ISBN 9789395369046

https://adventpublishing.in

RECENT TRENDS IN FISHERIES AND AQUACULTURE - VOL. I

Edited by - Gaikwad & Pedge

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Jaiprakash M. Gaikwad

Sudarshan S. Pedge

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Recent Trends in Fisheries and Aquaculture: Volume 1

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ISBN: 978-93-95369-04-6

First printing edition: November 2022

BD|#AP0224|8.26x11.70|98p|

Cover design by Mr. Jyotiram Shinde

Printed in Republic of India

Published by Advent Publishing

007, Aspen, Building No 01,
Mohan Highlands, Badlapur East
421503 (MS) INDIA

Phone: +91-92-092-80-738

E-mail: info@adventpublishing.in

Website: https://adventpublishing.in

Recent Trends in Fisheries and Aquaculture: Volume 1

Edited By

Dr. Jaiprakash M. Gaikwad
H. O. D. Department of Fisheries
Shri. Shivaji College Parbhani, Dist. Parbhani
431 401 Maharashtra, INDIA

Dr. Sudarshan S. Pedge
H. O. D. Zoology
Kai. Rasika Mahavidyalaya Deoni, Dist. Latur
413 519 Maharashtra, INDIA

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Preface

Fishery administrations and private sector industries now accord a place of importance to aquaculture, albeit after several decades of hesitation or downright skepticism. Though for farmers in many Asian countries aquaculture has been a way of life for centuries, its status in the context of global food production, aquatic resource management and socio-economic development of rural areas remained until recently a matter of debate. The scenario has changed radically with changes in world fisheries and the spectacular success of certain types of aquaculture enterprises. Development and donor agencies now consider it a priority area, and several scientific and technical institutions are presently involved in research on a number of aspects of aquaculture. Aquaculture workshops, symposia, conferences and expositions have become very frequent. All these have contributed to the recognition of some of the basic needs and problems of this new and emerging industry. The three world conferences on aquaculture – the Food and Agriculture Organization (FAO) Technical Conference on Aquaculture held in Kyoto, Japan, in 1976 (FAO, 1976), the subsequent World Conference on Aquaculture in Venice, Italy, in 1981 (Bilio *et al.*, 1986) and the Conference on Aquaculture in the Third Millennium, held in Bangkok, Thailand, in February 2000 (Subasinghe *et al.*, 2001, Pillayi *et al.*, 2004) – highlighted the importance of (i) the development and improvement of technology through research, (ii) the training of personnel and (iii) the dissemination of information in strategies for the rapid and orderly development of the sector. Many present day aquaculture practices are based on biological studies with only limited involvement of other concerned disciplines. This major handicap is now being increasingly understood, and aquaculture has come to be recognized as a multidisciplinary science, although expertise in the associated disciplines continues to be scarce. Farm management, which is an interdisciplinary science in itself, has yet to be developed for application in aquaculture. There are very significant communication gaps, and access to existing experience and information is extremely difficult. It is believed that large scale application of the present technologies despite all their deficiencies will result in much greater production, if only sufficient numbers of adequately trained and experienced personnel are available. All scientists, academicians, researchers, and students working in the fields of biology, freshwater and marine water fisheries among other fields, will find this book quite valuable.

This book with valuable book chapters from eminent scientists, academicians, and researchers will surely be a part of utmost information for the coming new research taken by the researchers in the field of fishery and aquaculture and other disciplines in the future.

Editors

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CHAPTER ONE

Effect of non-steroidal anti-inflammatory drug, Ibuprofen on biochemical profile of common carp (*Cyprinus carpoio*)

C.S. Kurhade[a] and U.M Jayabhaye[b]

[a]Department of Zoology, Nutan College Umri, Dist. Nanded, M.S., India
[b]Department of Zoology, Rajiv Gandhi Mahavidyalaya, Mudkhed, Dist. Nanded, M.S., India.
Corresponding author(s) *Email* address: cskurhade@gmail.com; jayabhaye_um@rediffmail.com

Abstract

The present investigation deals with the effect of ibuprofen on biochemical profile of *Cyprinus carpio*. For this investigation fishes are divided into four groups. According to Sub-lethal toxicity tests, fish exposed to $1/10^{th}$ of the LC_{50} value of ibuprofen. Ibuprofen's 96-h LC_{50} *Cyprinus carpio* was 12.75 mg/l. As a result, fish were exposed to 0.25, 0.50 and 1 mg/l concentration of ibuprofen. Control fish were maintained without any treatment in natural water. The biochemical estimation methods were used to investigate the effect of ibuprofen on the biochemical contents of *Cyprinus carpio's* liver, gills and brain, namely proteins, carbohydrates and lipids. Ibuprofen administration caused significant decrease proteins, carbohydrates and lipids in all tissues. The percentage reduction in the carbohydrate level in the liver, gills and brain tissues of fish was high with the increase of exposure duration. Based on these results, it is inferred that the lipid content was declined in all the tissues of *Cyprinus carpio* in groups.

Keywords: Ibuprofen, Cyprinus carpio, Carbohydrates, Proteins, Lipids, Biochemical.

Introduction

Pharmaceutical drugs are the newest group of pollutants in all aquatic ecosystems. Pharmaceuticals can also end up in the environment due to improper disposal, sludge fertilizer run off, reclaimed waste water irrigation and sewage leaks. Non- steroidal anti- inflammatory drugs (NSAIDs) are among the most prevalent pharmaceuticals found in the aquatic environment. NSAIDs have been found in surface water and they may persist in the environment for a long time (Corcoran *et al.* 2010) Because of their inherent therapeutic characteristics, they have a proclivity to bio-accumulate in creatures other than humans, potentially causing consequences on the biota of aquatic and terrestrial ecosystems (*Halling-Sorensen, 1998*).

Ibuprofen is widely used in human and veterinary medicine and as a result, it has been found in water in many countries. Ibuprofen (IBP) has an effective anti-inflammatory, antipyretic and analgesic action. It is used to relieve muscle pain and various other

inflammatory disorders. A number of studies have described the toxic effects of trace concentrations of ibuprofen. Mendez-Arriaga *et al*. (2008) described ibuprofen induced toxicity in rainbow trout (Oncorhynchus mykiss) oxidative damage to lipids, proteins and DNA and adverse effects on enzymatic antioxidant defense mechanism in aerobic organisms have been used in recent years as biomarkers for monitoring environmental pollution (Valavanidis *et al*. 2006). Bio indicators can be used to evaluate the toxic impact of contaminants present in water bodies. Toxicity studies in teleost fishes are necessary in order to understand the mechanisms of action of contaminants and to predict and evaluate their toxicity, particularly when contamination is chronic. The common carp *C.carpio* is commonly used as a bio indicator species (Huang *et al*. 2007).

The aim of this study was to investigate the harmful effect of ibuprofen on common carp (*Cyprinus carpio*) under experimental conditions, with a focus on biochemical abnormalities.

Materials and Methods

Fish Preparation and adaptation

A 21 day experiment was carried out within July to September 2019 in India by using common carp (*C. carpio*) as the test organism. Fish were caught from fish hatchery ponds located in Nanded, Maharashtra, India and transferred to the laboratory. Two weeks before the experiment, fish with an average body weight of 30±5 g; and average body length of 16.1±1.02 cm were stocked in aquaria (with volume of 140 liters of water) and aeration was provided with an air pump for 24 h. for adaptation. After the adaptation period, fish of similar mean weight were separated and survival test was performed with three replications: 20 fish were used in each replication, at a density of 3.5g L-1. Aeration was provided at all times and a photo period of 12:12 (L:D) was used. Fish were fed at the rate of 1% body weight and 50% of water was exchanged daily.

Test compound

Ibuprofen was chosen as a toxicant for this study partly because of the possibility of metabolic consequences. Due to their low water solubility, analytical grade ibuprofen was acquired from Fischer Scientific India Pvt. Ltd, India and 0.2ml/l was used to make the stock solution at varying concentrations of 5, 10, 15, 20, 25 and 30 mg/l.

Acute Toxicity Study

Median lethal concentration

Test systems consisting in 120×80×40-cm glass tanks filled with water reconstituted from the following salts: $NaHCO_3$ (174 mg/L), $MgSO_4$ (120 mg/L), KCl (8 mg/L) and $CaSO_4.2H_2O$ (120 mg/L) were maintained at room temperature with constant aeration and a natural light/dark photoperiod. Static systems were used, and no food was provided to specimens during the exposure period.

To establish the target concentration to be used in evaluating biochemical analysis of tissue, the median lethal concentration (LC_{50}) of ibuprofen was determined. To this end, six experimental systems containing different proportions of ibuprofen 5,10,15,20,25 and 30 in reconstituted water and a seventh ibuprofen free control system were set up, and ten carp

randomly selected from the stock using the random number method and were placed in each system. 10 fish per each group were used in the LC_{50} determination.

Duration of the exposure period was 96 h, at the end of which the number of dead specimens in each system was counted. The assay was carried out in quintuplicate. The 96-h LC_{50} of ibuprofen and its 95 % confidence limits ($P<0.05$) were estimated by Probit analysis.

According to sub-lethal toxicity tests, fish exposed to 1/10th of the LC_{50} value of ibuprofen. Ibuprofen's 96-h LC_{50} in *Cyprinus carpio* was 12.75 mg/l. As a result, during this experiment, fish were exposed to ibuprofen doses of 0.25, 0.50 and 1 mg/l. In natural water, control fish were kept without any treatment.

Experimental groups and dosage

After being starved for 24 h, fish (n=40) were gathered and then randomly distributed into 4 glass aquarium. Each aquarium contained 10 fish and 25L of test solution, with three tanks used in each treatment group. Detailed information about the exposure groups is listed in the following:

Groups	Dosage
Group I	Control fishes
Group II	Fishes exposed to ibuprofen (0.25 mg/L) for 21 days.
Group III	Fishes exposed to ibuprofen (0.50 mg/L) for 21 days
Group IV	Fishes exposed to ibuprofen (1 mg/L) for 21 days

From the above four groups, except group I, the remaining three groups of fishes were exposed to their respective sub-lethal concentration of ibuprofen for 24, 48, 72, 96 hrs, 10 days and 20 days respectively. Group I was maintained as control. All the groups received the same type of food and other conditions were maintained similarly. At the end of each exposure period, fishes were sacrificed and tissues such as gill, liver and brain were dissected and removed. The tissues (10 mg) were homogenized in 80% methanol, centrifuged at 3500 rpm for 15 minutes and the clear supernatant was used for the analysis of biochemical parameters.

Biochemical Study

The following estimation methods have been applied to study the effect of ibuprofen on biochemical contests ie. Soluble proteins, carbohydrates and lipids in the liver, gills and brain of fish *Cyprinus carpio*.

Estimation of protein

Total protein concentration was estimated by the method of Lowry *et al*. (1951), based on the following principle. Proteins in the sample form a complex with copper ions. The amino acids containing aromatic groups, tyrosin and tryptophane, present in copper protein complex react with Folin Cliocalteu phenol reagent to give blue colour due to the reduction of

phosphomolybdate. The intensity of the colour developed is proportional to the concentration of protein present in the sample. The value is expressed as mg/g of tissues.

Estimation of carbohydrate

The quantitative estimation of carbohydrate in the tissues was done following the method by Hedg's and Hofreiter, (1962). Carbohydrates are first hydrolyzed into simple sugars using dilute hydrochloric acid. In hot acidic medium glucose is dehydrated to hydroxymethyl furfural. This compound forms with anthrone, a green coloured product with an absorption maximum at 630 nm.

Estimation of lipid

The lipid was estimated by using method of Richmond, (1973) based on the following principle.

Cholesterol esterase is a protein that hydrolyses cholesterol esters into free cholesterol and fatty acids. Lipids react with sulphuric acid to form carbonium ions which subsequently react with the vanillin phosphate ester to yield a purple complex that is measured photometrically at 540 nm. The intensity of the colour is proportional to the total lipids concentration.

Statistical analysis

The data were obtained from at least three different experiments and statistically analysed and presented as mean ± SEM. Mean values of the control and the treated samples were then compared using one way ANOVA using Statistical Package of Microsoft Excel's software with $p < 0.05$ regarded as statistically significant.

Results and Discussion

Determination of LC_{50}

24, 48, 72 and 96 h median lethal concentrations (LC_{50}) of ibuprofen for *C. carpio* are shown in Figure.1. The probit numerical values along with their 95% confidence intervals are also presented in Tables 2-4. The 96-h LC_{50} of ibuprofen in *C. carpio* was 12.75 mg/L.

Table.1. Median lethal concentrations (24-96 h LC_{50}) of ibuprofen for *C. carpio*

NSAIDs (mg/L)	Mean LC_{50} values			
	24 h	48 h	72 h	96 h
Ibuprofen	33.87	28.33	18.83	12.75

According to 96h LC_{50}=12.75 mg/L of ibuprofen in *C. carpio*, Fish were exposed to nominal concentrating of 0.25, 0.50 and 1 mg/L of ibuprofen to study the biochemical alterations.

Effect of ibuprofen on Protein content

The amount of protein in the tissues estimated after exposing the fishes to different exposure periods of the ibuprofen drug are presented in Table 2. Ibuprofen administration caused significant decrease proteins in all tissues. After 7, 14 and 21 days, the protein contents in the liver, gills and brain of control fishes observed similar to that of initial control fish. When fishes exposed to ibuprofen (0.25 and 0.5 mg/L) no remarkable change in protein contents has been observed up to 7 and 14 days. However, after 21 days remarkable depletion has been observed.

In liver, the protein content is decreased after 7, 14 and 21 days from 14.9 to 10.50 (-29.5%), 14.8 to 10 (-32.4%) and from 14.9 to 9.55 (-35.9%) mg/g tissue when fish was exposed to 1 mg/L ibuprofen.

The protein content in gills also significant changes observed when fish was exposed to 1 mg/L ibuprofen during 7, 14 and 21 days of exposure. The protein content was decreased -25.1%, -30.3% and -44.3% after 7, 14 and 21 days exposed to 1 mg/L ibuprofen. The decline in the protein level was higher in gills when compared to liver and brain.

Table 2: Effect of ibuprofen in the Liver, Gills and Brain tissue of fish *Cyprinus carpio* with reference to protein contents (mg/g) (Values are mean + SE and % changes)

Tissue	Day of Exposure	Experimental Groups			
		Group I (Control)	Group II (0.25 mg/L)	Group III (0.5 mg/L)	Group IV (1 mg/L)
Liver	7	14.9+0.67	14.6±0.55 (-2.01)	12.6+0.87 (-15.4)	10.50+0.55 (-29.5)
	14	14.8+0.14	13.9+0.65 (-6.01)	13.7+0.55 (-7.4)	10.0+0.43 (-32.4)
	21	14.9+0.73	14.0+0.42 (-6.04)	12.9+0.33 (-13.4)	9.55+0.57 (-35.97)
Gills	7	18.3+0.74	17.4+0.53 (-4.91)	15.5+0.45 (-15.3)	13.7+0.33 (-25.1)
	14	18.1+0.95	17.9+0.56 (-1.10)	16.3+0.67 (9.9)	12.6+0.56 (-30.3)
	21	18.5+0.14	16.3+0.88 (-11.8)	14.9+0.43 (-19.4)	10.3+0.66 (-44.3)
Brain	7	16.4+0.66	16.1+0.99 (-1.8)	14.2+0.56 (-13.4)	12.0+0.59 (-26.8)
	14	16.8+0.43	15.3+0.23 (-6.7)	14.0+0.55 (16.6)	11.5+0.56 (-31.5)
	21	16.3+0.87	15.9+0.53 (-2.45)	13.5+0.58 (-17.1)	9.56+0.43 (41.3)

Table 3: Effect of ibuprofen in the Liver, Gills and Brain tissue of fish *Cyprinus carpio* with reference to carbohydrate contents (mg/g) (Values are mean + SE and % changes)

Tissue	Day of Exposure	Experimental Groups			
		Group I (Control)	Group II (0.25 mg/L)	Group III (0.5 mg/L)	Group IV (1 mg/L)
Liver	7	13.5+0.87	13.0+0.11 (-3.7)	12.7+0.54 (-5.9)	11.6+0.88 (-14.0)
	14	14.0+0.13	13.3+0.55 (-5)	13.0+0.55 (-7.14)	11.4+0.76 (-18.5)
	21	13.6+0.63	12.2+0.34 (-10.2)	11.6+0.65 (-14.7)	9.49+0.34 (-30.2)
Gills	7	9.3+0.84	9.1+0.45 (-2.1)	8.9+0.13 (-4.3)	7.9+0.87 (-15)
	14	8.8+0.98	7.9+0.44 (-10.2)	7.0+0.87 (-20.45)	6.67+0.76 (-24.2)
	21	8.3+0.64	7.3+0.67 (-12.0)	6.9+0.56 (16.8)	5.90+0.87 (-28.9)
Brain	7	11.5+0.73	11.3+0.42 (-1.7)	10.5+0.56 (-8.69)	8.0+0.55 (-30.4)
	14	12.8+0.53	12.0+0.67 (-6.25)	9.4+0.88 (-26.5)	8.2+0.49 (-35.9)
	21	11.9+0.52	10.2+0.44 (-11.7)	8.4+0.44 (-29.4)	7.8+0.99 (-34.4)

Effect of ibuprofen on carbohydrate content

The maximum value of carbohydrate content in *C. carpio* liver occurred as 14 mg/g (+0.13) in control group. The carbohydrate content during 7th day of ibuprofen exposure in 0.25, 0.5 and 1 mg/L concentrated animals was 13+0.11, 12.7+0.54 and 11.6+0.88 respectively. The carbohydrate content was declined from -3.7% (7th day of exposure) to -10.2% (21st day of exposure) in 0.25 mg/L treated animals, and also declined from -14% (7th day of exposure) to -30% (21st day of exposure) in 1 mg/mL treated animals.

The carbohydrate content in gills of 1mg/L treated in different exposure period also declined. During 7th day exposure the declined content of carbohydrates is -2.1%, 04.3% and -15% in 0.25, 0.5 and 1 mg/L treated fishes respectively. When compared to control the level of carbohydrate is decreased -28.9% during the 21st day of ibuprofen exposed animals treated with 1 mg/L concentration.

There is no significant changes in carbohydrate levels up to 7 days ibuprofen exposed fishes. In the brain tissue, the levels of carbohydrate was decreased to -6.25% (0.25 mg/L), -26.5% (0.5 mg/L) and -35.9% (1 mg/L) for 14 days ibuprofen exposed fish, whereas, it was decreased to -11.7% (0.25 mg/L), -29.4% (0.5 mg/L) and -34.4% (1 mg/L) for 21 days ibuprofen exposed fish.

Due to the continuous exposure to the sub lethal concentrations of ibuprofen, the fish, *C. carpio*, has developed a stress and reduction in food intake resulted in the depletion of liver, gills and brain carbohydrate from the beginning till the termination of the experiment. The percentage reduction in the carbohydrate level in both the liver, gills and brain tissues of ibuprofen exposed fish was high with the increase of exposure duration.

Table 4: Effect of ibuprofen in the Liver, Gills and Brain tissue of fish *Cyprinus carpio* with reference to lipid contents (mg/g) (Values are mean + SE and % changes)

Tissue	Day of Exposure	Experimental Groups			
		Group I (Control)	Group II (0.25 mg/L)	Group III (0.5 mg/L)	Group IV (1 mg/L)
Liver	7	11.2+0.67	10.5+0.45 (-6.25)	9.6+0.65 (-14.2)	8.1+0.54 (-27.6)
	14	12.6+0.73	11.5+0.71 (-8.73)	10.4+0.54 (-17.4)	7.4+0.66 (-41.2)
	21	11.4+0.52	10.5+0.12 (-7.89)	9.4+0.5 (-17.5)	5.3+0.33 (-53.5)
Gills	7	8.5+0.82	7.0+0.55 (-17.6)	6.3+0.44 (-25.8)	6.0+0.77 (-29.4)
	14	9.4+0.53	8.8+0.53 (-6.3)	7.9+0.66 (-15.9)	5.5+0.34 (-41.4)
	21	9.1+0.49	8.5+0.11 (-6.59)	6.8+0.55 (-25.2)	4.8+0.47 (-47.2)
Brain	7	18.1+0.21	16.8+0.32 (-7.18)	13.4+0.2 (-25.9)	11.4+0.4 (-37.0)
	14	15.4+0.78	14.0+0.76 (-9.09)	11.4+0.77 (-25.9)	9.2+0.55 (-40.2)
	21	14.7+0.51	12.6+0.55 (14.2)	9.4+0.32 (-36.0)	7.5+0.56 (-48.9)

Effect of ibuprofen on lipid content

The results of the comparisons of the lipid content in the liver, gills and brain of the *C. carpio* during three exposure periods are furnished in Table -4.

