The results (Table 2) of total suspended solids analysis indicate that waters with a high concentration of total suspended solids can be analyzed with slightly better precision than waters with a lower concentration of total suspended solids.

The accuracy of procedures can be checked by adding a known amount of the substance to be measured to distilled water, analyzing the resulting standard solution, and determining how close the measured value approaches the true value (represented by the concentration of the

standard solution). It is again more desirable to determine the accuracy of a method by measurements involving natural water. This can be achieved by determining the concentration of the substance in natural water and then adding a known amount of the substance to the natural water and determining the percentage recovery. This technique, called spike recovery, is illustrated for the determination of total ammonia nitrogen. A water had a measured total ammonia nitrogen concentration of 1.51 mg/ liter. An ammonia nitrogen spike of 1.0 mg/L was added to the sample to provide a concentration of 2.51 mg/L of total ammonia nitrogen. Replicate determinations were made and the obtained were given in Table 3.

Table 2. Illustration of evaluation of precision of total suspended solids analysis

Replicate	Total Suspended Solids		
	Sample A	Sample B	Sample C
1	18.0	65.6	155.6
2	16.8	64.4	152.0
3	17.8	64.5	159.1
4	18.0	63.1	155.8
5	17.5	64.1	157.2
6	18.8	66.9	150.3
7	19.0	63.0	160.5
Mean	18.0	64.5	155.8
Standard deviation	0.75	1.38	3.64
95% confidence interval	1.83	3.36	8.92
Coefficient of variation (%)	4.17	2.13	2.34

Table 3. Illustration of evaluation of accuracy of total ammonia nitrogen analysis

Replicate	Total ammonia nitrogen (mg/l)
1	2.50
2	2.39
3	2.35
4	2.45
5	2.53
6	2.40
7	2.51
Mean	2.45

$$\text{Recovery} = \frac{2.45}{1.51 + 1.00} \times 100 = 97.6\%$$

We may state that for water containing 2.51 mg/liter total ammonia nitrogen, the recovery was 97.6%. The percent recovery is a good approximation of accuracy, but the true concentration of substance can never be known with absolute certainly.

Obviously, an analyst cannot afford to make a large number of repetitive measurements, conduct a spike recovery for each sample, or analyze a standard solution with each sample. The analyst can and should make periodic checks of precision and accuracy. For example, about 5 to 10% of the samples should be analyzed in duplicate. If the duplicate measurements do not agree with the known precision of the method, the results are not reliable and the problem in the technique must be located and corrected. Similarly, periodic checks of accuracy should be made with spike recovery tests or by analyses of standard solutions.

For colorimetric methods, calibration graphs must be prepared by measuring the absorbance of known concentrations of the substance being measured and plotting the results. These graphs should be verified frequently by analyzing known concentrations of the substance in question. It is important to understand that the common practice of making duplicate or triplicate analyses of all samples is essentially worthless. Analysts should not waste time and reagents on checking every sample, and duplicate analyses provide no estimate of accuracy.

Quality Control Charts

A more refined quality control procedure involves use of quality control charts, and use of quality control charts is highly recommended for monitoring programs. Charts for maintaining quality control were originally developed for manufacturing, but they can be adapted for use by laboratories that conduct water analyses. A quality control chart consists of a graph on which the vertical scale represents the results and the horizontal scale indicates the sequence of the results (time). Warning and control limits and the averages of the statistical measures under consideration are indicated on the graph. The results are plotted over time and from these plots it can be ascertained if precision and accuracy are acceptable. The most commonly used quality control charts are range charts to reveal the control of precision and means charts to reveal the control of accuracy. The greatest value of quality control charts is that trends of change in precision and accuracy over time may be detected.

Range Control Charts

A range control chart for replicate measurements is made by calculating a mean range (R), a warning limit (WL), and a control limit (CL). A minimum of 20 range values (difference between the lowest and highest values in replicate analyses of a sample) is used to make the chart. The factors for computing control and warning limits on range control charts are as follow:

Number of replicates (n)	Factors for control limits (D_4)
2	3.27
3	2.58
4	2.28
5	2.12
6	2.00

The range values should be obtained during normal laboratory operation over a period of several days. For water quality monitoring, it is sufficient to base the chart on duplicate analyses (n = 2; D_4 = 3.27). An example of a set of duplicate analyses of 25 total ammonia nitrogen samples is provided in Table 3. Calculations of R, CL, and WL are provided below:

The necessary equations are:

$$\overline{R} = \sum R/n$$

$$CL = D_4 (\overline{R})$$

$$WL = 0.67 (\overline{D_4 R} - \overline{R}) + \overline{R}$$

The analyst should measure about 10% of samples in duplicate. The range is determined for each of the duplicate analyses and plotted on the range chart. If the ranges for the duplicates remain below WL, the analysis is in control of precision. A single value above WL suggests a problem, and steps should be taken to determine if a problem exists. Of course, range values above the control limit should be a signal to stop the analyses and find the source of the problem. All data collected for quality control should be plotted on the chart and the chart updated as necessary.

Table 4. Results of duplicate total ammonia nitrogen analyses used to prepare a quality control chart for precision

Date	Total ammonia nitrogen (mg/lit)		
	Result 1	Result 2	Range
July 2	0.51	0.47	0.04
July 3	0.25	0.20	0.05
July 4	0.11	0.09	0.02
July 5	1.05	0.92	0.13
July 6	0.82	0.95	0.13
July 9	0.75	0.74	0.01
July 10	0.44	0.44	0.00
July 11	0.36	0.38	0.02
July 12	2.13	2.05	0.08
July 13	1.50	1.55	0.05

July 16	0.09	0.06	0.03
July 17	0.35	0.37	0.02
July 18	0.50	0.54	0.04
July 19	0.62	0.58	0.04
July 22	1.00	0.92	0.08
July 23	0.78	0.71	0.07
July 24	0.98	0.92	0.06
July 25	0.68	0.72	0.04
July 26	1.25	1.31	0.06
July 29	0.05	0.05	0.00
July 30	1.33	1.25	0.08
July 31	1.62	1.74	0.12
August 3	0.45	0.42	0.03
August 4	0.62	0.66	0.04
August 5	0.80	0.75	0.05

$\overline{R} = 1.29 \div 25 = 0.052$ mg/L

CL = (3.27)(0.052) = 0.17 mg/L

WL = (0.67)[(3.27)(0.052) – 0.052] + 0.052 = 0.131 mg/L

The values for R, CL, and WL are plotted on a chart (Fig.1).

