

**STUDY OF MICROALGAE FEED IN SHRIMP HATCHERY –
Thalassiosira
(Project – Report)**

Submitted in partial fulfilment of the requirements for the award of
Bachelor of Science degree in Biotechnology

By

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APRIL - 2021



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BONAFIDE CERTIFICATE

This is to certify that this Project Report is the bonafide work of SUBRAJA K (38770016) who carried out the project entitled “**(STUDY OF MICROALGAE FEED IN SHRIMP HATCHERY – Thalassiosira)**” under our supervision from

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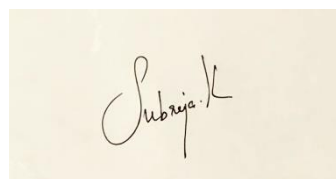
DECLARATION

I **SUBRAJA K (38770016)** hereby declare that the Project Report entitled

“STUDY OF MICROALGAE FEED IN SHRIMP HATCHERY - Thalassiosira” done by me under the guidance of **Dr. Masilamani Selvam** at **Sathyabama Institute of Science and Technology, Chennai** is submitted in partial fulfilment of the requirements for the award of Bachelor of Science degree in Biotechnology.

DATE: 07/04/2021

PLACE: Chennai

A rectangular box containing a handwritten signature in black ink. The signature appears to be 'Subraja K' written in a cursive style.

SIGNATURE OF THE CANDIDATE

ACKNOWLEDGEMENT

I am pleased to acknowledge my sincere thanks to Board of Management of SATHYABAMA for their kind encouragement in doing this project and for completing it successfully. I am grateful to them.

I convey my thanks to Dr.V.Ramesh Kumar, Head of the Department, Dept. of Biotechnology for providing me necessary support and details at the right time during the progressive reviews.

I would like to express my sincere and deep sense of gratitude to my Project Guide Dr. Masilamani Selvam for his valuable guidance, suggestions and constant encouragement that paved way for the successful completion of my project work.

I wish to express my thanks to all Teaching and Non-teaching staff members of the Department of Biotechnology who were helpful in many ways for the completion of the project.

I would also like convey my deepest appreciation and profound gratitude to BBC shrimp hatcheries , Azhagankuppam , Marakkanam , Villupuram for authorizing me to use workspace and providing me with the essential resources to accomplish my projects .

ABSTRACT

Shrimp is a valuable marine food resource which is quite high in protein and other nutritional values. Shrimp requires good export marketing techniques.

Litopenaeus vannamei, formerly *Penaeus vannamei* (White leg shrimp) has greatly elevate mass yielding of shrimp fries concealed by hatchery conditions. The culturing of the microalgae -Thalassiosira in the prepared F2 medium under optimal conditions. The starter pure culture of thalassiosira is commercially available, which is then cultured through serial dilution from 250ml to 10 ton, from indoor to outdoor lab eventually. The sea water used for the media preparation should be filtered properly with salinity of 30‰ and pH – 8.2-8.7. The F2 medium main components are Sodium nitrate, ortho phosphate, E.D.T.A, FeCl₃ and trace metals. Following these process by the time of day 8, the thalassiosira is healthily cultured and ready to be fed for the shrimp seeds .Then the observation is done closely. During Post larvae stage, alongside probiotics, microparticulated commercial shrimp feed is given which is enhanced with fish meal, brewer's yeast, soy lecithin, wheat by-products, and fish oil along with many nutritional values. Further regarding packing and exporting process is also studied wherein the feed artemia is used. The salt water used for artemia is cultivation should be of pH 8.0, and the temperature around 28°C to 30°C and salinity rate of 25-35 ppt. The aim and main motive of this study is to evaluate and enhance the nutrients required for the shrimp through the microalgae feed- Thalassiosira, thereby knowing their importance and immune responses to diseases and attacks.

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LIST OF ABBREVIATIONS

1. BHA- Butylated hydroxyanisole
2. BHT - Butylated hydroxytoluene
3. CoCl_2 - Cobaltous chloride
4. Cu – Copper
5. CuSO_4 - Cupric sulphate
6. FRP – Fibre reinforced Plastic
7. Fe – Iron
8. GCMS – Gas Chromatography Mass Spectroscopy
9. GMO – Genetically Modified Organisms
10. Mn – Manganese
11. MT – Metric Tonne
12. m^3 - cubic meter
13. μm – Micrometer
14. MnCl_2 - Manganous chloride
15. Na_2MoO_4 - Sodium molybdate
16. ppt – Parts Per Trillion
17. ppm – Parts Per Million
18. SEPA – Scottish Environment Protection Agency
19. UV/Vis spectroscopy – Ultraviolet Visible Spectroscopy
20. Zn – Zinc
21. ZnSO_4 - Zinc sulphate

CHAPTER 1

1.1 INTRODUCTION

Thalassiosira weissflogi is a widely used and distributed unicellular microalgae. It is a diatom found in almost in many regions of the world and types of environment like marine, back waters, fresh water environments too. *Thalassiosira pseudonana*, a microalgae, is extensively used in aquaculture. In terms of technology and economics, extracting microalgae biomass from culture medium is a critical concern. This diatom is of size around 4-32µm and is in short cylindrical in shape. These diatoms either occur as single cells or as groups ingrained in a gelatinous matrix. *Thalassiosira* is known to survive and thrive at high salinity environment and can be easily cultured even in poor quality water. *Thalassiosira* is not a toxic diatom on its own but when it gets incorporated with other species of microalgae can cause algal bloom and other issues such as red tides. The reproduction can be either asexual or sexual. The sexual mode of reproduction can be induced or prompted by external factors and environments such as temperature, pH of the water, light intensity etc. *Thalassiosira* is the most widely used feed for shrimp especially during shrimp larval stages.

1.2 Aquaculture

Aquaculture defines to the rearing of aquatic/marine organisms under controlled or semi controlled conditions. (R.R. Stickney et al, 1994)

Another mainly used and majorly recognized definition proposed by FAO of UN is “the farming of an aquatic organisms, including fish, molluscs, crustaceans, and aquatic plants “(FAO, Agriculture production statistics 1974-1993).

Aquaculture in the public sector refers to the development of aquatic animals to complement or develop recreational and commercial fishing. Aquaculture has become more common in recent years as a means of assisting in the recovery of threatened and endangered species. Those projects are currently public-sector initiatives, though private-sector participation is being considered. Recovery programmes for aquatic threatened and endangered species may emerge in many more countries as global awareness of

endangered species issues expands. Enhancement stocking programmes aimed at releasing juvenile animals to restore populations of marine animals that have been depleted due to overfishing go hand in hand with efforts to recover endangered species.

The majority of aquaculture production is used directly for human use, with public aquaculture playing a minor or non-existent role in many countries. However, private aquaculture is about more than just human food production. Bait and ornamental fishes, as well as invertebrates, are produced in some areas by well-developed private sector aquaculture.

According to the newly released data, world aquaculture production of food fish has almost reached 62.7 million tons in 2011, up by 6.2% approximately from 59 million tons in 2010. The estimated value of farmed food fish in USD 130 billion. Farmed aquatic algae production in 2011 was 21 million tons, worth around USD 5.5 billion. The 2010 world production level in new data release, due largely to the downward adjustment of 2010 production by India, the world's 2nd largest aquaculture producer.

Aquaculture contributed 40.1% to the world total fish production, and almost all the seaweeds production (FAO Fisheries and Aquaculture department, March 2013)

1.3 Aquaculture & it's type

To be more descriptive, aquaculture is the skilful process of raising aquatic animals for human consumption. If we replace plants or animals with fish, we're on the same track as agriculture. Food can now be grown in coastal marine waters and the open ocean, thanks to rising global demand for delectable seafood delicacies and advancements in technology.

1.3.1 Mariculture

Seawater is needed for marine culture. The ocean, a segmented portion of the ocean, or ponds containing seawater are used for this form of aquaculture. Molluscs, shellfish, prawns, and seaweed are some of the

marine species that are widely bred in these areas of the oceans. Seaweeds are another important part of mariculture. Seaweed collagen, for example, is used in some facial creams, while mollusc pearls are hand-picked and crafted into beautiful fashion accessories.

1.3.2 Fish Farming

Fish farming is, of course, the most basic method of aquaculture. Fish are carefully bred as part of the process. It can be done in any freshwater, but it is better done in seawater. This method of breeding aids in the development of a food and consumption source. Fish farming is a relatively simple process that requires only the right water conditions and temperatures. This method of farming does not need a larger space because the breeding organisms, such as tilapia, are much too small.

1.3.3 Alga culture

Alga culture is the farming of algae. Algae, as we all know, are microbial species with animal and plant-like characteristics. They are harvested in high quantities in order to sustain economic viability. Algae are used in a range of methods. The source of energy for smartphones is an example of such an application.

1.3.4 Open-net pen and cage systems

Another form of aquaculture that is implanted offshore and in freshwater lakes is open-net pen and cage systems. The fish are first placed in net cages ranging in size from 6 to 60 cubic feet (pens) in the water. Water habitats become purified of toxins, wastes, and parasites as a result of the high ingestion of fish in the pens. Predacious animals and larger fish are attracted to the fish in the net cages, and they get stuck in the nets.

1.3.5 Shrimp farming

Shrimps and prawns are two common names for decapod crustaceans belonging to the Penaeidae and Palaemonidae families. However, a significant difference exists between the 2 groups in recent aquaculture literature. Freshwater Palaemonids are called prawns, while marine penaeids are called shrimps. Shrimps are found in coastal waters in more than 50 species/varieties, with a broad range of distribution in both tropical and temperate habitats. The majority are too small to be used for cultivation or human use. Shrimp farming, or the supervised cultivation of shrimp in salt- or brackish-water ponds, has sparked a lot of interest in the United States in recent years. Because of their rapid growth and high market value, shrimp tend to be especially attractive for artificial cultivation.

Shrimp farming methods take advantage of those shrimp's ability to thrive and develop quickly in shallow estuarine waters. The shrimp farmer changes the natural environment by building dams, resulting in a significant rise in the amount of shrimp harvested from estuarine areas. A shrimp farmer can be able to monitor the market by using proper time schedule creation of his stocks, so that plenty of live bait shrimp of the right size can be harvested when demand is at its highest.

Shrimp and other marine animal culture is a significant industry in some Southeast Asian maritime countries. Shrimp farming in the United States, on the other hand, is still in its early stages, and further study is needed to assess its biological and economic viability.

Despite an agreement reached at a world conference on the biology and culture of shrimps and prawns held in Mexico City in 1967 to limit the term "prawn" to freshwater species and "shrimps" to marine and brackish water counterparts, these two terms are used interchangeably in many publications. (Dr. Balasubramanian et al, 2018)

There is no distinction between shrimp and prawns on a scientific and technical level. That being said, in the Indian framework, we currently adhere to the FAO consensus and refer to both marine salt water and brackish water species as 'shrimp'. (Dr. Balasubramanian et al, 2018)

The three main types of prawns and shrimps are;

Penaeid,

Caridean, and

Stenopodean shrimp

Penaeid shrimps - Penaeid shrimps represent approximately all the aquacultured shrimps (of the genus *Penaeus*). The first three pereopods, are chelate, and they are all of the same size and shape. The pleuron of the abdominal part of the second segment aligns with the pleuron of the third abdominal segment, but not with the pleuron of the first. The females of this species lay their eggs directly in the water.

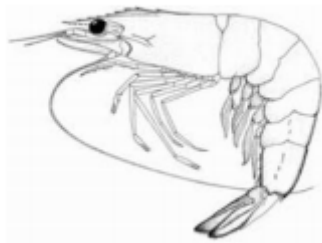


Figure 1 Penaeid shrimp

Caridean shrimps - The pleura of the second abdominal segment overlaps itself to the first and third abdominal segments, so the third pair of this section is not chelate. The eggs are borne by the female until the process of hatching to take place.

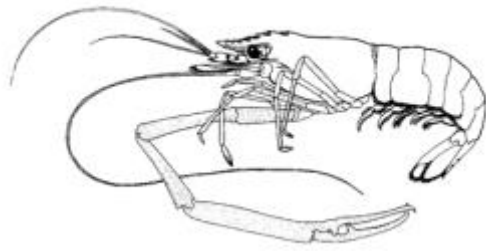


Figure 2 Caridean shrimp

Stenopodean shrimp – This kind of shrimp is the less popular and known. The third walking leg is chelate and very long, the female carries eggs, and the pleura of the second abdomen is close to that of penaeid shrimps, which were first described in 1798. There are 29 species in the genus *Penaeus*. This genus of shrimp has always gotten a lot of attention because they are commercially harvested and financially lucrative to a multitude of nations.

