

**ASSESSMENT OF TOXICITY EFFECTS OF IRON AND
POLYSTYRENE MICROPLASTICS IN A MARINE POLYCHAETE
EMBRYO (*NAMALYCASTIS JAYA*)**

PROJECT REPORT

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

By

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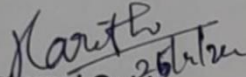
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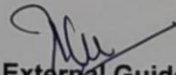
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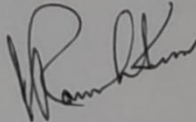
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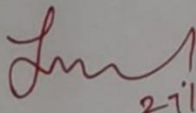


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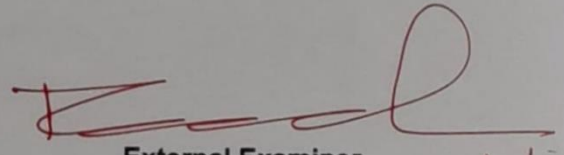
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ACKNOWLEDGEMENT

I am pleased to acknowledge my sincere thanks to **THE BOARD OF MANAGEMENT OF SATHYABAMA INSTITUTE OF SCIENCE AND TECHNOLOGY** for their encouragement in doing this project and for completing it successfully. I am grateful to them.

I convey my thanks to **Dr. REKHA CHAKRAVARTHI, Dean, School of Science and Humanities** and **Dr. V. RAMESH KUMAR, M.Phil., Ph.D., Head of the Department, Department of Biotechnology** for providing me necessary support during the progressive reviews.

I would like to express my sincere and deep sense of gratitude to my Project Guide **Ms. S. HARITHA (Assistant Professor) and Dr. M. RAVI (Assistant Professor Research) AT CENTRE OF OCEAN RESEARCH** for his valuable guidance, suggestions and constant encouragement 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.

ABSTRACT

Microplastics and heavy metals are emerging as global pollutants in the marine ecosystem and they pose a great threat to marine organisms. They originate from primary and secondary sources. Due to various degradation and ageing, these microplastics can adsorb chemical substances, persistent organic pollutants (POPs) such as polychlorinated biphenyls (PCBs), polycyclic aromatic hydrocarbons (PAHs), and pesticides such as dichlorodiphenyltrichloroethane (DDT) or hexachlorobenzene (HCB) and some heavy metals and cause adverse effects. Microplastics that absorb heavy metals have been ingested into marine organisms. The ingested pollutants can be toxic to polychaete egg cell division. The accumulated pollutants can cause internal or external damage in cell wall of egg. Microplastics increase the chance of heavy metal damages. Therefore, this study aimed to identify the toxicity effects of iron and microplastics in a model species polychaete worm egg (*Namalycastis jaya*). The polychaetes were exposed to Iron and microplastics alone for an acute period of 24 and 48 hours. Morphological cell division analysis was performed to identify the cell death due to highest concentration of microplastics 10^{-5} exposure. The results indicated that toxic effects of microplastics increased cell/egg death.

Keywords: Microplastics, *Namalycastis jaya*, Heavy metals, cell death.

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LIST OF ABBREVIATION

POPs	Persistent Organic Pollutants
PCBs	Polychlorinated Biphenyls
PAHs	Polycyclic Aromatic Hydrocarbons
DDT	Dichlorodiphenyltrichloroethane
HCB	Hexchlorobenzene
MP	Microplastics
Fe	Iron
CDOM	Chromophonic Dissolving Organic Matter
FM	Fertilization Membrane
PE	Polyethylene
PP	Polypropylene
PS	Polystyrene
PET	polyethylene terephthalate
PVC	polyvinyl chloride
LDPE	low density polyethylene
HDPE	high-density polyethylene

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CHAPTER-1

INTRODUCTION

1.1 MARINE ENVIRONMENT:

The term "marine environment" describes every type of plant, animal, and creature found in the earth's oceans and seas. Marine animals are divided into four different taxonomic groups: these include cetaceans (whale, dolphin, porpoises), pinnipeds (seals, sea lions, walruses), sirens (manatees, dugongs), and marine fish pets (polar bear, sea otter). According to the World Register of Marine Species, worms, the total number of marine species known is approximately more than 2,42,000. However, there are an estimated 14 to 1.6 million marine species on earth. Marine worms are any worms that are found in marine environments. Marine worms are classified into six phyla. Platyhelminthes (flatworms), Nematode (roundworms), Annelida (worms), Chaetognatha (arrow worms), Hemichordate (fish eggs) and Phoronida (horseshoe worms). One of the most abundant marine creatures are these segmented earthworms, which may be seen swimming freely close to the surface, living in the deep ocean, or burrowing into the sand and mud of shores. Marine worms are long, narrow-bodied invertebrate mollusks. They possess neurological, circulatory, and digestive systems. Their bodies are coated with a stiff yet flexible collagen outer layer. Brightly colored, multisegmented worms known as Polychaetes may be found in all ocean settings, from the deepest abysses to the shallow estuaries and rocky coastlines, and they can be seen swimming freely in open water.

1.2 MARINE POLLUTION:

The introduction of human substances into the marine environment that have an adverse effect, such as the depletion of biological resources, hazards to human health, obstructions to marine activities like fishing, degradation of seawater use, and reduction of facilities, is known as marine pollution. Marine pollution occurs when human-produced or dispersed substances, such as smaller particles, noise, invasive organisms, industrial, agricultural, and domestic trash, are released into the ocean and cause harmful effects in the marine environment. Although shipping also makes a substantial contribution, land-based activities lead to the majority of this waste (80%). The continental shelf is particularly susceptible to contamination since the majority of inputs originate from the land and are carried down by rivers, sewage, or the atmosphere. Particles of iron, carbonic acid, nitrogen, silicon, Sulphur, pesticides, or dust are introduced into the ocean by air pollution, which is another contributing factor. Pollution frequently originates from non-point sources like agricultural runoff, debris carried by the wind, and dust. The primary cause of these nonpoint sources is the runoff that flows into the ocean through rivers. However, wind-blown debris and dust can also contribute to pollution as they settle in the oceans. The pathways through which pollution spreads include direct discharge, inland runoff, pollution from ships, air pollution, and the possibility of pollution from deep-sea mining.