The mean lipid content of the liver, gills and brain of *C. carpio* recorded during the three different durations of exposure was analysed. In the case of control and treated fished the data collected on 21st day showed significant variation in the lipid content of the three different tissues of the fish.

In liver, the lipid levels are decreasing with increasing the exposure days and concentration of ibuprofen. In group II (0.25 mg/L ibuprofen treated) animals, at 7th day of exposure, the lipid levels decreased 6% compared to control, at 14th day of exposure, decreased 8% and at 21st day of exposure decreased 7%, when compared to control fishes. There is a high significant reduction in lipid content in group IV animals (1mg/L ibuprofen treated) then group III animals (0.5 mg/L ibuprofen treated). In group IV animals, the lipid content is declined with the day of exposure is increasing in liver. 27%, 41% and 53% lipid content declined during the 7th, 14th and 21st day of exposure in group IV animals.

In gills, the lipid content in group II animals is 7.0+0.55, 8.8+0.53 and 8.5+0.11 mg/g tissue at 7th, 14th and 21st day ibuprofen exposed animals respectively. When compared to control fishes, the lipid levels are decreased during the 7th, 14th and 21st day of exposure in group II animals. There was high decrease in lipid content in gills in group IV animals at 21st day of exposure. In group IV animals, at 7th day of ibuprofen exposure the lipid content decreased to 29%, while during 14th day and 21st day of exposure the lipid content is decreased to 41% and 47% respectively in gills.

In brain, the lipid content at 7th day of exposure, in group I, group II, group III and group IV are 18.1+0.21, 16.8+0.32, 13.4+0.2, and 11.4+0.4 respectively and it was decreased from

7.18% to 37%. During the 14th day of exposure, the lipid content in gills is decreased 9%, 25% and 40% in group II, III and IV animals respectively whereas, during the 21st day of exposure, it was decreased 14%, 36% and 48.9% in group II, III and IV animals respectively. Based on these results, it is concluded that, the lipid content was declined in all the tissues of *C. carpio* in group IV animals at 21st day of exposure of ibuprofen.

Proteins are involved in major physiological events therefore the assessment of the protein content can be considered as a diagnostic tool to determine the physiological phases of organism. Proteins are highly sensitive to heavy metal poisoning (Jacobs *et al.*, 1977). Depletion of protein content has been observed in the liver, gills and brain of the fish *C. caprio* as a result of ibuprofen toxicity. When an animal is under toxic stress, diversification of energy occurs to accomplish the impending energy demands and hence the protein level is depleted (Neff, 1985).

The depletion of total protein content may be due to breakdown of protein into free amino acid under the effect ibuprofen at the lower exposure period (Shakoori *et al*., 1994).

Vutukuru, (2005) reported that there is an appreciable decline in different biochemical constituents in various tissues in fresh water fish, Labeo rohita under chromium stress. Kannan *et al.* (2010) reported the decreased protein content on gill, brain and muscle of Mystus vittatus when exposed to mercuric chloride. The decreased trend of protein content in various tissues of C. mrigala in the present study may be due to metabolic utilization of keto acids in the synthesis of glucose or for the osmotic and ionic regulation.

Carbohydrate is an essential component of living cells and sources of energy for animals. The results of the present findings showed a significant decrease in carbohydrate content in all the tissues studied. Shazia Quadir *et al.* (2014) observed a significant decrease in glucose content by the exposure of Imidacloprid. Tissue specific depletion of carbohydrates as observed in the present study may be due to its rapid utilization to meet the energy demands under the impact of drug.

In the present study, an initial decrease in the level of total carbohydrate has been noticed in the liver, gills and brain tissues. The decrease in carbohydrate content in the gills, liver and brain may be due to glucose utilization to meet excess energy demand imposed by severe anaerobic stress of mercury intoxication (Margarat *et al*., 1999).

Lipid is an important normal body constituent used in the structure of cell membranes, synthesis of bile acid and synthesis of steroid hormones. Remia *et al*. (2008) reported that the decline of lipid may be due to utilization of fatty deposits instead of glucose for energy purpose of the fish, Tilapia mossambica on exposed to Monocrotophos. Mohsen Abdel – Tawwab *et al*. (2013) reported that the significant decrease in lipid content.

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CHAPTER TWO

A review on nutritional requirements in fishes: a perspective in aquaculture

P. Subavathy*[1], K. Chitra[2], G. Amala Jothi Grace[3] and S. Alwin David[4]

*[1]P.G. and Research Department of Zoology, St.Mary's College (Autonomous), Thoothukudi – 628001, Tamil Nadu, India

[2]P.G. and Research Department of Microbiology, Sadakathullah Appa College (Autonomous), Tirunelveli – 627011

[3]Department of Chemistry, St.Mary's College (Autonomous), Thoothukudi – 628001, Tamil Nadu, India

[4]Department of Chemistry, V.O Chidambaram College, Thoothukudi – 628008, Tamil Nadu, India

Corresponding author(s) *Email* address: subavathy23@gmail.com

Abstract

The aquaculture sector as a whole has expanded rapidly during the past few decades. It has started to change from an art to a science during this time, but even today; this transformation is far from complete. Feeds and feeding are essential components in the culture of aquatic animals, just like in other types of animal husbandry. The cost of feed is thought to be the largest ongoing expense in aquaculture, frequently ranging from 30% to 60%, depending on the scale of the operation. The growth and health of the industry depend on any feed cost reduction, whether it comes from diet research, better husbandry, or other direct or indirect methods. Simple feed formulas, the use of unconventional feedstuffs, and feed processing have all received attention. In this regard, the features of feeds and feeding that are crucial to the aquaculture sector, particularly those connected to feeds and feeding in relation to the environment and the aquafeed sector.

Keywords: Nutritional Components, Carbohydrate, Protein, Lipid, Vitamins, Minerals

Introduction

At the moment, aquaculture is expanding globally, and it is anticipated that it will increasingly make up for the lack of aquatic food supplies. The only alternative for the improvement of fisheries resources and the rehabilitation of ecosystems is thought to be aquaculture activities (Okechi, 2004). Diets that are prepared or manufactured might be either full or supplemental. Complete diets give fish all the nutrients they need for optimum growth and health, including protein, carbs, lipids, vitamins, and minerals. The majority of commercial diets contain the important elements, such as protein, fat, carbohydrate, ash, phosphorous, water, minerals, and vitamins, in amounts between 18 and 50 percent, 10 to 25 percent, 15-20 percent, 8.5 percent, 1.5 percent and 10 percent respectively. Aquatic species cultured in

indoor systems or confined cages may not have access to natural meals, thus their nutritional needs can only be met by the addition of additional feeds that have been nutritionally enriched.

(Craig and Helfrich, 2013).

In animal production systems, a healthy, high-quality product can only be produced economically with good nutrition. Nutrition is important in fish farming (aquaculture), where feed typically accounts for around 50% of the variable production costs. Recent years have seen a significant advancement in fish nutrition thanks to the creation of new, balanced commercial diets that support ideal fish growth and health. The creation of novel species-specific diet formulations aids the aquaculture sector as it grows to meet the demand for more readily available, secure, and high-quality fish and seafood products (Steven Craig, 2017).

Fish diets typically contain a lot of protein. Foods for fingerlings and fry usually include more crude protein than 50%. Dietary protein levels drop as fish mature and their development rate slows. When it comes to crude protein, grow-out diets frequently reach or even surpass 40%, whereas maintenance diets may only have 25–35% of it. As fish grow, the protein content of the meal must be reduced, and the particle size must also be altered. Due to their tiny mouth pieces, many fish need live food when they are hatching. Some fish are big enough to be put on a fry diet right away without having to worry about the cost and labour associated with live foods.

The majority of fish need dietary ascorbic acid (vitamin C). This becomes crucial if fish are raised in a dimly lighted environment where algae cannot thrive or if they are housed in such close quarters that they are unable to eat any natural food sources that may be present in the water. To stabilise the vitamin and lengthen storage, ascorbic acid should be phoshorylated before being added to fish diets. Fish foods should also contain vitamins A, D, E, and B complex. Vitamin E concentrations are frequently insufficient, especially in meals high in fat. Vitamin supplementation appears to be less significant if fish are kept in natural systems with algae and phytoplankton and stocking rates are not excessively high, likely due to the availability of natural food items (Sangipran Baishya *et al*., 2012).

Carbohydrates

The most affordable and practical sources of energy for fish diets are carbohydrates. Carbohydrates are included in aquaculture diets even if they are not necessary since they lower feed costs and have a binding effect on feed production. In the extrusion manufacture of floating feeds, dietary starches like cassava starch are employed. Growth is aided by carbohydrates, which also act as precursors to several amino acids and nucleic acids. Additionally, the cheapest source of dietary energy is carbohydrate. Carbohydrates save protein in nutrition because less protein will be utilized for energy. The liver might grow and store glycogen if there are too many carbohydrates in the diet. A diet containing no more than 12% digestible carbs is often advised. Most of the energy is provided by fats and proteins in fish diets (Parker, 2011).

Protein

Peptide bonds hold together the lengthy sequences of amino acids that make up proteins. Nitrogen is a component of all proteins and all amino acids. In actuality, determining nitrogen content is a way to determine protein content. Proteins are broken down for energy, and the by products are nitrogenous. These are excreted by fish in their urine, faeces, and gills. In fish ponds, these nitrogen by products can be problematic. The main factor to consider when making fish feed is protein. The most costly and crucial elements that affect how well cultured species thrive are involved with fish feed (Deng *et al*., 2011).

The amino acids included in dietary proteins are essentially what are needed to meet the requirement for protein in fish diets. The essential amino acids Arginine, Valine, Histidine, Isoleucine, Leucine, Lysine, Methionine, Threonine, Tryptophan, and Phenylalanine are those that fish cannot synthesise. The digestibility of protein tends to decrease as dietary carbohydrates rise. Additionally, warming during drying or processing lowers the nutritional value of proteins. However, inadequate heating of soybean meal reduces the amount of protein available. In comparison to warm-blooded land animals, fish have far higher protein needs. Fish require less protein as they get older. Although sources of animal protein are more expensive than those from plants, they are typically thought to be of higher quality. Combining different sources of protein improves results in diets (Pandey, 2013).

Fish are unable to utilize sources of nitrogen that are not proteins. Urea and diammonium citrate, which even non-ruminant animals can utilize to a certain extent, are non-protein nitrogen sources that are of no use as fish feed. In fact, nonprotein nitrogen at excessive concentrations can be hazardous. A decline in weight growth is a sign of a protein shortage or essential amino acid deficiency. However, some specific amino acid deficiency diseases show up as illnesses. When provided diets, salmonid cataracts, particularly those of rainbow trout, lack tryptophan or methionine. Some salmonids also develop scoliosis, a lateral curvature of the spinal column, as a result of a tryptophan shortage. Tryptophan shortage in trout affects how the minerals calcium, magnesium, sodium, and potassium are metabolised. Protein and energy should be balanced in fish diets. Energy shortages or surpluses slow growth rates. Protein is converted into energy when nutritional energy is insufficient. Feed consumption declines when there is an excess of dietary energy, which lowers the intake of the essential levels of protein for growth (Abowei and Ekubo, 2011).

Lipid

Each gram of fat contains 2.5 times the energy in a gram of carbohydrates or proteins.

The digestion of animal fats and highly saturated fats is reduced. On the other side, there is a risk of oxidation in highly unsaturated fats, fats that fish can assimilate quickly which could lead to feed spoiling. Most fish diets frequently include antioxidants to stop fats from being rancid while being stored. Dietary fats offer essential fatty acids (EFA), which are necessary for healthy growth and development in addition to being a significant source of energy for fish. These fatty acids are not synthesised by fish. Additionally, dietary fats help the body absorb fat-soluble vitamins. Freshwater fish need a supply of linoleic and linolenic acid in their diet. Both of these fatty acids have 18 carbons (Parker, 2011).

Vitamins

Organic substances called vitamins are necessary for healthy growth, reproduction, and diet-related functions. They participate in several chemical processes that occur throughout the body. Because fish have a straightforward digestive system, there is a clear requirement for vitamin supplements in fish diets. Fish have vitamin needs similar to non-ruminant animals like pigs and chickens. The two types of vitamins are water soluble and fat soluble.

Thiamine, Riboflavin, Pyridoxine, Pantothenic Acid, Niacin, Biotin, Folate, Vitamin B_{12}, Choline, Myoinositol, and Vitamin C are among the water-soluble vitamins. The activities of choline, myoinositol, and vitamin C are diverse. Connective tissue, the bone matrix, and wound healing all benefit from vitamin C. Additionally, it aids in the absorption of iron from the intestine and aids in stopping the peroxidation of tissues lipids. The majority of water-soluble vitamins function as coenzymes in metabolic activities within the body. A biological catalyst is an enzyme. Most enzymes are proteins, and each biological reaction requires a different enzyme. Coenzymes then collaborate with or comprise an enzyme.

Vitamins A, D, E, and K are fat-soluble vitamins. Along with the fats in the diet, fat-soluble vitamins are absorbed in the intestine. Unlike vitamins that are water soluble, fat soluble vitamins can be kept in body tissues. Hypervitaminosis is a hazardous illness that can be brought on by excessive quantities in the diet. The fat-soluble vitamins have very specialised roles to play. Vitamin A is essential for healthy vision, development, reproduction, infection resistance, and skin preservation. Fish can utilise betacarotene as a precursor to vitamin A, much like many land animals can. The body needs calcium and phosphorus to be mobilised, transported, absorbed, and used. It functions using two hormones produced by the parathyroid, an endocrine gland. Vitamin E working with selenium, protects cells against adverse effects of oxidation. Vitamin K is required for the normal blood clotting process (Parker, 2011).

Minerals

Minerals including calcium (Ca), magnesium (Mg), sodium (Na), potassium (K), iron (Fe), zinc (Zn), copper (Cu), and selenium (Fe) can all be absorbed by fish directly from the water (Se). As a result, the diet's need for minerals decreases. However, this also makes it difficult and ineffective to do studies on dietary mineral requirements. The majority of scientists concur that fish need all the nutrients that other creatures do. Minerals are separated into two categories: macrominerals and microminerals, depending on whether they require or employ an animal. In the body, macrominerals are found in comparatively high concentrations.

The macrominerals include Calcium (Ca), Chlorine (Cl), Magnesium (Mg), Phosphorous (P), Potassium (K) and Sodium (Na). The growth and development of the skeleton is most directly influenced by calcium and phosphorous, although they also have a role in a number of other metabolic processes. Fish's gills and skin directly take up calcium from the water. The water chemistry determines the need for calcium (Athithan *et al*., 2013).

Magnesium works as a cofactor with numerous enzymes. Either the water or the feed will satisfy the nutritional need. Magnesium deficiency results in anorexia, stunted growth, lethargy, vertebral malformations, cell deterioration, and convulsions. Chlorine, sodium, and potassium are electrolytes. In the fluid surrounding the cells, sodium and chlorine are present.

Potassium is an intracellular cation that resides inside cells. Deficit indicators are hard to manifest because of how prevalent these substances are in the environment. Even though microminerals are only found in trace amounts in fish bodies, they are nonetheless vital to their wellbeing. The microminerals include Copper (Cu), Iodine (I), Iron (Fe), Manganese (Mn), Selenium (Se) and Zinc (Zn).

Many enzymes include copper, which is necessary for their action. Copper can be hazardous at quantities of 0.8 to 1.0 m per litre of water, despite being essential for fish health.

Fish can tolerate copper in feed better than they can in water. Iodine is required for the thyroid gland to produce hormones. Iodine can be found in both water and food for fish. A deficit causes the thyroid gland to enlarge, similar to how it does in terrestrial animals and produces goitre (Halver and Hardy, 1985).

The synthesis of heme compounds requires iron. These substances include oxygen. Feed is regarded as the main source of iron due to the low iron content of natural streams. Anemia is a condition brought on by iron deficiency. Iron can be harmful at high concentrations and result in stunted growth, diarrhoea, liver damage, and even death. Manganese performs an enzyme- or cofactor-related function. Although it can be absorbed from the water, it is absorbed from the feed more effectively. Reduced growth and skeletal deformities result from a deficit. Cells and membranes are shielded against peroxide danger by selenium. Deficits in selenium result in slower growth.

Some species need both selenium and vitamin E to fend off muscular degeneration. When dietary selenium exceeds 13 to 15 mg per kg of dry feed, it turns toxic and causes stunted growth, ineffective feed utilisation, and eventual death. In addition, zinc is found in many enzymes. Zinc from food is absorbed more effectively than zinc from water. Dietary calcium, phosphorus, and the type of protein with phytic acid all have an impact on the use of zinc. Reduced growth, cataracts, fin and skin erosions, dwarfism, and even mortality can result from a zinc shortage. Although there is scant evidence, other trace minerals like fluoride and chromium might be significant (NRC, 1993).

Different types of feed

Commercially produced milled fish food is typically offered as flakes, pellets, or semi-moist pellets. The most comprehensive diets are usually those in pellet form. They are prepared, and if sold as a complete meal, each particle should contain the same amount of nourishment. Negative aspects include the possibility of rapid sinking if the pellet isn't extruded. The size of the pellets is also crucial. Some fish, particularly young fish of many species, may not be able to consume a particle that is small enough to be manufactured. A very little pellet may not be appropriate for larger animals. Diets that are semi-moist are fluffy and little. Although many of them are pricey, they frequently consist of high-quality diets and might be a great option for some species. Since they are soft enough for extremely small fish to ingest, flakes have long been used widely in the ornamental fish market. Additionally, they sank slowly. Unfortunately, the volume needed to satisfy the animals nutritional needs could be overly large.

The technology involved in raising living foods is advancing quickly. This is benefiting larval rearing, which is sometimes a barrier to the marketing of "novel" species. The smallest

live food commonly utilised for larval rearing is called rotifers. Brine shrimp that have just hatched are more substantial but still fairly little, and they are frequently utilised in fish hatcheries. High-quality nourishment can be obtained from cultured live foods, but caution must be exercised to prevent the spread of infectious diseases. The possibility of introducing diseases when using wild-caught food is also there (Sangipran Baishya *et al*., 2012).

Conclusion

It is advised to eat fish twice a week since it is a nutrient-dense component of the human diet and because it contains long chain polyunsaturated n-3 fatty acids. These fats are crucial for human nutrition and have been shown to be involved in numerous metabolic processes. They play important roles in cell membranes, the cardiovascular system, the brain, and the nervous system, among other things, and have anti-inflammatory actions, reduce platelet aggregation, and other functions. Additionally, the benefits of fish's proteins, peptides, and amino acids for health have only lately come to light. Additionally, fish is a great provider of nutrients like Vitamin D, selenium, phosphorus, and calcium. It is important to note that when taking into account factors of health related to nutrition, it is impossible to focus one group of nutrients separately. Most probably the discussed effects of fish on human health are due to the consumption of the fish as a whole and hence the combination of all present nutrients.

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CHAPTER THREE

Principles of transgenic technology and its applications in fisheries

Shivaji G. Jetithor [1], Datta A. Nalle[2]

[1]Department of Fishery Science, Yeshwantrao Chavan Mahavidyalaya, Tuljapur Dist. Osmanabad.