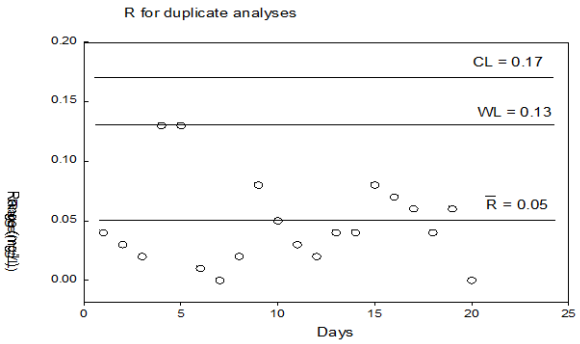


Fig 1. Range chart for control of precision in total ammonia nitrogen analysis

Means Control Chart

A means control chart allows evaluation of control on accuracy. A common way of making means control charts is to make about 20 measurements on a standard solution of the variable of interest over a period of several days during normal laboratory operation. The mean and standard deviation of these measurements is determined, and the upper and lower warning and control limits are taken as ± 2 standard deviations and ± 3 standard deviations, respectively. For example, suppose that twenty measured values for a total phosphorus

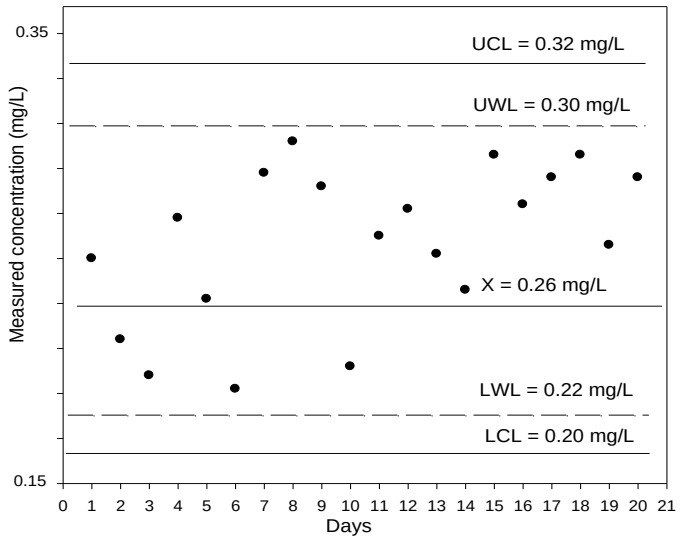
standard have an average of 0.26 mg/L with a standard deviation of ± 0.02 mg/L. A plot of the limits is shown in Fig 2. The limits will be as follows:

Upper control limit	0.32 mg/L
Upper warning limit	0.30 mg/L
Mean	0.26 mg/L
Lower warning limit	0.22 mg/L
Lower control limit	0.20 mg/L

Alternatively, percentage recovery values can be used to make a means control chart. Suppose that percentage recovery values for total ammonia nitrogen averaged 95.0 with a standard deviation of ± 2.5. The limits would be as follows:

Upper control limit	102.5 %
Upper warning limit	100.0 %
Mean	95.0 %
Lower warning limit	90.0 %
Lower control limit	87.5 %

The analyst should, at intervals, analyze a standard solution or conduct a percentage recovery trial. The results of these analyses or trials should then be plotted on the mean control chart. Interpretation of the means control chart is the same as explained above for use of the range control chart.



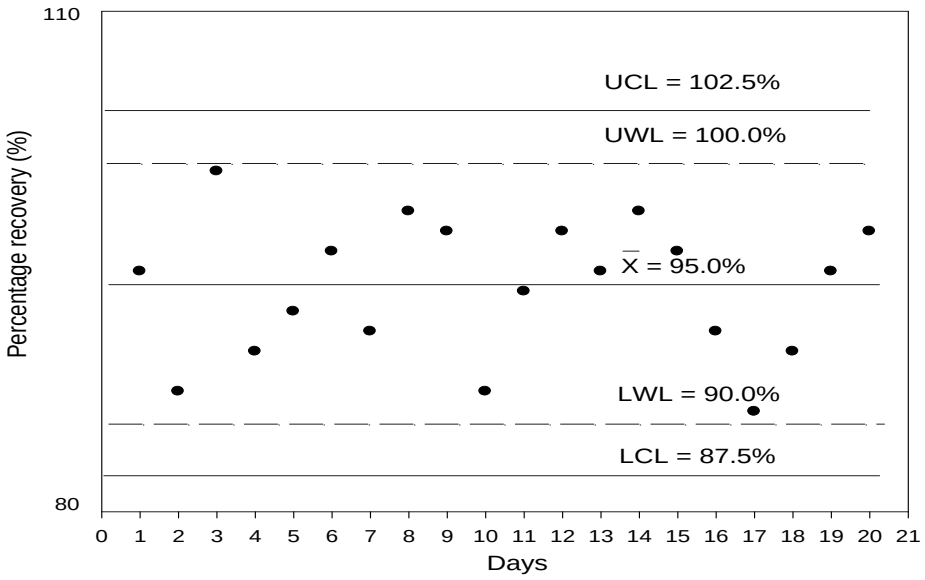


Fig.2. Means control chart for the control of accuracy of total phosphorus analyses based on analyses of a standard solution.