1.3 HEAVY METAL POLLUTION:

Although heavy metals are necessary for life and often found in nature, they may accumulate in organisms and become hazardous. In modern times, metallic chemical elements and metalloids that are hazardous to humans as well as the environment are referred to as "heavy metals." The most common heavy metals found in the environment include lead, copper, nickel, chromium, mercury, cadmium, and arsenic. The term "heavy metals" refers to metallic substances having extremely high densities when separated from

water. Heavy metal contamination is a major environmental problem in the modern world. Rapid industrialization leads to industry development, which continually increases the concentration of metals and creates serious pollution difficulties. This is because industrial waste products are incorrectly disposed of, ending up in land areas and water bodies (Dixit *et al.* 2015). The primary components of the earth's crust are heavy metals. Heavy metals are strong environmental pollutants that cannot fully biodegrade and must instead transform into harmless forms (Coral *et al.*, 2005). These metals are directly linked to problems with biological toxicity and environmental contamination due to their strong inhibitory effects on biodegradation processes. (Nagajyoti *et al.*, 2010; Gupta *et al.*, 2014). Metals are often used as cofactors in many enzymatic and metabolic processes, and they are required in small amounts as nutrients. Higher concentrations of these metals, however, may be exceedingly hazardous or suppressive to all living forms, including humans, animals, plants, and microbes. Despite the fact that a relatively small number of metals, such as cadmium (Cd), arsenic (As), and mercury (Hg), are exceedingly deadly at very low quantities (Altus and Bartha 1993; Nes 1990), These can also enter the body through food, water, or the air and accumulate over time (Lenntech 2004, Deeb, and Altahali 2009). The accumulation of lethal metals in the human body can lead to numerous health issues such as abnormal growth and development, neuromuscular abnormalities, mental illnesses, and metabolic malfunction (Thiele 1995; Chandra *et al.* 2011). The main sources of toxic metals in the environment are a range of activities and processes, such as the burning and release of toxic waste into the atmosphere, as well as excessive emissions of wastewater and effluents that contaminate soils and surface waters with hazardous chemicals and other pollutants. It has become harmful to the diversity of life on Earth because dangerous metals are constantly utilized in anthropogenic activities such as electroplating, painting, tanning, textiles and dyes, papermaking, mining, and others. (Dhal *et al.*, 2013; Yadav *et al.*, 2017). These industrial processes regularly release large amounts of dangerous metals, which raises the risk of environmental contamination. Therefore, to protect the environment, their removal or remediation is

necessary. (Dhal *et al.* 2013; Velma *et al.* 2009). As a result, biological remediation using microbes and plants is often regarded as an ecologically benign, safe, inexpensive, and long-lasting solution for the elimination of hazardous metals when compared to standard treatment technologies. However, in comparison to biological treatment methods, which are easy to use and do not create secondary pollutants, traditional systems have certain limitations, such as high operating costs, maintenance needs, the development of harmful byproducts, or inefficiency (Vargas *et al.* 2012). Chandra *et al.* (2011). Bioremediation and phytoremediation are the techniques that employ microorganisms and plants to decrease and detoxify very harmful pollutants into safe and less destructive forms. (Jain *et al.* 2014).

1.4 MICROPLASTIC POLLUTION:

The utilization and production of plastic have experienced a significant increase in recent years, primarily due to its cost-effectiveness. The most prominent synthetic plastics are polyethylene (PE), polypropylene (PP), polystyrene (PS), polyethylene terephthalate (PET), polyvinyl chloride (PVC), low density polyethylene (LDPE) and high-density polyethylene (HDPE) and constitute 90% of the worldwide plastic production. Consequently, there has been a rise in the disposal of non-recycled synthetic plastic polymers in both terrestrial and aquatic ecosystems. Microplastics, which are defined as small plastic fragments with dimensions equal to or less than 5 mm, are particularly prevalent in marine habitats. Various marine organisms, such as corals, plankton, marine invertebrates, fish, and whales, ultimately entering the food chain, can ingest these minuscule plastic particles. This direct consumption of plastic polymers poses a substantial threat to marine organisms and indirectly affects the ecosystem by adsorbing other marine pollutants. Microplastics, due to their high surface area-to-volume ratio, readily absorb hydrophobic pollutants from the aquatic system. Consequently, the pollution caused by microplastics has become a concerning issue, primarily due to its detrimental effects on marine health and biodiversity. Microplastics are introduced into the aquatic ecosystem primarily through domestic runoff,

which contains microbeads and fragments of microplastics. Additionally, the fragmentation of large plastic trash contributes to this issue. Plastic manufacturing industries further exacerbate the problem by releasing plastics in the form of pellets and resin powders, which are produced through air-blasting techniques. These plastics ultimately contaminate the aquatic environment. Furthermore, coastal activities such as fishing practices, aquatourism activities, and marine industries also serve as sources of microplastic pollution in the marine ecosystem. Microplastic particles persist in the marine ecosystem and, due to their small size, are often mistaken for food by various marine organisms such as corals, phytoplankton, zooplankton, sea urchins, lobsters, and fish. This leads to their ingestion and subsequent transfer to higher trophic levels. The impact of microplastics on marine life is a significant concern as they can result in entanglement and ingestion, posing a threat to the survival of marine species. These microplastic fragments primarily originate from terrestrial sources, posing a great threat to coastal ecosystems like coral reefs due to pollution. (Subhankar Chatterjee.2019).

1.5 POLYCHAETE:

Polychaetes are marine annelid worms that can help with aquaculture diversification. Bristle worms, or polychaetes, are a type of annelid worm that live in marine environments. There are 8,000 species in the world, with 153 of those being found in India. Sensitivity to reduced oxygen levels and accumulation of heavy metal pollution indicators has been viable and commercially attempted, but intensive production has progressed only in a few countries around the world. Imported or harvested marine polychaetes are used in the non-producing nations. A significant and ongoing investment in research has improved the knowledge of some species' nutritional needs and reproductive processes. Recent research has revealed new technical advancements that have the potential to significantly increase productivity and stimulate the production of polychaetes. It was determined that a few species of marine worms would make excellent subjects for integrated

multitrophic aquaculture. The development of cost-effective aquaculture techniques Polychaetes are marine multi-segmented worms with significant ecological and biotechnological importance. Polychaete applications in various fields, as well as their cultural prospects, are briefly discussed. Their function as "stress indicators" in environmental impact assessment studies is substantial. They also contributed significantly to the sea fishing industry's bait supply and provided a very good live feed for aquaculture. This article elaborates on the characteristics of polychaetes that are diagnostic. Polychaetes are abundant in many seabed habitats and play an important role in the upkeep of benthic communities. They are found throughout marine ecosystems and can reach high densities there. A great number of polychaetes are sessile, tube-dwelling organisms that are commonly gregarious and can create primary biogenic structures or alter the abiotic properties of the substrate that already exists. They are active builders of forest-like structures that provide new substrates for other benthic species, influence habitat conditions, and regulate ecosystem functioning. Therefore, the engineers of animal forests are said to be polychaetes. This chapter reports the main evidence of polychaetes acting as habitat formers on sedimentary and rocky bottoms, examines the time-scale variations of biogenic structures, and illustrates the threats and sensitivity of polychaete forests to help inform decisions about coastal ecosystem management and protection. Polychaetes are abundant in most marine habitats and dominate many neonatal communities. The functional classification of guilds based on identity and taxonomy connects community structure and ecological function. Molecular genetic analyses have placed siboglinids, echiurans, and myzostomes in the Annelida class, while osmotic siboglinids are currently included in the functional groups. Understanding the mechanics of encounters has made it evident how feeding schedules and locomotion are related. The distinction between surface and subsurface feeding is unclear, and the prevalence of bilateral symmetry is explained by new analyses of burrowing mechanisms. Different digestive strategies further support the dichotomy between microscopic deposits and suspension and macrophage predators, herbivores, and omnivores. Juveniles and small worms are

frequently limited to larger, richer foods and digestible organic matter, suggesting that deposit feeding is primarily reserved for worms larger than 1 cm. The confusing microphage-microscopic dichotomy applies well to larger worms.