[2]Department of Zoology & Fishery Science, Rajarshi Shahu Mahavidyalaya (Autonomous) Latur (Maharashtra)

Corresponding author(s) Email address: shivajijetithor@gmail.com, iprometheous007@gmail.com

Abstract

A transgenic animal is one that carries a foreign gene that has been deliberately inserted into its genome. The foreign gene is constructed using recombinant DNA methodology. In addition to the gene itself, the DNA usually includes other sequences to enable it to be incorporated into the DNA of the host and to be expressed correctly by the cells of the host. Gene transfer into fish embryo is being performed in several species (trout, salmon, carps, tilapia, medaka, goldfish, zebrafish, loach, catfish, etc.). In most cases, pronuclei are not visible and microinjection must be done into the cytoplasm of early embryos. Several million copies of the gene are generally injected. In medaka, transgenesis was attempted by injection of the foreign gene into the nucleus of oocyte. Several reports indicate that the injected DNA was rapidly replicated in the early phase of embryo development, regardless of the origin and the sequence of the foreign DNA. The survival of the injected embryos was reasonably good and a large number reached maturity. The proportion of transgenic animals ranged from 1 to 50% or more, according to species and to experimentators. The reasons for this discrepancy have not been elucidated. In all species, the transgenic animals were mosaic. The copy number of the foreign DNA was different in the various tissues of an animal and a proportion lower than 50% of F1 offsprings received the gene from their parents. This suggests that the foreign DNA was integrated into the fish genome at the two cells stage or later. An examination of the integrated DNA in different cell types of an animal revealed that integration occurred mainly during early development. The transgene was found essentially unrearranged in the fish genome of the founders and offsprings. The transgenes were therefore stably transmitted to progeny in a Mendelian fashion. Southern blot analysis revealed the presence of possible junction fragments and also of minor bands which may result from a rearrangement of the injected DNA. In all species, the integrated DNA appeared mainly as random end-to-end concatemers. In adult trout blood cells, a small proportion of the foreign DNA was maintained in the form of non-integrated concatemers, as judged by the existence of end fragments. The transgenes were generally only poorly expressed.

Recent Trends in Fisheries and Aquaculture - Vol. 1 – Gaikwad et al. (Eds.) ISBN: 978-93-95369-04-6

The majority of the injected gene constructs contained essentially mammalian or higher vertebrates' sequences. The comparison of the expression efficiency of these constructs in transfected fish and mammalian cells indicates that some of the mammalian DNA sequences are most efficiently understood by the fish cell machinery. Chloramphenicol acetyl transferase gene under the control of promoters from Rous sarcoma virus, and human cytomegalovirus, was expressed in several tissues of transgenic fish. Chicken delta-crystallin gene was expressed in several tissues of transgenic fish

Gene Transfer Technology for Development of Transgenic Fishes

The most commonly used methods in fish biotechnology are chromosome manipulation and hormone treatments, which can be produced triploid, tetraploid, haploid, gynogenic and androgenetic fish.

Other popular methods of gene transfer in fish are microinjection, electroporation of sperms, electroporation of eggs and incubation of sperms. Following are the main steps in gene transfer for development of transgenic fish.

A. *Preparation of DNA Construct*

Desired transgene should be a recombinant gene or DNA construct, which is constructed in plasmid that contains an appropriate promoter-enhancer element and a structural DNA sequence.

The foreign genes are typically introduced with strong genetic signals, promoters and/or enhancers, which enable the foreign genes to be expressed at very high levels continuously (or constitutively), effectively placing those genes outside the normal metabolic regulation of the cell, and of the transgenic organism resulting from the trans-formed cell.

There are three main types of transgenes

(1) Gain-of-Function

These transgenes are able to increase particular function in transgenic individual after their expression. For example growth hormone genes from mammal and fish linked to appropriate promoter-enhancer element and a structural DNA sequence to produce GH transgene.

This GH transgene when express in transgenic individuals increases production of growth hormone leading to enhanced growth of transgenic animal.

(2) Reporter Function

These transgenes are able to identify and measure the strength of promoter-enhancer element.

(3) Loss of Function

This transgene is not yet used for modification of transgenic fish. Such transgenes are used for interfering with the expression of host genes. The promoter-enhancer elements of transgenes are linked to a growth hormone gene of fish. Hence transgenic fish contain extra DNA sequences that are originally derived from same species. Gene construct is then

introduced into fertilized egg or embryo, so that transgene be linked to genome of each cell of egg or embryo.

B. Gene Transfer by Microinjection

Microinjection is most successfully and widely used technique for gene transfer in fish. One method of microinjection technique involves the use of fine injection needle for introducing DNA into cut site in the cell. While doing so it destroys those cells that are in direct contact with the injected DNA. To ensure the integration of the DNA it should be injected to intact cells close to the cut site. The injection apparatus consists of a dissecting stereomicroscope and two micromanipulators, one with a glass micro-needle for delivering transgene and other with a micropipette for holding fish embryo in place.

The success of microinjection technique depends on the nature of egg chorion. The soft chorion facilitates the microinjection while the thick chorion limits the ability to visualize the target for injection of DNA. In many fishes (Atlantic salmon and rainbow trout) the egg chorion gets tough and hard just after the fertilization or to contact with the water and provides a difficulty in injecting the DNA.

But using the following methods can solve this problem

(1) By using the micropyle (an opening on the egg surface for sperm entry during the fertilization) for inserting the injecting needle.

(2) By using microsurgery for making an opening on the chorion.

(3) By digesting the chorion with enzymes.

(4) By using 1mM glutathione for initiating fertilization and reducing hardness of chorion.

(5) By direct injection to the unfertilized eggs. Another technique of gene transfer is intra-nuclear microinjection, which involves direct physical approach using a fine needle to deliver DNA into cell or even nuclei.

To facilitate rate of microinjection protoplast with partially reformed cell wall may be attached to a solid support with artificially bound substrate -without damaging the cells. Solid support may be of either glass cover slips or slides.

Steps of Microinjection Technique

(1) Desired eggs and sperms are stored separately at the optimum conditions.

(2) Add water and sperms and initiate the fertilization.

(3) Ten minutes after the fertilization, eggs are dechorionated by trypsinization.

(4) Fertilized eggs are microinjected with desired DNA just within a few hours of fertilization. DNA is released into the center of the germinal disc to the first cleavage in dechorionated eggs. The time available for microinjection is first 25 minutes and that too between fertilization and first cleavage.

(5) After microinjection the embryos are incubated in water until hatching takes place.

Survival rates of microinjected fish embryos are seem to be about 30-80% depending on the fish species.

Advantages of Microinjection Technique

This technique has the following merits:

(1) Optimum quantity of DNA can be delivered per cell, increasing chances for integrative transformation.

(2) The delivery of DNA is precise, even into nuclei of target cell again improving chances for integrative transformation.

(3) The small structure can be injected.

(4) It is a direct physical approach; hence it is a host range independent.

Disadvantages of Microinjection Technique:

(1) A single cell can be injected at a time; hence it is time consuming process.

(2) It requires sophisticated instruments and specialized skills.

(3) Limited embryonic time restricts injection to more eggs and a low transformation rate.

C. Gene Transfer by Electroporation

It is a simple, fast, efficient and convenient method for transferring gene. This method involves an electrical pulse to deliver DNA into cells (Fig. 43.3). The cells are exposed to a short electrical shock, which make the cell membrane temporarily permeable to DNA.

The desired DNA fragment is placed in direct contact of protoplast membrane, which enters into the cell upon electric shock. Hole may be created as a result and stabilized by a favourable dipole interaction with electric field.

Electroporation involves a chain of electrical pulses for permeation of cell membrane, thereby allowing the entry of DNA into fertilized eggs. The rate of DNA integration in electroporated embryo is more than 25% is the surviving rate, which is slightly higher in comparison of microinjected ones.

Advantages of Electroporation Technique:

(1) It allows simultaneous entry of DNA constructs.

(2) It is more suitable method for those species, which has very small eggs for microinjection.
(3) This method does not require specialized skill.

TRASGENIC FISHES

Antifreeze Protein Gene Transfer:

1. Many teleost inhabiting icy marine water in the Polar Regions produce antifreeze glycoproteins (AFGPs) or antifreeze proteins (AFPs) in their sera to protect them from freezing. This protein lowers the freezing temperature of solution without altering its melting temperature.

2. Thermal hysteresis, the difference between the freezing and melting temperature, is a unique property of these proteins. AFPs and AFGP have been demonstrated to bind to ice crystals and inhibit ice crystal growth.

3. Despite their similar antifreeze properties, these proteins are quite different in their protein structures. There are one type of AFGP and three types of AFP. Recently fourth type of AFP has also been reported in longhorn sculpin.

4. The Atlantic salmon Salmo salar, lacks any of these AGFPs or AFPs gene(s) and are unable to survive in sub-zero sea water temperature. An inability to tolerate temperature below – 0.6 °C to – 0.80 °C is one of the major problems of sea cage farming in Northern Atlantic coast. Hew and his co-workers developed antifreeze-resistant Atlantic salmon containing the AFP or AFGP genes using gene transfer technology.

5. They used genomic clone (2A-7) encoding the major liver-type AFP (wflAFP-6, previously known as (HPLC-6) from the winter flounder (Pleuronectus amaricanus) was used as a candidate for gene transfer.

6. Flounder AFPs belonged to the type I AFPs that are small polypeptides and high in alanine and helical content . Flounder AFPs is multi-gene family of 80-100 copies encoding two different isoforms, namely the liver type and skin type AFPs.

7. The liver type AFPs such as wflAFP-6 or wflAFP-8 (HPLC-8), are synthesized exclusively in the liver as prepro AFPs. In contrast the skin-type AFPs, including wfsAFP-2 and wfsAFP-3, are expressed widely in many peripheral tissues as intracellular mature AFPs

Growth Hormone Gene Transfer

1. Recently scientists have developed an “all fish” growth hormone model.

2. They have cloned and sequenced the grass carp and common carp carbonic anhydrase (CA) gene and growth hormone gene Hew et al., (1992).

3. The grass CA gene (beta-actin) promoter has been linked to a grass carp growth hormone cDNA to form a high efficiency expression vector called pCAZ.

4. Using the CAT gene as receptor gene, a pCA grass carp growth hormone was microinjected into fertilized, non-activated common carp via the micropyle, generating “all fish” transgenic carp.

5. The presence of transgene was detected by reverse transcriptase PCR and Northern blotting. These transgenic fish showed about 137% high growth rate of the control.

6. Gene constructs consisting of human growth hormone (hGH) gene driven by promoter/regulatory sequence of mouse metallothionein (mMT), viral thymidine kinase (vTK), rat cholecystokinin (rCCK), or chicken beta-actin (cBA) gene were injected into the cytoplasm of fertilized medaka eggs via the micropyle. More than 49% of the injected embryos survived at hatching. Up to 26% of the survivors showed integration of the introduced gene construct, as determined by polymerase chain reaction analysis and subsequent confirmation by Southern blot hybridization of the genomic DNA. A significant fraction of F1 progeny, derived from crosses between transgenic founders and the nontransgenic individuals, inherited the transgene. Expression of hGH gene was also observed in some of the P1 founders and F1 transgenic progeny carrying mMT-hCG or cBA-hGH gene.

7. Furthermore, the growth performance of the P1 mMT-hGH and cBA-hGH transgenic founders and F1 cBA-hGH F1 transgenic progeny was significantly greater than their full

sibling, nontransgenic individuals. In addition to the microinjection experiment, a gene construct containing the long-terminal repeat (LTR) sequence of avian Rous sarcoma virus (RSV) and rainbow trout (rt) GH2 cDNA was introduced into embryos of medaka by electroporation using an exponential decay electroporator. Approximately 70% of the electroporated embryos survived at hatching, and 20% of the survived individuals integrated RSVLTR-rtGH2 cDNA into their genomes. These two techniques will greatly enhance the ability to study regulation of gene expression in transgenic animals during differentiation and development.

8. Growth hormone (GH) transgenic fish often exhibit remarkable transformations in growth rate and other phenotypes relative to wild-type.

9. The 5750A transgenic coho salmon strain exhibits strong sexually dimorphic growth, with females possessing growth stimulation at a level typical of that seen for both sexes in other strains harbouring the same gene construct (e.g. M77), while males display a modest level of growth stimulation. GH mRNA levels were significantly higher in females than in males of the 5750A strain but equivalent in the M77 strain, indicating sex and transgene insertion locus altered transgene expression.

10. We found that acute estradiol treatments did not influence GH expression in either strain (5750A and M77) or the transgene promoter (metallothionein-B), suggesting that estradiol level was not a significant factor influencing transgene activity. The feminization of XX and XY fish of the 5750A and M77 strains generated all-female groups and resulted in equalized growth of the two genetic sexes, suggesting that the presence of the Y chromosome was not directly capable of influencing the GH transgene-mediated growth in a physiological female conditions. These data suggest that the difference in growth rate seen between the sexes in the 5750A strain arises from non-estradiol-mediated sex influences on gene regulation at the transgene locus. This study shows how genetic factors and transgene insertion sites can influence transgene expression with significant consequent effects on phenotype. (1. 2,3.4, and 5)

Disease-resistance Gene Transfer

1. In China scientists piloted a gene contributing resistance to the grass carp haemorrhagic virus (GCHV).

2. Eleven different gene fragments encoding protein was cloned and isolated from translation in vitro using GCHV genomic single gene fragments.

3. Based on the information of capsid protein SP6 and SP7 gene cDNA, 3 oligonucleotides were synthesized and fused with SV40 MT promoter and transferred into grass carp cytokine-induced killer (CIK) cells via a constructed expression vector and transfected with GCHV.

4. The result indicated that the mortalities were reduced by one order after challenge with the virus.

Applications of Transgenic Fish

Transgenic Fish may be better used for the following purposes:

(1) For increasing fish production to meet the growing due to demand of food due to increase in world population.

(2) For production of pharmaceutical and other industrial products from piscine origin.

(3) For development of transgenic native glow fish varieties for aquarium.

(4) As fish biosensors for monitoring aquatic pollution.

(5) For isolation of genes, promoters and synthesis of effective gene constructs.

(6) For researches in embryonic stem cells and in-vitro embryo production.

(7) For production of anti-freeze protein.

Environmental apprehensions about Transgenic Fish

The primary environmental concerns about releases of transgenic fish, for example, include competition with wild populations, movement of the transgene into the wild gene pool, and ecological disruptions due to changes in prey and other niche requirements in the transgenic variety versus the wild populations.

Transgenic Fish could intimidate Wild Populations

West Lafayette, Ind. — Purdue University researchers have found that releasing a transgenic fish to the wild could damage native populations even to the point of extinction. Transgenic fish could present a significant threat to native wildlife.

“Transgenic fish are typically larger than the native stock, and that can confer an advantage in attracting mates”, Muir says. “If, as in our experiments, the genetic change also reduces the offspring’s ability to survive, a transgenic animal could bring a wild population to extinction in 40 generations”.

Although at Canadian research facilities, elaborate precautions are being taken to prevent the release of transgenic fish into the environment. The fish are often raised in ponds covered with nets to keep birds out; enclosed by electric fences to keep muskrats, raccoons, and humans out; and the outlets are fitted with screened drains to prevent the loss of small fishes or eggs.

Gene Flow

One of the larger environmental concerns raised by transgenic fish is the possibility that a transgenic species raised in open water pens will escape and spread novel traits into the ecosystem by breeding with wild relatives, a biological process known as “gene flow.”

Gene flow between transgenic or conventionally bred fish and wild populations is an environmental concern, because it may present a threat to natural biodiversity.

Some researchers believe that the genetic differences introduced to a transgenic fish may impact its net fitness, a scientific term meaning an organism’s ability to survive and pass its genes to future generations.

The concept, which factors in characteristics such as the juvenile and adult viability of a fish, the number of eggs produced by a female, and the age at which a fish reaches sexual maturity, provides a useful barometer for discussing some gene flow scenarios.

According to one scientific model, if a transgenic fish escapes and mates with a wild fish, gene flow could follow one of three scenarios:

Purge Scenario

When the net fitness of a transgenic fish is lower than that of its wild relatives, natural selection will quickly purge from the wild population any novel gene(s) introduced by the transgenic fish. In theory, evidence of the novel trait will disappear from subsequent generations.

Spread Scenario

When the net fitness of a transgenic fish is equal to or higher than the net fitness of a wild mate, gene flow is likely to occur and the genes of the transgenic fish will spread through the wild population. This means evidence of the transgenic genome would persist in subsequent generations.

Trojan Gene Scenario

When the net fitness of a transgenic fish is altered such that the fish has enhanced mating success but reduced adult viability (i.e., chances of surviving long enough to mate), introduction of that fish into the wild population could result in a rapid decline of the wild population.

Essentially mating success would ensure the spread of the novel gene throughout the population, but the inability to survive would reduce the population size of subsequent generations and potentially lead to extinction.

A declining fish population would also have secondary impacts on other aquatic species that feed on, or otherwise depend on it. Populations unable to' successfully switch over to another food source, or those whose survival or reproduction depends directly on the declining population, would also suffer.

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CHAPTER FOUR

Crab Culture

Swarupa B. Jadhav*

Department of Zoology, Dayanand Science College, Latur. M.S., India

Corresponding author(s) *Email* address: swarupaspatil.123@gmail.com

Introduction

Crab is with protein, which is important for building and maintaining muscle. Crab also contains high level of omega – 3 fatty acids, vitamin, B12, and selenium. These nutrients play vital roles in improving general health while helping prevent a variety of chronic conditions.

The omega-3 fatty acids in crab provide many benefits related to heart health. These important nutrients may help lower triglycerides, reduce blood clotting.

Many of nutrients found in crab, including vitamin B12 and folate, help reduce the risk of vitamin deficiency anemia. People with vitamin deficiency anemia do not have enough healthy red blood cells and may experience fatigue or weakness.

Commercial Importance of Crab

The protein, Carbohydrate and lipid contents were found to be higher in hard shell than that of soft –shell crabs of Portunus sanguinolentus and reported10 essential amino acids in Portunus sanguinolentus among which, 8 individual essential amino acids were from hard shell crabs and 7 amino acids from soft shell crabs. Cheliped muscle recorded the highest value for protein and the lowest value for the total ash in both sexes of fresh water crab (Sudananautes africanus africanus). Means values for protein (35.0%), lipid (2.9%) carbohydrate (11.53%) and ash (49.55%) in the three-spot swimming card Ovalipes punctatus. The higher value of carbohydrate and fat in the bigger size group, while protein and moisture contents decreased slightly in crab Podophthalmus vigil. Crabs are consumed not only to fulfill the nutrient requirements but also to cure diseases.

Crabs have recreational values, such as – fishing large and small crabs and keeping colourful crabs in aquarium. More colourful indo-chinese potamid crabs of the genus Demanietta are sold in the market for aquariums. Crabs are one the most diversified crustaceans in the world. Excluding few poisonous crabs of the sea, many of them are eaten by humans as well as other living beings and also, they become food for many organisms. They play a significant role in the fishery wealth of many nations and are an important protein source. Crabs are consumed in many parts of the world.

The ecological role through their important position in the food web. They provide prey for many invertebrates and vertebrates and in turn feed on a variety of plant materials as competitor to the other small herbivores, small fishes, prawns and invertebrates. crabs are

Recent Trends in Fisheries and Aquaculture - Vol. 1 – Gaikwad et al. (Eds.) ISBN: 978-93-95369-04-6

predated by a diversity of organisms, chiefly otter but also fish, young crocodiles, monitor lizards, mongooses, civets, drills and birds such as storks and kingfishers. There is a difference in the size of crabs eaten by these species, trout feeding on the smaller individuals while otters (and other predators such as eels) catch larger individuals from the stream bed.

In India, the crab fishery is fast developing with a vast scope for the meat due to its delicacy and nutritional richness. The commercially important crabs found are Scylla serrate, S. tranquebarica, Portunus sanguinolentus, P. pelagicus, Podophthalamus vigil, Charbdis feriata, C. lucifera, C. natator C. granulate and C. truncate All these species are exploited in aquaculture. In some parts of the world, crabs are used in the form of staple food. In South indian region of India, due to the speedy growth rate, high meat yield, and excellent palatability and resistance to the pathogens has led to the development of rapidly increasing aquaculture industry of different species of crabs.

Freshwater crabs are an important protein source and are sold in many parts of the world. Muscles of the freshwater crab contain a substantial amount of nutrients in particular water content (male = 79.31 ± 2.30%), female crab contain (77.63+ 0.56%) protein (male=77.47+ 6.11%, female=63.28+3.62%) magnesium (male=51.48+16.10mg/g) Female=39.73+6.99mg/g) calcium (male=25.50+6.98 mg/g, female=39.73+6.99 mg/g). protein, carbohydrate, lipid, moisture and ash and minerals of calcium, magnesium, potassium sodium, iron, copper and zinc were maximum in cephalothorax and minimum in swimming and walking legs of fresh water crab Spiralothelphusa hydrodroma. Maximum protein content in females (23.47%) when compared to males (21.53%) and berried females (20.93%) the carbohydrate content was significantly higher in berried females (2.76%), and lesser in males (2.09%) and female (2.06%). The lipid contents were significantly higher in females (1.09%) and berrred females (1.05%) than males (0.32%).