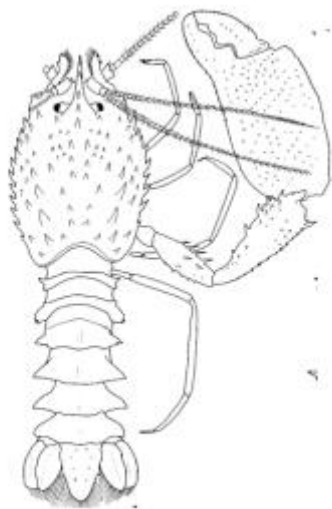


Figure 3 Stenopodean shrimp

1.3.6 Oyster Farming

Oyster farming is one of the most common styles of marine aquaculture in the United States. A total of 31 million pounds of oysters

were harvested in 2011, with a dockside value of \$135 million.
(National Marine Fisheries Services)

There exists 4 major types of oyster farming, they are;

1.3.6.1 Traditional bottom or bed culture

Oysters are raised on the intertidal zone's grounds, where they feed on the plankton present in the incoming tide. Gravel or hard packed sand beaches are common in these areas.

1.3.6.2 Rack & Bag oyster culture

Oysters are raised in plastic net bags suspended above the ground on steel racks, allowing water to flow easily around the oysters, allowing them to eat more rapidly and defend themselves from crabs and other predators. This method also allows oysters to be grown in places that would otherwise be too muddy for them to be grown directly on the intertidal grounds.

1.3.6.3 Suspended oyster culture

The suspended tray culture and the lantern net culture are alternative routes for this procedure to take place. The motive of this study is to allow oysters to develop in deeper, subtidal waters. The oysters are mounted in trays or nets suspended from floats or sub-tidal longlines so that they can hang below the water's surface 24 hours a day, enabling them to develop very quickly.

1.3.6.4 Inter-tidal longline culture

Adult oyster shells with baby oysters placed on them are inserted into the fibres of a rope in this form of suspension. Along this 300-600 foot chain, the shells are positioned every foot or two. At low tide, the rope is extended around the ground in the intertidal zone and pegged about a foot above the ground.

As a result, the total length of rope and shell is elevated above the natural bed, enabling the oysters to develop more quickly than if they would be buried in the mud or sand. Intertidal longline culture has seen the creation of new technologies which have proved to be sustainable.

Eel grass can now grow and maintain marine life such as crabs and other species thanks to modern systems that use SEPA bags and Flip bags. Due to their organic nature and ability for marine life to evolve among them, SEPA and Flip bags are the potential of intertidal longline cultivation.

1.3.7 Aquaculture / ornamental fish farming

Aquaculture is the cultivation of ornamental fishes. Ornamental fish culture is the activity of raising beautiful, colourful fish with a variety of characteristics in a confined aquatic environment. It is primarily grown by farmers and hobbyists. Living jewels are another term for ornamental fish. There are over 30,000 fish species recorded worldwide, with about 800 of them belonging to ornamental fishes. Freshwater is home to the majority of ornamental fish. They come under eight closely related families namely,

- Anabantidae,
- Callichthyidae,
- Characidae,
- Cichlidae,
- Cobitidae,
- Cyprinodontidae,

- Cyprinidae and
- Poeciliidae.

Fish in aquariums are divided into two groups: oviparous (egg layers) and ovo-viviparous (live bearers). The majority of aquarium species are egg layers, and external fertilisation is usually used.

Based on which, they are categorised accordingly;

- Egg buriers
- Mouth incubators
- Nest builders
- Egg carriers
- Egg scatter laying non adhesive eggs
- Egg scatter laying adhesive eggs

There are two types of ornamental species: indigenous and exotic. The access to an unlimited groups of indigenous species has aided the growth of the country's ornamental fish industry. Indigenous species are abundant mostly in eastern part states of West Bengal, Kerala, and Tamil Nadu.

To meet export demand, approximately 90% of invasive species (85% from northeast India) are gathered and cultivated. Approximately 100 native species are currently being reared as aquarium fish. Exotic species are also in high demand due to their colour, form, and appearance. The ornamental fish trade covers over 300 exotic species, but there is a higher demand for them. In India, around 200 species are bred. Kolkata accounts for 90% of India's exports, with Mumbai accounting for 8% and Chennai for 2%.

In fish culture, the aim is to maximise fish population per unit of culture space. Supplemental feeding with artificial diets is a healthy way to improve fish production. Supplementary diets, on the other hand, must be formulated in accordance with the essential nutritional requirements of the species, which include protein, lipid, and

carbohydrate and energy. Knowing the digestibility coefficients for ingredients is important for meeting the nutrient and energy requirements of fish by realistic feeding and for replacing one ingredient with another for economic reasons. A feed component will appear to be an excellent source of nutrients based on its chemical composition, but it will be of little use unless it can be digested and consumed by the target organisms.

1.4 Aquaculture Advantages

There are many aspects and points that stands for the pros of aquaculture and some of the main points are given below;

- They are the main source of rich animal protein's and micronutrients. Fish farming, also known as aquaculture, is a perfect source of protein. Fish, which is a result of aquaculture, meets the current need for animal protein for more than 1 billion people worldwide with limited resources. Micronutrients found in fish are also essential for children's proper cognitive and physical growth. As a result, aquaculture contributes to people's overall growth around the world.
- Plays a vital role in conserving the aquatic population. Aquaculture contributes to the conservation of wild aquatic plants and other organisms by preventing extinction due to unrestrained mistreatment.
- Strives to be the nurturer of ecosystem and the biodiversity. It is also another significant advantage of aquaculture. Aquaculture methods have led to the discovery of natural forms for various aquatic species and plants to coexist. As a consequence, aquaculture contributes to the elimination of environmental waste and the enhancement of ecosystem function.
- Gives out many employment opportunities. Aquaculture also offers a range of career choices. Aqua farming, as a new field, will generate a lot of job opportunities.

- By increasing and giving out employment opportunities in a community, results to the increase of the revenue in city, state level, and national level. This up brings the economic state of the country.
- Helps to balance the trade deficit of seafood. Which in turn helps to feed the growing population in all over the world.
- Aquaculture, especially mollusc and seaweed culture, may put a greater focus on pollution prevention in coastal waters.
- If a species can be created through aquaculture rather than fishing, it can help to reduce fishing pressure on wild stocks.
- People's nutritional requirements have also been met thanks to aquaculture. Fish is high in micronutrients, minerals, and proteins, all of which are important in your diet.
- Fish is becoming increasingly inexpensive and accessible to the general public as a result of increased demand.
- There has been a significant reduction in the burden on wild fisheries. Many fisheries all over the world have already reached unsustainable or unsafe levels.
- Ocean trawling, for example, does major harm to the ocean's substrate and can even destroy non-targeted animals. With the aid of aquaculture, these dangers are decreased.
- Aquaculture can also be less damaging to the environment than certain other conventional fishing methods.

1.5 Aquaculture disadvantages

- Other users of water bodies, such as lobstermen, fishermen, and migrating fish, can cause conflict through aquaculture farming process.
- Aquaculture can put undue strain on wild stocks used to produce high-protein feed pellets.
- Disease and parasites are amplified and transmitted to wild fish species through aquaculture.
- Excess nutrients (fish feed and waste), toxins, and antibiotics pollute water supplies in aquaculture.
- If farmed fish and native species interbreed, native gene pools can be jeopardised.
- Fishermen's livelihoods can be jeopardised by aquaculture farming.
- Due to its vulnerability to extreme weather, predators, disease, and global competition, aquaculture can be a risky business for small local communities.
- The intrinsic beauty of the coastline can also be harmed by aquaculture farming.
- Aquaculture can also affect the ecosystem in the following ways:
 1. Biodiversity is being lost.
 2. Fertility of wild fish has decreased.
 3. False reports of population growth due to emigration.
 4. Eutrophication occurs as a result of increased effluent in local waters.
 5. Water quality often deteriorated.

6. Aqua culturists are targeting the extinction of marine mammals and fowl.
 7. Fish meal stocks of pelagic fish are depleting.
 8. Non-native and potentially invasive fish have escaped.
 9. Land conversion has resulted in the destruction of ecologically sensitive areas.
 10. Wild stock has been genetically modified.
 11. Disease and parasite transfer from farmed fish to wild species.
- Dyes, antibiotics, PCBs, dioxin, and mercury can all be found in fish due to contaminated water and/or human inputs. Fish that is toxic to one person may not be toxic to another.
 - Since different genera, families, groups, orders, and phyla of seafood are consumed and processed in different ways, health claims must be controlled and warnings issued when appropriate.
 - Fish that are fundamental steps of commodity crops, many of which are GMO, have debatable health qualities that can make them inedible or undesirable to many domestic and foreign populations.
 - Current aquaculture methods, like land-based agriculture, will become unsustainable if a systems solution is not chosen, or if aquaculture systems become highly input-dependent.
 - The popular use of pelagic fish [fish that develop and mature rapidly and die young] in the fish-in-fish-out technique, for example, when breeding carnivorous fish, burdens natural systems and helps to offset the benefits of aquaculture.
 - Although aquaculture has the potential to provide a variety of benefits, these benefits can only be realised by resource management that is backed by reasonable and enforceable regulations.

- Aquaculture infrastructure construction would have an effect on natural wildlife, such as wetlands and mangrove swamps.
- The regional ecosystem will be harmed by raw wastewater discharges with a high organic load.
- Exotic species farming will add new pathogens to the new world.
- Shift of disease and parasites from captivity to wild animals

1.6 Shrimp Hatchery

1.6.1 Shrimp & its economic importance

Shrimp is one of the most valuable marine food resource with abundance of nutritional aspects as mentioned before. It has turned out to be the main target commodity for aqua farming and to enhance economical state of the country. Conventionally shrimp fry are captured and held in ponds, which is then later collected by shrimp fry collectors for stocking in grow-out ponds in shrimp farms. The hike in the completion of the life process of commercially demanded shrimps in captivity, like the *Penaeus monodon* (tiger shrimp), the *Penaeus japonicus* (Japanese kuruma ebi), the *Penaeus orientalis* (eastern shrimp) and the *Penaeus indicus* (banana shrimp), has greatly elevate mass yielding of shrimp fries concealed by hatchery conditions. The sterling growth performance of these hatchery-bred fry in grow-out pond farms staunchly displays that the shrimp hatcheries can answer the industry's demand for ample supply of shrimp fries for farming purposes. Despite the fact that , the commercial hatcheries are of so much cost effective and their outputs much more certain than before, we need to accept that the process and methods followed are still very experimental. Despite the sector's impressive growth, it has faced quite a lot of challenges, including viral diseases, marketing issues, and poor farm-level results. Shrimp farming has created millions of jobs and provided emerging

economies with foreign exchange. The production of farmed shrimp has increased significantly. Globally, farmed shrimp population rose from 10,000 metric tonnes to four million metric tonnes. The Indian shrimp farming/hatchery industries has also expanded significantly, from 50000 mt in 1990 to 600000 mt in 2016.(Dr.K.K.Vijayan 2018)