1.6 LIFE CYCLE OF POLYCHAETE:

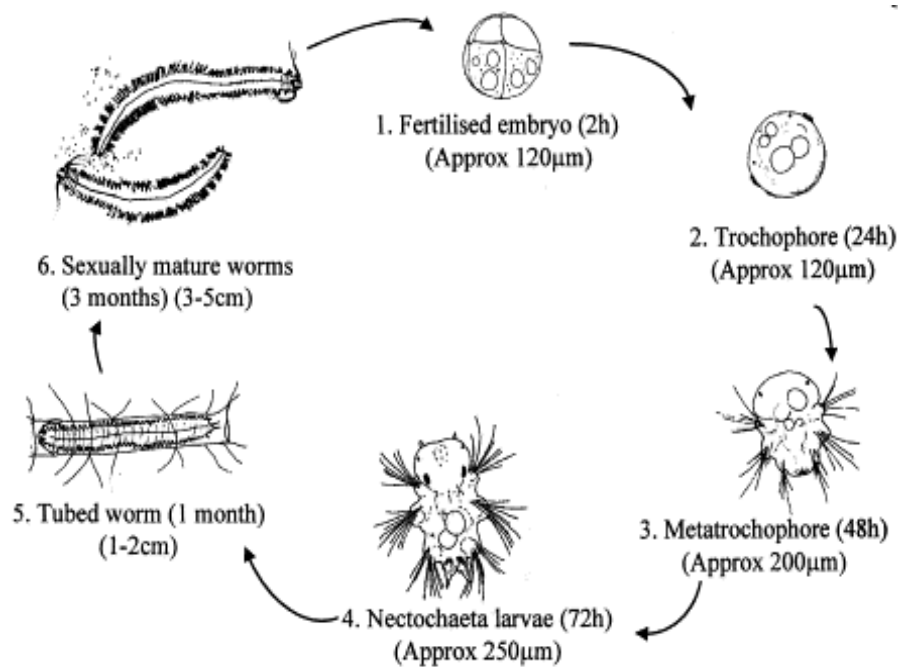


Fig 1.1 Larvae Development of Marine Polychaete.

Polychaetes have a wide variety of reproductive patterns. Males and females of several wayward species, including Nereids and Eunicids (which include beach worms), spill their gametes into the water, where fertilization occurs. Certain species that have been researched have populations that spawn only during specific periods of the year. For example, the Palolo Worm (*Eunice viridis*), a well-known worm that is native to Samoa, spawns in October or November at the start of the lunar quarter. At this point, the eyes and parapodia found in each mature worm in the population develop, and swimming chaetae form. When the new moon appears, they escape their

burrows and swim to the surface. In this area, the worms move quickly, and the males release their gametes into the water, which stimulates the females to release or deposit eggs. In order to ensure proper fertilization, spawning must synchronize, which is governed by environmental parameters like temperature, day length, or moon phases. Following fertilization, cell division begins, and a few hours later, a larva, or trochophore, has been developed. These pelagic animals can stay in the plankton for several months. They feed on other plankton organisms or reserves that were absorbed into the egg before it was shed. Following the completion of their larval development, the larvae sink to the sea bottom or settle on the coast due to a change in behavior. If all the necessary conditions are met, the larvae will metamorphose into adult form.

During breeding, some polychaetes, like some polynoids (scale worms), which are typically aggressive towards one another, form pairs. The male perches on top of the female and fertilizes the eggs as they are laid. The female then uses her parapodia to transfer the fertilized eggs beneath her back's scales. The worms then separate and become aggressive towards one another again. Underneath the scales, the eggs undergo development. Depending on the species, the larvae are eventually discharged into the plankton to finish their development. Certain serpulids exhibit a specialized structure on the underside of the operculum, which functions as a protective brood chamber for the larvae throughout their developmental stages. (Hutchings, p.1972).

The three phases that larval polychaetes typically go through are the trochophore, metatrochophore, and nectochaete stages. Trochophores are larvae with ciliated bodies. The prototroch, a band of cilia, is used for feeding and locomotion. Larvae of trochophores often have a large anterior and a tapering posterior. The larva's anterior region is referred to as the episphere, while its posterior region is termed the hyposphere. Typically, they are pelagic. Usually, two or three segments have developed by the time the metatrochophore stage occurs. By this developmental stage, parapodial

development (with or without setae) may be seen. Neuropodia and notopodia are terms used to describe the dorsal and ventral podia, respectively. The anterior-most portion is known as the prostomium. The peristomium is located just behind the prostomium. The head is made up of these two anterior regions. Both antennae and eyes develop in the prostomium. In the peristomium, the mouth, palps, or tentacular cirri develop. Planktotrophic larvae can grow a mouth and stomach while within plankton. Others depend on yolk chemicals for survival and are lecithotrophic. The segments of a larva that have grown setae are referred to as setigers. The pygidium is the most posterior section. New segments emerge gradually from the pygidium. Necrochaetes are advanced larvae. There are usually many more setigers in nematochaetes than in metatrochophores. The larvae may no longer be pelagic and may have lost their ciliation, depending on the species. After settling, larvae may undergo a metamorphosis to become juvenile worms, or they may proceed through a benthic stage known as the erpochaete stage. (Shanks, A., 2001.)

1.7 NAMALYCASTIS JAYA:

Namanereidinae (Polychaeta: Nereididae) is one of the most effective brackish water colonizers. The subfamily is now considered to consist of three genera: *Namalycastis* Hartman, 1959; *Namanereis* Chamberlin, 1919; and *Lycastoides* Johnson, 1903. *Namalycastis* is now the most diverse genus in *Namanereidinae*, as well as one of the most effective polychaetes in contaminated coastal environments. The species is often seen in littoral or supralittoral habitats, together with decaying plants and other organic-rich environments, or near the shore. *Namalycastis* species exhibit minute morphological diversity, which may provide difficulty for taxonomists. This is in part due to their simpler body structure, which lacks a notopodium and has fewer chaetae compared to other *Nereididae*. A new species of *Namalycastis* from the southwest coast of India was based on morphological examinations and phylogenetic analysis utilizing both mitochondrial and nuclear loci to confirm the species' originality. *Namalycastis jaya* is a brackish-water

polychaete worm from the Nereididae family found on Kerala's southern coast in southwest India. The worm is named after Tamil Nadu Chief Minister J. Jayalalithaa, who helped to improve the state's education system. The holotype displays a body widest around the mid-anterior region, gradually tapering towards both ends. Its small antennae are distally pointed and aligned with the mid-palps. Brown pigmentation is present on the front and back upper portions of the body. The triangular prostomium has a deep anterior cleft and a longitudinal groove running from tip to rear, slightly indented laterally. Two pairs of black eyes are arranged obliquely, with the rear pair notably smaller. Tentacle cirri extends backward to the second segment. The jaws feature eight teeth: four-sub terminal and four unsheathed proximally. The acicular neuropodial ligule is bilobed, with the superior lobe larger. Dorsal cirri lengthen towards the rear. Typically, there are fewer than four sesquigomph spinigers in the mid-body neuropodial supraacicular fascicle. Notochaetae are absent in all parapodia. Heterogomph chaetae have a normal boss length. Various types of chaetae exhibit finely or roughly serrated blades in different body segments. The pygidium has a black, multi-incised rim and two lateral anal cirri. *Namalycastis jaya sp. n.* shows resemblances to *Namalycastis elobeyensis* Glasby, 1999, and *N. hawaiiensis* Johnson, 1903, as they all lack notochaetae. The name *Namalycastis jaya sp. n.* is most likely derived from the Dondra population and the species' more widespread distribution in southern India and Sri Lanka.

