LETHAL AND SUBLETHAL TOXICITY OF DELTAMETHRIN ON FINGERLINGS OF *Catla catla* (HAMILTON)

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BY

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CERTIFICATE

This is to certify that the thesis entitled "Lethal and sublethal toxicity of deltamethrin on fingerlings of Catla catla (HAMILTON)" submitted by Miss. Livi Wilson., I.D. No.MFK 1111 in partial fulfillment of the requirements for the award of Master of Fisheries Science in Aquatic Environment Management of the Karnataka Veterinary, Animal and Fisheries Sciences University, Bidar is a record of bonafide research work carried out by her during the period of her study in this University under my guidance and supervision and the thesis has not previously formed the basis for the award of any degree, diploma associate ship, fellowship or other similar titles.

MANGALORE
AUGUST, 2013

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Dedicated

To My

Beloved Parents & Brother

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(LIVI WILSON)

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Introduction

I. INTRODUCTION

Aquatic ecosystems around the world face serious threats from anthropogenic contaminants. Contaminants from industry, agriculture, urban runoff, and other sources have found their way into these environments, affecting all levels of biological organization, from the individual to the entire ecosystem. The global population growth observed in the recent decades coupled with continued technological advancement and increase in the generation of new industrial products, including the manufacture of chemicals such as fertilizers and pesticides, has led to an expansion in the levels of xenobiotic compounds in aquatic ecosystems (Jesus and Carvalho, 2008).

The pollution of rivers and lakes with chemicals of anthropogenic origin have adverse effects such as the waters become unsuitable for drinking, irrigation, fish cultivation and other household purposes. Also, animal communities living in them may suffer seriously. Massive fish kills are recorded rather frequently, and changes in the population of the fauna as a consequence of sublethal effects on ecologically important species (Koprucu and Aydin, 2004).

Pesticides are used extensively in agriculture and industry because they are easy to apply, cost effective, and it is the only practical method of controlling the pests. However, benefits of pesticides are not derived without consequences. They are one of the most potentially harmful chemicals and are released into the environment by direct applications,

spraying, atmospheric deposition, and surface runoff. Given the fact that, insecticides are not selective and affect non target species readily as target organisms, however, it isn't surprising that a chemical that acts on the insect will elicit similar effects in higher forms of life (Dogan and Can, 2011).

Aquatic toxicology is the study of the effects of manufactured chemicals, anthropogenic and natural materials and their activities on aquatic organisms at various levels of organization, from sub cellular through individual organisms to communities and ecosystems. Acute toxicity is a significant reduction in survival of the exposed organisms within a relatively short time and is expressed as the species specific median lethal concentration (LC_{50}). Chronic toxicity effects can occur at exposure levels far below the concentration that causes lethality. The concentrations of pesticides in surface waters generally range far below lethal concentrations for aquatic organisms. However, sublethal concentrations have adverse effects which may result from exposure of aquatic organisms to pesticides at that concentration in the environment (Das and Mukherjee, 2003).

Pesticides in the environment may be used as a model for the study of environmental toxicology, because they contaminate air, land and water, causing adverse effects ranging from bacteria to humans. It is proved beyond doubt that these chemicals are toxic to aquatic invertebrates and fishes (Santos *et al.*, 2007). The effects of the use of pesticides are recognized worldwide and aggravated by misuse since part of this material is accumulated in plants and soil and much of it is transported to the rivers by rain (Tsuda *et al.*, 1995; Wilson and Tisdell, 2001). Contamination of water bodies with large amounts of pesticides lead to fish

mortality or starvation by destruction of food organisms, and many toxicants have been shown to affect growth rate, reproduction and behavior, with evidence of tissue damage (Van Der Oost , 2003 and Srivastav *et al.*, 2002). The poisoning of fish by pesticides can be acute or chronic and in general acute poisoning causes mass mortality. However, pollution is often a chronic process, apparently without any visible damage but sometimes producing several sublethal effects (Rodrigues, 2003).

In most of the aquatic organisms, toxic action of the chemicals is manifested either by overstimulation or depression of respiratory activity. One of the early symptoms of acute poisoning is the failure of respiratory metabolism. Pesticides affect the oxygen uptake, food consumption, ammonia excretion rate and oxygen : nitrogen ratio, the changes in these parameters can be used as biodetectors in monitoring the physiological effects of pesticides and the oxygen consumption pattern to indicate the possible mapping of metabolic pathways influenced by the pesticide stress.