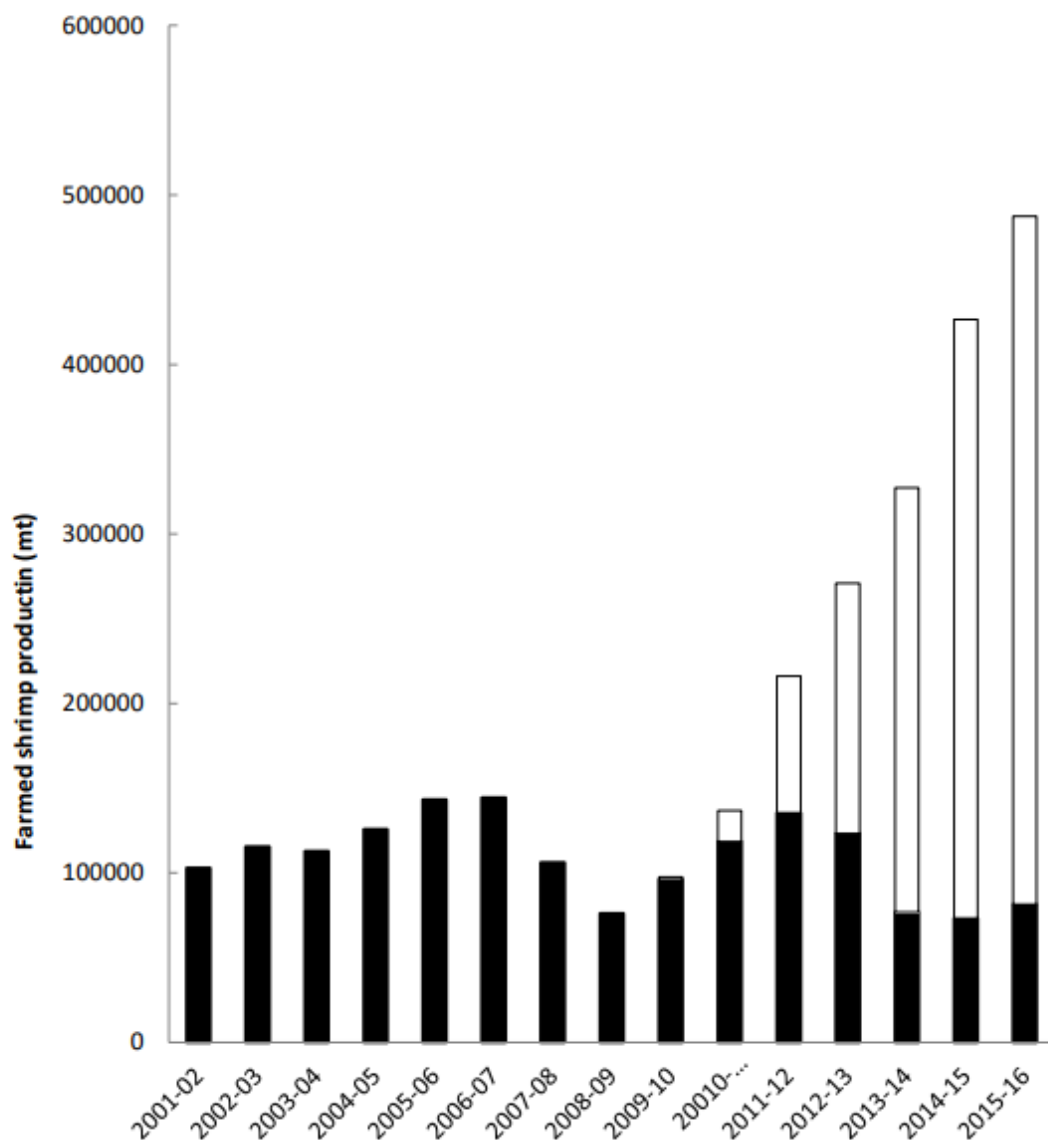


Figure 4 Farmed shrimp production in India

1.6.2 General life history of Shrimps

The vast majority of shrimp grown in salt-water ponds in the Far East marine shrimp that live the maximum amount of time in shallow, brackish estuaries. They've adapted well to their surroundings and can withstand significant changes in salinity and temperature. Female shrimp freely spawn ones eggs in the ocean, each delivering hundreds of thousands of eggs. The tiny shrimp migrate toward the coast after hatching and going through several larval stages. When young shrimp reach shallow, brackish shore waters, they settle to the bottom and develop quickly. When maturity approaches, they eventually migrate offshore into the ocean's spawning grounds, and the life cycle is repeated. Depending on the species, the life cycle can be anywhere from 1 to 3 years, and the overall length can be anywhere from 4 to 12 inches. There are four species of marine shrimp found along the Gulf and South Atlantic coasts of the United States that may be ideal for pond culture.

The white (*Penaeus setiferus*), pink (*Penaeus duorarum*), brown (*Penaeus aztecus*), and Caribbean brown (*Penaeus brasiliensis*) shrimps are all similar in appearance and habits to their Southeast Asian relatives, the cultured shrimp. In addition to marine shrimp, there are many types of broad river shrimp (*Macrobrachium* spp.) that should be considered for pond culture in the southern United States. These shrimp can be found in both fresh and brackish water. The eggs aren't released immediately into the water; instead, they stay attached to the female's "tail" before they hatch into young shrimp.

1.6.3 Hatchery design and construction

The main points and aspects to be considered while hatchery construction and to have an ideal hatchery with all the optimal conditions are given below;

- Choosing a suitable location.
- Hatchery design effectiveness and efficiency.
- Hatchery technician experience.
- A high level of operational management performance.

1.6.3.1 Selection of location

It is critical to conduct a comprehensive feasibility study to assess the suitability of the proposed site prior to the establishment of any shrimp hatchery. Water quality, spawner availability, and site usability are the key parameters used.

Since it is expensive to change sites after major financial investments have been made, the requirements for hatchery site selection must be strictly followed when developing large-scale modern hatcheries. Smaller hatcheries, on the other hand, have a more versatile site selection process than larger ones.

The main criteria for the selection of suitable locations are as follows

- Availability of sea water supply; In a hatchery, the sea water should be clean, clear, and relatively silt-free. Water quality should be consistent, with little variation in salinity. Suitable sites are generally located in sandy and rocky shore habitats where the sea water is clean, clear, and of good quality all year. Wetlands and stagnant shores where the liquid becomes brackish amid heavy rains or due to turmoil are not ideal for hatcheries. Ignore river mouths, where sudden decreases in salinity are normal during heavy rains.
- Attainability of spawners: The availability of spawners located near site has a significant benefit in terms of maintaining a stable supply

of spawners and lowering transportation costs, which might regulates the degree of spawning.

- Ease of access to power source: Electric power is required to operate the hatchery's equipment and other ecosystem services. Since some aquatic pumps and air filters can be powered directly by portable generators, the shrimp hatchery can be run in areas where electricity is not available.
- Freshwater supply: Freshwater is needed for constant hatchery operations such as salt concentrations adjustment, equipment servicing, and domestic consumption.
- Climatic conditions: A hatchery can indeed be installed in any climate as long as the requisite breeding standards are fulfilled. Both commercial hatcheries, on the other hand, make full use of nature as a source of energy and a source of high-quality water.
- Accessibility: A hatchery should ideally be located near ongoing shrimp farming operations so that if the shrimp larvae produced can be shipped and distributed to the grow-out ponds with ease. As a result, the location chosen for the hatchery must be conveniently accessible in order to promote contact and distribution.

1.6.3.2 Design effectiveness and efficiency

In general, there have been two hatchery programs in use. The large-tank hatchery, invented in Japan, was always commonly used in many Asian countries, including Taiwan, Thailand, the Philippines, and Indonesia. The small tank hatchery, that further originated in Galveston, Texas, is used throughout the Philippines, as well as Malaysia and Thailand to a lesser degree.

SIZE OF HATCHERY; In general, a hatchery's size should be determined by its functional requirements as well as its economic quality. Hatchery practise can be classified into three groups based on the size of the operation, the amount of product produced, and the amount of money invested:

- small-scale,
- medium-scale, and
- Large-scale hatcheries.

BASIC HATCHERY FACILITIES; when planning a hatchery, make sure to leave enough room for the rearing and tools for managing that will be needed. An active functional shrimp hatchery should have the following vital facilities:

- Maturation tanks -Maturation tanks may be round, rectangular, or oval - shaped. The tank size can range from 5 to 40 tonnes, and the depth can be anywhere between 1.2 and 2 metres. Bottom substrate isn't needed in the tank if the shrimps are held for less than 5 weeks.
- Spawning tanks -The bottom of the spawning tank should be horizontal or conical in shape. The amount of water that can be held varies from 50 litres to 1.5 tonnes. Fiberglass, Plexiglass, acrylic, or marine plywood may be used to build the tank. These spawning tanks are being used to house gravid females until they are ready to spawn.
- Rearing tanks for larvae; the freshly hatched larvae are reared in two types of rearing tanks they are

- i. Small tank system
- ii. Big tank system

- Live feed culture tanks-The scale of the tanks used in large scale production of live food organisms typically ranges from 1 to 20 tonnes. Fiberglass, polyethylene, marine plywood, or concrete may be used to build the tanks. The total tank capacity for live food culture is roughly 20% of the overall tank capacity for larval breeding on average.
- Filtration and water storage tanks - The water storage tanks are usually raised to make sure that water is delivered to the hatchery by force of gravity. The potential of this tank should be at least 20% than that of the larval rearing tanks. To withstand the water load, these tanks are usually made of reinforced concrete.

When the water used is polluted, a water treatment panel and a sand filter unit must be mounted. The filter chamber is usually built next to the holding tank. The filters used are mainly of two types

- i. Gravity filter
- ii. Reversing filter

- Aeration equipment - During the entire larval rearing process, aeration is important for maintaining an adequate rate of dissolved oxygen in the water, establishing balanced temperature of the water in the water column by turbulence, and reducing ammonia levels in the water.
- Supply of sea water and pipping system throughout.
- Marine pumps

1.7 *Penaeus vannamei* (Pacific white shrimp)

The production of farmed shrimp has increased significantly over the last 25 years, from nearly 50000 metric tonnes in 1990 to 600000 mt in 2016. The most common farmed shrimp in the world are *Penaeus monodon* (tiger shrimp) and Pacific white shrimp *Penaeus vannamei* (Pacific white shrimp).

Despite the dominance of *P. monodon*, global shrimp aquaculture has changed drastically to *P. vannamei* since 2001 owing to the availability of disease-resistant stock. To control the production process successfully and maximise the profitability and efficiency of farming, it is critical to have a basic and solid understanding of the physiology and biology of the species to be harvested.

Taxonomic Classification *Penaeus vannamei*

Kingdom	Animalia
Phylum	Arthropoda
Subphylum	Crustacea
Class	Malacostraca
Subclass	Eumalacostraca
Superorder	Eucardia
Order	Decapoda
Suborder	Dendrobranchiata
Superfamily	Penaeoidea
Family	Penaeidae
Genus	<i>Penaeus</i>
Species	<i>Penaeus vannamei</i>

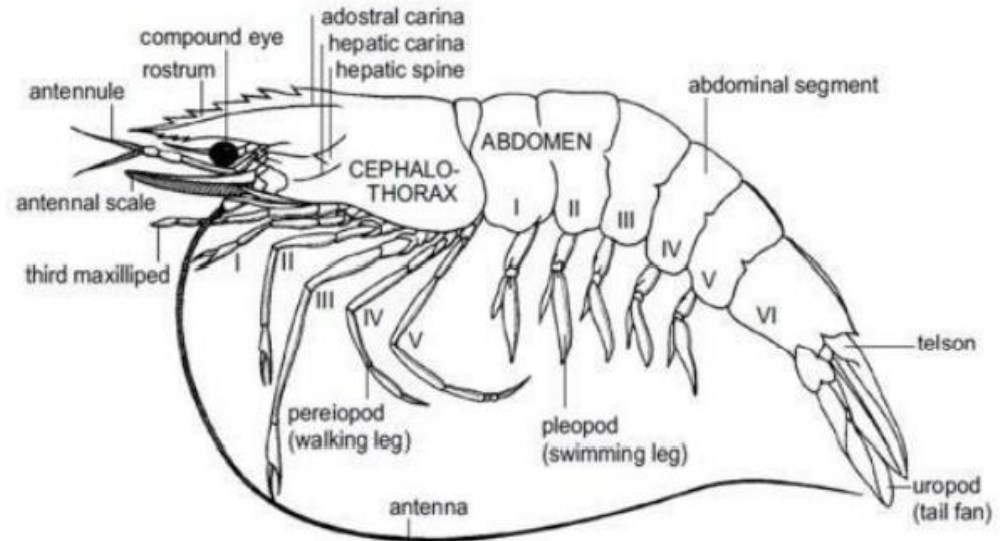


Figure 5 External morphology of P. vannamei

Coloration is primarily translucent white, but that can differ considerably on the substratum, feed, and turbidity of the water. With 7-10 dorsal and 2-4 ventral teeth, the rostrum is moderately long. Petasma is symmetrical and moderately open in mature males. The spermatophores array is made up of sperm mass encased in a sheath.

Thelycum is open in mature females. With a maximum CL of 9 cm, the maximum size is 23 cm. According to studies conducted, females usually grow faster and are larger than males. This kind of shrimp is native to the Eastern Pacific coast, from Sonora, Mexico in the north, all the way down to Tumbes, Peru in the south. (Dr.Balasubramanian et al, 2018)

They are extremely salt-resistant which is known as euryhaline and can tolerate salinities of 0 to 55 ppt. Post larvae migrate inshore to splurge their juvenile, adolescent, and sub-adult stages in coastal estuaries, lagoons, or mangrove areas, whereas adults live and give rise in the open ocean.

Males reach sexual maturity at 20 g and reach a total length approximately of 187 mm. Females are heavier compared to that of male shrimps, with a length of 23 mm, and achieve sexual maturity at the age of 6-7 months, when they weigh around 28g.

EXTERNAL FRAMEWORK OF *P.vannamei*

P.vannamei has the usual morphology like that of penaeid shrimp. The animal's body is divided into 19 parts.

- The cephalic region has the first 5 pairs,
- The thoracic region has the next 8 pairs, and
- The abdominal region has the last 6 pairs.

The cephalothorax is formed when the head and thoracic regions are joined together. The telson is the last part of the body after the abdomen, and it is surrounded by two pairs of uropod, which is collectively called as the 'tail' part.

LIFECYCLE of *P.vannamei*

Penaeus vannamei has a life cycle that is close to that of a typical penaeid shrimp. The adults live in the sea and reach reproductive maturity there. *P. vannamei* females develop faster than males, and an adult female weighing 35 to 45 g lays 100000 to 250000 eggs.