1.8 ENVIRONMENTAL IMPORTANCE OF POLYCHAETE:

Polychaetes are essential components of the marine ecosystem. Polychaetes are excellent bio-indicators of organic pollution due to their enormous variety, abundance, and functional significance. Significant function in food networks. They are Burrowers Polychaetes' burrowing and feeding activities improve sedimentary processes while releasing hydrophobic organic pollutants. Polychaetes play a crucial role in organic matter burial because they regulate the quantity of organic carbon in the

ocean and atmosphere. Important Polychaetes have long been considered the natural option for representative species due to their small size, short life cycles, and simplicity of management. Polychaetes' life cycle characteristics make them ideal candidates for serving as indicator species. Estuarine polychaetes performed the most significant ecological services, namely sediment bioturbation and organic matter fragmentation. Sediment stabilization, nitrogen cycling, and secondary production. Polychaetes on the ocean floor contribute to the conversion of organic detritus into carbon dioxide, which is then carried to the surface as dissolved water. Marine plant plankton absorb CO₂ and use photosynthesis to generate carbohydrates or food, releasing oxygen in the process. Polychaetes also serve as a food supply for over a hundred creatures, including fish and larvae. Polychaete worms play an important role in the fishing business, which supplies food for humans, metaphorically depicting a dinner plate in the twenty-first century. They form a crucial link in marine food webs. Because of their high calorific value and high protein content, both adult and larval polychaetes constitute the primary food source for many commercially significant fish. Polychaetes, like other benthos, respond strongly to environmental contamination, and investigating how they withstand contaminated habitats might give useful information about how to overcome these pollutants. An effective approach to marine pollution control management may be developed. Help with nutrient cycling in the marine environment. Polychaetes are the most numerous group of invertebrates in estuarine environments (Rodrigues *et al.*, 2011), serving as both a food source and a key component of estuary and coastal food webs. Many fish, crustaceans, and birds have their main species. Transferring pollutants to higher trophic levels (Lewis and Watson, 2012; Serrano *et al.*, 2003) Furthermore, because polychaetes come in close contact with sediments, they are particularly vulnerable to hazardous elements in both the substrate and the surrounding pore water (Banta and Andersen, 2003; Scaps, 2002). Polychaetes are useful models in toxicological investigations and serve as vital watchdogs in the evaluation of polluted sediments (Dean, 2008). Polychaetes are employed as aqua feed for the majority of aquaculture species as well as bioremediation agents for

organic waste. Polychaetes used on broodstock and crustaceans enhance gonadal maturation and the endocrine system of white prawns, overcoming the scarcity of young animals and ensuring aquaculture crop output. Reliable product. Industrial feed dominates the polychaete culture. Microalgae have critical nutrients and a desirable shape, making them perfect for polychaete larval growth. Homemade food is less appetizing owing to its complicated composition, but it may be used to complement the polychaetes' diet. Wastes with a high organic matter concentration are competitive as food for polychaetes, although they may be deficient in some nutrients.

CHAPTER-2

REVIEW OF LITERATURE

The study reveals that *Nereis virens* spawn in mid-water, with epitoke changes like enlarged parapodia and natatory chaetae. Females spawn without these changes. Premature spawning can be induced by an artificial temperature rise, and the process is influenced by the lunar period and temperature rise. Gravid worms have a rigid swimming method, with flagella vanes and features defining the spermatozoon. Mature oocytes bear microvilli, and some express a jellylike layer after fertilization. Unit membraned vesicles occur in the periphery of mature oocytes. Monotrochophore, trochophore, and nectochaete larvae differ from other Nereids. (Bass, N.R., and Brafield, A.E., 1972).

The study examined the larval development of *Marphysa gravelyi* from Pulicat Lake in South India using light and scanning electron microscopes. Adults have an elongated, reddish-brown body and live in burrows at the lake's bottom. They create benthic jelly masses, where embryos live and are protected until larval development is complete. The author discusses larval development in four stages: prototrochophore, early metatrochophore, late metatrochophore, and nectochaeta. Larval development takes eight to ten days, with synchrony indicating larvae within the jelly mass. (Malathi, E., Sunder Raj, S. K., Mercy Bai, P. 2011).

The study analyzed *Namalycastis jaya* sp., a species found in Kerala, southwest India. It shares characteristics with two other species, *Namalycastis elobeyensis* Glasby, 1999, and *Namalycastis hawaiiensis* Johnson, 1903. *Namalycastis jaya* differs from *N. elobeyensis* by its shorter antennae, uneven eye size, bilobed acicular, neuropodial ligule, and multi-incised pygidium rim. It also has fewer teeth on the serrated blades of sub-neuro acicular falcigers and finely serrated falcigers in posterior segments. The author discusses the study using molecular phylogenetics for the first

time in *Namalycastis* to support population monophyly and species designation. The morphological analysis was supported by nuclear and mitochondrial data, suggesting the specimens may belong to an unidentified species, *Namalycastis jaya* sp. n. The examined nereidid taxa form a monophyletic group. (Magesh, M., Kvist, S., et al. 2012).

The article is a comprehensive review of the use of polychaetes as indicator species of marine pollution. The study discusses the various ways in which polychaetes can be used as indicators of pollution, including their use in monitoring programmes and their ability to detect contaminants in sediment and water samples. The author also describes the importance of selecting appropriate species of polychaetes for indicator purposes and provides examples of their use in various countries around the world. Overall, the article provides valuable insights into the potential use of polychaetes in monitoring and assessing marine pollution. (Dean, Harlan K. et al., 2008).

The study discusses the harmful effects of heavy metals on the environment. The authors explore the source of heavy metal pollution, the effects of heavy metals on living organisms, and the various methods used to minimize their negative impact. The article also discusses the role of regulations and policies in controlling heavy metal pollution. Overall, the article provides an informative overview of the dangers of heavy metal pollution and the importance of taking steps to mitigate its effects. (Rajeswari, T. Raja Nambuuru Sailaja et al., 2014).

The study examined Heavy metal contamination in coastal waters as a growing environmental concern, causing cell damage in humans who consume them. The laor or wawo worm, a common food source in Ambon, can absorb heavy metals. The study analyzing heavy metal bioaccumulation in these worms revealed fluctuating distributions in water columns and wawo worms from three different locations. The study found a significant relationship between heavy metal content in water columns and wawo worms, with 42.4% of marine worms affected by the water's heavy metal

content, while the rest (57.6%) was regulated by other variables. (Liline, S., & Rumahlatu, D. 2019).

This study confirmed the absorption of microplastics by the deposit-feeding polychaete *Namalycastis sp.* in the estuary region. Samples were collected twice a month from six locations between November 2016 and November 2017. Microplastics were recovered from the polychaete samples and examined using a LUMOS Fourier transform infrared microscope (μ -FTIR). Out of the 3277 pieces, 99.79% were filaments, and 84.71% were transparent. The majority of microplastics detected were polypropylene and polyamide. The study found that microplastics predominated at sample stations 3 and 4, likely due to influxes from aquaculture and the open ocean. This provides baseline data on the fate of microplastics consumed by benthic species within the estuarine food web. (Hamzah, Siti Rabaah, et al., 2021).