Classification of crab

Phylum - Arthropoda

Subphylum - Mandibulata.

Class - Crustacea.

Subclass - Malacostraca

Order - Decapoda

Infraorder - Brachyura

Family - Portunidae

Genus - Portrunus

Species - pelagicus.

External Morphology of Crab

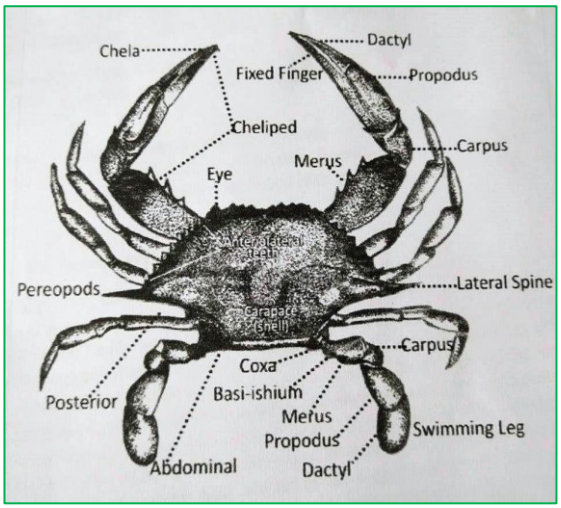

Fig. 1: External morphology of crab

1) The thoracic legs of a crab are used for walking. In certain crabs, including the blue crab, the last pair of thoracic legs is flattened and paddle – shaped and used for swimming.

2) Crabs have a very small tail, which they keep tucked underneath their body. Due to its small size, these tails and its appendages cannot be used for locomotion.

3) One pair of leg on each of these last five thoracic segments. The first pair is modified as chelipeds, of claws, while the remaining four pairs are adapted for walking and last pair for swimming.

4) only the last five segments of thorax are readily be visible and attachment of the thoracic legs to the exoskeleton is clearly apparent.

5) Ventrally, the boundary between head and thorax is well marked.

6) Hard carapace covers the head and thorax dorsally.

7) in the anterior portion of the cephalothorax of a crab are the mouth parts, grouped around the opening to the esophagus.

8) These mouth parts are generally similar to those of shrimps and lobsters.

9) The outermost pair is the third maxillipeds, used for holding food.

10) Under and in front of these two more pairs of maxilipeds and two pairs of maxillae, also used for holding food, and a pair of mandibles, or jaws, which push the food into the esophagus.

11) Sexes are separate and development is indirect.

Types of Crab

1. Rock Crabs

Carapace broadly oval or hexagonal; front not produced in form of a rostrum but having a central tooth; anterolateral margins toothed; lateral spines not strongly developed; antennules folding lengthwise.

Found only in northern part. This family comprises 1 genus, Cancer linnaeus.

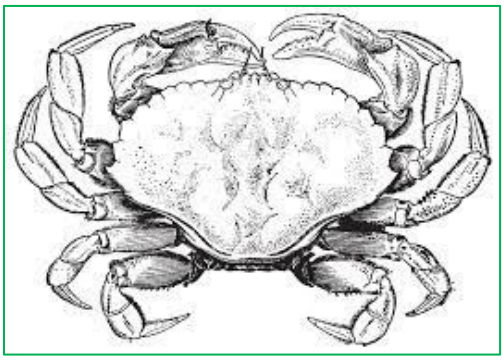

Fig. 2: Rock Crab

2) Land Crabs

Carapace transversely oval, not strongly depressed, anterolateral margins strongly arched, not divided into teeth or lobes; fronto – orbital margins, (between outer orbital angles) very much shorter than greatest width of carapace; third maxillipeds gaping noticeably, exposing the mandibles; dactyls of walking legs ridged and spiny. Live on land.

Fig. 3: Land Crab

3) Golden Crabs

Carapace hexagonal; dorsal surface relatively smooth to granular; frontal margin with 4 teeth; anterolateral margins distinctly convex, each with 3 to 5 low, sometimes indistinct teeth. Dactylus of walking legs T-Shaped in cross section. Male abdominal segments 3 to 5 fused, functionally immovable. But sutures are visible.

Fig. 4: Golden Crab

Different methods of crab culture

1) Cage culture (Suspended or fixed type)

Cage Design

Size of cages 1 m (L) x 1m (W) x 20 cm (H), which can be partitioned into nine equal compartments. Each of these cages should be provided with a lid to prevent the excape of crabs. A gap of 2.5 cm at the sides of cages to enable free movement of water through the cages. But no gap should be provided at the bottom.

Stocking density for fattening of crab in cages should be 9 crabs/m2. Different types of feeds such as trash fish, mussel, chicken waste, clams can be given to the crabs.

The term fattening means when underweight soft – Shelled crablets are stocked and reared for a few weeks until their gonad develops.

The cages could be made without cells inside. But the survival would reduce in this method due to cannibalism. These cages can either be suspender in backwaters or mangrove areas. Cages could also be made as fixed types in ponds, mangrove areas or coastal regions.

Cage Maintenance

1) Repair the damages in the cages immediately when it happens.

2) Deploy (move) the cages where there is mild water current.

3) If algal growth as found on the crabs, clean them using a brush.

4) Clean the cages as frequently as possible using brushes enabling free movement of water inside.

Fig. 5: Crab Culture in Cages

2) Pen culture in Mangrove areas

The pens could be constructed using locally available bamboo splits. These strips should be move 1-1.5 m deep into the soil to keep the crabs inside and predators outside. The pen could be 100 to 150 m^2, Mangrove trees in the Centre of the pen provide shade for the crabs. Roughly 1000 to 1500 crabs of 100g each could be stocked per pen.

The crabs could be fed once a day with low – cost fishes, mussels, clams, snails. The crabs when reach about 400g or more could be harvested. After 4–7-month harvesting could be done. The survival rate of 47 to 50% could be expected. This system is eco-friendly. The loss could be mainly due to cannibalism and escape of crabs. Lower stocking density is suggested to be a remedy.

3) Pond culture in mangrove areas

Ponds could be constructed around mangrove plants maximum pond area of 100 m2 is suitable for this type of culture. A canal should be dug around the edge of pond. Size of canal of 1m wide and 0.5 m deep in which water will be available even during low tide. The Centre of the pond forms mangrove vegetation, which the crabs would use during low tide.

Polythene netting could be used to prevent the escape of the crabs. Feeding with low-value fishes, mangrove snails, clam, mussels etc.

4) Pen Culture In Ponds

Several units of pen of 4 x 4 x 2.5 m could be made inside the ponds using bamboo strips. Which move 1-1.5m deep into the soil to prevent the escape of the crabs by burrowing.

Fig. 6: Crab Culture in Pen

5) Crab Culture in Pond

Pond size of 0.5 to 1 acre is most suitable for crab culture. Sandy soils with a mixture of 50% clay are ideal for culture of these crabs. Water inlet system and an outlet system should be provided.

The pond should be constructed in such a way that it should hold 3.5 to 4 feet of water towards the inlet and 4.5 to 5 feet towards the outlet.

Mechanism of water exchange should be there in order to remove left over organic food material and to remove excretory material. A fencing of nylon net could be done to prevent the

escape of the crabs during night time. Fencing should be supported with split bamboos of 1.5 m height around the pond periphery for preventing escape of the crabs from climbing. Stocking density should be 1 crab/m^2.

Fig. 7: Crab Culture in pond

Harvesting of Crab

Before next moulting when the shell becomes sufficiently hardened the crabs are harvested. The harvesting is done by draining the pond and using scoop nets. And ring nets with baits.

Harvesting should be done in the early morning hours or evening to prevent mortality of crabs due to overheating of water at noon time. In a year 9 to 10 cycles of fattening can be taken from a pond. The harvesting of crabs can be effectively done in tide-fed ponds. As mud crabs tend to swim against the incoming water. They can be caught with the help of a scoop net and also by hand picking. The expected survival rate would be 70 to 80%.

Fig. 8: Harvesting of Crab

Packing of Crab

The first pair of largest legs with pincers (Chelate legs) of each crab should be firmly tied up to the body by nylon thread to avoid fighting among them.

The method of tying a live crab is as follows

A stick is firmly placed on the carapace for instant arrest of its movement and the thread is placed in between the frontal portion of the body and chelate legs. After keeping the chelate legs in folding posture, the thread is coiled around their fingers (chelae) and both the ends of

thread are put into a double knot at the posterior end of the crab. Wet seaweeds are kept in between the packed layers of the crabs to enhance moist and cool condition during transport from place to place. The tied-up crabs are washed with fresh seawater and packed either in bamboo baskets or in perforated thermocol boxes for export purposes.

Fig. 9: Packing of Crab

CHAPTER FIVE

Fish and shellfish nutrition

Ishrat Parveen Mohd. Bari

Department of Zoology, Sharda College, Parbhani M.S., India

Corresponding author(s) *Email* address: Ishratparveen70@gmail.com

Abstract

Fishes and shellfishes are the most important aquatic creature which are full of nutrition for human consumption they are having large number of nutrients. This chapter includes about their nutrition, nutritional requirements, growth, food consumption and type of food and feeding.

This study deals with the study of macro and micro nutrients, their requirement in different fishes and shell fishes (vitamin and mineral requirement), growth (factors influencing growth and methods for determining growth)

Introduction

Fishes are most successful and diverse group of vertebrates; they constitute economically very important groups of animals. They are poikilothermic i.e. they can maintain their body temperature according to the environment.

Fish need energy to maintain basic metabolic activities and to support growth, reproduction, activity, and health. Proteins, carbohydrates, and lipids (the macronutrients) provide this energy and also some essential nutrients. Micronutrients (vitamins and minerals) do not contain calories but are also required for good performance. For fish to thrive, there are essential nutrients that need to be included in their diet in varying amounts depending on species. Proteins, carbohydrates, and lipids are the three main categories these nutrients fall under. The ratio of proteins to carbohydrates to lipids changes depending on fish species, age, size, and feeding habit whether it's a carnivore, herbivore, or omnivore.

Fish do not need carbohydrates in their diet, but they are a cheap source of energy. In fact, too many carbs can deter proper growth, as fish are not able to readily digest carbohydrates like land animals do. However, there is variation by species to the amount of carbohydrate a fish can tolerate without suffering negative side effects. Carnivores tend to use this energy source less efficiently than herbivores or omnivores.

Adult fish, however, can tolerate as much as 40 percent carbohydrate in their diet, seemingly without ill effects, although 25 percent is better. Most of the carbohydrate in fish food comes in the form of starches (from grains) that are used to bind the food and prevent it from rapidly disintegrating in water.

Recent Trends in Fisheries and Aquaculture - Vol. 1 – Gaikwad et al. (Eds.) ISBN: 978-93-95369-04-6

Proteins are composed of various combinations of amino acids. Fish require essential amino acids in proteins for growth, tissue repair, general health, and reproduction. Protein quality affects fish performance. The amount and types of amino acids in a protein source determine its quality. Some protein sources like fish meal are high quality but also very expensive. Less expensive protein sources may be used if they meet the essential amino requirements of the fish.

There are "essential" amino acids, which must be provided through the diet, and "dispensable" amino acids that the fish can absorb or synthesize from sources other than their diet. There are about ten known essential and dispensable amino acids that are necessary for fish health.

Essential Amino Acids

Arginine, Histidine, Isoleucine, Leucine, Lysine, Methionine, Phenylalanine, Threonine, Tryptophan, Valine

Dispensable Amino Acids

Alanine, Asparagine, Aspartic Acid, Cystine, Glutamic Acid, Glutamine, Glycine, Proline, Serine, Tyrosine

Protein requirements also vary widely based on the species of fish. Good quality protein is the most expensive part of the components of fish food. However, protein is a key element required for good health and growth in all the fish species. Herbivores need 15 percent to 30 percent protein in their diet, while carnivores need at least 45 percent protein. For vigorous, healthy growth, young fish require a diet that is composed of at least 50 percent protein.

Lipids are energy-dense and contain essential fatty acids (n-3 and n-6 types) and fat-soluble vitamins (A, D, E, and K) that fish require for normal growth, health, and reproduction. Crustaceans, molluscs, and sometimes young fish also require phospholipids (such as soybean lecithin). Fatty acid requirements differ by species: herbivorous and omnivorous fish can usually perform well on plant oils (that have shorter-chain fatty acids), but carnivorous species often need longer-chain fatty acids (such as those found in marine fish oil).

Unlike proteins and lipids, carbohydrates do not contain essential nutrients, but they are a cheap energy source. Carbohydrates such as starch are also needed to produce floating pellets because they expand during extrusion ("cooking").

Fiber is the non-digestible form of carbohydrate (cellulose and lignin). Although small quantities of fiber are important in the diet to aid in digestion, they should not be too high. Carnivores are not able to digest fiber well, and should not have more than 4 percent fiber in their diet. To remain healthy, herbivorous fish should have between 5 percent and 10 percent fiber in their diet.

Vitamins are organic compounds required in small amounts for normal growth, health and play an important key role in the health of fish. They are also required for metabolism of other nutrients. Many of the water-soluble vitamins act as co-enzymes. Key vitamins needed for good health are A, D3, E, K, B1, B2, B3, B5, B6, B12, Biotin, Choline, Folacin and Inositol. Vitamin C (Ascorbic Acid) is important for its anti-oxidant and anti-inflammatory activity. Their absence can cause number of diseases in different species of fishes like Lack of

vitamin A can cause spinal deformities and stunted growth in young developing fish. Anytime a fish is under stress, the need for vitamin A is increased, which can mean the difference between falling prey to disease and remaining healthy. Vitamins E and A are key factors in maintaining fish in top breeding condition. Vitamin K is critical for proper blood clotting.

Vitamins B1, B2, and B6 are important for normal growth. Good digestion requires an adequate number of vitamins B3 and C. Vitamin C is also needed for healthy bones and teeth, which are important in all species of fish. Both vitamins B5 and Inositol are key factors in metabolism. Lack of Folacin also known as Folic Acid reduces the formation of blood cells and can cause anaemia.

Vitamin requirements in fish diets (per kg feed)

Ascorbic acid: 100-150mg/ kg for carp and channel catfish, 300 mg/kg for salmon and trout

Choline: Trout and salmon 600-800mg/kg diet; carp 500-600mg / kg diet;

Inositol: In fish diets 200-400 mg/ kg

Thiamine: Channel catfish require about 1mg/kg, salmon and trout require10-12mg/ kg, carp 2-3 mg / kg.

Riboflavin: Salmon and trout 10-12mg; carp 4-7mg; channel catfish 10mg;

Pyridoxine: In fish diets 10-20mg/ kg, prawn diets 30- 50mg/ kg

Pantothenic Acid: Salmon and trout 40-50 mg; carp 30-40mg; channel catfish 2S-30mg;

Niacin: in general, 50-100 mg/ kg; carp 25-30mg;

Biotin: 0.4 to 1.0mg/ kg diet for fish

Folic acid: Range between 5-10mg/ kg

Cyanocobalamine (B12): 0.015 to 0.02 mg/kg dry diet;

Vitamin A: Fish 1000-2000 I.U/ kg;

Vitamin D: Fish requires between 1000 and 1000 l.U.,

Vitamin E: In fish dietary level recommend ed range from 30-50mg/kg diet

Vitamin K: Dietary level in fish feeds: 10mg/kg; prawn fees 5-20mg/kg

Minerals are inorganic substances that are needed for the same purposes as like vitamins. Minerals are important for healthy cells, immune systems, metabolic enzymes, bones, teeth, and even for maintaining healthy scales. Some minerals such as calcium are directly obtained by fish through gills and skin or both, while others are made available from natural food and ingested detritus. There are more than 20 recognized mineral for performing essential functions in the body. The minerals required by fish are calcium, phosphorus, magnesium, sodium, potassium, chlorine, and sulphur along with a number of trace elements such as cobalt, copper, iodine, iron, manganese, selenium, zinc, aluminium, chromium and vanadium. The key minerals fish need in bulk are calcium and phosphorus. Calcium plays a major role in blood clotting, muscle function and proper nerve impulse transmission. Phosphorus is involved in energy transformation, permeability of cellular membrane and general control of reproduction and growth. Calcium is found in hard water and can be absorbed through the gills, and phosphorus is found in live underwater plants.

Mineral requirements in diets (per kg feed)

Calcium (g): trout and salmon 0.2-0.3 common carp 0.28, red sea bream 3.4; Japanese eel 2.7; Indian and Chinese carps 5-18

Phosphorus (g): trout and salmon 7-8; common carp 6-7; tilapia 9; red sea bream 6.8; Japanese Magnesium (g): trout and salmon 0.5-0.7; carp 0.4-0.5; fish (in general) 0.5; prawns 0.8-1.0

Copper (mg): trout and salmon 3; channel catfish 1.5; fish (in general) 1-4

Manganese (mg): trout and salmon 12-13; carp 4; fish (in general) 20-25

Zinc (mg): trout and salmon 15-30; fish (in general) 30- 100

Iron (mg): carp 150, fish (in general) 50-100

Cobalt (mg): fish (in general) 5-10

Selenium (mg): trout and salmon 0.1-0.4

Sodium (g): fish (in general) 1-3

Potassium (g): fish (in general) 1-3

Sulphur (g): fish 3-5, Chlorine (mg): fish 1-5

Growth in Fishes

Growth is one of the basic characteristics of living organisms and a bio-energetic process and is defined as a change in its length and weight over a period of time. It indicates the health of the individual. The growth and age of a fish are closely related to each other and depends on several factors. The rate of growth varies from species to species. It may vary for the same fish; different parts of the body or even different organs have different rates of growth.

The two parameters (length and weight) exhibit growth of a fish. The growth in length indicates long term change, whereas growth in weight is more subject to seasonal variation. There are the following types of growth,

- Absolute growth: means the highest or perfect growth of fish from embryonic to senescence period.
- Relative growth: means comparison of growth from one life period to another. For obvious reasons growth is never similar during any two life periods.
- Isometric growth: means fish having equality of measure, having the plane of projection equally inclined to three perpendicular axes at right angles to one another. If the fish is following the cube law (A fish which doubles its length increases by eight times in weight), the growth is called isometric.
- Allometric growth: it is lopsided (one side lower or smaller than the other) growth. There may be various pattern of this type of growth. For example, several fish grow more in length than width and weight.

Factors influencing growth of a fish

- Temperature
- Photoperiod

- Quantity and quality of food available
- Dissolved oxygen
- Ammonia in water
- Salinity
- Age and stage of maturity of fish
- Inter-specific and intra-specific competition
- Stocking density
- Disease

Condition factor or Ponderal Index

Condition factor is generally used by fish biologist as an indication of the health of a fish population. The condition factor or Ponderal index, or coefficient of correlation expresses the condition of a fish, such as the degree of well-being, relative robustness, plumpness or fatness in numerical terms. The condition factor used to determine from length and weight of the fish.

Ponderal index or condition factor $k=W/L^3$

Where L is length in CMS and W is weight of fish in grams. The cube of length is taken because the growth in weight is proportionate to the growth in volume.

A high value of K shows that plenty of food is available to support development of fish. The value of K differs with season and influenced by maturity and spawning. The value of K is maximum during spawning season.

Method for Determining Growth

Growth in fishes can be determined by counting annual or daily rings that are formed on hard parts such as scales, otolith, vertebrae etc. The annular rings are formed due to seasonal variations in temperature and availability of food in the environment. Another direct method of recording growth is by tagging and recapture, but it is expensive and recovery of tagged fishes is meagre.

Direct method

Growth rate of a fish can be determined directly by rearing the fish under controlled conditions. For this eggs or larva of known age are kept in experimental pond. Length and weight of each are measured at known intervals of time for calculating growth rate.

Fish marking and tagging

In this method fishes are marked or tagged after the length and weight for identification and are than released in the natural habitat. After the few months these fishes are recaptured and measured again. The change in size during the interval gives the growth rate.

Food Consumption

Food is one of the most important external signals in fish that stimulates its feeding behaviour and growth. The intake of food is the main factor determining efficiency and cost, maximizing production efficiency in fish.

Fish do not consume all the food items they come across Fish are selective in the choice of food that contain the necessary nutrients for the survival, growth and reproduction.