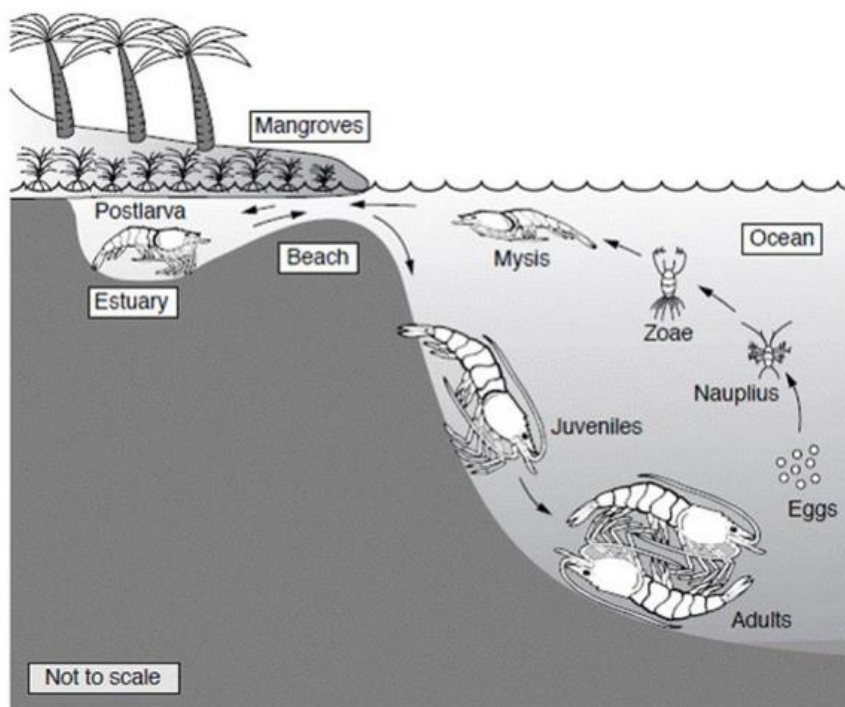


Figure 6 Lifecycle of penaeid shrimp (Rosenberry, 2009)

CHAPTER 2: Literature Survey

Microalgae has a lot of promise as a substitute for aquaculture or animal feed. Its complex nutritional profile will provide animals with balanced nutrition. Reduces feed consumption, resulting in better development and disease prevention. For a sustainable future, microalgae companies favour feed processing. To meet the demands of an ever-increasing population, aquaculture and meat animal rearing have increased dramatically. Using small fish and agricultural commodities for feed production will result in resource erosion and food competition, alike. Microalgae may be the next alternative solution for animal and aquatic feed production that is both economically viable and environmentally viable. In terms of protein, omega 3 fatty acids, and carotenoids, microalgae are more nutritious than typical sources of animal and aquatic feed such as millet, grammes, and other small fishes. Aside from being nutritious, they contain anti-oxidant, anti-microbial, and disease-preventive molecules that can help animals and fish live longer lives. It is important to develop mass production strategies for low-value commodities such as feed. The analysis highlights the nutritional and physiological importance of microalgae to animals and aquatic species, as well as the companies who produce algal biomass feed. (Gnanasekaran Dineshbabu et al 2019)

Microalgae can thrive in a variety of wastewaters, including those generated in aquaculture as a result of hatcheries and farming operations. These species can eliminate toxins from their growing atmosphere, and the biomass they generate can be used in other ways. Such lignocellulosic biomass lipids rich in polyunsaturated fatty acids and other essential micronutrients, and it can be used as a live feed for zooplankton in shrimp farming or fisheries. (Raghdah et al, 2018)

Commercial shrimp culture in low-salinity water has been achieved with great success. Long-term research on the growth response of shrimp culture in freshwater are, however, lacking. *Penaeus vannamei* were cultured in freshwater of 0 ppt at three different densities such as 90, 130, and 180 shrimp m⁻², and the growth, length–weight relationship, and condition factor data were compared. Weight gain findings indicated that the freshwater culture method was viable, with density effects

($P < 0.05$). The inverse relationship between growth rates and density ($P < 0.05$, $R^2 = 0.68$) was observed. With variations between treatments ($P < 0.05$), survival declined as density increased. (Marcelo Araneda et al 2008)

For artificial rearing, several hatcheries employ live microalgae and algae powder; although, the effects of these dietary changes on the rearing process and bacterial communities are unknown. The aim of this study was to see how algae live and algal powder affected the larval culture of *Penaeus monodon*. For the first time, the influence of live microalgae and algae powder on the traits of the bacterial population in the rearing water and larvae of *Penaeus monodon* at various stages of development were investigated. Feeding live algae rather than algae powder may enhance bacterial diversity and stabilization, as well as facilitate larval metamorphosis, survival, and digestive efficiency. In the zoea stage and the PL stage, the bacterial population in the rearing water had negative and positive associations with $\text{NH}_4\text{-N}$ and $\text{NO}_2\text{-N}$, respectively. The effects of two forms of live algae which are *Chaetoceros muelleri*, *Thalassiosira weissflogii* and *Spirulina* powder on the environmental parameters of water, the digestive enzymes production of larvae, and the bacterial population from the zoea stage to the post larval (PL) stage were investigated in this report.

The findings showed that feeding *Chaetoceros muelleri* to larvae increased the enzymatic activity of amylase and the survival rate of the larvae, as well as reducing the concentrations of $\text{NH}_4\text{-N}$, $\text{NO}_2\text{-N}$, and the amount of *Vibrio* in the water ($P < 0.05$). The enzymatic activity of lipase and pepsin, as well as the percentage of metamorphosis ($P < 0.05$), were significantly improved by feeding *Thalassiosira weissflogii*. It was discovered that introducing live microalgae to larvae enhanced the abundance of the bacterial population. In the algal solutions of *C. muelleri* and *T. weissflogii*, Cyanobacteria and Patescibacteria were the most prevalent bacteria. Two phyla, Bacteroidetes and Proteobacteria, dominated the entire rearing climate. At the zoea point, *Virgulinella fragilis*, a bacteria found in the liquid of *C. muelleri* and *T. weissflogii*, makes up a large portion of the bacteria in the rearing water. $\text{NH}_4\text{-N}$ and $\text{NO}_2\text{-N}$ had substantial negative correlations with the bacterial community in the zoea stage ($P < 0.05$), while $\text{NH}_4\text{-N}$ and $\text{NO}_2\text{-N}$ had significant positive correlations

with the bacterial community in the PL stage (P.05). Finally, by improving the external water environment, enhancing digestion, and controlling the bacterial population, feeding larvae with live microalgae could increase the percentage of metamorphosis and survival rates of larvae. (Yapeng Tang et al 2020)

To test their effects on juvenile shrimp development, survival, and structure, researchers supplemented either whole algae or algae fractions to a standardized control diet (*Litopenaeus vannamei*). Diatom (*Thalassiosira weissflogii*) and *Nannochloropsis* species were freeze-dried and divided into a carotenoid segment and a residual fraction using acetone extraction. Individually, whole diatoms (*T. weissflogii*), whole *Nannochloropsis*, a mixture including whole diatoms and *Nannochloropsis*, their carotenoid portion, or their residue fraction are added to a control diet to develop seven diets. Shrimp fed diets containing whole diatoms, entire *Nannochloropsis*, a mixture of both the two, or their residue proportions had higher (P 0.05) growth (0.57 to 0.64 g week⁻¹) and survival rates (93.8 to 100%) than most of those fed the control diet (0.46 g week⁻¹; 79.2 percent). Since the addition of microalgae decreased the levels of crude protein, crude lipid, and gross energy in the control diet, this enhanced growth effect may not be due to the macronutrient composition of the microalgae. Shrimp fed diets containing the carotenoid fraction of diatoms and *Nannochloropsis* (0.54 and 0.47 g week⁻¹) grew at a similar pace (P > 0.05) to shrimp fed diets containing the carotenoid fraction of diatoms and *Nannochloropsis* (0.54 and 0.47 g week⁻¹). The content of fatty acids and astaxanthin in shrimp tail muscles was also increased when whole algae was added. The increased growth rates point to the possible benefits of using dried algae as a feed additive, as well as the advantages of growing phytoplankton in shrimp ponds. (Z.Y.Ju et al 2009)

Thalassiosira weissflogii is an orientated diatom plant, a single-celled microalga. It can be located in both coastal and inland waters in several parts of the world. *Thalassiosira weissflogii* is non-toxic, but it's been linked to other microalgae that trigger algal blooms or red tide in the past. *Thalassiosira weissflogii* is used to feed shrimp and shellfish larvae in aquaculture hatcheries. It is preferred for this reason

over the other commercially produced microalgae, *Chaetoceros* and *Tetraselmis*, due to its relatively high size, which allows it to be used at later stages of larval growth. It's accessible as an "Instant Algae" culture that can be processed at 0-4 degrees Celsius. Polysaccharides from diatoms, such as laminarin, have anti-inflammatory and immunostimulatory properties. *Thalassiosira weissflogii* has been shown to be a potential anti medication in the treatment of cancer, neurological disorders, and ageing, as well as for the production of biopharmaceuticals for various bacterial infections. The anti-inflammatory effect of *Thalassiosira weissflogii* extraction was assessed using albumin denaturation, membrane stabilisation, and a proteinase inhibition assay in the current report. Agar well diffusion was used to test the antibacterial activity of a methanolic extract of the diatom *T. weissflogii* against various species such as *E. coli*, *S. aureus*, and *B. subtilis*. *T. weissflogii* had 12 mm, 19 mm, and 17 mm of resistance to *E. coli*, *S. aureus*, and *B. subtilis*, respectively. These were contrasted to a median inhibitory zone of 18.6 mm for *E. coli*, 15.2 mm for *S. aureus*, and 26 mm for *B. subtilis*, as measured by 30 g/ml streptomycin. They identified pigment, phenolic, and tocopherol among the chemical constituents. Numerous characterization techniques such as UV-vis Spectroscopy and GCMS were used to evaluate and characterise the secondary metabolites of the Diatom *Thalassiosira weissflogii*. *T. weissflogii* methanolic crude extract was extracted and tested for anti-inflammatory and antimicrobial activity in vitro in this sample. In vivo models can be used to assess anti-inflammatory behaviours in the future.(A Asha shalini et al 2018)

CHAPTER 3 AIM & SCOPE

3.1 AIM

The present study was undertaken with the following objectives:

- Culturing of *Thalassiosira* as feed for shrimp
- To understand the provide optimal conditions for the microalgae culture
- Feeding the cultured microalgae to shrimp
- To closely observe the growth and stages of shrimp
- Enhance the growth along with probiotics and microparticulated feed.
- Packing and exporting of shrimps under optimal conditions

3.2 SCOPE

- This study helps us to describe the different methods used to generate and apply live food organisms, as well as how they are used in shrimp hatcheries as most efficient feeds.
- Also makes us understand the conditioning and environment required for the culturing of microalgae.
- Provides a brief framework about the architecture of a shrimp hatchery and the process that takes place.

CHAPTER 4 MATERIALS & METHODOLOGY

4.1 Equipment & Glass wares

- Autoclave
- Titanium water heater
- Personal Protective Equipment
- Conical flasks – 250ml,1l

- Aerating pumps
- Dissolved oxygen probe
- Inverted microscope
- Polypropylene sediment filter

4.2 Chemicals & commercial products

1. Potassium permanganate used for sterilization purpose



Figure 7 Potassium permanganate solution

2. f/2 Medium

Table 1; Composition of F2 medium

I.	Sodium Nitrate	75gm/ml
	Ortho phosphate	5gm/ml
II.	E.D.T.A	4.36gm/ml
	FeCl ₃	3.15gm/ml

	Trace metals	1ml each
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III.	Sodium silicate	35gm/ml
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IV.	Vitamin	5ml/each
	Thiamine	20gm/ml
	D-Biotin	0.1gm/ml
	Cyno cobalamin	0.1gm/ml

Cupric sulphate (CuSO ₄)	10gm/ml
Zinc sulphate (ZnSO ₄)	22gm/ml
Cobaltous chloride (CoCl ₂)	10gm/ml
Manganous chloride (MnCl ₂)	180g/ml
Sodium molybdate (Na ₂ MoO ₄)	6gm/ml

Table 2; Trace metals composition

2) BioSphere's microparticulated shrimp feed

Main and vital ingredients are;

1. Fish meal
2. Brewer's yeast
3. Soy lecithin
4. Wheat by-products
5. Fish oil

Nutrient	PL 150 Feed
Moisture (%)	8,0
Protein (%)	55,0

Lipids (%)	10,0
Ash (%)	10,0
Fiber (%)	1,5
Calcium (%)	0,5
Sodium (%)	2,2
Phosphorous (%)	1,6
Vitamin A (1μ/kg)	15.000
Vitamin C(ppm)	200
Vitamin D3(1μ/kg)	2.000
Vitamin E (ppm)	200
n-3 HUFA (mg/g)	20,0

Table 3: Composition of BioSphere feed

Additives; Antioxidants: E321 Butylated hydroxytoluene (BHT) 67,5ppm, E320 Butylated hydroxyanisole (BHA) 75ppm,

Trace elements : 3b202 Iodine, Calcium iodate , anhydrous 2,5ppm , 3b8.14 hydroxyl-analogue of selenomethionine 0,2ppm , E6 Zinc- Zn, Zinc chelate of amino acids 100ppm , E 5 manganese-Mn , Manganese chelate of amino acids 50ppm , E 4 Copper- Cu , Cupric chelate of aminoacids hydrate 25ppm , E 1 iron –Fe , Ferrous chelate of amino acids 200ppm , vitamins and provitamins : 3a672a Vitamin A 25000 IU/kg , E671 Vitamin D3 2000 IU/kg.

3) Pro Hatch's probiotics

HATCHPRO is a specifically & scientifically formulated blend of non-pathogenic and pure microbial cultures of *Bacillus subtilis*, *Bacillus licheniformis*, *Bacillus megaterium*, *Bacillus polymyxa*, *Bacillus pumilus*, *Bacillus coagulans* and *Bacillus clausii* enriched with Nitrifying and Denitrifying Bacteria and *Saccharomyces cerevisiae* in a stable, compatible and nutritive base for efficient water conditioning in shrimp hatcheries.