In India, currently 231 pesticides are registered and about 27 pesticides are banned and 13 are under restricted use in 2010. India produces 90,000 metric tons of pesticides a year. The production of pesticides started in India in 1952 with the establishment of a plant for the production of BHC near Calcutta. Presently India is the second largest manufacturer of pesticides in Asia after China and ranks twelfth globally (Mathur, 1999). There has been a steady growth in the production of technical grade pesticides in India, from 5,000 metric tons in 1958 to 102,240 metric tons in 1998. In 1996–97 the demand for pesticides in terms of value was estimated to be around Rs. 22 billion (USD 0.5 billion), which is about 2% of the

total world market (Md. Wasim *et al.*, 2009). The demand of various types of pesticides for use in agriculture during 2006-07 was 43718 MT (technical grade). The pattern of pesticide usage in India is different from that of the world in general. In India 76% of the pesticide used is insecticide, as against 44% globally (Mathur, 1999). The use of herbicides and fungicides is correspondingly less. The main use of pesticides in India is for cotton crops (45%), followed by paddy and wheat.

Genarally, pesticides are classified into organochlorine pesticides, organophosphate pesticides, carbamates, synthetic-pyrethroid pesticides, microbial insecticides, and insect growth regulators. Organochlorine pesticides are the synthetic organic pesticides that are earliest discovered and used. They are broad-spectrum pesticides having long residual effect and relatively low toxicity. However, due to their stable chemical nature, they are hard to break down in the natural environment. Prolonged use in large quantities will easily lead to environmental pollution and accumulation in mammals, resulting in cumulative poisoning or damage. Organochlorine pesticides are therefore banned under general circumstances and gradually replaced by other pesticides. DDT, Dieldrin, Heptachlor, Chlordane and Endosulfan are some examples of organochlorine pesticides.

Organophosphate pesticides are characterized by their multiple functions and the capacity of controlling a broad spectrum of pests. They are nerve poisons that can be used not only as stomach poison but also as contact poison and fumigant. These pesticides are biodegradable, cause minimum environmental pollution and slow pest resistance. Malathion, Paraoxon and Parathion are examples of some organophosphate pesticides.

Carbamate pesticides work on the same principle as organophosphate pesticides by affecting the transmission of nerve signals resulting in the death of the pest by poisoning. They can be used as stomach and contact poisons as well as fumigants. Moreover, as their molecular structures are largely similar to that of natural organic substances, they can be degraded easily in a natural manner with minimum environmental pollution. Carbofuran is an example of carbamate pesticide.

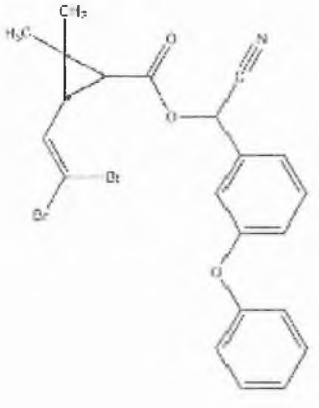
Synthetic-Pyrethroid pesticides are pesticides synthesized by imitating the structure of natural pyrethrins. They are comparatively more stable with longer residual effects than natural pyrethrins. Synthetic-pyrethroid pesticides are highly toxic to insects but slightly toxic to mammals. Deltamethrin, Allethrin and Permethrin are examples of synthetic-pyrethroid pesticides.

Microbial insecticides control pests by means of pathogenic micro-organisms including bacteria, fungus and viruses. *Bacillus thuringiensis israelensis* (*Bti*) is an example of microbial insecticide.

Insect growth regulators are compounds developed by copying insect juvenile hormone. The main functions are to interfere with the growth and hatching of larvae into adults, and to prevent the formation of exoskeleton so as to prevent the growth of the insect. As its ability to live as a living organism is curtailed, the insect may die eventually as well as the whole insect population. Methoprene is an example of insect growth regulator.

Deltamethrin is a pyrethroid pesticide which partially mimics natural chemicals produced by certain chrysanthemum flowers and which have an insecticidal or insect repellent effect. In pure form it exists as odourless white to beige crystals. In its pure crystalline form it is relatively stable but in contact with water, soil or sunlight it is generally rapidly broken down. It kills insects by disrupting signals in the insect's nervous system.