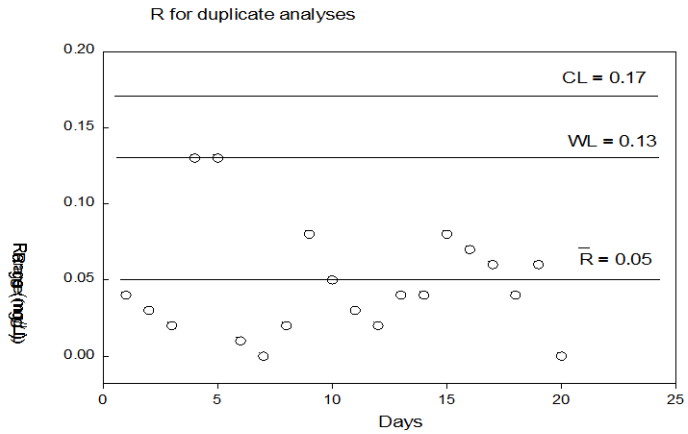


Fig.3. Means control chart for the control of accuracy of total ammonia nitrogen analyses based on spike recovery trials.

Standard solutions

In any analytical lab, it is essential to maintain stocks of various reagents in solutions. Standard solutions are those which have known concentration of some chemical.

Primary standards contain an accurately known amount of an analyte and its strength need not be checked. A primary reagent must have a known stoichiometry, a known purity (or assay), and be stable during long-term storage both in solid and solution form. Solutions of primary standards generally are prepared in class A volumetric glassware to minimize determinate errors. Even so, the relative error in preparing a primary standard is typically ±0.1%. eg: sodium carbonate, potassium dichromate, potassium hydrogen phthalate.

A secondary standard is one which has to be standardised , i.e purity has to established relative to primary standard. eg: Sulphuric acid, Hydrochloric acid, NaOH

Preparing Stock Solutions

A stock solution is prepared by weighing out an appropriate portion of a pure solid or by measuring out an appropriate volume of a pure liquid and diluting to a known volume. Exactly how this is done depends on the required concentration units.

eg: to prepare 1L 1000ppm K solution from KCl

Mol wt of KCl - 74.5g
Atomic mass of K- 39g
Therefore, 74.5/39= 1.91 g of KCl dissolved in 1L will contain 1000ppm K.

Preparing Solutions by Dilution

Solutions with small concentrations are often prepared by diluting a more concentrated stock solution. A known volume of the stock solution is transferred to a new container and brought to a new volume. Since the total amount of solute is the same before and after dilution, we know that

$$C\text{o} \times V\text{o} = C\text{d} \times V\text{d}$$

where *C*o is the concentration of the stock solution, *V*o is the volume of the stock, solution being diluted, *C*d is the concentration of the dilute solution, and *V*d is the volume of the dilute solution.

CHAPTER 15

SOIL ANALYSIS METHODS

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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 1/2" to 3/4" slice of soil from 8-10 spots, scattered uniformly over the area (preferably a zig-zag pattern) should be collected.
- The depth to which samples should be obtained for analysis depends on the land use. Proper sampling tools should be used. Any of the tools such as tube auger, screw type auger, post hole 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 post hole 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 khurpi. 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 and 60-90 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.

Precautions

- Ensure that soil samples are not taken immediately after rains, irrigation, fertilisation
- Do not sample sites near to/ along an irrigation canal, lateral drain, bounds, farm yard manure pits, shady trees, roads etc.
- Sampling in summer season should be done only after scraping the while crust patches.

1. Soil reaction

The soil reaction (pH) is meant to express the acidity or alkalinity of soil. The pH is very important property of the soil because it determines the capacity for the growth of hytoplankton, availability of nutrients and influences microbial activity and physical properties of a soil. The pH of a solution, a term introduced by Sorrensen has been defined as the negative logarithm of the hydrogen ion activity

$$pH = - \log a^{H+}$$

Where, a^{H+} represents to the acidity of H^+ ions which refers strictly to a true solution in which the ions are completely dissociated. But in soil-water system the dissociation is not complete as in true solution.

Measurement of pH

There are two main methods to determine pH of solution

(i) Colorimetric method

(ii) Potentiometric method

Colorimetric method

This method is based on the assumption that an indicator gives the same colour in two different solutions having same pH. Of the colorimetric methods, the most commonly used one is Kuhn's colorimetric method.

Principle

The underlying principle of the method being that when a soil suspension is shaken vigorously with very pure barium sulphate, the later flocculates the soil colloids and leaves a clear and colourless solution. If the indicator which is not absorbed by the soil is present, its colour will denote the soil reaction. This colour is compared with Lovibond colour disc to know the pH of soil. The amount of $BaSO_4$ necessary to give a clear suspension depends upon the amount of colloids present. For loam and heavy soils it is necessary to reduce the quantity of soil used.

Procedure

Place a one cm thick layer of neutral $BaSO_4$ in a 50 ml clean dry test tube. Then add 10 g of air dry soil sample and 25 ml of distilled water. Shake vigorously for about a minute and keep it for settling for about half-an hour. Take out 10 ml of supernatant water and determine pH value colorimetrically, by comparing colour with that of colour charts, colour discs etc.

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.

P***rinciple***

If a metallic rod is dipped in water or in a solution of one of its salts, it is found to acquire an electric charge which reaches a maximum value after some time. This is due to the fact that either the metal gives ions to the solution or takes ions from the solution. An electric potential is thus developed due to the differences in the electric charges of the rod and the

surrounding solution. This is called electrode potential. If we can find the electrode potential developed by dipping it in the solution, we can calculate pH. Such an electrode is called `Half Cell' and is called indicator electrode. It is not practicable to find the E.M.F of this half-cell and therefore, coupled with another half-cell of constant value which is called reference electrode.

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^{o}C and 4.02 at 35^{o}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 22^{o}C: Dissolve 3.81 g of borax in distilled water and dilute to 1 litre.