Generally, hunger simulates the behavioural response of feeding fish. When feed is available, fish may initially feed at a faster rate and slowly decrease or stop with a gradual decline of appetite. Feeding behaviour despite being influenced by intrinsic factors is extremely influenced by ecological or extrinsic factors like stress, temperature, hypoxia etc influence food ingestion and feeding behaviour in fish.

Food and Feeding Habits in Fish

There are four basic eating groups among fish: carnivores, herbivores, omnivores and limnivores. Each group of fish needs to be fed in a particular way. Carnivores need at least 45% of protein in their food, without which they become severely malnourished. Although many of the prepared foods are spiked with extra protein to help such fish, carnivores fed live food like worms. Recommended food for the carnivores would be: - Earthworms, Red worms, Tubifex worms and Daphnia. - Larvae of mosquitoes or fruit flies. - Oysters, shrimps, clams and other fish.

Herbivorous fish are those that will use plant materials as their food, their feeding habit may range from microvegetation to microvegetation.

Recommended foods for this variety are: - Cucumber, peas and potatoes, Vegetable flakes, Algal flakes will also be a favourite among this kind of fish.

Limnivore fish feed mainly on algae and on the microorganisms in aquarium. These kinds of fish are constantly eating, and can be given pellets and algae-based foods. Proper feeding practices are a matter of habit. The type of food, the culture conditions and the individual fish will all affect the quantity of food that provide. In nature, there may be times when an adult fish starves for a day or two, or even for longer periods of time. The younger fish need more frequent feedings than the older ones. The fry has their own feeding needs.

Shellfish

Shellfish is a major component of global seafood production. As the name suggests, shellfish are animals that dwell in water and have a shell or shell-like exterior. They have joint body structure. Body is divisible into head, thorax and abdomen. It has jointed appendages. They are mainly filter feeder. Body is covered by a mantle and shell.

They can be divided into two groups: crustaceans and molluscs. Crustaceans are invertebrates with segmented bodies, protected by hard shells made of chitin, and include shrimp, lobster, crayfish and crab. Molluscs are invertebrates with soft bodies, divided into foot and visceral section. Shellfish are not actually fish, but are simply water- dwelling

animals. Crustaceans include shrimp, crayfish, crab, and lobster, while clams, scallops, oysters, and mussels are examples of molluscs.

Most shellfish live in saltwater, but the name also refers to species found in freshwater. These are low in calories and rich sources of lean protein, healthy fats, and many micronutrients. shellfish are rich in iron, zinc, magnesium, and vitamin B12.

They mainly eat slow-moving or stationary bottom-dwelling animals such as molluscs, smaller crabs and worms. They also eat plant material, small fish.

Shellfish are importance as Economically, Ecology, Nutritional, and Environmental value. Economical- important as ornamental value, lime production, raw materials for poultry fish feed, pearl production, and other jewellery items. Ecologically shellfishes play a key role for the balance of nature by filtering water. Nutritionally shellfish is a store house of nutrient. Good source of protein, lipids, vitamin, minerals etc. Environmentally shellfishes provide excellent habitat for Juvenile fish and other crustaceans.

The primary difference between the fish and shell fish is that the fish have bones, while shellfish are bone-free invertebrates. They also have organs and methods of motion to support their survival.

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CHAPTER SIX

Protozoan parasites in fishes

Deshmukh Shaziya Sultana K.A

Department of Fishery Science, Dyanopasak College, Parbhani (M.S) India

Corresponding author(s) *Email* address: deshmukhshaziya39@gmail.com

Introduction

Most of the commonly encountered fish parasites are protozoan. Protozoans are simple single celled, eukaryotic organism of the kingdom Protista. It includes members of the phyla Ciliophora (Cilliate), Sarcomastigophora, (Flagellates), Microsporea and Apicomplexa with regards to fish parasites. Many of which are freely in the aquatic environment. There are more than 65,000 described species of protozoa and around 10,000 parasitic species (5600 Myxosporidians and allies, 2500 ciliates, 1800 flagellates). More than 2400 parasitize fishes (Lucky and Ernest, 1994).

The single cell protozoan organisms composed of a plasma membrane or pellicle, cytoplasm, one to several nuclei; many have specialized structures that aid in locomotion, food capture, attachment or protection. In protozoan the functions of ingestion, digestion, gas exchange, and osmotic regulation are performed by various organelles of the cell instead of by separate organs composed of many tissues as occurs in multi-cellular animals. Protozoa usually range in size 10 to 100 µ which is why a microscope is required to see them.

According to Klinger and Francis-Floyd protozoa are a vast assemblage of eukaryotic organisms and that most of the commonly encountered fish parasites are protozoa, which with practice, are the easiest to identify and easiest to control. In general protozoa are one of the major sectors of fish parasites that have been long neglected because of its inherent difficulty in studying compared to other larger parasites. Among protozoa, ecto and endo parasitic protozoa occupy a very important sector as one of the hazardous threats to fish health. These parasites attack the fish, causing massive destruction of skin and gill epithelium. Even moderate infection of these organisms on small fish may prove a fatal disease, since the infection may cause the fish to stop feeding.

Most of the commonly encountered fish parasites are protozoans. Protozoans are single-celled organism, many of which are free- living in the aquatic environment. Typically, no intermediate host is required for the parasite to reproduce and life cycle is direct. They can reproduce asexually and their numbers increase very fast.

Consequently, they can build up to very high numbers when fish are crowded causing weight loss, debilitation and mortality. The majority of the fish parasites, typically, protozoan are present in large numbers either on the surface of the fish within the gills or both. When

Recent Trends in Fisheries and Aquaculture - Vol. 1 – Gaikwad et al. (Eds.) ISBN: 978-93-95369-04-6

they are present in the gills, they cause problems with respiration and death will commonly occur when additional stress are present in the aquatic environment. Protozoan parasites on the skin, fins or scale only (i.e., not affecting the gills) usually do not result in death, unless they are accompanied by a secondary bacterial infection.

The common protozoan ectoparasites of fish are *Ichthyophathirius multifiliis* (Fouquet, 1876); *Chilodonella sp*. **(**Strand, 1928); *Chilodonella piscicola* (Zacharias, 1894); *Chilodonella hexasticha* (Kiernik, 1909); *Cryptocaryon irritans* (Brown, 1951); *Trichodina domerguei* (Wallengren, 1897); *Trichodina sp*. (Ehrenberg, 1838); *Trichodina truttae* (Muller, 1937); *Glossatella sp*. (Buetschli, 1889); *Glossatella glabra* (Roth, 1909); *Tetrahymena corlissi* (Thompson, 1955); *Tetrahymena pyriformis* (Ehrenberg, 1830); *Ichthybodo necator* (Henneguy, 1883) *Piscinoodinium pillulare* (Schaperclaus, 1954); *Oodinium ocellatum* (Brown, 1931); *Amyloodinium limneticum* (Jacobs, 1946); *Plistophora typicalis* (Gurley, 1893) and *Kudoa histolytica* (Perard , 1928).

The main groups of protozoa that cause fish disease are:

- Mastigophora/Flagellates
- Myxozoa
- Ciliates

Mastigophora/Flagellates

A flagellate is an organism with one or more whip-like organelles called flagella. Some cells in animals may be flagellate, for instance the spermatozoa of most phyla. Many protists take the form of single-celled flagellates.

Flagellates are protozoa that possess one to eight whips like flagella, although the majority have one to four. However, a few, which are not parasitic on fish, may have more than eight flagella. The flagella are used both for locomotion and as attachment organelles.

Protozoan of the Order Kinetoplastida are a diverse and unique group of flagellates that include some of the most commonly studied organisms in the world. The order contains a number of parasitic, ecto commensal, and free-living species that occur over a wide range of habitats. The Order Kinetoplastida consists of two suborders, the exclusively parasitic Suborder Trypanosomatina and the Suborder Bodonina.

In general flagellated protozoan are small parasites that can infect fish externally and internally. Because of their small size, they move in whip like or jerky motion. The most pathogenic species belong to the genera Trypanosoma, Ichthyobodo, Cryptobia.

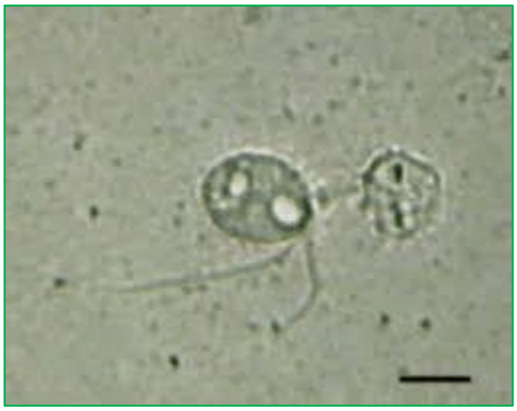


Plate 1 - *Ichthyobodo spp.*

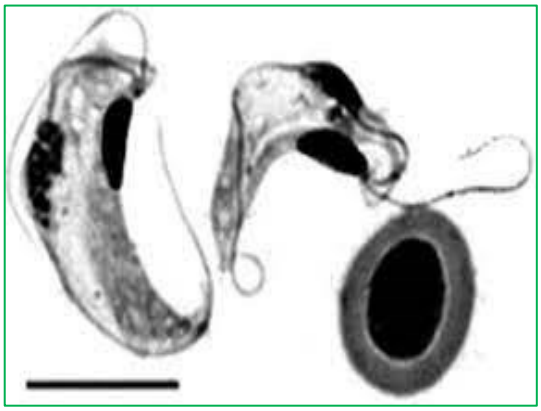


Plate 2 - *Cryptobia spp.*

Myxozoa

Beside ciliates and flagellates other protozoan groups are Microsporea and Myxosporea. These are all obligate parasites, many of which infest (external) and infect (internal) fishes. The myxosporidians are found as ectoparasites and endoparasites in various marine and fresh water fishes, a large number of species have been reported from many fish species.

According to Lom and Dykova, 1992, the myxozoan parasites have been known since the early 19th century. More than 1,200 species of myxosporean parasites have been described based exclusively on the spore stage in the vertebrate host, mainly fish and about 40 'species' of actinosporean parasites, based on the spore stage in the invertebrate host, mainly oligochaetes.

White or yellowish nodules may appear on infected organs. Chronic wasting disease is common among intestinal myxozoans such as with Chloromyxum. "Whirling disease" caused by Myxobolus cerebralis has been a serious problem in salmonid culture. Elimination of the affected fish and disinfection of the environment is the best control of myxozoan.

Van Wyk, 1968 in their observation several myxosporean infections of cultured fish were reported to be pathogenic. Most notorious is the whirling disease of trout, manifested by skeletal deformities, which is also claimed to have been introduced with rainbow trout into South Africa. According to Rukyani, 1990, heavy infection of carp gills with Myxobolus koi caused fusion through epithelial hypertrophy, and congestion; rupture of cysts caused inflammation. Damage to the gills by dense infestation resulted in respiratory problems; fish were swimming near the surface with distended opercula

The most pathogenic species belong to the genera Ceratomyxa, Myxobolus, Myxidium, Spaherospora, Enteromyxum, Kudoa, Tetracapsuloides and Sphaerospora. In freshwater fish the most significant diseases are whirling disease, PKD, sphaerosporosis and ceratomyxosis (produced by Ceratomyxa shasta). Myxosporea reported from cultured marine fish include species of the genera Ceratomyxa, Enteromyxum, Kudoa, Lepthoteca, Sphaerospora and Sinuolinea.

Plate 3 - *Whirling disease*

Plate 4 – *Myxosporean infection in C. carpio*

Ciliates

The ciliates are a group of protozoan characterized by the presence of hair-like organelles called cilia, which are identical in structure to eukaryotic flagella, but typically shorter and present in much larger numbers with a different undulating pattern than flagella. Cilia occur in all members of the group (although the peculiar Suctoria only have them for part of the life cycle) and are variously used in swimming, crawling, attachment, feeding, and sensation.

Most of protozoan parasites are ciliates. Ciliates have a direct life cycle and many are common inhabitants of pond-reared fish. Ciliary protozoans are found in large number of fishes. They usually attack on gills and skin, but some of them are also found under the skin.

These are protozoa that move by means of cilia, although some become sedentary when mature. Generally they possess two types of nuclei large conspicuous macronucleus and a small inconspicuous micronucleus. Asexual reproduction is by binary fission or multiple fissions in cysts, and sexual reproduction is by conjugation. Nutrition may be either holozoic or saprozoic. The cytostome or mouth usually lies in a depression called the peristome, which is characterized and defined by cilia. The ciliary movement around the cytostome aids in guiding food into the cytostome. In some ciliates the cytopharynx is lined with rod-like supportive structures called trichites and the entire structure is called a "pharyngeal basket".

Ciliates are one of the most important groups of protists, common almost everywhere there is water - in lakes, ponds, oceans, rivers, and soils. Ciliates have many ectosymbiotic and endosymbiotic members, as well as some obligate and opportunistic parasites. Ciliates are large single cells, a few reaching 2 mm in length, and are some of the most complex protozoans in structure. Member of this phylum have cilia in at least one stage of the life cycle. They have two types of nucleus, micro nucleus and macronucleus. Most of fish parasites can separate in three classes; Kinetofragminophorea, oligohynenophorea and Prostomatea.

Member of the class Kinetofragminiphorea have oral ciliature slightly distinct from body ciliature such as chillodonella spp. only two species C. piscicola and C. hexasticha are pathogenic for fish. Chilodonella spp attach on fish skin and gills. Oral apparatus of the member of class Oligohymenophorea usually well-defined and oral ciliature is distict from somatic ciliature such as Ichthyophthirius multifiliis, Trichodina spp, Tetrahymena spp and Epistylis spp.

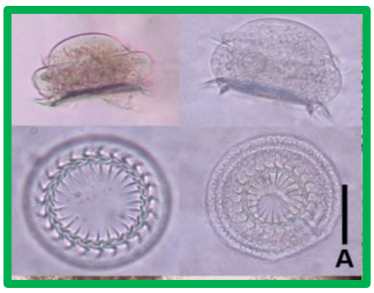


Plate 5 – *A – Trichodina spp.*

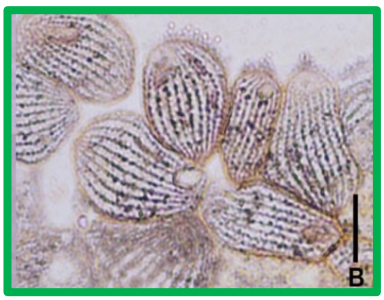


Plate 5 – *B – Chillodinella spp.*

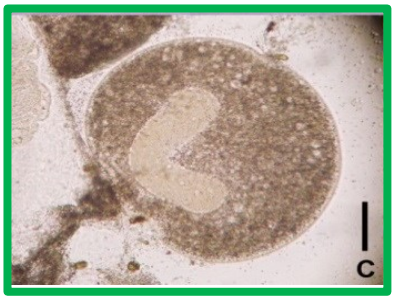


Plate 5 – *C - Ichthyophthirius multifiliis*

CHAPTER SEVEN

Aquarium fishes and maintenance

Shivaji B. Ubarhande

Department of Zoology, Rajarshi Shahu Arts, Commerce and Science College, Pathri, Phulambri Aurangabad M.S., India

Corresponding author(s) *Email* address: shivaji.ubarhande@gmail.com

Introduction

Aquarium means an artifical tank for keeping live aquatic plants and animals (fishes) for the entertenment of human being.

History

Glass aquaria concept is originated when blowing of glass was invented at the time 300 B.C in China. During "T"ang Dynasty (A.D 650) first packed badge gold colour fishes were kept. In 1596 Chang Ch'en-te wrote a book how to kept goldfish in tanks. In the year 1853 Landon Zoological gardens built first public Aquarium in the world. Modern style aquaria were developed in 19th century.

Types of aquaria

Mostly there are three types of aquaria

1) Home aquaria

Home aquaria is basically construct for dacoation of home office hostels,shops and etc and in this small ornamental fishes were kept.

2) Public aquaria

This type of Aquaria is kept in public places like exhibition, moll, Bridge, Building, Church, Room, Courtyard, Cowdung, Crop, Market, Railway station,Air-port etc.Small and medium sizeof fishes arekept in it.

3) Research and study aquaria

At higher studies the big size aquaria is used for research studies(experimental purpose) as well as to study at school and college .

Requirements for aquarium

1 Aquarium

2) Aquarium accessories

3) Aquarium fishes

4) Fish feed

5) Setting up a home aquarium

6) Diseases of aquarium fishes

1) Aquarium

In market various firms are manufacture the readymade aquarium for home, office, hostels, shops and etc. all standard 10-gallon tanks have approximately the same dimensions (20 x 10 x 12 inches) and weigh about 111 pounds when filled with gravel and water. For public aquaria, an 18 gallon aquarium of size 60x30x38 inches and for research purpose, 30x20x12 standard size.

Construction of aquarium is in fully glass, plastic and fiber glass. In glass aquarium all side with bottom and ends of the glass sheets cemented together using silicon rubber cement. In plastic aquarium the flat and coloured perpex cement together used to bind the corners and sides. In fiber glass, used for the front, back and sides. it is hard type of glass and produced in varieties in colour and non-corrosive.

2) Aquarium Accessories

a) Sand/Gravels: Small coloured stones and pebbles placed in the bottom of the aquarium, this sand or pebbles are helpful for growing of aquatic plants. These gravels are available at any local pet shop.

b) Light Arrangement: For more decoration the florescent light is recommended for home as well as public aquaria. Direct sunlight enhances the algal growth and finally water gets become green. Florescent light enhance the beauty of gravels and pearls and helpful for the growth of plants.

c) Temperature: Aquarium temperature is an important factor in maintenance for tropical ornamental fishes, the temperature should be maintained within the range 18- 30 oC low in temperature makes fishes sluggish and more susceptible to disease and too high in temperature decreases the oxygen level in water.to maintain the temperature heating is necessary for that heater (electric)is used. The underwater lighting helps to heat the water as well as providing light.

d) Air Pumps/Air aerator Electric air pumps works as a filtration system in that the air should be force fully insert into aquarium tank and it helps to clear the water and oxygenated

e) Filters: In aquarium two types of filters

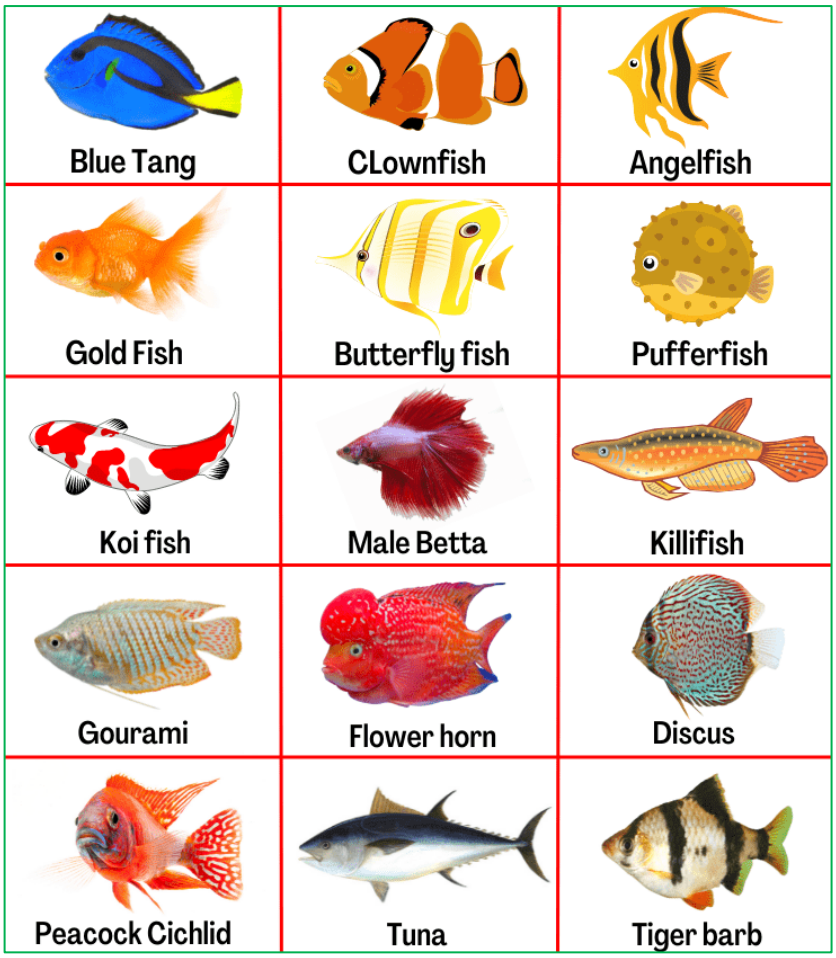
1) Side filter and 2) basement filter side filter with the help of air pump the waste material of fishes passing through the medium are filtered off, keeping the water clear, and freely circulating.