4) Artemia

4.3 Methodology

4.3.1 Culturing of *Thalassiosira* as feed for shrimp

Though there are many microalgae that can be used as feed, the most efficient ones according to external and internal factors are 1. *Chaetoceros* sp. 2. *Thalassiosira* sp. *Thalassiosira* is vastly used in most south Asian countries compared to other microalgae.

STEP 1: Culture of *Thalassiosira* is commercially available, which is then cultivated by pure culture method using F2 medium and serial dilution in lab. The glassware used are autoclaved and sterilized beforehand. The lab should be cleaned and remain hygienic. The temperature of the lab is to be maintained around 20-23°C. To prevent any entrance of contaminant, the entrance of lab has potassium permanganate solution in which we are supposed to wash our exposed parts and any product that we take inside the lab.

STEP 2: (DAY1) the pure culture bought is transferred into a 250ml conical flask containing filtered sea water with provided aeration of dissolved oxygen. The sea water has to be filtered well with the use of polypropylene sediment filters in order to avoid any contaminations, the salinity of the water should be around 30‰. And pH – 8.2 -8.7

STEP 3: (DAY 2)

Right before continuing with serial dilution, F 2 medium is prepared with the required composition that's been mentioned in table 1 .Using the titanium rode, water along with the medium is heated to 20 and 25 °C. Then the culture in the 250ml conical flask is transferred into 1l jar containing the medium. The 1l jar should be autoclaved for 30mins at 121°C under 15lbs pressure. Aeration is provided through dissolved oxygen probe continuously and light is provided accordingly.

STEP 4: (DAY3)

Serial dilution is done from 1 l to 20 l sterilized can. The required optimum condition that are mentioned in the above steps are provided and left for observation for 24hrs. With a temperature of 23°C.

STEP 5: (DAY 4)

The colour change is observed to a yellowish brown which indicates the healthy algal bloom. It is then observed under microscope to check the live cells and dead, and also for any protozoan and other contaminations.

STEP 6: (DAY 5)

Serial dilution is done from 20l to 1 ton tank through the help of motor pump. The required optimum conditions and aerations are provided accordingly. FRP (Fibre – reinforced plastic) tanks are used. FRP tanks are designed to be lightweight whilst also offering a high degree of strength. Since FRP tanks resist rust and corrosion, they don't need to be painted or re-clad. Heat, tension, conductivity, and harsh weather conditions does n't bother it. As compared to concrete, it is more slip resistant, making it ideal for industrial applications and even more ideal for shrimp farming sectors.

STEP 7: (DAY 6)

The culture is then transferred from 1 ton to 10 ton tank, which is either made up of FRP or even charcoal sheets can be used as they are cost efficient. Then it is observed under optimum conditions for 48 hours

After DAY 8, the microalgae culture is ready to be fed for the shrimp Nauplius

The tanks used for the Nauplius should have the required optimum conditions such as,

1. Minimum light exposure
2. 30 - 35 °C.
3. pH 7.5
4. When Nauplius kept under the required conditions in the thalassiosira feed for 2 days approximately it'll evolve into zoea.

After zoea stage, while entering PL stage the shrimps are fed with probiotics and commercial feeds for further enhancement. In this case, we've used BioSphere's microparticulated feed along with Pro Hatch's probiotics.

The main components of BioSpheres PL 150 feed used especially from zoea stage to Post larvae stage contains of

1. Fish meal
2. Brewer's yeast
3. Soy lecithin
4. Wheat by-products
5. Fish oil

Stage	Amount (g/10 lakhs)
PI 1	25
PI 2	30
PI 5	50
PI 8	70
PI 10	100
PI 12	120

Table 4; Dosage of BioSphere feed given to respective stages

4.3.2 Probiotics used for shrimp

Probiotics is mainly used to control and compete with pathogenic bacteria as well as to promote the growth of the cultured organisms which in our case is shrimp especially during post larval stage.

BENEFITS OF USING PROBIOTICS

Inhibits growth of pathogenic bacteria and increases disease resistant. Maintains water hygiene and makes water clear by digesting the pathogens. Keeps the tanks free from toxin materials and malodorous. Highly effective in both fresh water and sea water. Leaves no residue and hence environment friendly.

HATCHPRO is a specifically & scientifically formulated blend of non-pathogenic and pure microbial cultures of *Bacillus subtilis*, *Bacillus lichiniformis*, *Bacillus megaterium*, *Bacillus polymyxa*, *Bacillus pumilus*, *Bacillus coagulans* and *Bacillus clausii* enriched with Nitrifying and Denitrifying Bacteria and *Saccharomyces cerevisiae* in a stable, compatible and nutritive base for efficient water conditioning in shrimp hatcheries.

Total viable count – NLT 8×10^9 CFU per gram

Under Normal Conditions: Day before stocking: 2 grams per MT of culture water

Day before stocking: 2 grams per MT of culture water

N5 to PL1: 1 gram per MT of culture water

PL2 to PL12: 0.5 gram per MT of culture water

4.3.3 Packing & Exporting

Packing is usually done at the respective area allotted for the process, Since it has to be clean and composed. The temperature of the packing area should be around 28°C .The ideal temperature for travelling and for retaining the metabolic functions of shrimp seeds is required to be approximately 26°C. Usually polyethylene covers are used for packing. Double polyethylene packing is done in order to prevent any leaking and to retain the atmosphere inside the packing to remain constant. The cover is filled up with dissolved oxygen. And are exported to the respective farms within 24 – 48 hrs duration at max.

4.3.4 Shrimp feed – For packing & exporting

The nauplii of the brine shrimp *Artemia* are the most commonly used food item in the live diets used in the larviculture of fish and shellfish. About 2000 mt of dry *Artemia* cysts are sold each year for on-site hatching into 0.4 mm nauplii around the world. Evidently, the small branchiopod crustacean *Artemia*'s exceptional ability to

form dormant embryos, also known as "cysts," can account for a large part of its reputation as an easy, suitable, or superior larval source of food.

Artemiacysts are cysts made up of latent *Artemia* embryos and a three-layered cyst shell. When hatching conditions are ideal. The embryos break free from the shell and hatch into nauplii, which are used to feed larvae or fry of commercially valuable species. (L.C.Lim et al, 2002). The alveolar layer, the hard outer shell of the *Artemia* cyst, can be completely removed using a chemical procedure known as decapsulation. (Sorgeloos, Bossuyt, Lavens. Leger, Vanhaecke & Versichele 1983). The decapsulated cysts might be used right away, or dehydrated in brine solution for long-term storage (brine cysts), or dried for longer-term storage (dried cysts). The advantages of decapsulation include disinfection of the cysts, improved hatching of the cysts into nauplii. Higher energy content of the nauplii. And no risk of fish larvae suffering from gut obstruction due to the ingestion of the cyst shell (Bengtson, Leger & Sorgeloos 1991).

While packing instead of algal feed or any other commercial feed, *Artemia* is used. The process of cultivation of *Artemia* is given below.

1. Wash and clean the tank with hypochlorite. Rinse the tank walls with water after disinfecting.
2. Use filtered seawater. Salinity – 25-35 ppt. pH: 8.0
3. Incubate up to 2.5g of cysts per litre water. Apply vigorous aeration from the bottom of the tank.
4. Always maintain water temperature at 28°C to 30°C, do not exceed 30°C. Optimal temperature: 29°C. Provide continuous artificial or natural Light. (Minimum 2000 lux at the water surface)

CHAPTER 5 RESULTS & DISCUSSIONS

5.1 STEP 2: (DAY1)

The culture has been successfully transferred to a 250ml conical flask. And the set-up is kept under the optimal atmosphere.



Figure 8 Day 1 of microalgae culture

5.2 STEP 3: (DAY 2)



Figure 9 Day 2 of microalgae culture

5.3 STEP 4 : (DAY3)



Figure 10 Day 3 of microalgae culture

5.4 Step 5 : (Day 4)

After 24 hours of leaving the culture undisturbed. The colour change is clearly observed when compared to the culture of day 3



Figure 11 Day 4 of the algal culture

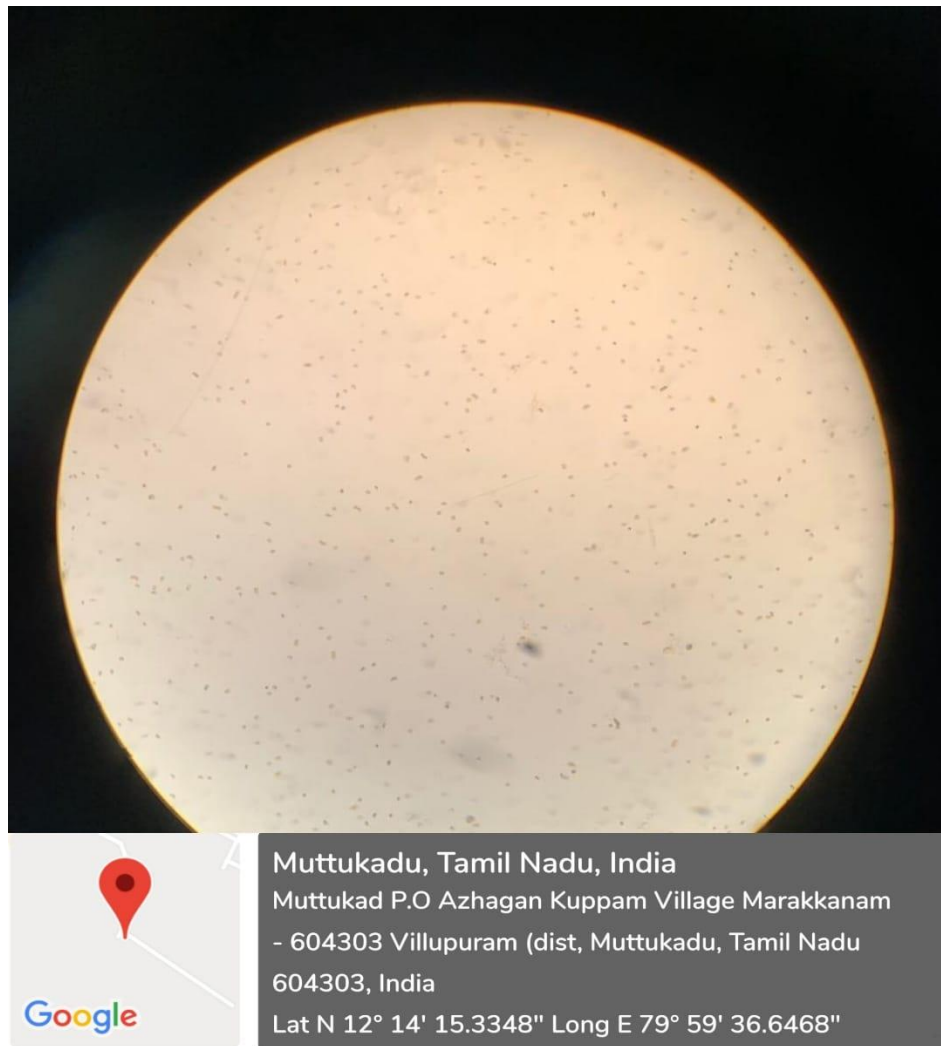


Figure 12 Microscopic view of the Thalassiosira from Day 4 of culturing

It is observed and it shows that the culture is free from any kind of contaminants or protozoans.

5.5 STEP 6 : (DAY5)

The cultures are further moved to outdoor labs from indoor lab due its high capacity. The culture is being successfully transferred from 20l can to 1ton tank with the help of motor.



Figure 14 Day 5 of culture (outdoor lab)(a)



Figure 13 Day 5 of culture (outdoor lab)(b)

5.6 Step 7: (Day 8)

The culture is then provided with the following conditions.

- Minimum light exposure
- Temperature around 30 - 35 °C.
- pH 7.5

The brownish colour indicates a healthy algal bloom and also proves to be ready to be used as a stable feed for Nauplius and the following stages.



Figure 15 Day 8 of the culture

5.6 Step 8

The prepared *Thalassiosira* feed is transferred into a pond containing Nauplius through motor. The pond is designed to prevent excessive lighting and other external factors as in temperature, insects etc than the required amount. They are constructed very securely and contaminant-free. The temperature is kept under checks of regular intervals. When Nauplius kept under the required conditions in the *thalassiosira* feed for 2 days approximately it'll evolve into zoea.