(c) Standard buffer tablets/ 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 which 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)

2. Determination of electrical conductivity

Electrical conductivity (E.C) is commonly used for indicating the total concentration of the ionized constituents of solutions. It is closely related to the sum of cations (or anions) as determined chemically and usually correlates closely with total dissolved solids. As the soluble salts content controls the osmotic pressure of soil solution, highly saline soils reduce the water availability due to high osmotic pressure and also reduce availability of other nutrients. A fairly quantitative estimate of the salt content of solutions extracted from soils can be made from their electrical conductance. It is a rapid and reasonably precise determination that does not alter or consume any of the samples.

Principle

When water is added to the soil, the soluble salts gets 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's 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 centimeter.

Instrumentation

Conductivity meter

Materials and Reagents

1. Glass beaker
2. Glass rod
3. 0.02M potassium chloride - Dissolve 1.4912 g of KCl in distilled water and dilute to one litre. The specific conductance of this solution at 25^{o}C 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 KCl 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 salts/ 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

3. Estimation of organic matter

Organic matter in a mineral soil is regarded as an index of its fertility status. Organic matter is a direct source of nutrient elements and the release of which depends upon microbial activity and by affecting the cation exchange capacity. The initial soil in pond bottoms usually is low in organic matter content. The organic matter from a nearly constructed pond is often in the form of soil humus and not highly reactive. Once the pond is filled with water, organic matter from uneaten feed, application of manure, dead plankton and fish/prawn excrement continually reaches the pond bottom. Organic matter does not degrade completely and it tends to accumulate slowly in pond bottoms. The organic matter content of soils can be obtained by organic carbon estimation.

Determination of organic carbon of soil can be done by dry combustion and wet digestion methods. The dry combustion method is most accurate, but it is time consuming and cannot be applied to soils containing carbonates. Wet combustion methods are suitable for use in soils containing carbonates, but the application of a correction factor is required to compensate for the incomplete oxidation of the organic matter. The rapid titration method of Walkley and Black 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 diphenyl amine indicator till the bright blue colour changes to light green colour.

Reactions in digestion:

$K_2Cr_2O_7 + 4H_2SO_4 \rightarrow K_2SO_4 + Cr_2(SO_4)_3 + 4H_2O + 3(O)$

$3C + 6(O) \rightarrow 3CO_2$

$2K_2Cr_2O_7 + 8H_2SO_4 + 3C \rightarrow 2K_2SO_4 + 2Cr_2(SO_4)_3 + 8H_2O + 3CO_2$

Reactions in Titration

$K_2Cr_2O_7 + H_2SO_4 + 6FeSO_4 \rightarrow K_2SO_4 + Cr_2(SO_4)_3 + 3\,Fe_2(SO_4)_3 + 7\,H_2O$

Reagents

(a) **1N potassium dichromate solution :** Dissolve 49.04g 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 $AgSO_4$ in 100 ml of conc. H_2SO_4.

(c) **85% orthophosphoric acid** (H_3PO_4)**:** 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) **1N 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 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 min with intermittent shaking. After digestion about 100 ml of distilled water is added followed by 5-10 ml of H_3PO_4. 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 conducted without soil sample.

Observations and Calculations

1ml of 1N ferrous ammonium sulphate = 0.003g of carbon

Organic carbon (%) = (Blank titration value - Sample titration value) X 0.003/100*Normality of ferrous ammonium sulphate

% organic matter in soil = Organic carbon X 1.724

4. Determination of available nitrogen in soils

The inorganic form of nitrogen (N) constitutes a very small fraction of total N in most soils and it is this form which is available to phytoplankton. Although total soil nitrogen content of a mineral soil gives some idea of its supplying power, the practical value of reliable methods providing an index of the availability of soil N has long been appreciated. In upland

soils available form of N which predominates is nitrate (NO_3) while in the submerged or flooded soils ammonium (NH_4) predominates. Sometimes nitrite may be detected also, but generally its magnitude is small that it could be ignored in the determination of available nitrogen. Among different methods of available soil N, the alkaline permanganate method of Subbiah and Asija which includes the easily oxidisable organic nitrogen, has been reported to have good correlation with productivity of brackishwater ponds.

Principle

A Known weight of soil is mixed with excess of alkaline potassium permanganate and distilled, where by NH_4 - N is released (from the oxidisable organic matter) in the form of ammonia gas. The liberated ammonia is collected in boric acid with mixed indicator and titrated against standard acid.

Reactions:

$(NH_4)_2SO_4 + 2NaOH \rightarrow Na_2SO_4 + NH_4OH$

$2NH_4OH + H_3BO_3 \rightarrow (NH_4)_2B_4O_7 + {}_2O(NH_4)_2B_4O_7 + H_2SO_4 + 5H_2O \rightarrow (NH_4)_2SO_4 + 4H_3BO_3$

Reagents

(a) **0.32% potassium permanganate:** Dissolve 3.2 g of $KMnO_4$ crystals in distilled water and make up the volume up to 1 litre.

(b) **2.5% sodium hydroxide:** Dissolve 25 g of pure NaOH pellets in 1 litre of distilled water.

(c) **liquid paraffin:** Commercially available.

(d) **0.02 N sulphuric acid:** Dilute 30 ml of Conc. H_2SO_4 to 1 litre with distilled water to get approximately 1N stock solution. To make 0.02 N H_2SO_4 , take 20 ml of this stock solution and dilute to one litre with distilled water. Standardise this solution against 0.02N Na_2CO_3 using methyl orange as indicator.

(e) **4% Boric acid:** Dissolve 40 g of boric acid in distilled water and make up the volume to 1 litre.

(f) **Bromocresol green and methyl red mixed indicator:** About 99 mg of Bromocresol green and 66 mg of methyl red indicator are dissolved in 100 ml of ethyl alcohol. This will give 0.1% mixed indicator. The colour of the indicator is mild blue pink. The pH should be between 4.7 - 5.0. 5 ml of mixed indicator should be added for every litre of boric acid.

Procedure

Take 10 g of air dried soil sample in 800 ml distillation flask, add 100 ml of 0.32% $KMnO_4$ solution. To that contents add 1 ml of paraffin wax and few glass beads. Attach the flask to distillation set and add 100 ml of 2.5% NaOH and close the flask. Then start distillation and collect the distillate in 20 ml of boric acid. After collecting 100 ml distillate the boric acid is titrated against N/20 standard H_2SO_4, till the green colour of indicator changes to pink colour at the end point. A blank titration is also conducted without soil sample.