Structural formula



Deltamethrin has been extensively used in households, agriculture and veterinary practices. The main advantages of pyrethroids that made them successively replacing organophosphate pesticides are their photostability, high effectiveness even in low concentration, easy disintegration and low toxicity to birds and mammals. Deltamethrin is used as a pesticide applied to the foliage of plants and crops, as a topical treatment for pets and farm animals and as a prescribed medicine to treat sea lice infestations on farmed fish.

Deltamethrin is widely used in intensive carp farming system for the treatment of external parasites mostly Argulus, Lernae, Paradactylogyrus and Dactylogyrus. They are applied to kill selected life stages of parasites which are present in water. It is released to the environment mainly through its application to crops and animals as an agricultural pesticide

and as a treatment for sea lice. Also, it may enter the environment during its manufacture, transportation and storage.

Table 1: Physical and chemical characteristics of deltamethrin

Common name	Deltamethrin
Classification	Pyrethroid
Trade name	Butox
Chemical name	(S)-α-cyano-3-phenoxybenzyl (1R,3R)-3-(2,2-dibromovinyl)-2,2-dimethylcyclopropanecarboxylate (IUPAC)
Molecular formula	$C_{22}H_{19}Br_2NO_3$
Molecular weight	505.2
Appearance	Off-white solid powder
Solubility	Water, kerosene, isoalkanes, acetone 500g/l, benzene 450 g/l, dimethyl sulfoxide 450 g/l, cyclohexanone 750 g/l, dioxane 900 g/l all at room temperature, toluene 250 g/l

Fish and various other aquatic organisms are extremely susceptible to pyrethroids as the 96 h LC_{50} value determined in laboratory tests, generally lies below 10 µg/L. Deltamethrin

[(s)-a-cyano-3-phenoxybenzyl (R1-R2)-3-(2,2dibromvinyl)-2,2dimethylcyclo propan carboxylate] is one of the most important widely used pyrethroids pesticide. The application of pyrethroid as insecticide and antiparasitary preparations has been accepted on a large scale for agricultural purposes during last 10–15 years. It is already known that pyrethroid insecticide is highly toxic to fish and various other aquatic organisms (Mittal *et al.*, 1994). Sublethal biological responses of pyrethroids include behavioral changes, reduced growth, immune system effects, endocrine effects including decrease of reproductive success, histopathological and biochemical changes (Werner and Moran, 2008). Disturbance of the non-specific immune system is connected with decreased production of leucocytes. Changes of colours and integrity of body surface develop during the weeks of exposure (El-Sayed and Saad, 2007). Fingerlings of Indian carp change shape of their bodies in sublethal exposure to pyrethroids. They become lean towards the abdominal position compared to the control fish and they seem to be under stress (Marigoudar *et al.*, 2009).

Among aquatic vertebrates, fishes as sentinels of pollution studies are gaining importance during the last few decades. Fishes are poikilotherms and are capable of providing an imprint of physicochemical factors and anthropogenic materials in their structural, functional and behavioral patterns. An assessment of the impact of aquatic pollution would be possible only when the deleterious effects are documented employing sentinel species. Fish has been largely used to evaluate the quality of aquatic systems as bioindicators of environmental pollutants. Toxicity tests allow the determination of toxic effects, providing direct evidence of the biological responses of aquatic organisms to contaminants. Due to the fact that organisms from different species vary in their sensitivity towards chemical

substances, it is difficult to set standards for protection of species with regard to pollutants in the environment. Extrapolation from one species to another is, therefore, difficult if their relative sensitivities are not known (Van Straalen *et al*., 1994).

The species of Indian major carp *Catla catla,* is commonly known as catla. It is a Gangetic carp, distributed in rivers of the Indo-Gangetic system in India, Bangladesh, Pakistan and in the rivers of Myanmar that drain into the Bay of Bengal. It also occurs in southern river system and cultured in ponds throughout India. It is a very popular food fish cultured in India. The fish has wide occurrence and distribution, found in freshwater rivers, reservoirs, lakes, jheels, bheels and ponds in India. *Catla catla* is a tropical fish; hardy in nature; its natural temperature range is 18-30 °C. Fish prefers deep pools for good growth, breeds during the south-west monsoon (May-September) in water temperature range of 24-31°C.