2) Basement filter is another type of filter in which under gravel filter where the water is filtered through the aquarium compost.

f) Aquarium cover: Normally the cover are made from aluminium, metal, wooden who reflect the light fairly well along with grooved rubber strip to prevent the dust, reduce excess heat and evaporation, stop jumping out of te fishes from aquarium.

g) Back ground poster: For more decorative of home aquaria back ground poster is very important .Plastic toys are also use to decorate the aquarium.

3) Aquarium fishes



4) Fish feed: For the better and natural growth of aquarium fish balanced diet is required, live as well as dry food is available in the market or we can make at home. Live foods i.e., Chironomus, Mosquito larva, earthworm, blood worms, Freshwater shrimps, phytoplankton, daphnia, cyclops, algae and aquatic plants. In addition to this supplementary food in the form of protein from liver, beef, animal waste. Commercially prepared diet food contain balanced diet which is useful for growth of fish.

5) Aquarium fish diseases

1) Ichthyophthirius (White spot)

White Spot diseases are very common to aquarium fishes but it can be kill fishes if proper treatment is not done. It causes when the temperature of water goes down and white spots occur on whole body of fish and rapidly spread with in few days to all fishes. Eaching to infected fishes are the symptoms, fishes rub their body to aquatic plants or any object to reduce their irrection.

Preventive measure: 5% aqueous solution of methylene blue used to treat the infected fishes for 7 to 10 days and remove harmful aquatic plants from aquaria.

2) Costiasis: Costiasis is a protozoan disease caused by protozoan parasite *costia sp.* which feed on mucus of skin of the fish. Fishes become lethargic and respire rapidly.

Preventive measure: keep infected fishes for 10 to 15 min., in 2.5% salt water solution every day.

3) Saprolenia (cotton wool): Saprolenia is a fungal disease is present in all aquarium water but attacks only where the fish skin is damaged where the parasite well grows at the skin wound producing a cotton wool like growth.

Preventive measure: 5% aqueous solution of methylene blue used to treat the infected fishes for 7 to 10 days.

4) Fin Rot/Gill Rot/Tail Rot: It is a bacterial disease attacks on fins, gills and tail and inflammation and destruction of tissue takes places and spread all over the body and causes death.

Preventive measure: A week solution of acriflavine or Penicillin 1/160 gallon is used to cure the diseases.

5) Fluke infection

Gyroductylus and Dactylogyrus

It is cause by trematode worms and grows on body as well as gills respectively in that diseses the body get pale, wide open pale gills and torn slimy fins.

Preventive measure: 5% aqueous solution of Methylene blue used to treat the infected fishes for 7 to 10 days.

6) Setting up an aquarium

i. Visit to place where you have to set up an aquarium
ii. Decide the size of home/commercial aquarium
iii. Procuring tank, stand, gravels, heater, air pump, decoration material, proper light connection and etc.
iv. Fill the tank up to 90% and stay for two days to find the leakage if any
v. Wash the sand, gavels, stone, plastic plants, decorative material from rusting
vi. Wash and dry the tank after two days
vii. Kept of the material in the tank
viii. Proper placement of heater, sand, gavels, stone, plastic plants, decorative material air pump, filter and etc.
ix. Fill the tank and switching the lights
x. Cover the aquarium
xi. Keep monitoring the aquarium for a week i.e. temp and leakage
xii. Add colorful aquarium fishes in aquaria

Side air

Filter silicon

Gun air pump

Tools for making aquarium

Equipment of home aquarium

Equipment of home aquarium

Different size and shape of aquarium

Decoration of home aquarium - 1

Aquarium fishes

Aquarium aquatic plants

Aquatic aquarium plants

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CHAPTER EIGHT

Role of aquaculture in organic farming in Marathwada region (Maharashtra)

Jayvardhan V. Balkhande

Department of Zoology, Digambarrao Bindu ACS College, Bhokar Dist. Nanded. (M.S.)

Corresponding author(s) *Email* address: cageculture2014@gmail.com

Abstract

Fisheries play a crucial role in the economy of India. India ranked number 2 in the world in inland fisheries next to China. Maharashtra is one of the important states in inland fish production as well as marine fish production. Processing of fish leads to enormous amounts of waste. It is estimated that fish processing waste after filleting accounts for approximately 75% of the total fish weight. About 30% of the total fish weight remains as waste in the form of skins and bones during preparation of fish fillets. If we use fish composting technology for the management of fish waste, it can generate organic fertilizer along with employment opportunity to the fish farmers as well as fish sellers. This papers deals with the role of Aquaculture in organic farming in Marathwada.

Keywords: aquaculture, fish waste, organic fertilizer, India

Introduction

Fish is one of the important sources of high-quality protein and provides at least 2.9 billion people with around 15% of their dietary protein (FAO, 2009). Aquaculture is a growing industry that strives to optimize and intensify traditional fish rearing techniques to supply much needed protein for humans, but also meet the needs of luxury demand and it contributed 51.7 million tonnes to total world production, including 31.6 million tons from inland waters (FAO, 2009). Fish and fish oils are also a balanced source of protein components and essential fatty acids contents in human diets (Lovell, 1991). Fish and fishery products are of substantial social and economic importance and are worth around US$170 billion annually and employ up to 47.5 million people worldwide (FAO, 2009).

Fishing generates large quantities of waste daily in fish markets and fish processing industries (canneries, fresh and frozen fish processing plants, etc.). Fish remains have also been traditionally used as fertilizer, given their wealth of nutritive elements (principally N and P) and their rapid decomposition. Composting initiatives using fish offal derived mainly from aquiculture have been carried out in various parts of the world in search of alternative and viable techniques for transforming fish waste into useful agricultural products [Frederick et.al (1989), Liao *et.al* (1997) and Kinnunen *et.al* (2005)].

Indian share in the global production has reached 4.36 percent with 9.92 percent share in inland and 2.8 percent in marine in 2009. For inland sector India is ranked second after China (Wagay, 2012).

Though fish is available in all season, during the post monsoon season vast quantity of fish waste along with non-edible related waste are generated both in village market and city fish market. The fish sellers just concentrate on selling of the fishes but they are avoiding to proper management of these fish waste generated from the fishes, as this waste material generated from fishes poses a big problem. As the fish waste contains rich proteins, it cannot be kept beyond 24 hours. After 24 hours fish started to spoil, disgusting smell comes from the fish. Hence preparation of organic fertilizer from fish waste is one of the great outputs to enhancement of the soil fertility.

Table 1: State-wise area of small, medium and large reservoirs (ha)

State	Small	Medium	Large	Total
Tamil Nadu	315,941	19,577	23,222	358,740
Karnataka	228,657	29,078	179,556	437,291
Madhya Pradesh	172,575	149,259	138,550	460,384
Andhra Pradesh	201,927	66,429	190,151	458,507
Maharashtra	**119,515**	**39,161**	**115,054**	**273,750**
Gujarat	84,124	57,748	144,358	286,230
Bihar	12,461	12,523	71,711	96,695
Orissa	66,047	12,748	119,403	198,198
Kerala	7,975	15,500	6,160	29,635
Uttar Pradesh	218,651	44,993	71,196	334,840
Rajasthan	54,231	49,827	49,386	153,444
West Bengal	732	4,600	10,400	15,732
North eastern state	2,239	5,835	----	8,074
Himachal Pradesh	200	----	41,364	41,564
Haryana	282	----	----	282
Total	**1,485,557**	**507,298**	**1,160,511**	**3,153,366**

From: (Vass and Sugunan 2011)

Why organic farming through Aquaculture in Marathwada

Marathwada is one of the most important parts of Maharashtra state. Fresh water resources of Marathwada are rivers, reservoirs, ponds, lakes, etc. out of them river Godavari is the important river and many projects are constructed in the basin of river Godavari and its tributaries such as Jayakwadi (Aurangabad), Mazalgaon (Beed), Purna Yeldari (Parbhani), Lower Terna (Beed), Purna Siddheshwar (Hingoli), Manar (Nanded) and Manjra (Latur) etc. and many more are being constructed in this river system. These projects are used to perform various activities such as irrigation, agriculture, domestic, industrial and aquaculture etc. culture as well as capture fisheries are performed here. Indian major carps i.e. Catla catla, Labeo rohita and Cirrhinus mrigala and Exotic carp along with some other important fishes were cultured in these water bodies.

Challenges

The Organic farming sector faces a number of constraints despite its seemingly bright prospects and high potential for expansion and continued growth. There is also an urgent need

to consider the practical foundation on which to establish a sustainable organic farming sector to ensure sustainable development.

The following issues will face by Aquaculture for organic farming sector:

1. Lack of technologies and qualified technicians.

2. Fish farmers and sellers were not interested to store the waste material from fishes.

3. Disgusting smell of the fish waste is one of the challenges for storage.

4. It requires time and some management.

Fish waste composting benefits and applications

Fish wastes have long been used as fertilizers can be directly applied to soil (Olsen and Olsen, 2011). Their high contents in nutrients particularly N and P, (Arvanitoyannis and Kassaveti, 2008).

There are various types of commercial fish-based fertilizers, namely:

Fish emulsions which are obtained from the liquid fraction remaining preparing fish flour.

Fish hydrolysates, which retain the full properties of the starting material because they are not obtained by extracting oil. Rather, they are produced by cold enzymatic hydrolysis, which breaks fish materials into more simple protein complexes.

Fish flour which is obtained by extracting oil. Initially used as fertilizer, fish flour became a major ingredient of animal feed for pigs and poultry in the mid-XX century at the expense of previous agricultural uses .However, fish flour is still used in some commercial fertilizers.

Fish compost obtained by aerobic fermentation which reduces the volume of the waste and implies a thermophilic phase with temperatures above 55 ºC for a few days which ensure complete hygienization of the product (Marta Illera Vives, 2015). Fish waste compost contains about 1.18% N, 0.48% phosphorous and 0.58% potassium (Balkhande, 2020). With proper disposal decomposition, fish remains can be turned “waste” into wealth, not only for environment and agriculture, but also for fishery industry. Fish waste composting can aid in pollution abatement and building fairly good ecological environment, protecting water resources from contamination. Moreover, it’s beneficial to increase soil nutrient content, restrain some plant disease, decrease parasites and eliminate weed seeds. As organic fertilizer, high quality fish waste composting also has marketable value, which can increase extra income opportunity. Besides, it also helps to end the wastes of recyclable raw ingredients through returning nutrition’s back to agriculture. In virtue of all these advantages, fish waste compost can be applied into farm, garden, vegetable production, field crops, trees and landscapes as soil amendment and fertilizer supplement to increase soil organic matter and nutrients, promote moisture holding ability, and then enhance production and quality.

If this technology can accept by the fish sellers as well as fish farmers in this area hence they will have double benefit. This sector not only provides cheapest source of protein but also generate employment opportunities among the fish related people.

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CHAPTER NINE

Chinese Hatchery

Shivaji B. Ubarhande

Department of Zoology, Rajarshi Shahu Arts, Commerce and Science College, Pathri, Phulambri Aurangabad M.S., India

Corresponding author(s) *Email* address: shivaji.ubarhande@gmail.com

Introduction

Chinese circular fish seed hatchery is also called ECO-HATCHERY was introduced in India in the year 1985. The Chinese circular hatchery is a hatching system is economically sustainable and capable of maintaining optimum environmental condition required for the production of quality fish seed in India.

The Chinese circular hatchery is circular in shape and includes four main components.

1) Overhead Tank

2) Spawning Tank

3) Incubation/Hatching Pool

4) Spawn Collection Unit

1) Over-head tank

The water capacity of overhead tank varies as per the requirement and water supply to overhead tank by pumping water through well or tube well and water from overhead tank to all system regulated by plastic pipes.

The main purpose of overhead tank is to store the water to the spawning and hatching unit. The floor of the tank is 2.6 to 3.0m above ground level and the inside dimension is about 3.5mx2.0mx2.0m having a capacity of 15000 lit.

2) Spawning pool

Spawning Pool is a circular cement tank of 4 to 5 m diameter and this size may be various as per the requirement with a total inside depth at the periphery is 1.2m to 1.5m. Where slope down to the center is 1.5m. It is provided with centrally located outlet pipe leading to the incubation chamber. It is also provided with diagonally fitted inlet pipes (Duck Mouth shape) to the pool at 15 to 16 places equally spaced and fixed at the angle of 45° and which are connected with a main pipe from the overhead tank. Inflow of water in the spawning pool creates a current of water which stimulates riverine conditions.

Recent Trends in Fisheries and Aquaculture - Vol. 1 – Gaikwad et al. (Eds.) ISBN: 978-93-95369-04-6

This arrangement of inlet pipes produces a centrifugal force and help in the smooth of fertilized eggs through centrally transmitted in to the hatching pool.

A number of fine sowers are fitted to the pool with helps in aeration and cooling the water. Function of spawning pool is to provide an optimum environment to the injected brooders as it is available in natural breeding grounds for successful spawning. The ratio of brooders for breeding is 1:2 as per the weight one female: two male and the artificial hormonal injection used for brooders are Ovatide or Ovaprine

3) Incubation/Hatching Pool

As per the requirement and depending of the frequency of operation two or three incubation pool. The diameter of hatching pool is 1.5m and consists of 2 chambers. The outer chamber diameter is 2.0m Cement wall and another circular wall fixed with nylon screen at 0.5 m diameter from the outer wall. The inner chamber is provided with 5cm diameter vertical outlet with holes at different height for getting extra amount of water.

Water supply pipes are fitted from the spawning pools by 7.5cm pipe line and then bifurcated into two are three depending on the number of hatching pools which are further connected to mouth on the floor of hatching pools. It avoids setting of the eggs at the bottom and also provides oxygenation.

4) Spawn Collection Unit

Hatching is completed between 12 to 16 hours (duration of one operation is of 4 days) depending on the water temperature hatching are allowed to remain in incubation pool till the yock sac is absorbed from the outlet the spawn pass into receiving pond. The rate of flow of water from hatching pool is 0.2 to 0.4 m/sec. Ones the yock sac is completely absorbed the spawn received at the lower elevation than the hatching pool so as to drain out the spawn with water from the gravity.

After transfer of spawn in to receiving pond where cloth hapa is fixed with the help of bamboo poles spawn are collected to hatching pool, measured and transfer to nursery pond for rearing.

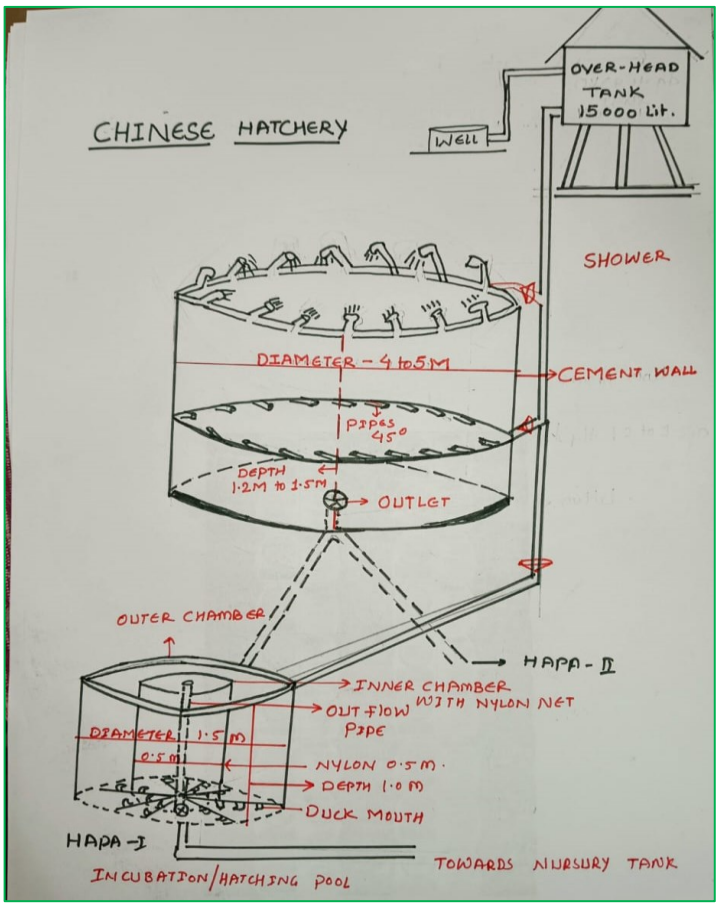
Advantages and disadvantages of Chinese hatchery

Advantages

i. Chinese hatchery is an Eco-Hatchery system and very easy to operate
ii. One or two persons can operate the Chinese hatchery system.
iii. Hatching can produce huge quantity of eggs at a time and hatching survival is about 90 to 95 % hence better chance of sympathetic breeding
iv. In another method of breeding we have to kill the fish for pituitary exact hence in this method no need to kill the fish instead that the hormonal injection is used for breeding purpose (Ovatide or Ovaprine) and also the ratio of fish is reduced 1:2 (One female : two male).
v. This system is designed for fish breeding and incubation.
vi. The duration of one operation for hatching is 4 days. It can be repeated after a period of 4 days.
vii. Major carps, minor carp, grass carp, silver carp are more suitable for breeding.

Disadvantage

1. At initial stage higher construction cost and its non-portable nature
2. Chinese hatchery requires more amount of water.
3. Need permanent source of water either well, tube well or nearby any dam.



Incubation/hatching pool

Chinese circular hatchery

Hatching hapa

Overhead tank

Acknowledgement

Author is thankful to the Principal, Rajarshi Shahu arts, commerce and Science College, Owner/Manager of Chinese circular hatchery center Paithan for valuable suggestions, photograph and guidance.

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CHAPTER TEN

Impact of microplastics in marine ecosystem: a review

Prasenjit Maity and Joydev Maity*

Department of Fishery Sciences, Vidyasagar University, Midnapore – 721102, (WB), India

Corresponding author(s) *Email* address: jmaity@mail.vidyasagar.ac.in

Abstract

Microplastic is one of the substances that is polluting not just the world's coasts but also freshwater bodies because people utilize the oceans as trash cans. Microplastics are particles that are less than 5 mm in size. Microplastics are microscopic plastic grains that are used as cleaners in cosmetics, hand cleaners, and air-blasting. These pollutants are currently present in practically all maritime ecosystems. Plastics' durability makes them very resistant to disintegration, and via indiscriminate disposal, they end up in the aquatic environment. Today, it is a growing scientific concern since these microparticles are generally accessible to a variety of aquatic creatures and are eventually transported along the food chain. Ingestion of alternative microparticles has the possibility of harming humans by altering genetics and increasing the chance of developing cancer, obesity, and reproduction. Given the serious threat that microplastics pose to coastal ecosystems and public health, it is vital to reduce the overuse of microplastics and implement suitable rules and policies to regulate the origins of plastic litter. We will be able to remove our oceans' garbage in the future by implementing biodegradable plastic procedures and pushing plastic awareness programs through different information and social media.

Keywords: microplastic, microplastic effect, microbeads, microplastic in the ocean, plastic

Introduction

Plastics are synthetic organic polymers made by polymerizing monomers taken from petroleum and other sources. They are lightweight, affordable, and long-lasting materials that can be readily sculpted into a wide range of items. It may be used in a wide range of applications Plastics have achieved a vital role in contemporary life and are currently widespread. (Halden RU, 2010). With the discovery of vulcanized rubber and polystyrene in 1839, the squeezing of plastics was constrained. In 1907, Bakelite, the very first fully synthetic polymer, was invented in Belgium. Plastic production began in the 1940s and has continued to grow ever since. The 1920s saw the start of the plastics industry, which took off after the 1940s. Global plastic production in 2014 was 20 times greater than it was in 1964. (Neufeld *et al.* 2016). In 2010, 4.8 to 12.7 million metric tonnes of land-based plastic garbage from 192 coastal countries totaled approximately 275 million metric tonnes (Jambeck *et al.* 2015). Furthermore, as marine plastic waste degrades and fragments, dangerous secondary microplastic is created in the water. 60 to 99 million metric tonnes of microplastic trash were

produced in 2015. (Lebreton and Andrady 2019). Plastic output expanded significantly from the 1950s, from 1.7 million tonnes to 360 million tonnes in 2018 (Plastics Europe, 2019). It is also the most rapidly growing type of urban rubbish, accounting for 60-80% of marine debris (Moore CJ, 2008).