Observations and calculations

1ml of 1N H_2SO_4 = 0.014g N

Titre Value = y ml

10 g soil contains = y * 0.014g N*Normality of H_2SO_4

100 g soil contains = y* 0.14g N*Normality of H_2SO_4

The amount of available N mg/100g = 140 * y * Normality of H_2SO_4

5. Determination of available phosphorus in soils

Phosphorus (P) in soil occurs as orthophosphate in different forms and combinations. A small portion of total phosphorus is available to phytoplankton. A wide variety of soil chemical tests are being employed for the extraction of phosphates. The choice for a suitable method depends largely on the nature and properties of soils.

Principle

The pH of the extracting solution is kept nearly constant at 8.5. This solution extracts P from calcium phosphates by lowering the Ca concentration by causing precipitation of calcium as $CaCO_3$ and thereby increasing P concentration in solution (based on solubility product principle). In acid soils containing aluminium and iron phosphates, P concentration in solution increases as the pH rises. Secondary precipitation reactions in acid and calcareous soils are reduced to a minimum as Al, Ca and Fe concentration remain at low level in the soil extract. The extract containing available P on treatment with acidic molybdate gives phosphomolybdate which is on reduction with $SnCl_2$ develops characteristic blue colour. This intensity of blue colour depends upon the P concentration of the solution which can be measured at 660 nm by spectronic - 20.

Reagents

A. **Standard P solution (1000 ppm):** Dissolve 4.390 g dried KH_2PO_4 in 400 ml distilled water; add 25 ml of 7 N H_2SO_4 and make up to 1 litre.

B. **Stannous chloride stock solution:** 10 g of crystalline stannous chloride dissolved in 25 ml HCl by volume. The contents are warmed. Store this solution in amber colour glass bottle under a 1 cm of mineral oil to protect from oxygen and light.

Dilute stannous chloride (0.05N) : 0.5 ml of stock $SnCl_2$ solution is diluted to 66 ml with distilled water.

C. 2.5% sulphomolybdic acid: 25 g of ammonium molybdate is dissolved in 200 ml of distilled water at 60^oC. In another glass container, dilute 275 ml of phosphorus free concentrated H_2SO_4 to 750 ml with distilled water. After both the solution have cooled down add ammonium molybdate solution to the dilute H_2SO_4 slowly by constant stirring. Cool down the mixture to room temperature, make up the volume to 1 litre with distilled water and store in amber coloured bottle.

D. **Sodium bicarbonate solution (0.5M) (Olsen's reagent):** Dissolve 42g of $NaHCO_3$ in distilled water and make up the volume to 1 litre. Adjust the pH of solution to 8.5 by NaOH.

E. **Activated charcoal:** Washed with 0.5 M $NaHCO_3$ and dilute HCl. After washing with HCl, distilled water washings should be continued till the leachate is chloride free.

F. **2,4 Dinitrophenol indicator:** 250 mg of Dinitrophenol is dissolved in distilled water and make up the volume to 100 ml.

G. **2N H_2SO_4 :** 5.4 ml of 36N H_2SO_4 is dissolved in distilled water and diluted to one litre.

Procedure

Preparation of standard curve:

0, 0.5, 1, 2, 4, 6, 8, and 10 ml 5 ppm `P' solution is transferred to 50 ml volumetric flasks. 5 ml of Olsen's reagent is added followed by 5 ml of sulphomolybdic acid in each flask and little amount of distilled water is added. Then 1 to 2 drops of 2,4 dinitro phenol indicator is added to each flask and yellow colour is developed. Then 2 N H_2SO_4 is added drop wise in each volumetric flask until the yellow colour disappears. (Then the pH of test solution is at

3). Now add 1 ml of 0.05 N $SnCl_2$ in each flask and make up the volume to 50 ml with distilled water. Then the solutions are kept for reading colour intensity within 12 minutes of preparation. A standard curve is drawn between concentration of P and absorbance.

Preparation of soil extract:

Take 5 g of soil in 150 ml conical flask and add 50 ml of Olsen's reagent followed by 1 or 2 g of Darco-G-60 (free of phosphorus). Shake the contents for 30 min in mechanical shaker. After shaking filter the solution with Whatman No. 40 filter paper. If the solution is still coloured, add some more amount of Darco-G-60 and the contents are shaken and the solution is filtered. Take 2 ml of phosphorus extract in to 50 ml volumetric flask, add 5 ml of sulphomolybdic acid and 1-2 drops of 2,4 dinitrophenol indicator. Add 2N H_2SO_4 drop wise until the yellow colour disappears. Then 1 ml of 0.05 N $SnCl_2$ is added, make up the volume to 50 ml. Colour intensity is measured by spectronic 20 and phosphorus concentration is obtained from standard curve.

Observations and Calculations

Phosphorus concentration = y ppm (ug/ml) from reading

Dilution factor = 50/2*50/5=250

1 g soil contains = y *250ug p

p in mg/100g = y *25

6. Determination of available potassium in soil

The term available k incorporates both exchangeable and water soluble forms of the nutrient in soil. The readily exchangeable plus water soluble potassium is determined in the neutral normal ammonium acetate extract of soil.

Principle

The ammonium ion provides a sharp and rapid separation from exchangeable complex while the other cations bring about a gradual replacement of either lesser or greater amount of k which generally increases with the period of contact. The estimation of the in the extract is carried out with the help of flame photometer. Chemical methods being rather elaborate and time consuming are not suitable for soil testing purpose.

Reagents

1. **Neutral normal ammonium acetate:** Dilute 114 ml of glacial acetic acid (99.5%) with distilled water to a volume of 1 litre. Add 138 ml of conc. NH_4OH and add water to get a pH 7 and dilute to 2 lit with distilled water. Alternatively dissolve ammonium acetate crystals (27.08g) in 400 ml of distilled water and dilute to 1 litre and adjust pH to 7.0

2. **KCl stock solution (1000ppm K):** Dissolve 1.908 g of A.R grade KCl (dried at 60^oC for 1 hr) in distilled water and make upto 1 litre to get 1000ppm K.