Catla is an important candidate species in the intensive carp farming system in India. Mostly the major carps are prone to different parasitic diseases like Argulosis, Lernaeosis, Paradactylogyrosis and Dactylogyrosis in the intensive culture systems. Deltamethrin is widely used for the treatment of these parasitic diseases. An excess use of chemicals for controlling parasitic diseases is to be avoided. Little research has been done on the effect of this chemical in sublethal concentrations on catla fingerlings.

With the above background the objectives of the study were

- To assess the lethal toxicity of pesticide deltamethrin on fingerlings of *Catla catla*.

- To study the sublethal effects of deltamethrin on fingerlings of *Catla catla* in terms of oxygen consumption rate, food consumption rate, ammonia excretion rate and oxygen: nitrogen ratio under laboratory condition.

Review of literature

II. REVIEW OF LITERATURE

Due to rapid increase in the industrialization, urbanization, agriculture and human population, the pollution of aquatic ecosystem has become a universal phenomenon in the present day world (Belazutshi and Raghuprasad, 2008). Aquatic environment is the ultimate sink for all pollutants where they are going to affect the zones more than their counterparts in the two environs of land and water. The agriculture and aquaculture ventures necessitate the use of chemicals and these chemicals as contaminants damage the living inhabitants (Rand and Petrocelli, 1985). The pollution of aquatic environment with wide array of xenobiotic compounds has become a menace to the aquatic flora and fauna and is a problem of immediate concern. These contaminants are let out into the water bodies from industrial and agricultural areas and as most of them are highly persistent, their levels fast reach to life threatening in terms of both space and time (Brack *et al*., 2002; Diez *et al*., 2002).

Pesticides are recognized worldwide as a veritable means of controlling pests, at the same time such chemicals are highly toxic to other species in the environment (Venkateswara Rao, 2006). Presently, there is an increasing concern world over, on the indiscriminate use of such chemicals that result in environmental pollution and toxicity risk to non-target organisms (Velisek *et al.,* 2006). Non-target organisms have been the most vulnerable ones to pesticide poisoning, with deleterious effects ranging from outright death to subtle physiological disorders (Matsumura *et al.,* 1972). Sometimes, even very low concentrations of pesticides are found to be toxic to non-target organisms (Kilkis *et al*., 1981). Pesticides may exert their toxic effects in various forms ranging from alteration within a single cell,

whole organism or even changes in whole population (Giari *et al.*, 2008). When aquatic habitats receive pesticides, there can be substantial perturbation to the ecosystem. A major goal in many ecotoxicological studies has been to determine the perturbative effects of pesticides on ecosystem-level parameters including species diversity, primary productivity and abiotic conditions (e.g. pH and dissolved oxygen). However, there is a growing appreciation that lower (*i.e.* sublethal) concentrations of pesticides can alter a wide range of individual traits including changes in neurotransmitters, hormones, immune response, reproduction, physiology, morphology and behavior. The changes in behavior includes reduced foraging and changes in swimming ability, predator detection, learning and social interactions; reviewed in Weis *et al.* (2001).

Fishes are good bio-indicators of environmental pollution monitoring and can play significant roles in assessing potential risk associated with contamination in aquatic environment, since they are directly exposed to chemicals resulting from agricultural production or indirectly through food chain of ecosystem (Lakra and Nagpure, 2009). Aquatic contamination of the pesticides causes acute and chronic poisoning of fish and other organism. The early life stages of fish, like eggs and larvae are particularly sensitive to contaminant (Fiumann, 1993). The toxicity tests measure the integrated responses to the possible acute or chronic effects of contaminants (Watts and Pascoe, 2000). Chronic toxicity test data is generally more reliable, provides responses related to a complete or part of life cycle of the test-species (Nascimento *et al.,* 2000). The reduced fitness and growth of the fish occurs at sublethal levels depending on exposure time, toxicity and concentrations of the chemical substances involved (Lanno and Dixon, 1994). The factors decreasing fish growth

consist of disorder in feeding behaviors, decrease in feeding rate, dysfunction in metabolism process and waste of energy to overcome the stress caused by pesticide exposure (Tripathi *et al.*, 2003). In the last few decades, acute toxicity tests based on mortality were widely used as indicators of toxicity. However, in the environment most of the exposures of organisms to pollutants are at sublethal levels, so these acute toxicity tests have restricted value in practice (Maltby and Naylor, 1990).