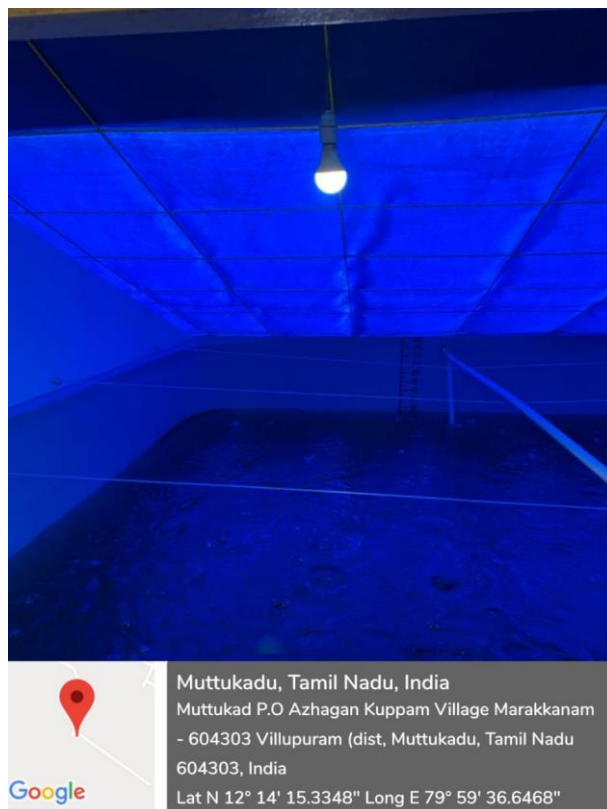


Figure 16 Structure of shrimp pond

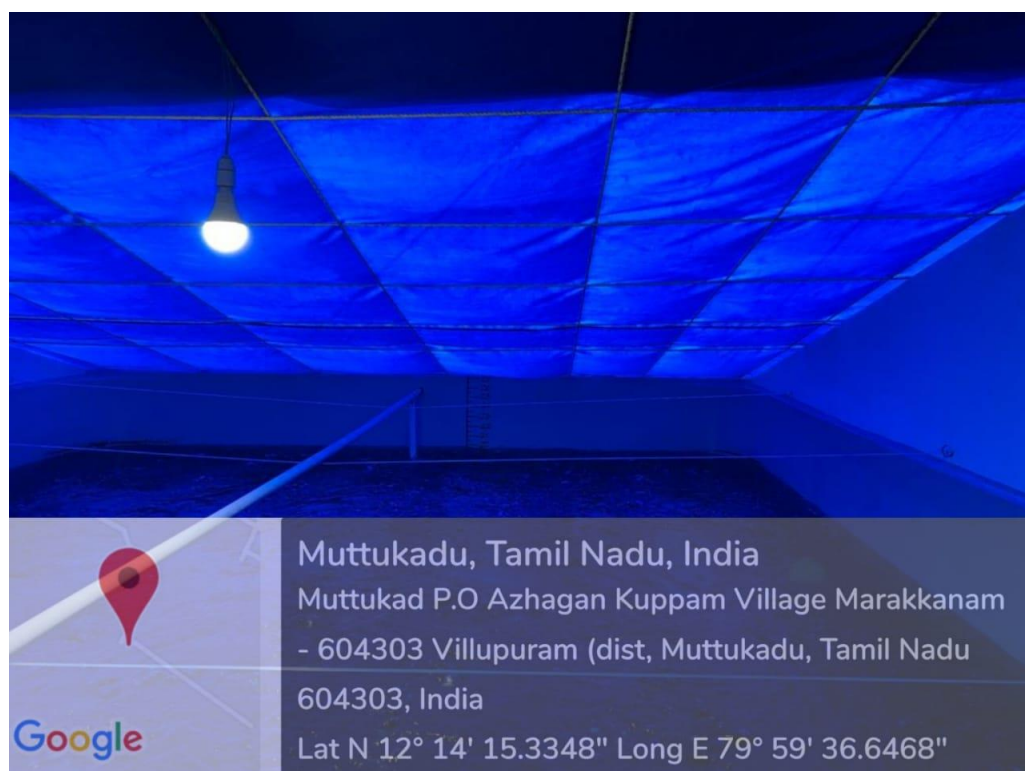


Figure 17 Shrimp pond for zoea stage



Figure 18 Zoea stage

5.7 Step 9 – Microparticulates commercial feed and probiotic

Once the shrimps enter the post larval stage known as PL stage, they are fed with BioSphere's microparticulated feed to enhance their values. Along with probiotic of Pro Hatch companies to increase and intensify their immune system and protect from any protozoan entry.



Figure 19 BioSphere feed



Figure 20 Pro Hatch probiotic

The composition and benefits regarding the microparticulated feed has been mentioned before in the chapter 4.2. And the dosage given at the respective stage is given in detail (table 4). Accordingly the shrimps are fed, 6 times per day.

The probiotics are fed by the following,

- ◆ Under Normal Conditions: Day before stocking: 2 grams per MT of culture water
- ◆ Day before stocking: 2 grams per MT of culture water
- ◆ N5 to PL1 : 1 gram per MT of culture water
- ◆ PL2 to PL12: 0.5 gram per MT of culture water