What is microplastic?

Plastic has been identified as the most prevalent source of marine contamination in our seas and Great Lakes. Plastic debris occurs in a variety of shapes and sizes, but those less than five millimeters long (about the size of a sesame seed) are known as "microplastics." Microplastics are found in a variety of environments, including larger plastic garbage that degrades into smaller and smaller particles. Furthermore, microbeads, a kind of microplastic, are very small particles of synthetic polyethylene plastic used as exfoliants in a variety of cleansers and kinds of toothpaste. These small particles easily get through water filtration systems and end up in the ocean and Great Lakes, posing a threat to aquatic life. (NOAA, 2017). Plastic compounds break down into tiny bits over time. Due to changing environmental circumstances, larger plastic trash progressively degrades into little bits ranging in size from meters to nanometers. Microplastics are fragmented plastics with a size of less than 5 mm. (GESAMP, 2016).

Microplastics (MPs) have been detected as little plastic parts no greater than five millimeters in size with no set bottom limit in aquatic ecology, they are highly hardy. These are invisible to the naked eye but may be found in a range of habitats such as lakes, rivers, oceans, sea ice, and snow. Wildlife digestive systems, respiratory structures, and tissues include birds, mammals, reptiles, fish, and shellfish, among other things. (Depledge MH et al, 2013).

Categories of plastic debris

Shape, size, colour, kind of polymer, origin (such as from the land, sea, or air), and other characteristics of plastic trash can be used to describe and narrate it. detritus from sewage or fishing), as well as its original use (e.g., Rope, packing They are also divided into groups according to origin depending on whether the source is primary or secondary Particles are that size because they were made to be that size (primary) if they came about as a result of the degradation of microplastics (secondary).

The four groups of plastics listed by Barnes et al. (Barnes DK et al, 2009)

a) Macro plastics are big plastic particles (>20 mm) like plastic containers.

b) Virgin plastic, which is a type of big plastic particle resin granules, which typically range in size from 5 to 20 mm.

d) Microplastics are tiny pieces of plastic that are generally less than that result from the degradation of macro plastics in less than 5mm.

d) Tiny microplastic particles known as nano plastics range in size from 0.2-2mm in size.

Mega-debris (>100mm) is another name for this type of debris used for substantial detritus, such as discarded fishing nets.

Source of microplastic

Microplastics are difficult for the human eye to see. Although their potentially detrimental effects may be less visible, their discharge into the oceans might nevertheless have significant repercussions. The accumulating of microplastics in the food chain and/or adsorption raises questions about human health. when plastic is exposed to toxicants and moves through the environment. (Eriksen, M *et al,* 2014).

The world's oceans are being contaminated by primary and secondary microplastics. As a result of the usage of various definitions and interpretations (Lassen *et al.*, 2015), we selected the following definitions as suggested by Norwegian research. (Sundt, P, 2014).

1. *Primary microplastic:* Plastics that are introduced into the environment directly as tiny particles are referred to as primary microplastics. Products like cleaning agents in toiletries and cosmetics might optionally include them (e.g., shower gels). Additionally, they may result from the erosion of thick plastic. Objects throughout production, usage, or maintenance, such as tire wear during transportation, of the washing-induced erosion of synthetic fabrics. Tiny particles are directly discharged into the environment. Are estimated to account for 15-31% of microplastics in the seas. Laundry of synthetic garments (35% of major microplastics); tyre abrasion from driving (28%); and purposely added microplastics in personal hygiene products, such as microbeads in face scrubs (2%). (Boucher, J. and Friot D., 2017)
2. *Secondary microplastic:* Secondary microplastics are microplastics that form when bigger plastic objects degrade into tiny plastic pieces in the marine environment. This occurs as a consequence of photodegradation and other environmental processes of improper disposal of garbage, such as discarded plastic bags or accidental losses such as fishing nets Given the fact that the roots of Secondary microplastics are difficult to identify due to their disintegration, and it is impossible to effectively quantify them. Determine how many of the microplastic shapes have now been transformed into microplastic. It's for as a result, the paper intends to concentrate on the measurement of primary microplastics. This results from the breakdown of bigger plastic items, such as plastic bags, bottles, or fishing nets. Microplastics contribute to 69-81% of microplastics discovered in the seas. (Boucher, J. and Friot D., 2017).

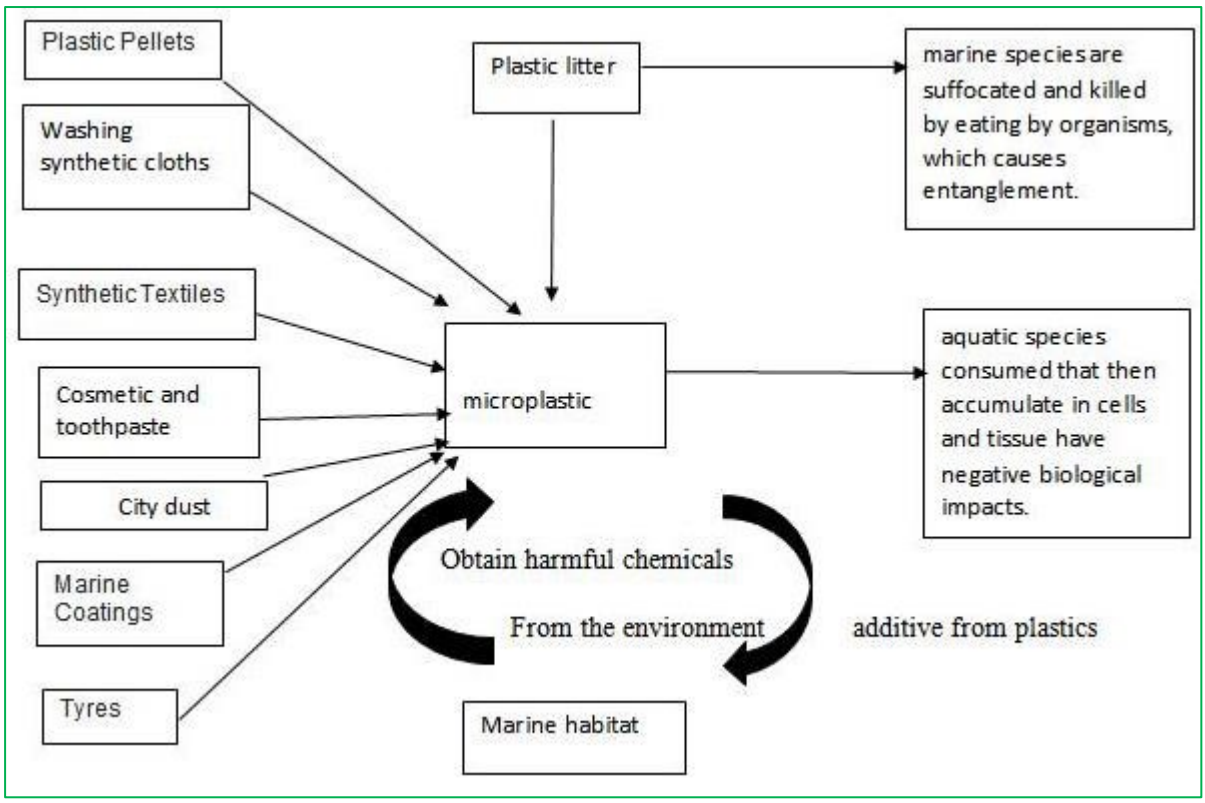

Figure 1: Various environmental microplastic sources and their effects on marine life

Composition of microplastic

Microplastics, defined as any plastic with a diameter of fewer than 5 centimeters, represent a severe danger to our marine ecosystem. According to experts, by 2050, our oceans will contain more plastic than fish. The five primary types of microplastics are described below.

1. *Microfibers*: microfibers may be found in both aquatic and terrestrial ecosystems, from the bottom of the Indian Ocean to American farms According to our findings, when synthetic coats are cleaned, the washing machine releases an average of 1,174 milligrams of microfibers. These microfibers then make their way to a nearby wastewater treatment plant, where up to 40% of them might wind up in rivers, lakes, and seas (depending on local wastewater treatment conditions). (Hartline, N.L, 2016).

2. *Microbeads*: Microbeads are non-biodegradable plastic particles with a diameter of less than one mm. Microbeads can be found in face cleansers, exfoliating soaps, and toothpaste. Microbeads, due to their small size, can get past treatment facilities and enter the Great Lakes. To put things in perspective, one tube of toothpaste can contain 300,000 microbeads. They are an issue because fish and other aquatic creatures mistake them for food. Because plastic is indigestible, it can block the intestines, resulting in famine and death.

3. *Fragments*: Fragments are tiny plastic fragments that break off from bigger plastic parts. Cutlery, lids, and single-use goods are common examples. The sun's UV radiation breaks down these shards into even smaller bits.

4. *Nurdles*: Small plastic pellets called "nurdles" are used to make plastic items. They are melted down by businesses to create moulds for plastic goods like container lids. Nurdles may

fall out of delivery trucks due to their size, especially with train carriages. These nurdles are subsequently pushed down storm drains, which eventually empty into the lake, by storms and precipitation. Fish and other aquatic creatures may confuse nurdles for food, just as pieces and microbeads.

5. *Foam*: Food containers, coffee cups, and packing materials all use Styrofoam. Styrofoam chemicals can contaminate food and drink, harming people's health. The exposure is increased when food is heated in a Styrofoam container. Styrofoam splits into smaller bits as fragments do. Styrofoam recycling is not common among local government.

Microplastic in marine organism

Analytical techniques for microplastics in foods were assessed by EFSA in 2016. In conclusion, the following phases are included in microplastics methods: biogenic matter extraction and degradation, visual detection, visual quantification (enumeration), confirmation, and/or or a description of the particles' plastic nature. If optical microscopy with a computer is employed, the low micrometer range lower size limit of detection. Essential elements Considerable measures must be taken to prevent sample contamination. Microplastics from the environment, clothing, tools, reagents, and/or analyses, and confirmation that the found particles are in fact microscopic plastics. Reference strategies. Microplastics in foods have not yet been sampled or analysed in detail.

Microplastic in bivalves

Few papers have been written about the presence of microplastics in marine bivalves, and those that have largely deal with wild-caught mussels. With the exception of Li *et al* (2016) in table no 1. The studies' sample sizes are rather moderate. Reports are only available for China, Brazil, Europe, and North America. Europe had the lowest concentrations of microplastics overall, with fewer than 0.5 particles per g of soft tissue. Newfoundland, Canada, had the highest concentrations, which were almost 100 times greater than the measurements made in Europe (Mathalon and Hill, 2014). The ambient particle concentration in the lab air was quite high in this most recent investigation, and blank samples showed that laboratory contamination may have contributed 25 tiny particles per gram of soft tissue. Adjusting would result in contents similar to those observed in bivalves given this setting. Out of China (Li *et al*., 2015)

Table 1: Microplastics found in bivalves that are intended for human consumption

species	location	Type of particles	The average number of particles per g soft tissue (SD)	reference
Mytilus edulis	Belgium France Netherlands	Partial fragments	0.20 (0.30); n=6 Size: 20–90 µm	Van Cauwenberghe et al., 2015
Crassostrea gigas	Germany	Fragments, spheroids	0.47 (0.16); n = 21 Size: 5–25 µm (55%), > 25 µm (45%)	Van Cauwenberghe and Janssen, 2014
Venerupis philippinarum	British Colombia	Fibres, films, fragments	Wild: 0.84 (0.85) Farmed: 1.13 (0.66) n= 54 Size: no data	Davidson and Dudas, 2016
Mytilus edulis	Germany	Fragments, spheroids	0.36 (0.07); n = 72 Size: 5–25 µm (85%), > 25 µm (15%) 0.47 (0.16); n = 21	Van Cauwenberghe et al., 2015
Perna perna	Brazil	Fragments	75% contained ≥ 1 particle; n = 30 Size: < 5000 µm	Santana et al., 2016
Mytilus edulis	China	Fibres Fragments	2.2 (0.9–4.6)c ; n~1100 Size: 33–4700 µm (fibres)	Li et al., 2016

only microfibres, spherical particles not quantified, values calculated from Mathalon and Hill (2014) using a weight of 4 g of soft tissue per mussel.

Microplastics in other invertebrates

Common shrimp (*Crangon crangon*) and Norway lobster (*Nephrops norvegicus*) have had their microplastic contents just recently discovered in the North Sea and Irish Sea's coastal waters. sampling areas for typical Belgian, French, Dutch, and Belgian-French coastal waterways, as well as the British Empire. The amount of microplastic particles per gramme varied from 0.03 to 1.92. depending on the location and the sampling date, wet weight

(Devriese et al., 2015). Peeling will eliminate the majority of the digestive system, the head, and the gills, which are thought to be where the majority of the microplastics are found. Peeling occasionally leaves the digestive tract behind, hence Devriese et al. (2015) suggested using as a peeling factor, 90%. Microplastics were found in the stomachs of Irish Sea Norway lobsters (Murray and Cowie, 2011; Welden and Cowie, 2016a). According to Murray and Cowie (2011), 83% of the animals were infected. Although only Welden & Cowie (2016a) assessed the gut's microplastic concentration and discovered samples from the vicinity of the Irish Sea, an average amount of microplastics per person of 0.40 mg to 0.80 mg is the maximum dosage. Because the digestive system is typically Norway lobster guts found to contain microplastics that need to be cleaned before ingestion. won't pose a significant hazard to human health. Ingestion of nylon and polyvinyl chloride (PVC) microplastic particles has been found in four species of sea cucumbers under laboratory circumstances, and echinoderms are widely consumed in some areas (Graham and Thompson, 2009). in most. In some circumstances, echinoderms' digestive tracts are consumed, but this only occurs in wild populations. Only one unidentified sea cucumber specimen had microplastics deep-sea samples of various species (Taylor et al., 2016).

Microplastic in fin fish

Although at relatively low concentrations of one to two items per fish, intake of microplastics has been documented in a sizable number of fish species used for human food from the Pacific, Atlantic, and Indian seas as well as the Mediterranean Sea. each person (Lusher 2015, GESAMP, 2016). As an illustration, microplastics have 11 of of the 20 most significant species that have been found in the gastro-intestinal tract, and species of fish that are important to world marine fisheries (FAO, 2016b). These kinds are Atlantic herring (*Clupea harengus*), chub mackerel (*Scomber japonicus*), and Japanese anchovy (*Engraulis japonicus*), mackerel (*Scomber scombrus*), and Atlantic cod Blue whiting (*Micromesistius)*, European pilchard (*Gadus morhua*), and pilchard (*Sardina pilchardus*) poutassou, king mackerel, and European sprat (*Sprattus sprattus*) (*Scomberomorus cavalla*) shortfin (*Decapterus macrosoma*) and amberstripe (*Scomberomorus spp* group) Scads from the Decapterus spp. family (*Decapterus muroadsi*) and Indian oil sardines (*Sardinella longiceps*), a species of Sardinella (Brate et al., 2016; Collard et al., 2017). Güven et al., 2017, Liboiron et al., 2016, Lusher, 2015, 2017, Foekema et al., 2013, Miranda and Freire de Carvalho-Souza, 2016, McHugh and Thompson, 2013, and Neves Rochman et al., 2015; Rummel et al., 2016b; Sulochanan; et al., 2015; Ory et al., 2017; Tanaka and Takada (2016).

Microplastic was found in roughly 30% of the different fish species studied (Possatto et al. 2011; Lusher et al. 2013). Microplastics were discovered in the bottom-feeding fish (Gerreidae) from a tropical estuary in northeastern Brazil. Mostly impacted was the stomach (Ramos et al. 2012). Direct intake as food or mistaken ingestion of prey items is the main method by which microplastics are exposed to the body. Microplastic (5 mm) buildup in fish guts causes starvation and malnutrition, which finally leads to fish mortality (Boerger et al.2010). According to these findings, larger microplastic beads are more detrimental to the marine fish population than smaller microplastic fragments since smaller microplastics can be eliminated through natural feces. aside from Fish consumption of various types of

microplastics investigated 83% of the lobsters were discovered to be Norway lobster contaminated with microfibers (Murray and Cowie 2011).

Microplastic in plankton

The photosynthetic phytoplankton uses energy from sunlight and inorganic CO2 to repair carbon. According to Nerland et al. (2014), microplastics pierce planktonic cell walls and membranes and lower the quantities of chlorophyll in green algae. The interactions between these microplastic particles were prompted by the fact that microplastic was everywhere in the water column and zooplankton, as well as the results of such interactions, important because zooplankton supplies a crucial resource energy transmission to trophic levels higher. as a result, it stored contaminants could transfer to the water. Through this method, greater trophic levels are attained, which poses a serious risk to the well-being of the marine ecosystem. Zooplankton includes numerous species, including various life cycle stages, and display a variety of feeding techniques (Wirtz 2012). Numerous zooplankton species, including copepods, shrimp, cladocerans, worms, ciliates, and polychaete larvae, have been found to consume microplastics in the Baltic Sea. (Setala et al). In the form of faecal pellets, the microplastics are ingested by zooplankton species, and this ejected material is subsequently available to pelagic and benthic aquatic creatures as well as to aquatic life columns. These feces are sinking above the surface of the water their presence in the column to the seafloor, which benthos (Setala et al. 2014). Ingestion of microplastics by pelagic copepods larvae of the genus Marenzelleria was reported to be the highest among zooplankton species. Ingested microparticles also may pass through the stomach of zooplanktons or may obstruct or concentrate in their digestive tracts, causing disruptions in feeding and digestion. In contrast to the coast crab Carcinus maenus, zooplankton kept polystyrene microplastic in their guts for up to 7 days, according to Cole et al (2013).'s report (Watts et al. 2014).

Microplastic in benthic organisms

A significant portion of the marine environment, about 98% of the total marine biota, is made up of the benthic community. Benthic invertebrates include blue crabs, oysters, and Microplastic ingestion in mussels, barnacles, and lobsters has all been documented (Nerland et al. 2014). 33.5% of microplastic particles were also found in the suspension-feeding benthic barnacles from the North Pacific (Goldstein and Goodwin 2013). Microplastics (less than 5 mm) appeared as tangled balls in the gut of lobsters (*Nephrops norvegicus*) in Clyde Bay (on Scotland's west coast) (Murray and Cowie 2011). It is assumed that when omnivorous lobsters ate benthic animals like crustaceans, polychaetes, and bivalves, the lobsters then absorbed microplastic through the feeding process (Murray and Cowie 2011). Sea urchin larvae have been discovered to consume microplastic particles in the size range of 10–40 M in laboratory settings, which is similar to the dimensions of their prey, which serves as their meal. The high lipid content of the benthic worm Arenicola marina makes it an essential component of marine food chains. Unfortunately, a large percentage of microplastics are also indirectly ingested by this worm when it is feeding (Wright et al. 2013).

Seabirds

Sea birds that fed at the water's surface, like albatrosses, shearwaters, petrels, and northern fulmars, swallowed microplastic that accumulated in their stomachs. About 30–35%

of the plastics detected in the sea birds were in the form of manufacturing pellets (Ryan 1987; Robards et al. 1995; Blight and Burger 1997). Seabirds can eliminate microplastics from their intestinal tracts by regurgitation, and *Larus glaucescens* has been found to do the same (Lindborg et al. 2012). *Larus glaucescens* was already discovered to regurgitate microplastics from its intestinal tracts in the same way that seabirds May (Lindborg et al. 2012).

Microplastic in coral reef

Coral reefs are geological formations that have the largest biodiversity diversity in the ocean environment and protect coastal areas from the ocean's destructive forces (Yap 2012). They cover 0.2% of the ocean's surface and are home to thousands of different animals, including one-third of all aquatic species (Barnette 2001). Corals consume a variety of other foods, such as phytoplankton, zooplankton, and other tiny animals that live in seawater, in addition to the energy they obtain through photosynthesis carried out by symbiotic organisms within their cells. Corals' primary food supply is phytoplankton, copepods, zooplankton, and other aquatic life forms that can consume microplastics (2–5 mm) in aquatic organisms. Because microplastics cannot be digested by corals and instead collect in their digestive systems, their health is directly impacted by microplastic contamination (Ferrier-Pages *et al*. 2003). Microplastics were found in low concentrations in marine environments, in the first study on microplastic ingestion in coral reefs in the vicinity of Australia's Great Barrier Reef (GRE, 18°31′S 146° 23′E). A feeding experiment on corals revealed that they confuse microplastics for food and can consume up to 50 g of plastic per square centimeter per hour, a pace that is comparable to the rates at which they consume plankton and species like Artemia nauplii. Mesenterial tissue within in the coral gut cavity was discovered to be the most impacted location following ingestion of large concentrations of microplastics by corals, which ultimately damages the corals' health (Hall *et al*. 2015).