Procedure

Preparation for standard curve:

0, 5, 10, 20, 30, 40, 50 ppm of K solution are prepared from standard stock solution .Each solution is fed to flame photometer and the readings are noted and a standard curve is prepared.

Preparation of soil extract:

5 g of soil is shaken with 25 ml of neutral normal ammonium acetate for 5 min and filtered immediately through a dry filter paper (Whatman No.1). First two ml of filtrate may be

rejected. The K concentration in the extract is determined by the flame photometer. In the same way water soluble K is estimated by shaking the soil with distilled water for one hour and estimated by flame photometer.

Observations and calculations

Exchangeable k = Ammonium acetate extractable K - water soluble K (mg/100 g soil).

7. Determination of soil texture

The pond soil consists of a mixture of inorganic soil particles of various sizes and organic matter in various stages of decay. The texture of a mud refers to the distribution by size group of particles comprising the mud. In order to assess the texture, a sample of mud is dried and subjected to a mechanical analysis. The proportion of larger particles may be determined by sieve analysis and the smaller particles by hydrometer and other techniques. It is an important soil property because it is closely related to the rate of water intake, water retaining power, the fertility, erosion, aeration and energy required to fill the soil. After the three types of particles are estimated, the soil texture is determined from the soil textural triangle given in the figure. Soil textural diagram is a diagram by means of which the textural name of soil may be determined from mechanical analysis.

Principle

The aim of textural analysis of soil is to determine the percentage of soil material contained in different size fractions and this can be done by means of mechanical analysis. Mechanical analysis consists essentially of two distinct operations, namely dispersion of the soil to ultimate soil particles and grading the dispersed particles according to their size groups.

Reagents

(a) **6% Hydrogen peroxide:** H_2O_2 is generally available at 30% concentration. Dilute 20 ml of this to 100 ml with distilled water before analysis.

(b) **2 N Hydrochloric acid :** Dilute 100 ml of concentrated HCl to 600 ml with distilled water to give approximately 2 N HCl.

(c) **2 N Sodium hydroxide:** Dissolve 40 g of NaOH in about 300 ml distilled water and dilute upto 500 ml with distilled water.

(d) **5% Silver nitrate:** Dissolve 5 g silver nitrate in 100 ml of distilled water.

Procedure

Take 20 g soil in a 500 ml beaker, add 250 ml of water and boil for 10 minutes, allow the suspension to settle and decant the supernatant water. Now, digest the soil with 35 ml of 6% H_2O_2 on a water bath adding more H_2O_2 till no frothing takes place. Add 30-35 ml of 2 N HCl and 100 ml of distilled water and allow to stand for 1 hour with occasional stirring to make the soil free from carbonates. Filter the soil and wash free of HCl with hot water by testing with $AgNO_3$ solution. Transfer the suspension to a suitable glass container, add 5 ml of 2 N NaOH and shake for half an hour. Transfer the content to a 1000 ml tall cylinder, make up the volume, shake for 1 minute and allow to stand. After 4 minutes lower a 20 ml pipette at 10 cm depth and collect 20 ml of the content, dry it in a 50 ml beaker and find out the weight of clay + silt. Repeat the same procedure after 6 hours to get the weight of clay alone.

Observations and calculations

If weight of clay + silt be x g
and that of clay only be y g
then, % of clay = y X 250
% of silt = (x-y) X 250
% of sand = 100 - (x X 250)

Percentage of sand, silt and clay in the principal textural classes (Based upon U.S. Dept. of Agric. Fraction System)

Textural name (Soil class)	Sand (%)	Silt (%)	Clay (%)
Sand	85-100	0-15	0-10
Loamy sand	70-90	0-30	0-15
Sandy loam	43-80	0-50	0-20
Loam	43-80	28-50	7-27
Silt loam	0-50	50-88	0-27
Sandy clay loam	45-80	0-28	20-35
Clay loam	20-45	15-23	27-40
Silty clay loam	0-20	40-73	27-40
Sandy clay	45-65	0-20	35-45
Silty clay	0-20	40-60	40-60
Clay	0-40	0-40	40-100

Size limits of soil separates
Sand - 0.05 to 2.0 mm
Silt - 0.05 to 0.002 mm
Clay - below 0.002 mm

CHAPTER 16

WATER ANALYSIS METHODS

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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.

Preservation of water samples

Parameter	Preservation
pH, CO_2, Alkalinity, Hardness	Add 5 ml/l of chloroform. Exclude light and air.
Dissolved Oxygen	Fix the Sample using two Winkler reagents, immediately. Exclude bubble
NH_3-N, NO_2-N, NO_3-N	Freeze or add 5ml/l of 2M H_2SO_4
PO_4-P	Add 5 ml/l of chloroform or 2M H_2SO_4
Phytoplankton	Lugols solution (5ml /500 ml)
Zooplankton	Formalin 4% (5 ml /50 ml)

1. 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 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.

2. 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 1.06 g anhydrous sodium carbonate in 1 litre distilled water.

(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 as $CaCO_3$) = Titre value x N of H_2SO_4 x 50 x1000 / Volume of sample

3. Turbidity: (Nephelometric method)

***Principle*:** Turbidity can be caused either by planktonic organisms or by suspended soil particles. Turbidity due to suspended soil particles be 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. The 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**:* Disoolve10 g hexamethylene tetramine in distilled water and dilute to 100 ml.

Mix 5 ml each of Solutions I and II. 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.

4. 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(d1). Now the disc is slowly lifted upward and the depth at which the disc reappears is noted (d2). The value (d1+d2)/2 in cm gives a measure of transparency.

5. Total Settleable solids

***Principle**:* This is a portion of organic and inorganic solids that settles in 1 h in an Imphoff 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.