The study discusses the microplastics pose a significant threat to marine ecosystems, affecting organisms at various stages of their life cycle and food web levels. Studies have been limited in understanding their effects on marine animal survival and development. A study examined the effects of polystyrene microbeads on the early stages of two invertebrate species in Mediterranean shallow waters: pelagic planktotrophic pluteus larvae of the sea urchin *Paracentrotus lividus* and filter-feeding sessile juveniles of the ascidian *Ciona robusta*. The study found that both species were highly efficient in ingesting microplastics, slowing down the metamorphosis of ascidian juveniles and altering the development of plutei. This study highlights the need for monitoring coastal invertebrate's populations due to their impact on sensitive life cycle stages and generation recruitment. (Messinetti, Silvia, et al., 2018).

The study explores the role of microplastics (MPs) in adsorbing toxic heavy metals from the environment, a growing environmental concern. The adsorption process is influenced by factors like pH, salinity, and metal concentration, with aging or biofouled MPs facilitating greater adsorption.

The author also discusses the abundance, distribution, adsorption, and desorption behavior of MPs for heavy metals and their combined toxic effects on flora and fauna. Understanding the interactions of MPs with heavy metals in different ecosystems is crucial for assessing their eco-toxic effects and regulating pollutants. (Khalid, Noreen, et al., 2021).

CHAPTER-3

AIM AND OBJECTIVES

3.1 AIM:

To assess of toxicity of Iron and Polystyrene microplastic in a marine Polychaeta embryo (*Namalycastis jaya*).

3.2 OBJECTIVES:

- To develop the life cycle of marine polychaetes worms.
- To assess the morphological changes on acute exposure to Microplastic.
- To assess the morphological changes on acute exposure to Heavy Metal (Iron).

CHAPTER-4

MATERIALS AND METHODS

4.1 SAMPLING SITE:

Muttukadu estuary (lat. 12°49'09.6"N long. 80°14'46.4"E), situated on the east coast of India, is located approximately 36 km south of Chennai, Tamil Nadu. The backwater runs at a right angle to the coast and expands over a distance of 15 km, providing an excellent site for commercial purposes (prawn hatchery, aquaculture) and recreational activities (boating, fishing, and tourism). The width of the estuary ranges from 800m to 1050m. The estuary is shallow, with the maximum depth being 2 m, while in most of the areas it is 1m or less. The temperature and salinity of this estuary range between 25-30°C and 32-34ppt, respectively. The landward side of the catchment area is a marsh wetland where the hydrological conditions are chiefly influenced by multiple anthropogenic pressures from human settlements, aquaculture, aquatic vegetation, industrial, agricultural, sewage waste, runoff, and suspended sediments. Many of these processes could potentially contribute to higher Chromophonic Dissolving Organic Matter (CDOM) contents, phytoplankton growth and accumulation, and detritus mineral sediments in the backwater system. Due to the high level of influence of freshwater inlets during monsoons, there are differences in the salinity of backwaters.

4.2 SAMPLING METHOD:

The polychaete egg mass samples are usually found in the sediments in the mangrove area. The samples of polychaetes egg mass were collected during the post monsoon at low tide in the sediment. The sediments were shoveled, and the egg mass was scooped out slowly so a whole egg would be obtained. The polychaete eggs were washed gently with seawater to wash away the leftover sediments, and placed into a zip-lock bag filled with seawater. Polychaete egg mass was placed in an icebox and transported to the laboratory.

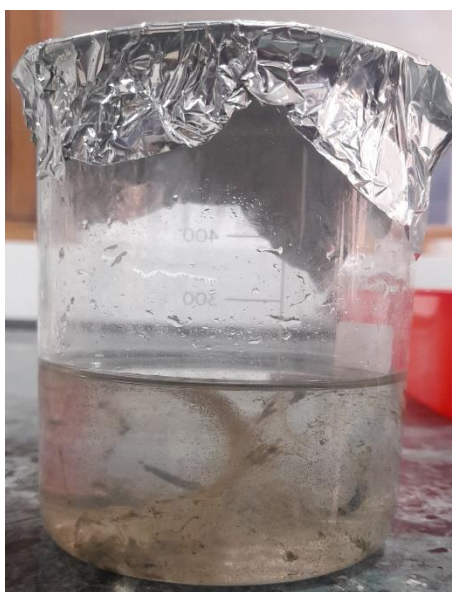


FIG 4.1 Sample Collection at Muttukadu Estuary

4.3 PREPARATION OF EXPOSURE MEDIA:

4.3.1 Microplastics Preparation:

Polystyrene Microplastics are prepared by diluting 10^{-5} concentration into 10^{-6} by taking 30 μL and diluting in 300 ml seawater using commercially purchased Fluorophores polystyrene, 1.0 μm from Invitrogen Thermo Fisher Scientific (Lee *et al.*, 2021).

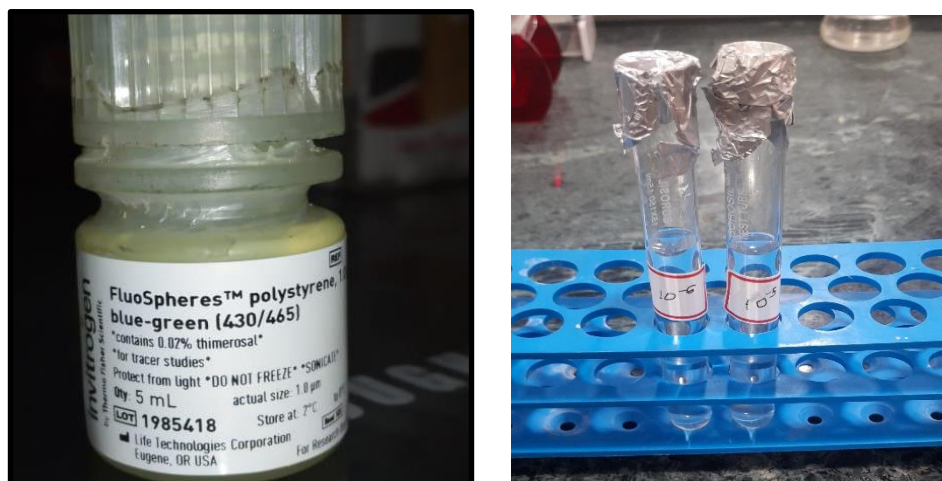


FIG 4.2 Polystyrene Microplastics Purchased From Invitrogen Thermo Fisher Scientific.

4.3.2 Heavy Metal (Iron) Preparation:

For worm eggs incubation, a heavy metal solution was prepared by dissolving 175 μg and 200 μg of iron (heavy metal) powder in 1000 ml of seawater as shown in Fig 4.3. The concentration of iron solution was prepared based on the research paper “Bioaccumulation of heavy metals in water, sediment, and tissues of major fisheries from Adyar estuary, southeast coast of India: An Ecotoxicological Impact of a Metropolitan City” by Rubalingeswari. N et al., 2021.