Since the 1960s, researchers interested in environmental studies have used a variety of physiological responses to account for the effect of pollutants on individual organisms and great efforts have been made to assess stress indices with predictive value. It is well recognized that acute exposures to pollutants that kill few organisms may or may not have ecological impact, whereas, chronic exposures that cause delays in development, diseases, reproductive malfunctions or decrease in probability of survival, might result in ecological consequences (Moriarty, 1988). In this sense, some behavioral and physiological integrative responses may be advantageous to account for the health condition of individual organisms and some of them could provide feasible explanations of higher organization levels. In general, behavioral and physiological responses that give relevant information on the environmental impact of chemicals are those that describe the performance, account for the health of individual organisms and indicate their chance of survival or long term opportunity to reproduce (Vaughn *et al.,* 1984). To evaluate the effects of pollutants on animal populations, communities and ecosystems, various methods have been developed ranging from the (sub) cellular to the ecosystem level of biological response. However, the predictive ability of measurements at higher levels of biological organization is limited, because

ecologically important effects (e.g. death or impaired organism function) have already occurred before they can be detected at population and community levels.

Biological methods are applied worldwide for evaluation of toxic effects of pollutants, their mixtures and sewage on various test-objects, their sensitive physiological, morphological indices and behavioral patterns. Acute methods for determination of lethal toxicity are widely distributed and standardized. They allow us to evaluate quickly the effects of toxicants on organisms. The criterion of lethal toxicity is mortality, the final response of an organism to a toxic effect (Kai Sun *et al.*, 1995; Kazlauskiene *et al.*, 1999). Over the last few decades, biomarkers at suborganismal levels of organization (biochemical, physiological and histological) have been considered to be viable measures of responses to stressors (Huggett *et al.*, 1992). In acute water pollution incidents, the physiological disturbances of fish are well known, e.g., respiratory distress, loss of locomotive ability and behavioral alterations. Such responses to environmental stressors have little value as biomarkers, because they are insensitive endpoints from the ecosystem perspective and give little information on environmental contamination. In an extreme case, death is indicative that the lethal threshold has been exceeded. In contrast, when the exposure is chronic or sublethal, biomarkers based on changes in physiological parameters within the natural homeostasis variability associated with biotic and abiotic parameters allow correlating those changes with the effects of exposure to pollutants (Handy and Depledge, 1999).

2.1. Pyrethroid pesticide

The pyrethroids which have emerged as a major class of highly active pesticides due to their high bio-efficacy and relatively low toxicity in comparison to organochlorine and organophosphorous pesticides (Werner *et al.*, 2002), are used to control pests worldwide in households, cereals, vegetable, cotton, tobacco, and other crops. They are also widely used for the control of ectoparasites of domestic animals. Pyrethrins are derived from the flowers of *Chrysanthemum cinerariaefolium* that have been used as insecticides for more than a century (LaForge and Markwood, 1938). Pyrethroids are structural derivatives of pyrethrins that have greater potency and environmental stability (Casida, 1980; Elliott and Janes, 1978). Pyrethroids are reported to degrade rapidly in the environment, however, in fact half-life ranges from 1 to 16 weeks (Kaneko *et al.*, 1978; Kidd and James, 1991). These are considered as effective insecticides due to their high insecticidal toxicity with low mammalian toxicity (Elliott *et al.*, 1974).

Pyrethrins are highly toxic to fish and tadpoles. They affect their skin touch receptors and balance organs (Tomlin, 1994). Pyrethroids have been reported to be extremely toxic to fish and some beneficial aquatic arthropods, for example, lobster and shrimp (Bradbury and Coats, 1989; Srivastav *et al.*, 1997). The commonly accepted mechanism of action of pyrethroids is the prolongation of the open state of voltage-dependent sodium channels in nerve tissue (Narahashi, 2000; Soderlund *et al.*, 2002; Vijverberg and Van den Bercken, 1990). The sodium channel is the primary physiological target of the pyrethroid insecticides

and is highly dependent on stereochemical structure (Milam *et al.*, 2000). By acting on the sodium channels to depolarize the pre-synaptic terminals, pyrethroid insecticides effectively paralyze organisms by severely limiting neuro-transmission (Salgado *et al.*, 1983). Pyrethroids have also been shown to inhibit ATPase enzyme production (Clark and Matsumura, 1982). Due to the lipophilic nature of pyrethroids, biological membranes and tissues readily take up pyrethroids. Exposed organisms may exhibit symptoms of hyperexcitation, tremors, convulsions, followed by lethargy and paralysis.