6. Total Suspended Solids (TSS) and Total Dissolved Solids

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 103oC to 105oC. 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.

Dry filter disc/crucible containing residues for at least 1h at 103°C-105°C in an oven. Cool in a dessicator 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)

7. Total Dissolved Solids

Evaporate the filtrate in dish to dryness on a steam bath. Dry for atleast 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)

8. Salinity

Principle: The salinity of sea water 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 Cl^-.

(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) : It can be used in the field .

Calculation

Chlorinity (ppt) = volume of $AgNO_3$ used for titration

Salinity (ppt) = 0.03 + 1.805 x Chlorinity (ppt)

9. Dissolved Oxygen

Principle: DO can be determined by Winkler's method. In this method a 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 reverts 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 $MnSO_4.4H_2O$ 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 $Na_2S_2O_3.5H_2O$ 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 $K_2Cr_2O_7$ in 1 lit of distilled water.

(f) **Starch solution(0.2%)**: Add 2.0 g starch and 30 ml 20% 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 :

$$\text{DO (ppm)} = \frac{N \times V_1 \times 8 \times 1000}{V_2}$$

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) = V_1 x 4**

Measurement of DO by DO meter: DO can also be measured by portable DO meter in field

10. Chemical Oxygen Demand

Principle: COD is a measure of organic matter and represents the amount of oxygen required to oxidize the organic matter by strong oxidizing chemicals (potassium dichromate) under acidic condition. The excess dichromate is titrated with standard ferrous ammonium sulphate using ferroin as an indicator. Mercuric sulphate is added to complex the chlorides, thereby effectively eliminating the chlorides interference.

Reagents:

(a) **0.05 N Potassium dichromate**: Dissolve 2.452 g dried, crystalline $K_2Cr_2O_7$ in distilled water and make up the volume to 1 litre.

(b) **0.05 N Ferrous ammonium sulphate (FAS)**: Dissolve 19.61 g of $Fe(NH_4)_2(SO_4)_2.6H_2O$ in 800 ml of distilled water containing 1 ml of conc. sulphuric acid and make up the volume to 1 litre.

(c) Mercuric sulphate

(d) **Ferroin indicator**: Dissolve 1.888 g of 1:10 phenanthrolin monohydrate and 0.70 g of $FeSO_4.7H_2O$ in 100 ml of distilled water.

Procedure: Pipette out 20 ml of water sample into a 125 ml Erlenmeyer flask. Add exactly 10 ml of 0.05 N $K_2Cr_2O_7$ solutions to the flask. Add 200 mg $HgSO_4$ for each 1000 mg per litre of chloride ($HgSO_4$: Cl :: 10 : 1). Swirl until the $HgSO_4$ is dissolved. Add carefully 30 ml of conc. H_2SO_4 Cover the flask with watch glass and allow to stand for 30 min. Add 15 ml of distilled water and 3 drops of ferroin indicator and titrate the whole reaction mixture with FAS of same normality. Prepare blank with 20 ml distilled water and repeat the same procedure.

Calculation:

$$\text{COD mg/l} = \frac{(B-S) \times N \times 8 \times 1000}{V}$$

B = Titre value for Blank in ml
S = Titre value for sample in ml
N = Normality of FAS
V = Volume of sample in ml

11. Biochemical Oxygen Demand

Principle: The sample of water or appropriate dilution is incubated for 5 days at 20^{o}C in the dark. The reduction in DO concentration during the incubation period yields a measure of the BOD.

Reagents: Use all the reagents required for the determination of DO.

Procedure: Collect three water samples from one site into BOD bottle following the procedure for DO. Determine the DO level in one of these samples, whilst the remaining two samples are firmly stoppered and placed in an incubator at 20^{o} C in the dark for 5 days. At the end of this time, the DO level is determined by the usual Winkler's titration.

Calculation:

Initial DO = D_o ppm

Final DO (after 5 days incubation) = D ppm

BOD (reduction in DO) = (D_o - D) ppm

In heavily polluted samples, it is necessary to dilute the sample with a known amount of clean, air saturated water, so as to obtain required dilution (almost 50%). Siphon out the

mixed sample into two sets of specially designed BOD bottles, one set for incubation and the other for determination of initial DO.

Calculation

Initial DO = D_o ppm

Final DO = D ppm

Reduction in DO = D_o - D = D_{r1} ppm

Dilution water initial DO = D_1 ppm

Final DO = D_2 ppm

Reduction in DO = D_1 - D_2 = D_{r2} ppm

Therefore reduction due to sample = D_{r1} - D_{r2} ppm = D_s

BOD (ppm) = D_s x Dilution factor

12. 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 catalyzer. The blue indophenol colour formed with ammonia is measured spectrophotometrically.

Reagents

(a) De-ionised water

(b) **Phenol solution**: Dissolve 20 g of analytical grade phenol in 200 ml of 95%v/v ethyl alcohol.

(c) **Sodium nitroprusside solutio**: 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 4 and 25 ml of reagent 5. Prepare fresh every day.

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 ml 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.

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 200ppm 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.

13. 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-naphthyl)- 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 6.07 g anhydrous, analytical grade potassium nitrite, KNO_2, (dried at 105 C for 1 hr) in distilled water. Add 1 ml 5 N NaOH and dilute to 1000 ml. This solution contains 1000 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 sulfanilamide 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.

14. Nitrate

Principle: Nitrate in water sample is reduced almost quantitatively to nitrite. The nitrite produced is determined by diazotising with sulfanilamide and coupling with NED to form a highly coloured azo dye which can be measured spectrophotometrically.

Reagents

(a) **Phenol solutions**: 23 g phenol in 500 ml of distilled water.

(b) **NaOH:** 1.25 g in 500 ml of distilled water.

(c) **Buffer reagent**: Mix equal volume of phenol solution and NaOH solution.

(d) **Copper sulphate solution**: 0.1 g in 1 litre distilled water.

(e) **Hydrazine sulphate**: 3.625 g in 500 ml of distilled water.