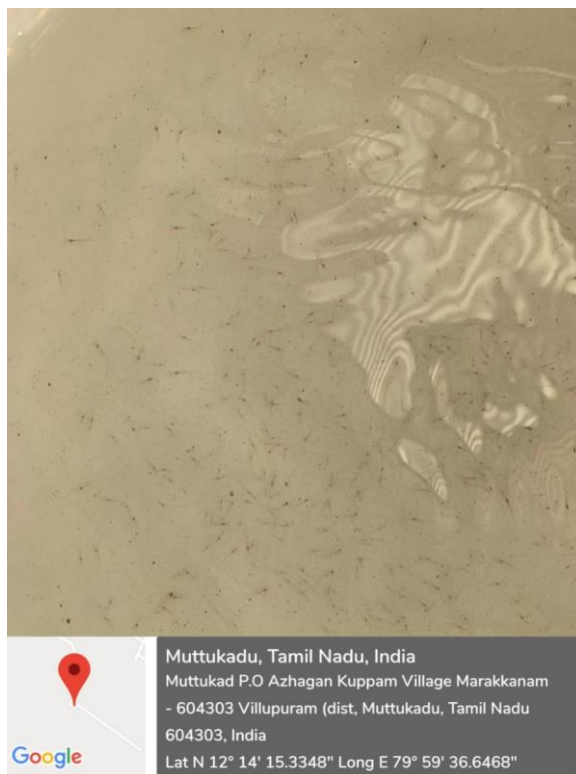


Figure 21 PL stage 8



Figure 22 PL stage 8

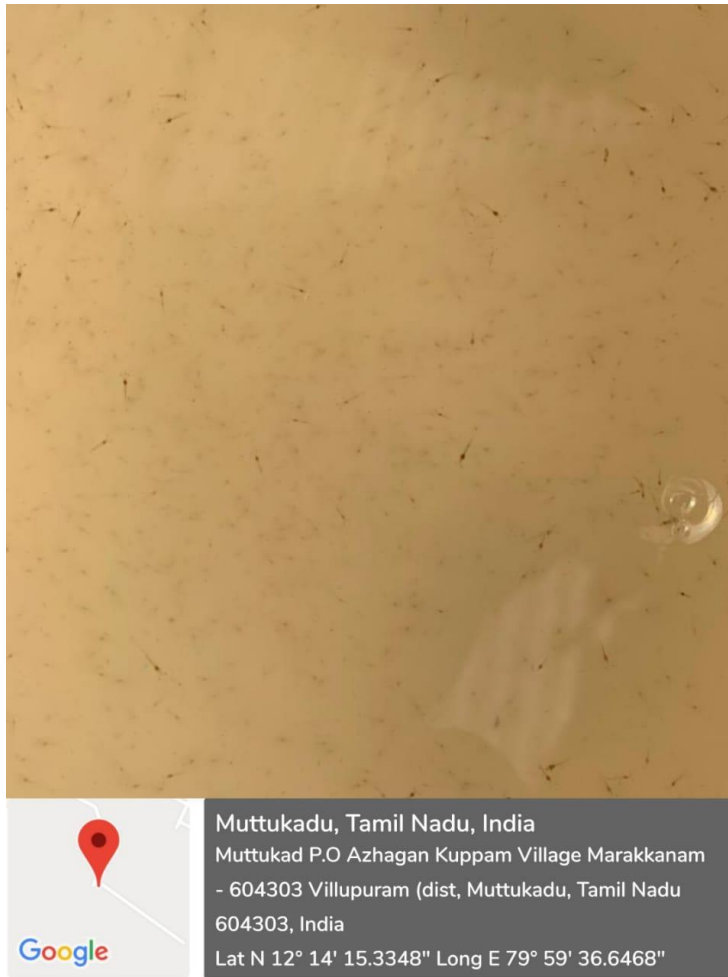


Figure 23 Pl stage 10

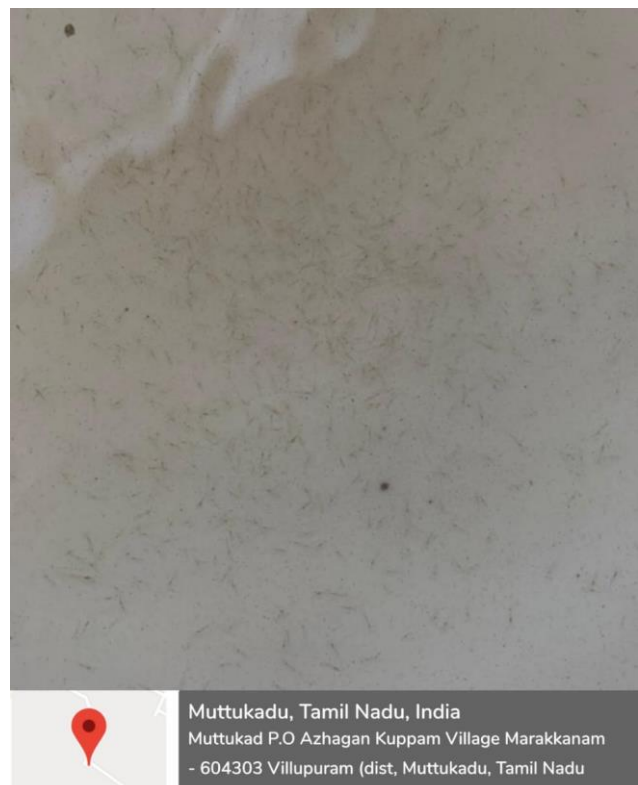


Figure 24 Pl Stage 10

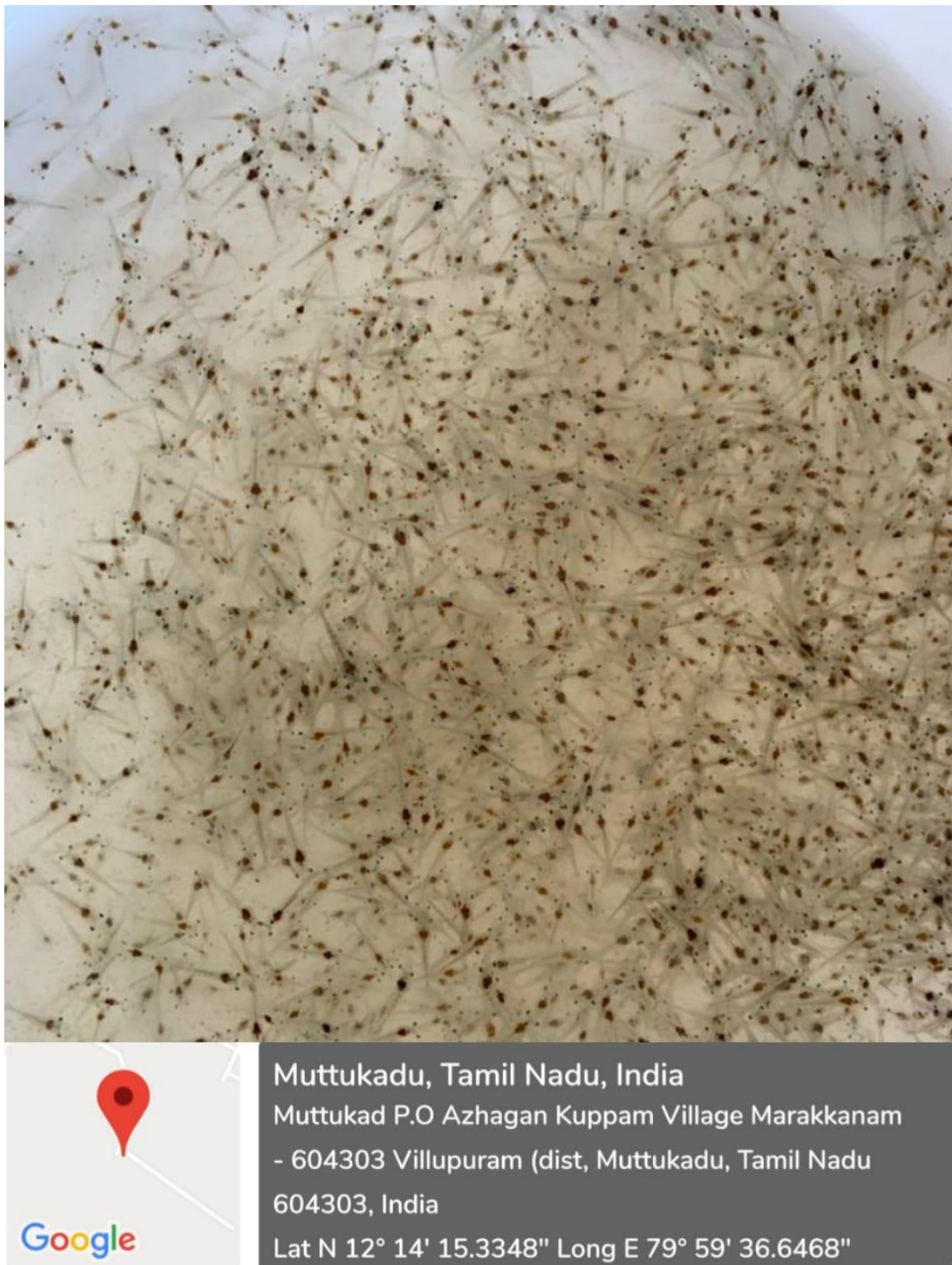


Figure 25 Pl stage 13

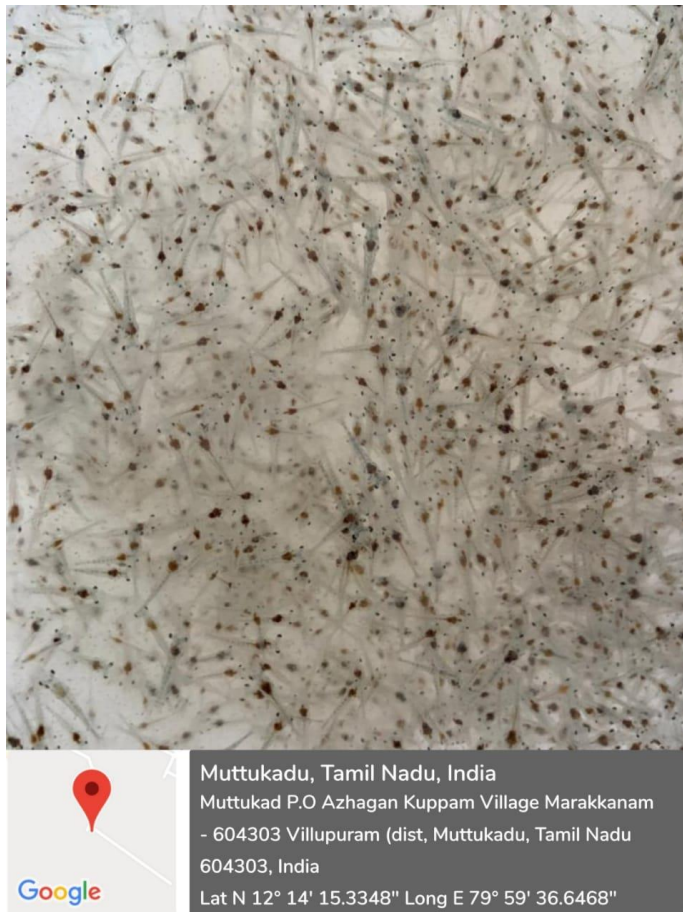


Figure 26 Pl stage 15

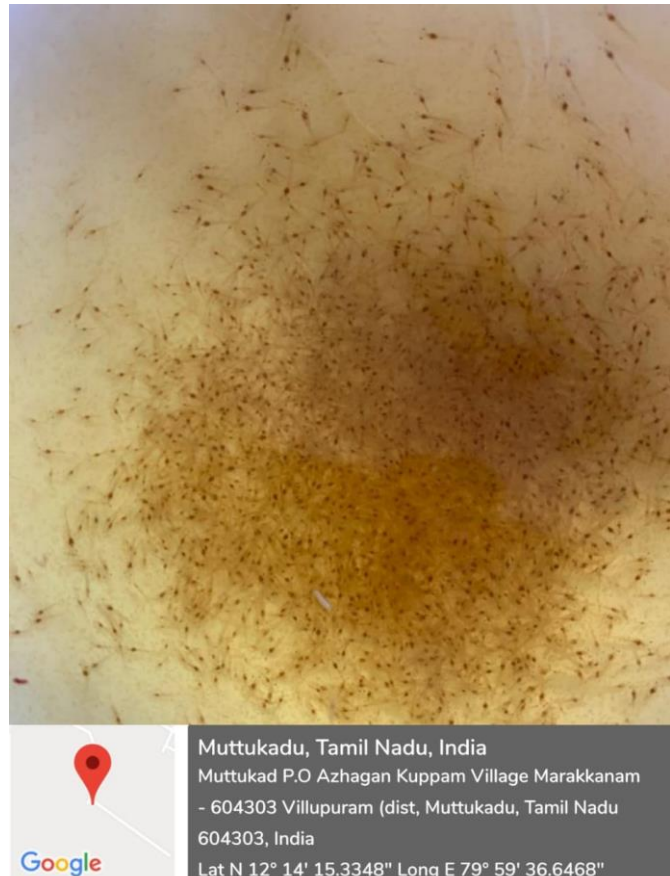


Figure 27 PL Stage 15

Once the shrimps attain the size of Size $\geq 10\text{mm}$ during the PL stage 15, it refers to a healthy growth. After which they are packed and exported to the demanded out-grown shrimp farms. The protocol and conditioning of the packing process is been explained briefly in chap 4.3.3.

Artemia is used as feed while packing as they are easily digestible compared to other feeds in an closed environment.



Figure 28 Sea water preparation for artemia

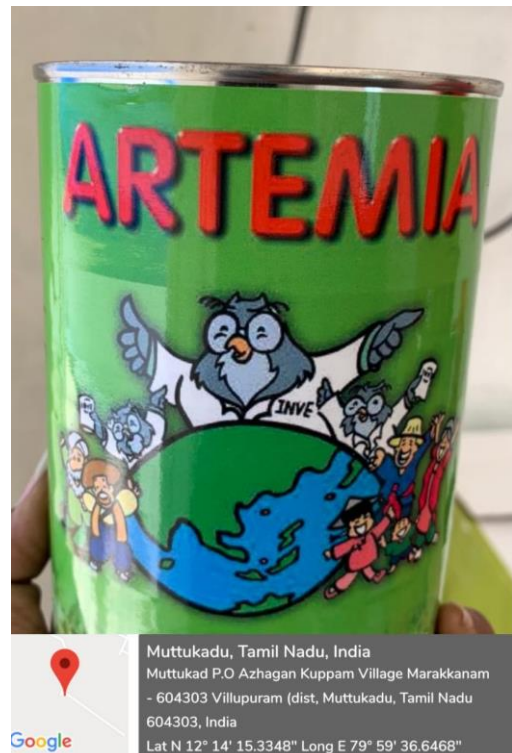


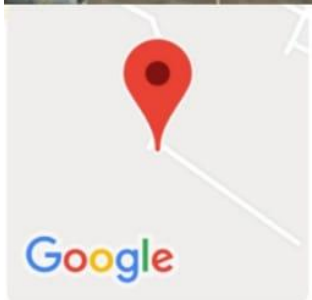
Figure 29 Artemia



Figure 30 Artemia



Figure 31 Culturing of Artemia



Muttukadu, Tamil Nadu, India
Muttukad P.O Azhagan Kuppam Village Marakkanam
- 604303 Villupuram (dist, Muttukadu, Tamil Nadu
604303, India
Lat N 12° 14' 15.3348" Long E 79° 59' 36.6468"