Various reproductive parameters were affected by different pyrethroids in fishes. 100% mortality reached in Medaka fish (*Oryzias latipes*) after 4 days exposure to 9.4 mg/L esfenvalerate and downward trend in fecundity and fertilization success with increasing esfenvalerate concentration (Werner *et al.*, 2002). Pyrethroids also caused changes in hematological parameters in different fishes. Cakmak and Girgin (2003) described decreased levels of hemoglobin, leukocytes, red blood cells, and mean corpuscular hemoglobin concentration with increased levels of mean cell volume in rainbow trout exposed to cypermethrin. Sopinska and Guz (1998) conducted experiments with another pyrethroid, permethrin and observed a decrease in total leukocyte count and neutrophil granulocyte count in carp. Anita *et al.* (2010) reported a decrease in protein content in the liver, muscle, brain and gill of the two carps, *Labeo rohita* and *Cirrhinus mrigala* exposed to sublethal concentration of fenvalerate. Talstar 10 EC, a synthetic pyrethroid changed the respiratory rhythm and inhibited the energy metabolism of Prussian carp and perch (Ponepal, et al 2010).

2.2. Lethal toxicity of deltamethrin

Acute toxicity data for deltamethrin in fish have been summarized in a report of the World Health Organization (WHO, 1990) and classified as highly toxic to fish, being in the LC_{50} <1.0 ppb. The potential hazard to fish is due to its heavy use in many aquatic larvicidal programs. Golow and Godzi (1994) reported 96 h Fish LC_{50} value for *Oreochromis niloticus* fingerlings as 14.50 mg/L. They concluded that deltamethrin was two times more toxic to the fish species compared to dieldrin. Bradbury and Coats (1989) have reviewed the toxicology of pyrethroids in mammals, birds, fishes, amphibians, and invertebrates (terrestrial and aquatic) and cited deltamethrin toxicity to Atlantic salmon (*Salmo salar*), mosquito fish (*Gambusia affinis*), and rainbow trout (*Oncorhynchus mykiss*)as 96 h LC_{50} values of between 0.50 and 1.97 mg/L. In addition, the 48 h LC_{50} value of deltamethrin for common carp (*Cyprinus carpio*) embryos and larvae were found as 0.213 and 0.074 ppb, respectively. The effects of deltamethrin on the sensitive early life stages of zebrafish (*Brachydanio rerio*), were examined by George and Nagel (1990). The development of larvae was influenced by deltamethrin. Hatchability of embryos was reduced in a dramatic way a 0.80 ppb and higher. The calculated LC_{50} value for deltamethrin at 35 days was 0.52 ppb.

Datta *et al.* (2002) observed that the hardness of water significantly reduced the toxicity of the pesticide deltamethrin to scale carp (*Cyprinus carpio* var. *communis*) fry. According to them 96 h LC_{50} value of deltamethrin, which was observed as 0.102 mg/L in soft water, increased to 0.8 mg/L in hard water. Prussian carp (*Carassius auratus gibelo*) exposed

to deltamethrin (2 ppb for 14 days) exhibited symptoms of induced hepatic, gonadal and renal toxicity (Staicu *et al.*, 2007).

Koprucu *et al.* (2006) also reported that European catfish (*Silurus glanis*) exposed to acute concentrations of deltamethrin higher than 0.50 µg/L showed abnormal behavior such as loss of equilibrium, hanging vertically in the water, rapid gill movement, erratic swimming, swimming at the water surface, air gulping and fading in color of fingerlings. Changes in behavioral patterns exhibited by fish were possibly to counteract aquatic hypoxia condition possibly caused by the agrochemical (Kind *et al.*, 2002).