FIG 4.3 Iron Solution

4.4 EXPERIMENTAL DESIGN:

Separation of eggs from eggs mass and each well contains 20 eggs in 6 well plate for per experimental groups: NC, the control group; T₁, Iron (heavy metal) (175 µg/ml concentration) T₂, Iron (heavy metal) (200 µg/ml concentration); T₃, Microplastics (10⁻⁵ particles/ml concentration); T₃, Microplastics 10⁻⁶ particles/ml concentration). The exposure experiments were conducted in triplicates for 24 hours and 48 hours. At each sampling time, polychaete eggs per each group was selected and was analyzed by microscope.

4.5 MICROSCOPE ANALYSIS:

The polychaete egg treated with iron and microplastic were analyzed at each time points (24hrs and 48 hrs.) of the experimental exposure, the samples test well was observed by advanced stereomicroscope with 40X magnification.

CHAPTER-5

RESULTS AND DISCUSSION

5.1 LIFE CYCLE OF *NAMALYCASTIS JAYA*:

Namalycastis jaya is a large-sized, reddish-brown worm with no sexual dimorphism. The description corresponds to Southern (1921). The fertilized eggs are enclosed inside a jelly mass, where indirect development takes place. The jelly masses were pear-shaped with a broad distal end and a narrower proximal end, which continued as a stalk for attachment. M. gravely produces polylecithal eggs, and hence the larvae are lecithotropic. There was an accumulation of fertilized eggs towards the central core of the jelly mass, where the jelly mass shows a thicker consistency. Peripheral to the central core, the gelatinous substance looks thinner, with an externally thin sheath. In an ever-changing brackish environment with many micro- and macro Particles, the jelly mass becomes encrusted with the particles as it ages from the first day through the final settlement of the larvae.



Figure 5.1 Egg Mass in Situ

STAGE I

Prototrochophore: the larvae were spherical and uniformly ciliated, measuring 200–220 μm . They were found to revolve in the jelly with the help of cilia. The larvae appeared opaque due to the retention of yolks accumulated during vitellogenesis. The larva utilizes this yolk for its nutrition, as it does not feed at this stage. The only observable feature at this stage is the appearance of cilia all over the surface. After 24 hours, the spherical prototrochophore larva became oval.

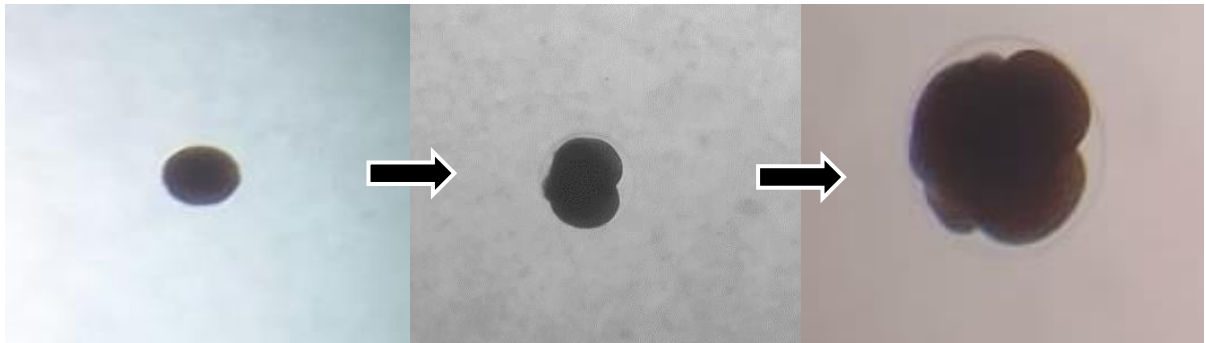
STAGE II

Early metatrochophore: the uniformly ciliated larva became oval and lost its ciliation in certain regions. At two-thirds the length of the body in a non-ciliated region, a pair of grooves appears. The first pair of parapodia develop in this region. A pair of eyes develop at the anterior dorsal lateral region. This also helped in the identification of the anterior and posterior regions of the larva. The anterior region of the larva was broader than the posterior end. The opacity of the larva reduces slightly. The parapodia are a simple structure with a single lobe, not a podium. A single seta is formed, which is an unjointed pointed seta. This is followed by another unjointed seta. The movement of the larva inside the jelly mass is restricted. The length of the larva continues to increase, and it enters the next stage.

STAGE III:

Late metatrochophore: the larva at this stage is twice the length of the prototrochophore larva. Posterior to the first pair of parapodia, the second pair starts appearing. The first pair of parapodia become prominent. The larva shows a kind of screwing rotary motion. Occasionally, it contracts and expands its body. The posterior end of the larva becomes narrower. About 36 hours after the appearance of the first pair of parapodia, the second pair of parapodia, which bear a single jointed seta, becomes prominently visible.

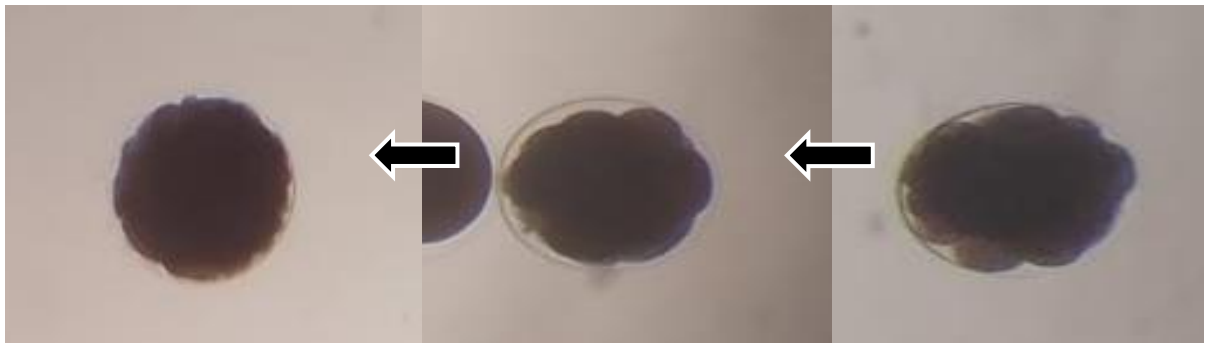
The cilia remains in the locomotor organ by which the larva moves in the jelly substance. The larva measures about 360–380 μm in length and 270–280 μm in width. The eyespots were well developed. This stage is called the late metatrochophore. Nectochaeta: the third pair of parapodia develops, and cilia disappear. A pair of lobular, fleshy, transparent anal styles are formed at the posterior end. This stage is marked by cephalization and the appearance of a pair of jaws. This stage corresponds to the nectochaeta stage. The body elongates and becomes twice as long as wide. The larva begins the creeping habit. The larva continues in this stage for another 24–48 hours until the jelly mass disintegrates. Following this, the larva settles into the sediment. The entire larval development takes 8–10 days. It is observed that there is a synchrony in the development of larvae inside the egg mass. There is also asynchrony in the development of larvae in all the jelly masses collected in a particular area at a particular time.



Fertilized embryo
(2hrs.)