Figure 32 Lab setup for artemia cultivation

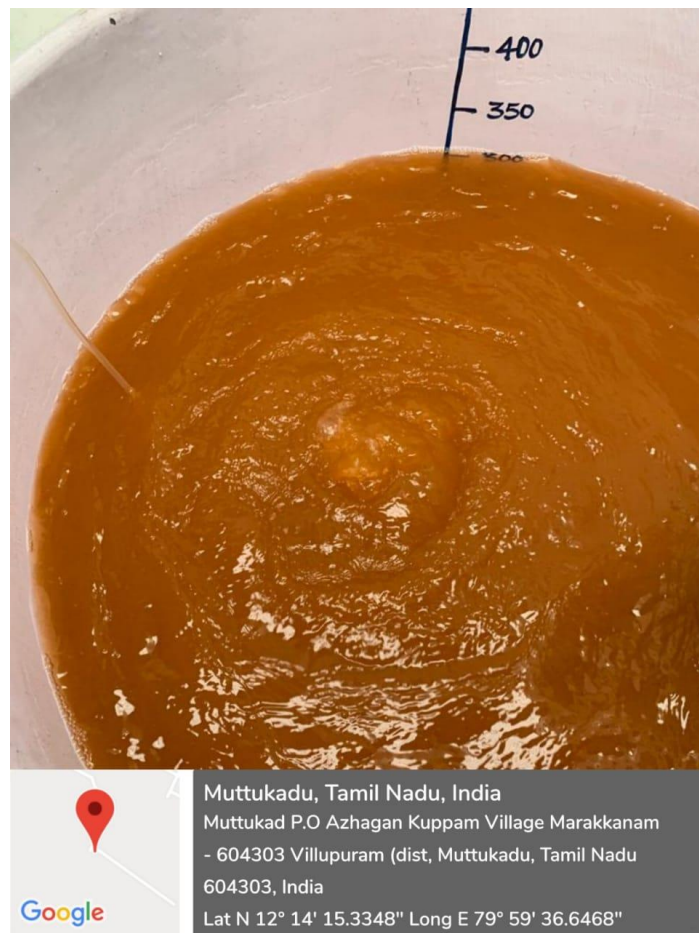


Figure 33 Artemia culture

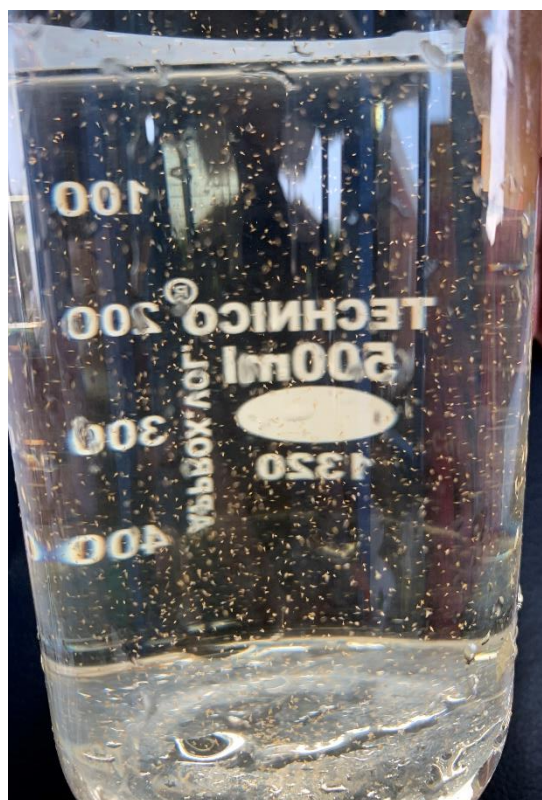


Figure 34 Artemia culture for packing



Figure 35 Instrument used for counting of shrimp

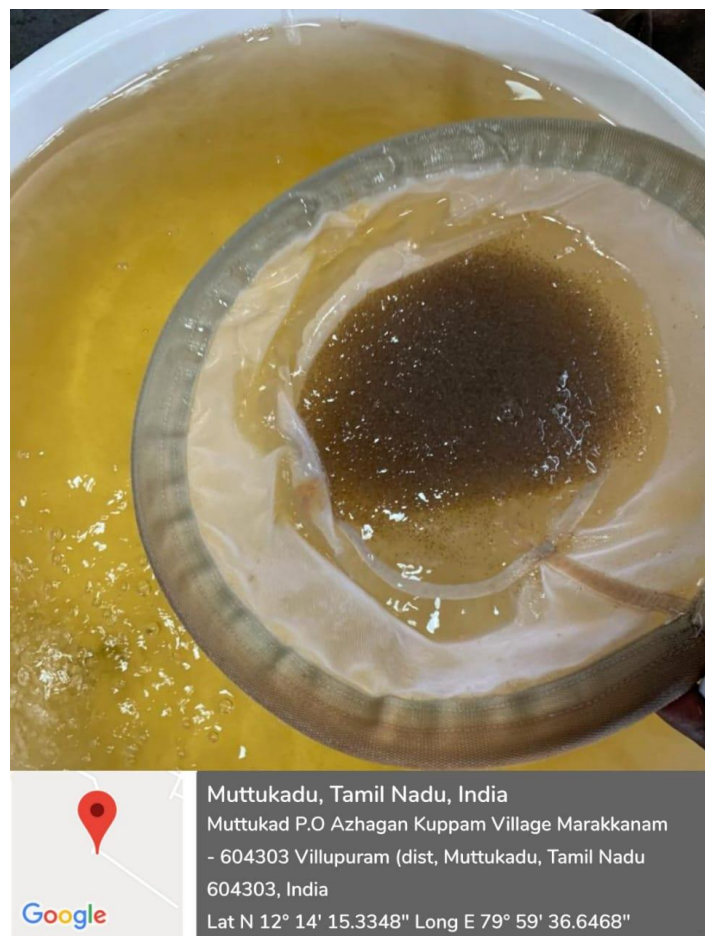


Figure 36 Packing process a



Figure 37 packing process b

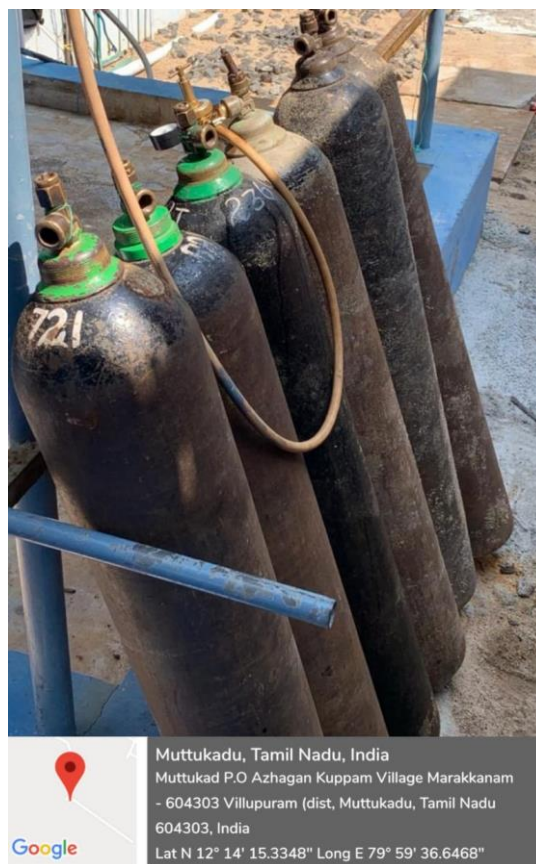


Figure 38 Dissolved oxygen for packing process

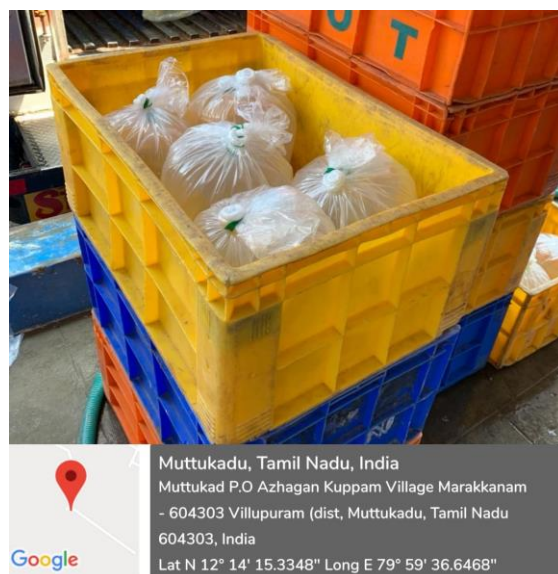


Figure 39 Packed shrimp for export

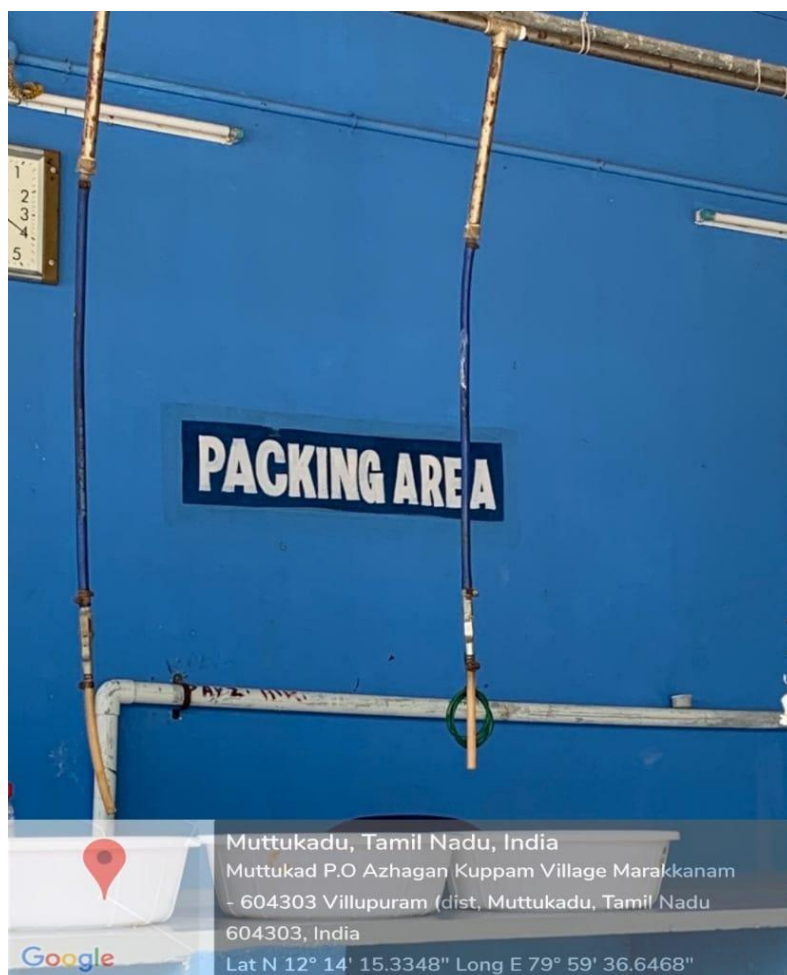


Figure 40 Packing area set-up

5.8 An outlook on the framework of shrimp hatchery



Figure 41 Widelook on *Thalassiosira* culturing pond

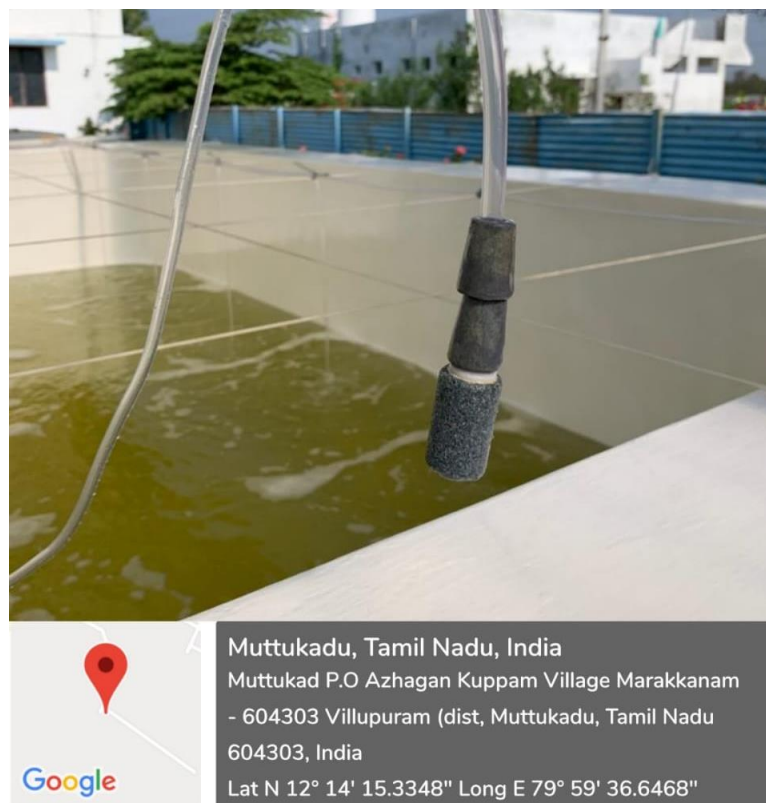


Figure 42 Dissolved oxygen probe for pond

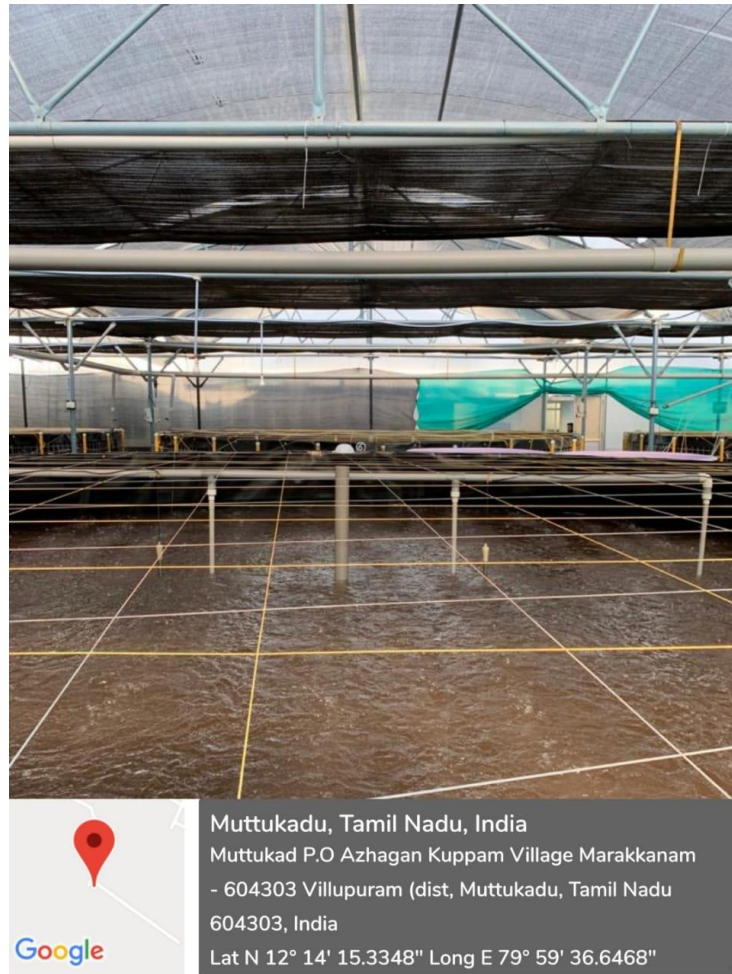


Figure 43 Using charcoal plated ponds instead of FRP

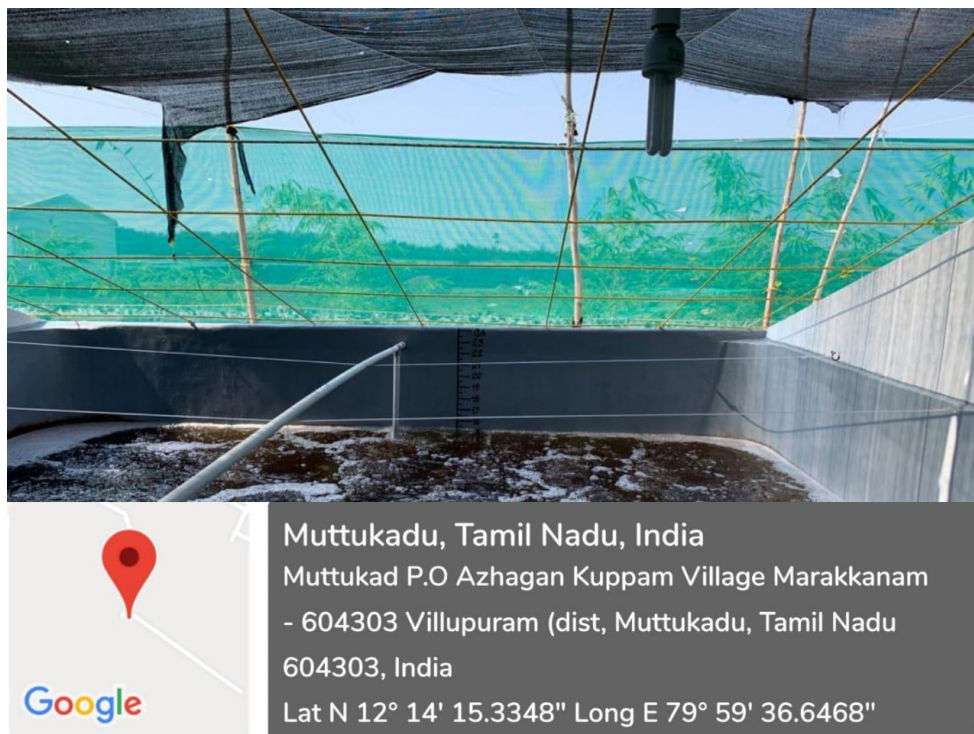


Figure 44 Post larval pond

CHAPTER 6 SUMMARY & CONCLUSION

Thalassiosira is a wide - ranging genus that includes species with a variety of anatomical trends, as currently described. While *Thalassiosira* has not been commonly reported as a vital element of genuinely freshwater systems, the genus is represented in the freshwater flora by a variety of morphology. It is difficult to classify *Thalassiosira* concisely due to the wide range of morphological variation present, and further research may lead to additional classification changes. (Hasle, 1978; Hasle and Lange, 1989). After feeding the shrimp with the cultivated microalgae, each and every stage such as Nauplius, zoea, mysis to post larvae are closely observed while providing with the ideal environment. Providing with optimum temperature, pH the growth is found to be healthy without any contaminant or protozoan being reported. By the time the shrimp reaches PL stage 15, they are ready to be exported to the demanding out-grown shrimp farm. The process of packing is been done with utmost consciousness regarding the environment that is been provided inside the package. The number of seeds per pack are counted by the instrument according to the required amount. The exporting should be done with 24hrs after packing, any delay in the process may lead to serious issues such as release of ammonia inside the package caused by the excreta of the shrimps. Also the feed given during packing is easily digestible which may also lead to deficient of the feed thereby attacking one and other.

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