Among the pyrethroids, deltamethrin is often the most toxic to crustaceans in comparative tests (Haya, 1989). The study by Weston *et al.* (2004) showed high toxicity of deltamethrin in freshwater sediment toxicity tests to amphipods. Muir *et al.* (1985) found sediments to be the major sink for deltamethrin in experimentally treated freshwater ponds, and showed that chironomid larvae exposed for 48 hrs accumulated radiolabelled deltamethrin from sediments 360 days after treatment. It is also highly toxic for aquatic invertebrates; the 48-h LC_{50} for *Daphnia* is 5 µg/L (WHO, 1990).

Amin and Hashem (2012) showed that deltamethrin is highly toxic to catfish (*Clarias gariepinus*) even in low concentration (0.75 µg/L) while sublethal concentration of deltamethrin was 1.5 µg/L as reported by Svobodova *et al.* (2003). This highly toxic effect of deltamethrin in catfish is due to the high rate of absorption of deltamethrin through the gills and lack of fish to the enzymes responsible for metabolism and detoxification of deltamethrin. Mittal *et al.* (1994) reported deltamethrin toxicity to guppy (*Poecilia reticulate*), as the most

toxic of the pyrethroids studied, LC_{50} as 0.016 ppb. Viran *et al.* (2003) estimated 48 h LC_{50} and LC_{99} values of deltamethrin in *P. reticulata* as 5.13 and 33.09 ppb, respectively.

2.3. Sublethal toxicity studies

2.3.1. Behavior of normal and exposed fish

Behavior provides a unique perspective linking the physiology and ecology of an organism and its environment (Little and Brewer, 2001). Behavior is both a sequence of quantifiable actions, operating through the central and peripheral nervous systems and the cumulative manifestation of genetic, biochemical and physiologic processes essential to life, such as feeding, reproduction and predator avoidance. Behavior allows an organism to adjust to external and internal stimuli in order to meet the challenge of surviving in a changing environment. Conversely, behavior is also the result of adaptations to environmental variables. Thus, behavior is a selective response that is constantly adapting through direct interaction with physical, chemical and social aspects of the environment (Kane *et al.*, 2005).

Susan *et al.* (2010) carried out a study on acute toxicity, behavioral changes in the three major Carps, *Labeo rohita* (Ham), *Catla catla* (Ham) and *Cirrhinus mrigala* (Ham) exposed to fenvalerate. They observed that, toxicant exposed fish showed anomalous behavior like surfacing phenomenon, irregular, erratic and darting swimming movements, hyper excitability, loss of equilibrium and hitting to the walls of the test tank before finally sinking to the bottom just before death. Chebbi *et al.* (2010) carried out a study on behavioral responses of the carp, *Cyprinus carpio* under quinolphos intoxication in sublethal doses. Fish

in toxic media exhibited irregular, erratic and darting swimming movements, hyper excitability and loss of equilibrium and sinking to the bottom. Caudal bending was the chief morphological alterations during the exposure tenures.

Galeb *et al.* (2011) studied the behavior of silver catfish (*Rhamdia quelen*) exposed to sublethal concentrations of deltamethrin and they have presented loss of balance, swimming alteration and dyspnea, keeping their mouths and opercula open. Post-mortem signs observed in the animals exposed to deltamethrin were mainly darkening of the surface of the body, tail and hemorrhagic spots on the body surface. Josef *et al.* (2011) carried out a study on behavioral responses of rainbow trout and common carp to pyrethroids (deltamethrin, cypermethrin and bifenthrin). They observed clinical symptoms like accelerated respiration, loss of movement and coordination, fish lying at the tank bottom and moving in one spot, subsequent short excitation periods with convulsions and movement in circles.

Srivastava *et al.* (2010) carried out a study on acute toxicity and behavioral responses of stinging catfish (*Heteropneustes fossilis*) to an organophosphate insecticide, dimethoate. *H. fossilis* showed behavioral changes like increased opercular movement, sluggish, lethargic and abnormal swimming and loss of buoyancy against dimethoate intoxication. The treated fishes also showed fading of their body color. Dube *et al.* (2010) studied the behavioral surveillance and oxygen consumption in the freshwater fish, *Labeo rohita* (Hamilton) exposed to sodium cyanide. Behavioral patterns were observed in $1/3^{rd}$ and $1/5^{th}$ sublethal concentrations for 1, 5, 10 and 15 days. Fish behaved irregular, erratic and drastic movements, followed by hyper excitability, loss of balance and finally settles to the bottom of the test chamber.