Marine mammals and turtles

One millimeter-diameter microplastic particles have been found in the fur seals and Hooker's sea lions (Goldsworthy *et al.* 1997; McMahon *et al.* 1999). Sea turtles, whales, harbour seals, and polar bears are just a few of the huge marine creatures that have been shown to be adversely affected by microplastic waste (Derraik 2002). Baleen whales, another marine mammal, were particularly vulnerable to microplastic contamination because they were involved in filtering species that filter seawater and that make it easier for microplastics to enter their systems (Fossi *et al.* 2012). Whales are also very susceptible to ingesting and accumulating microplastics in their stomach and intestine due to their high fat and lipid content. There have been numerous instances in recent months of stranded whales dying with large amounts of microplastic debris in their guts. A harbour seal's (*Phoca vitulina*) stomach and intestine were found to contain microplastics (Bravo Rebolledo *et al.* 2013). According to research by Nerland *et al.* (2014), around 60.5% of sea turtles in Brazil had microplastic buildup in their digestive tracts.

Transfer of microplastic into the food chain

The ability of pollutants to absorb from water and pass to additional trophic levels by biomagnifications is a severe threat as well as the problem with pollutants transference in the food chain. Because they have a reasonable area to volume ratio and are made of hazardous

additives and monomers, microplastics are good at removing hydrophobic contaminants from water sources (Mato *et al*. 2001; Thompson *et al*. 2007). Microplastics are able to partition very low amounts of persistent organic pollutants (POPs) that are present in marine ecosystems. The hydrophobicity of POPs is what causes them to absorb more readily in microplastic waste and when consumed by marine life, which allows them to move up the oceanic food chain. Numerous marine animals are harmed by the consumption of microplastics and microbeads, in addition to phytoplankton and corals, which also absorb their toxins. Microplastics exposed to toxic algae produce phycotoxins, which may have an indirect negative impact on human health and the economy. The majority of phytotoxins are delivered by the harmful alga and are then taken up by plankton or benthos (bivalves or crustaceans) through eating. For instance, algal toxins created by the exposure of microplastics that effectively accumulate in shellfish and can transfer in the food web and indirectly cause dangerous symptoms in people are the cause of diarrhetic and paralytic shellfish poisonings (DSP and PSP) (Teegarden and Cembella 1996; Mons *et al*. 1998; Campbell *et al*. 2005). Algal toxins are capable of building up in marine food webs and moving up the trophic chain. The following is a discussion of the impact of microplastic pollution in marine habitats, species by species.

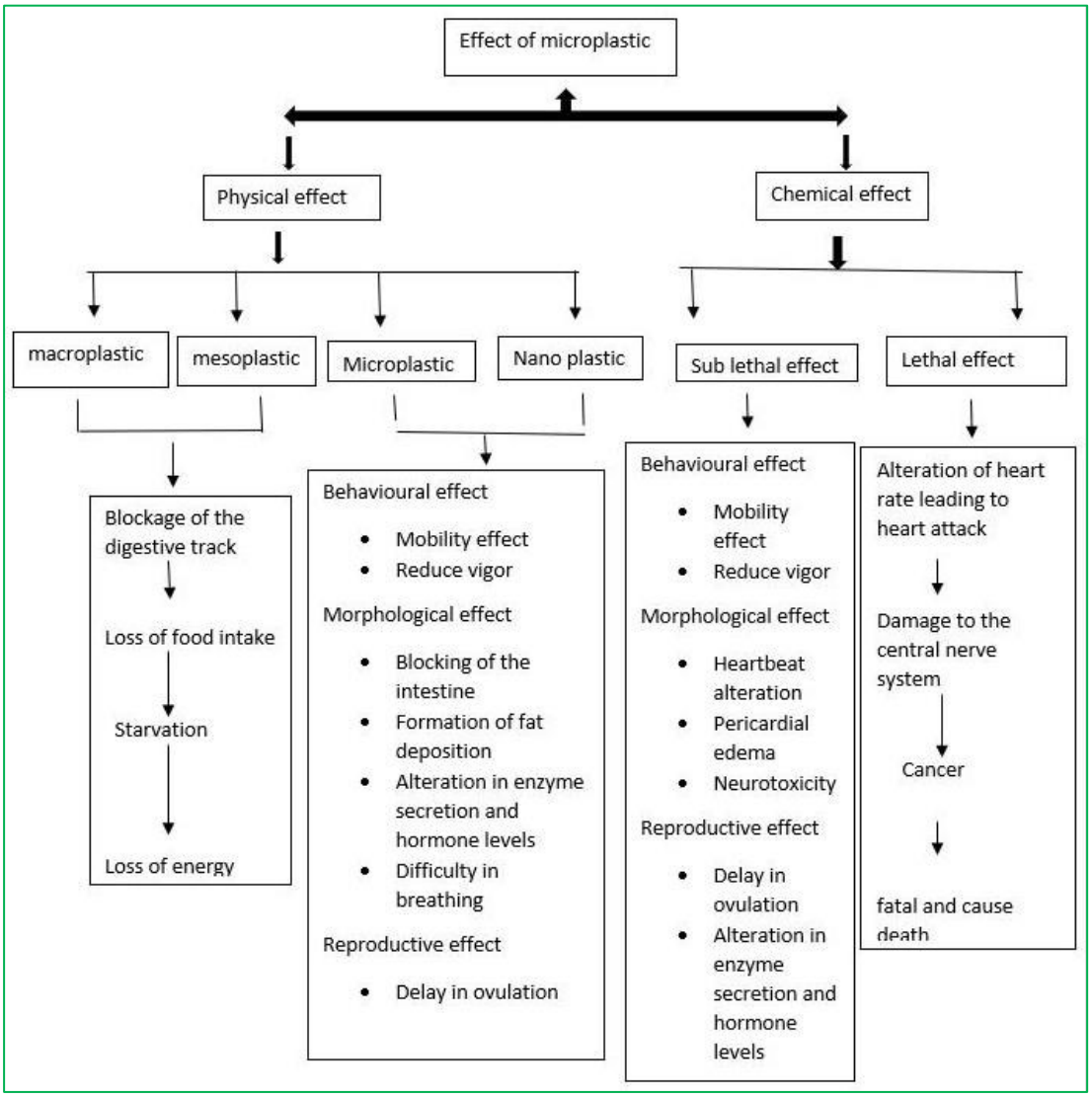

Figure 2: Negative effect of microplastic on animal health

Policies adopted by various organizations worldwide

A referendum on plastic bags is gaining popularity all across the world. Recently, California implemented a complete ban on single-use plastic bags by adopting the Prop 67 initiative (Plastic Pollution Coalition 2016). Similar to this, Scotland's policy banning plastic bags stopped about 650 million bags from entering the trash system (Plastic Pollution Coalition 2016). In order to limit plastic bags in Ireland, Lowenthal's bill was introduced, which mandated a minimum fine of ten cents on plastic shopping bags and a fine of four cents on recyclable plastic bags. The fine money was then donated to the Land and Water Conservation Fund for an environmental protection and conservation initiative (Plastic Pollution Coalition

Table no 2: different policies adopted to control plastic by a different country.

Country	State	Policy
USA	California	With the passage of the Prop. 67 bill, California became the inaugural state in the USA to outlaw single-use plastic shopping bags.
USA	San Francisco	Plastic water bottle sales are completely prohibited in San Francisco.
Ireland		When Lowenthal's measure was launched, each bag that stores provided to carry out foodstuffs and other purchases would cost a minimum of 10 cents, and 4 cents if the store had a recycling programme that qualified.
India		Environmental hazards of this kind have an impact not only on people but also on the entire ecosystem. To keep the environment safer for future generations, the government should replace paper or jute bags with plastic ones. The waste management system in India has been enhanced thanks to the government's initial response. More regularly should programmes like Swacch Bharat Abhyaan be undertaken.
France		To combat the world's growing problem of plastic pollution, the government passed a 'Plastic Ban' law in 2016, which declares that all plastic plates, cups, and utensils would be outlawed by 2020. France is the first country to outlaw all plastic-based consumer goods. This regulation also requires that replacements for these items be manufactured from biologically derived and compostable materials. The rule is also the outcome of a complete ban on the use of plastic shopping bags. By 2025, the law intends to slash the country's use of plastic bags in half.

The federal government of other nations, including Canada, Austria, Australia, Belgium, Luxembourg, the Netherlands, Germany, and Sweden, has likewise outlawed the use of microbeads in cosmetics (Perschbacher 2016). San Francisco becomes the first state in the United States to prohibit the use of plastic water bottles (Plastic Pollution Coalition 2016).

Microbeads are on the list of harmful compounds established by the Canadian Environmental Protection Act, and their usage is strictly prohibited. They also proposed restrictions prohibiting the manufacture, import, and export of microbead-containing personal care items (The Globe and Mail Newspaper 2016).

A number of grocery firms, including Johnson & Johnson, Livon, and L'Oréal, have committed to eliminating microfibers and microbeads from various products for personal care (Copeland 2015). Government should impose a "zero Table no 2: different policies adopted to control plastic by a different country tolerance" policy on this matter and mandate that businesses use starch or other biodegradable materials in place of nonbiodegradable ones. This biodegradable material will be broken down by bacteria and fungi, shortening the time that these polymers remain in the environment. The upgrading or recycling of plastic waste should be promoted in industries. The tertiary recycling of plastic, which involves breaking down plastic materials into smaller bits that can then be utilised as feedstock for the production of new petrochemicals, has recently come to light as one of the most advanced recycling procedures. To raise people's awareness of this potential hazard, more reviews on this subject should be written and more research should be done. The different policy adopts by the different countries for control of plastic pollution. The policy was taken by different country given in table no 2.

Conclusion

Microplastics in the world's oceans are in worrying condition because it is pervasive in the environment, have negative effects on marine life, and is transferred down the food chain, all of which are serious problems. At the global, national, and local levels, there is an urgent need to take serious action to solve the issue. Developing nations such as Pakistan, Bangladesh, Thailand, Vietnam, and India, the top contributors are Korea, China, Sri Lanka, and the Philippines of airborne plastic contamination in the ocean. Many developing nations haven't developed laws and guidelines to halt the pollution from microplastics. In order to monitor the long-term consequences of plastic litter in the environment, it is advised that local governments implement strict legal regulations. It is necessary to develop new scientific information on the contamination caused by microplastics for standard guidelines and conservation management the foundation for educational efforts is strengthened. The general public's understanding of microplastic pollution is crucial because it will influence how they consume plastic and, more crucially, since most people are still unaware of the harmful impacts of plastic pollution. It is vital to embrace various strategies and programs that may help raise public awareness of the chronic and long-term effects of plastic pollution. To reduce the pollution caused by microplastics, several socially engaged international organizations, such as the International Maritime Organization (IMO) and the United Nations Environment Programme (UNEP), should organize specific campaigns on a worldwide level. Finally, the plastics manufacturing sector needs to be responsible and handle its end-of-life products with care. Government should impose a "zero tolerance" policy on this matter and mandate that businesses use starch or other biodegradable materials in place of nonbiodegradable ones. This renewable component will then be broken down by bacteria and fungi, shortening the time that these polymers remain in the environment. The upgrading or recycling of plastic waste should be promoted in industries. The tertiary recycling of plastic, which involves breaking down

plastic materials into smaller bits that can then be utilised as feedstock for the production of new petrochemicals, has recently come to light as one of the most advanced recycling procedures. To raise people's awareness of this potential hazard, more studies on this subject should be written and more research should be done.

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CHAPTER ELEVEN

Freshwater designer pearl farming

V. Dabhade* and S. Poul

Department of Zoology, Madhavrao Patil College, Palam Dist. Parbhani M.S., India

Corresponding author(s) *Email* address: dabhadevarsha@gmai.com

Introduction

Mussels found in fresh and marine waters. Freshwater mussels found in river, lake, pond and tanks. These amazing animal, are essentially living water filters, moving as much as eight gallons (36.37 lit) of water per day through their internal filtration system. Filter water for planktons and feeds them therefore known to clear turbidity of water due to high filtration ability. Freshwater mussels can maintain normal metabolism even at levels of dissolved oxygen as low as 1mg/L and can tolerate even complete anoxia for as long as several weeks by simply closing the shells. Hence they are biological indictor water quality. Their life span of many decades hardy and harmless.

Micro-algae, Rotifers, Protozoans, Planktonic microorganism and organic detritus are the food of mussels. Mussels drawn water in through its incurrent siphon. It brought into branchial chamber by action of Cilla of gills for ciliary-mucus feeding. Finally water eats through excurrent siphon. The labial pals finally funnel the food into mouth, where digestion begins.

During filtration, accidentally sand or small crystal like objects enters into mussel. Mussels tries to send the foreign body out, but it cannot send out, it become irritant. To overcome this irritation, the mantle outer layer of the animal start to secrete shiny substances called as ‘nacre’ over the foreign body. This coating of the nacre over the object is the pearl or pearl object. The chances of getting naturally formed pearl in an oyster in 1 in 1000 and chances of death for divers was 50%. Hence to meet the demand for pearls and to conserve the population of mussels, pearl are cultured by inducing mussels to deposit nacre around the surgically implanted bio-compatible foreign body (bead or embossed image) of a particular shape and size into identified location in mussels.

Hyderabad is Pearl city, but not even single pearl is produced here. India import crude pearl from Japan Australia, Tahiti, Venezuela Indonesia and China. It processed and grade them before marketing. Pearl trade worth around 500 crores annually. Almost 40% sales attributed to tourists.

In Maharashtra, freshwater mussels are least studied and exploited for commercial use. There true identity is concealed by presence of less movement, sub-strata presence, slower growth rate, lower value as food. However, their intrinsic value for water purification as filter

Recent Trends in Fisheries and Aquaculture - Vol. 1 – Gaikwad et al. (Eds.) ISBN: 978-93-95369-04-6

feeders and their ability to form pearl objects make them interesting for the commercial aquaculture.

Reproduction

Gonads are paired and located either side intestine. Testes are creamy and ovaries are pale red in colour. Sperms released in water through exhalant siphon. Eggs shed into supra-bronchial chamber and carried to outer gill lamellae. Fertilized ovum passes the gill lamellae. Embryo develops into Glochidium larva. Glochidium larva has parasitic life for 60-70 days. It detached from host and growth further at water bottom. Hence it require bottom dwelling fishes like Mrigal or common carp to anchoring of Glochidia.

Scope and Limitation

It requires less water, space, food, labour and investment. Skill for pearl forming is easily acquire. The required material and skill are embossed images, which is easily available in market both online and offline. Other are image implantation in mantle cavity or near gonads. last is management of water quality. However, the limitations are it is started at small scale with designed pearl production and patient to be exercised through daily care of at least half an hour or culture period of one and half year round/oval pearls and 6-8 months for designer pearls.

Pearl Species

Pinctada fucata (Gould), div. margaritifera, (Linnaeus), div. Chemnitzii (Phillippi), div. Sugillata (Reeve), div. Anoamioides (Reeve) and div. Atropurpurea (Dunker) are the Oysters Pearls species are found along Indian Coast. There are 52 species of fresh water mussels belongs to two viz. Genus Lamellidens (Family Unionoidea) and Parreysia (Family Amblemidea). Only 3 out of 52 are used for pearl forming viz. Lamellidens marginalis L. corrianus and Parreysia corrugata.

Training Centre

Freshwater designer pearls production training is conducted for formers and at Inland Fisheries Unit (IFU) of University of Agriculture Sciences, Bangalore, Karnataka and Central Institute of Freshwater Aquaculture (ICAR-CIFA) Bhuvneshwar, Odisha. The sources from these institute, keeping the view of their growth and survival rate and ability to produce designer pearls, the water quality and plankton population are being maintained. The freshwater mussels can be reared in small water bodies the backyard with little daily care and less investment, as they do not need any supplementary food as fish culture. It is suitable for small and landless farmers and also for women in Self Help Groups. Efforts are on to come up with system adoptable for integrated forming system practiced by small and marginal formers. The of this chapter is production of designer pearl rather round or oval.

Advantages of Designer Pearl

A designer nucleus is easy to make by biocompatible materials like acrylic, mussel egg shell powder. Implantation of designers' nuclei is easy especially in mantle cavity. Value addition can made after extraction of designer pearl. There is no competition in designer pearl.

Any image which is in demand locally can be made like local deities, emblems, symbols etc. On other hand, in India round pearls are imported with risk about their genuineness. It is difficult to compete with round pearl imported from China. Formation time of designer pearl is less than round. Method of gonad implantation of round or oval nuclei is difficult to acquire by farmers. Availability of genuine round nuclei is difficult. Though, it can be made using shell powder and adhesives.

Stages of Culture of designer pearl

1. Collection and packing for transportation
2. Preparation of the pond
3. Labelling
4. Suspension of mussels
5. Stocking of mussels for acclimatization
6. Implantation
7. Recuperation
8. Post implantation culture in grow out ponds
9. Maintenance Water quality
10. Extraction and value addition

1. Collection and packing for transportation: From rivers, ponds, reservoir, tank mussels can be collected in summer. At spot wash it well; shift them in created aged clean ground freshwater. Keep here for atlas 5 hours. For packing spread a plastic sheet on the bottom of a cardboard perforated box, spread a layer of hey over it. Heap the washed mussels and then over with hey as the top layer, sprinkle the water and cover the box. Such packed box can transported for three days. Sprinkle the water intermittently. At destination keep mussels at quarantine in aerated water. After three of quarantine shift it to plankton rich water. Any 0.75 m depth tanks can be used for culture.

2. Preparation of the pond: On day first, apply 40gm of lime powder in the form of slurry to 1000 litre water. Second day, add 5 gm groundnut cake and 30gm cow dung in water. At day fourth, mix 10 gm cow dung and 5 gm groundnut cake. Repeat it on day six. Aim of this manure mixture is to enhance the plankton growth. Onward seventh day, one mussels can stock per 10 litre of water. 5 gm groundnut cake and 15 gm cow dung have to add at every 3rd day. If colour of water is green avoid the adding manure.

3. Implantation

i. Mantle cavity implantation for designer pearls
ii. Gonadal implantation for round or oval pearl
iii. Mantla tissue implantation for non-nucleated method (unattached irregular pearl/rice pearl) and nucleated method for unattached small round/oval pearl The required surgical tools are common extractor, forceps, graft cutter, shell opener, mussel holder, incision knife, spatula, ink filler, scissor, wooden small pegs, lamp, acrylic powder, engraver, different dies, magnifying lens, plastic sheet, gloves and mask. The embossed images (nuclei) with different bio-compatible material: The required dies like, rubber, metal acrylic, araldite material, handmade or made from mussel's shell can easily available on online as well offline

4. Labelling: Label all mussel's shell, to identify the image is embossed inside the mussels.

5. Suspension of mussels: For this farmer can use net bag attached with top or vertically arranged mussels in plastic trays or can use plastic trays with empty water bottle hence it won't sink down.

6. Stocking of mussels for acclimatization: Plastic tray can tie with empty water bottles to both sides of the tray by a plastic rope. Keep the setup in selected water body. In summer, net is must to shade the mussels. As scavengers, add 2-3 common carp. Check water parameter every day. After every 10-15 days change 25% water.

7. Recuperation: After every week aerated the aquarium. Observe for the nuclei vomiting, rejection or mortality of mussels.

8. Post implantation culture in grow out ponds and maintenance of Water quality: A week later, transfer mussels in prepared tank. Water has to maintain all parameter for next nine month.

9. Maintenance Water quality: The desired water parameters are like, 75 m depth, colour of water (green dark, green, brown, dark brown), neutral pH, 25-35 temperature, 25-30cm turbidity, >1mg/L DO, <0.2mg/L ammonia, 10mg/L nitrate etc

10. Extraction and value addition: Designer pearls can be embedded in gold or silver to form different jewellery.

The potential of the designer pearls forming the Govt of India notified the subsidy for production of designer pearl. This pearl forming will definitely enhance the economy of farmer's family.