2 Cell division

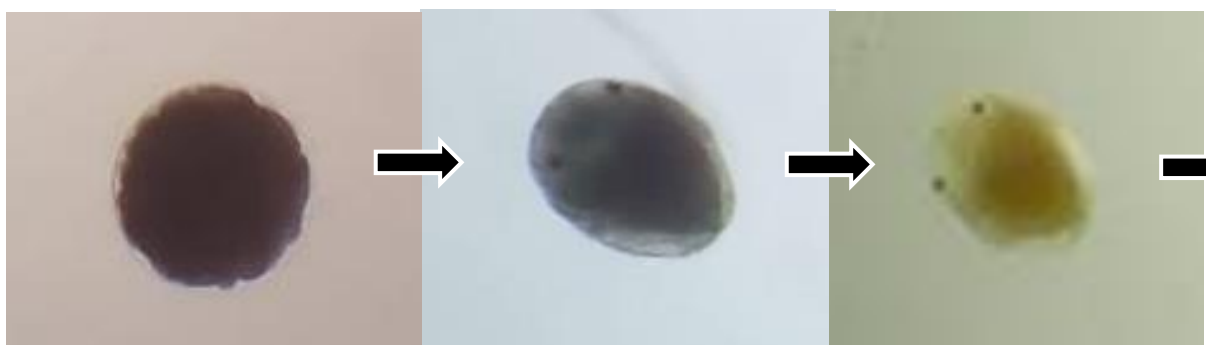
4 Cell division



32 Cell division

16 Cell division

8 Cell division



64 Cell division

Prototrochophore
(24hrs)

Early
metatrochophore

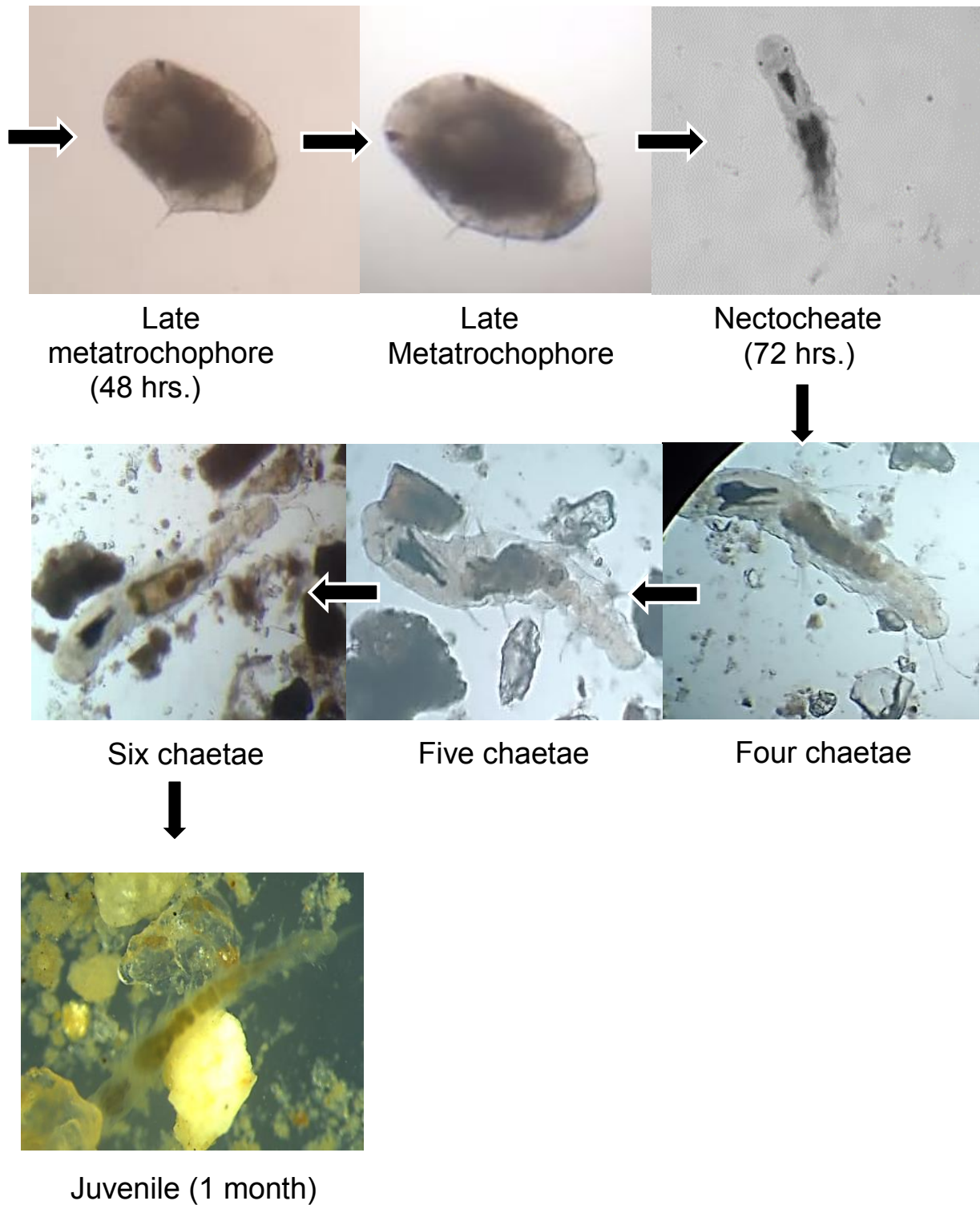


FIG 5.2: Lifecycle of *Namalycastis Jaya*

5.2 TOXIC EFFECT OF IRON ON THE DEVELOPMENTAL STAGES FERTILIZATION MEMBRANE (FM) PROTOTROCHOPHORE:

The percentage of successful development of the FM stage was 94.05 ± 2.09 in seawater. During the iron toxic study, there were no morphological changes in the Prototrochophore stage treated with 1mg/ml and 2mg/ml iron solution. (Fig 5.3a)

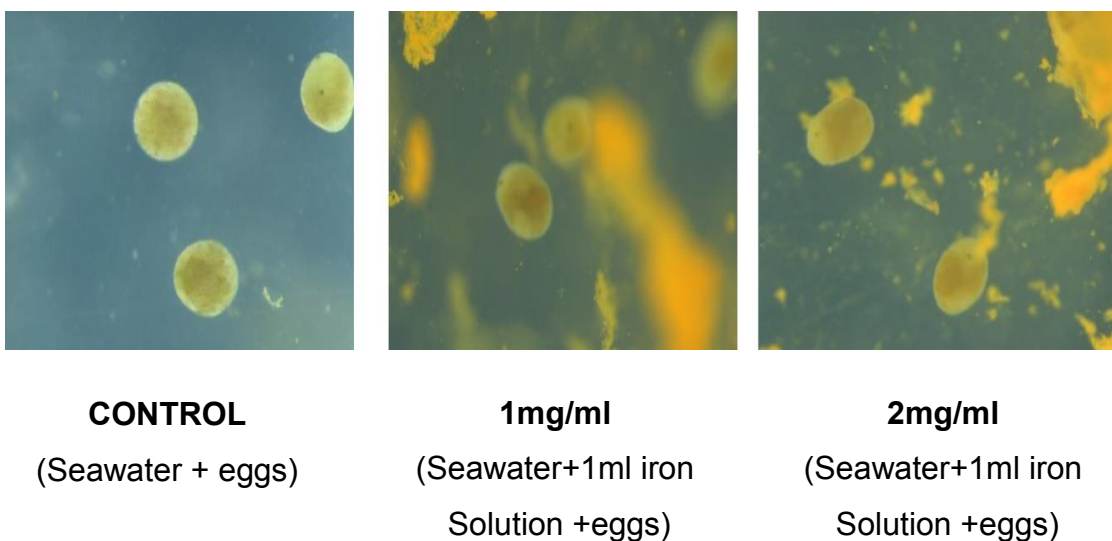


FIG 5.3a: Worm Egg Incubated With Iron Solution

5.3 TOXIC EFFECT OF MICROPLASTIC ON THE DEVELOPMENTAL STAGES FERTILIZATION MEMBRANE (FM) PROTOTROCHOPHORE:

During the microplastic study, 10^{-5} microplastic-treated groups were toxic, and it killed the polychaete embryo. After embryos death, cells started to infect with bacteria. There were no changes in the control group, and 10^{-6} microplastics treated. (Fig 5.3b)