(f) **Reducing agent**: 5 ml of copper sulphate solution to 5 ml of hydrazine sulphate.

(g) Acetone

(h) **Sulfanilamide:** Dissolve 5.0 g in 50 ml of conc. HCl and make up the volume to 500 ml.

(i) **NED**: Dissolve 0.5 g of NED in 500 ml of distilled water

(j) **Nitrate standard solutions**: Dissolve 7.214 g potassium nitrate, KNO_3 (AR dried at 105^oC) in 1000 ml distilled water. This final solution contains 1000 ppm NO_3^--N

Procedure: Take 10 ml of sample and add 0.4 ml of buffer and mix and then add 0.2 ml reducing agent and keep the tube in dark for 24 hours. Then add 0.4 ml of acetone and after 2 minutes add 0.2 ml of sulphanilamide. After 3 minutes, add 0.2 ml of NED solution and after 10 minutes, measure the absorbance at 540 nm in a spectrophotometer.

15. Total P and Dissolved reactive phosphorous

Principle*:* Ammonium molybdate and potassium antimony tartarate react in acid medium with orthophosphate to form a heteropoly acid- phosphomolybdic acid that is reduced to intensely coloured molybdenum blue by ascorbic acid.

Reagents

(a) **Sulphuric acid (5N)**: Dilutre 70 ml conc sulphuric acid to 500 ml.

(b) **Pottassium antimony tartarate**: Dissolve 1.3715 gm $K(SbO)C_4H_4O_6.1/2\ H_2O$ in 500 ml of distilled water.

(c) **Ammonium molybdate solution**: Dissolve 20 gm $(NH_4)_6\ Mo_7O_{24}.4H_2O$ in 500 ml of distilled water.

(d) **Ascorbic acid: 0.01M:** Dissolve 1.76 gm ascorbic acid in 100 ml of distilled water. Stable for 1 week at 4^oC.

(e) **Combined reagent:** Mix 50 ml of 5N H_2SO_4 + 5 ml Potassium antimony tartarate+ 15 ml ammonium molybdate reagents+ 30 ml ascorbic acid. If turbidity, shake until turbidity disappears. Stable for 4 hours.

(f) **Stock phosphate solution**: Dissolve 4.389 g anhydrous potassium di-hydrogen phosphate in distilled water and dilute to 1000 ml gives 1000 ppm of PO_4^{3-}-P.

Procedure*:* To 50 ml water sample in Erlenmeyer flask, add 0.05 ml (1 drop) phenolphthalein. If red colour appears, add 5 N sulphuric acid to discharge the colour. Add 8 ml combined reagent and mix thoroughly. After 10 minutes to 30 minute, measure absorbance of sample at 880 nm using reagent blank as the reference solution.

16. Total P

1. Add 8 ml of 4 % potassium persulfate solution into a 100 ml conical flask containing 40 ml of sample.
2. Mix well and cover the mouth of the flask with aluminium foil.
3. Place the conical flask in an autoclave for 15 minutes under a presence of 15 lbs.
4. After that, remove the flask, cool the contents and make up the volume to 60 ml with distilled water.
5. Then, follow the same procedure as mentioned above for inorganic phosphates.
6. Calculate the organic- P by deducting inorganic- P from total- P.

17. 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 Eriochrome 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** (EBT): 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 (0.01 M)**: 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 $MgCl_2.6H_2O$ 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.

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

Calculation:

$$\textbf{Total hardness (mg/l as } CaCO_3\textbf{)} = \frac{TV \times M \times 100 \times 1000}{S}$$

Where, T = Volume in ml of EDTA solution
M = Molarity of EDTA solution
S = Volume in ml of sample

18. 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 stadardize against 0.0250 *N* $Na_2S_2O_3$, using starch solution as indicator.

(c) **Standard sodium thiosulfate solution**, 0.0250N: Dissolve 6.205 g Na2S2O3.5H2O 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 sulfide present. Add distilled water, if necessary, to bring volume to about 20 ml. Add 2 ml 6N HCl. Pipet 200 ml sample into flask, discharging sample under solution surface. If iodine color disappears, add more iodine so that color remains. Back titrate with $Na_2S_2O_3$ solution as end point is approached, and continuing until blue color disappears.

Calculaltion

$$\text{mg } S^{2-}/L = \frac{[(A \times B)-(C \times D)] \times 16000}{\text{ml sample}}$$

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.

19. 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 Na_2HPO_4 and 46 g anhydrous KH_2PO_4 in distilled water. Combine with 100 ml distilled water in which 800 mg disodium ethylenediamine tetraacetate 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 – 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(1 + 3)H_2SO_4 and 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 1.106 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 µg Cl as Cl_2/ 1.00 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, 1.00 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.

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LIST OF EQUIPEMENT FOR SOIL AND WATER ANALYSIS

pH meter (Micro processor based): Rs.30,000/-

pH meter (digital): Rs. 10000/-

Salinometer: Rs. 10,000/-

Electrical conductivity meter: Rs.30,000/-

Portable Redox Meter (Imported): Rs.30,000/-

Portable Do meter (imported): Rs. 75,000/-

Portable Spectrophotometer – UV Visible Range (Imported): Rs. 3.7 lakh

Portable Multi-Parameter Water Quality Analyzer (Imported): Rs.3 lakh

Water Quality Analyser (Indegenous): Rs. 1,05,000

Flame photometer: Rs. 60,000

Nephelo turbidity meter: Rs. 20,000

Water bath: Rs.10,000/-

Hot plate: Rs.15,000/-

Quartz double distillation unit : Rs.70,000/-

Orbital shaker : Rs. 30,000/-

Acid Dispenser : Rs.20,000/-

Refrigerator: Rs. 30000

Magnetic Stirrer: Rs. 9000/-

Vortex Shaker: Rs. 7500/-

Spinwin Mcoo Micro Centrifuge: Rs. 7000/-

Hot Plate: Rs. 7000/-

Kjeltec N digestion and distillation System: Rs. 4 lakh

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