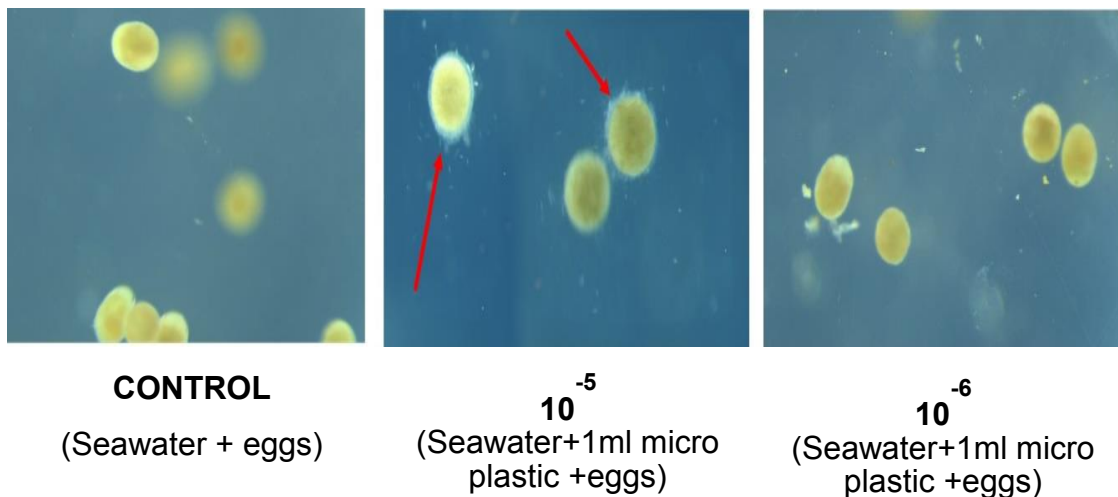


FIG 5.3b: Worm Egg Incubated With microplastic Solution

The range of salinity was also found to be convenient for *namalycastis jaya* worm larval development. The optimum range of salinity was also found similar to that of subfamily level, *N. littoralis* (6-21‰) (Ezhova 2011). On the other hand, at family level, such as *Hediste diadroma* could adapt to higher salinity of 27.5-30‰, which is usually unfavorable for fertilization of other Nereidid species (Junardi et al. 2014). The present study demonstrates that the water temperature of 25-29°C is favorable for *namalycastis jaya* worm fertilization and larval development. This confirms previous findings that polychaete larval development does not occur at 22°C, but normal larval development occurs at 27°C (Toso et al. 2020). Thorson. (1950) pointed out that the egg size in marine invertebrates is indicative of larval life and development pattern. This is shown by an empirical relationship in polychaetes. Small polychaete eggs (200-220 µm in diameter) develop into planktotrophic trochophores. Larvae from large eggs usually do not begin feeding until they become benthic. The jelly mass has been attributed with various functions, such as promoting successful fertilization (Sato & Osanai, 1996), protecting the embryos during early development (Vance, 1973; Schroeder & Hermans, 1975), supplying food for the developing embryos (Bookhout & Horn 1949; Sato et al. 1982) and limiting

the dispersal of the juveniles to keep them in a suitable habitat (Chapman,1965; Gibbs,1968; Sato et al. 1982; Nishihira et al. 1984).

The fertilization and developmental stages of *H. elegans* agrees with the data reported by Andrews and Anderson (1962), Marsden, and Anderson (1981). Within 24 hours, trochophore larvae were recognizable under 40X magnification. Because trochophore larvae were actively swimming by 24 hours. The heavy metal iron were tested for their toxicity toward early development of egg. It should be noted here that the environmental concentration of the heavy metal iron tested at one of the most polluted sites (adyar, Chennai, India) were 1.17, 2.24, 1.56, and 2.57 $\mu\text{g/g}$ (Rubalingeswari, N., et al, 2021). Our preliminary studies with metal concentration ranging from 1mg/ml and 2mg/ml showed no detectable changes in the development of *Namalycastis jaya* polychaete egg. It is important to note here that one recent study dealing with iron toxicity to developmental stages of *Namalycastis jaya* egg had tested iron concentrations ranging from 1mg/ml and 2mg/ml, but no significant change was discernible at lower metal concentrations with respect to the control (Xie et al., 2005), which is quite similar to our preliminary observations.

In this study, we evaluated the effects of polystyrene MPs on the development of *Namalycastis jaya* egg marine invertebrates. Our results add new important information about the effects of these emerging pollutants on early life stages of polychaete egg. During the microplastic study, 10^{-5} microplastic-treated groups were toxic, and it killed the polychaete embryo. After embryos death, cells started to infect with bacteria. There were no changes in the control group, and 10^{-6} microplastics treated.

SUMMARY AND CONCLUSION

The polychaetes eggs were developed by maintaining the eggs with suitable parameters like temperature, pH, and salinity on a daily basis to obtain the whole life cycle. For toxicity analysis, the polychaete eggs were collected from the Muttukadu estuary, Chennai, Tamil Nadu. The polychaete eggs were collected using scooping method. Polystyrene Microplastics prepared by diluting 10^{-5} concentration into 10^{-6} using commercially purchased Fluorophores polystyrene. Heavy metal solution prepared by dissolving 175 μg and 200 μg of iron powder in 1000 ml of seawater. The toxicity analysis involved separating eggs from their egg mass and exposing those to different concentrations of iron, and microplastics in 6-well plates. The exposure experiments were conducted in triplicate for 24 and 48 hours, with polychaete eggs selected at each sampling time. The polychaetes embryo were observed under stereomicroscope with 40X magnification.

In conclusion, the study highlights the critical role of microplastics and heavy metals as emerging global pollutants in marine ecosystems, posing significant threats to marine organisms. Through adsorption of chemical substances, including persistent organic pollutants and pesticides, microplastics can exacerbate the harmful effects of heavy metals, leading to bio magnification and potential toxicity to marine life. The research specifically focused on the effects of iron and microplastics on the egg development of the model species *Namalycastis jaya*, revealing that exposure to high concentrations of microplastics resulted in more pronounced adverse effects compared to separate exposure scenarios. These findings underscore the urgent need for comprehensive strategies to mitigate the proliferation of microplastics and heavy metals in marine environments, safeguarding the health and integrity of marine ecosystems and the organisms that inhabit them.

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