Imam *et al.* (2013) studied atrazine-induced hematological, behavioral and biochemical characteristics of African catfish (*Clarias gariepinus*) in sublethal doses. After exposure to atrazine some of the fishes exhibited hyperactivity characterized by rapid and erratic swimming or darting, partial loss of equilibrium, rapid pectoral fins and opercular movements, reduction in the feeding activity, fin haemorrhage and loss of some skin parts. Tiwari *et al.* (2012) carried out a study on the impact of cypermethrin on fingerlings of *Labeo rohita* in sublethal doses (1, 5, 10 and 15 days). Exposures to 0.176, 0.264, 0.352, and 0.440 ppb cypermethrin caused significant visible behavioral changes in the fingerlings of *L. rohita*. Immediately after treatment, fish showed body irritation, hyperactivity, during which fish became restless. After 30–60 minutes, their swimming activities were slowed down and irregular, jerky movements and loss of body equilibrium were observed. After some time, they tried to stay at upper water surface and loss of body equilibrium was pronounced. Finally their entire activity decreased, and they settled down at the base of aquaria.

2.3.2. Oxygen consumption rate

A change in respiration rate is one of the common physiological responses of organisms to toxicants and is easily detectable through changes in oxygen consumption rate, which is frequently used to evaluate the changes in metabolism under environmental deterioration. Respiratory activity of fish is often the first physiological response to be affected by the presence of contaminants in the aquatic environment. Although many biological early warning systems monitor abnormal opercular movement as an indicator of respiratory stress, a

more direct measurement of stress in this sense necessitates the quantification of oxygen consumed by the fish.

Pesticides in sublethal concentrations present in the aquatic environment are too low to cause rapid death directly, but may affect the functioning of the organism, disrupt normal behavior and reduce the fitness of natural population. In the aquatic environment one of the most important manifestation of the toxic action of chemical is the over stimulation or depression of respiratory activity. The changes in the respiratory activity of fish have been used by several investigators as an indicator of response to environmental stress. As aquatic organisms have their outer bodies and important organs such as gills almost entirely exposed to water, the effect of toxicants on the respiration is more pronounced (Premdas and Anderson, 1963; Ferguson *et al.,* 1966a).

Holden (1973) observed that, one of the earliest symptoms of acute pesticide poisoning is respiratory distress. This serves not only as a tool in evaluating the susceptibility or resistance potentiality of the animal, but also useful to correlate the behavior of the animal. The respiratory potential or oxygen consumption of an animal is the important physiological parameter to assess the toxic stress, because it is a valuable indicator of energy expenditure and metabolism (Prosser and Brown, 1977). Pesticides are indicated to cause respiratory distress or even failures by affecting respiratory centers of the brain or the tissue involved in breathing. The effect of toxicants on the respiration of fishes and invertebrates have received wide spread attention and were reviewed by Hughes (1976) and Wright (1978). The toxic

effects of pyrethroids on the metabolism particularly oxygen consumption have been reviewed by Kumaraguru and Beamish, 1983; Bradbury *et al.*, 1986; Baskaran, 1991.

Yuan *et al.* (2004) studied on sublethal effects of paraquat and malathion on the freshwater shrimp, *Macrobrachium nipponense*. They observed a decrease in weight-specific oxygen consumption for both the control and exposed group and found that the decrease was greater in exposed shrimp. The maximum oxygen consumption rate appeared after 4 d of exposure to 0.1 mg/L malathion. The next highest rate was observed after 4 d of exposure to 0.01 mg/L paraquat. The minimum oxygen consumption rate appeared after 1 d of exposure to 0.05 mg/L paraquat. However, the oxygen consumption rates for the treated groups were close to that of control group, which were significant after 14 d of exposure to 0.05 mg/L paraquat and to 0.1 mg/L malathion.

Palanivelu *et al.* (2009) studied on the effects of urea on survival, food utilization of freshwater fish, *Oreochromis mossambicus*. They found that, the consumption of oxygen in *O.mossambicus* diminished from 0.962 ± 0.208 to 0.645± 0.118 mg/g live fish/h when reared in the highest sublethal concentration of urea. Santos *et al.* (2006) conducted a study on the effects of naphthalene on metabolic rate and ammonia excretion of juvenile Pompano, *Trachinotus carolinus*. The 96 h LC_{50} at 24° C was 2.83 ppm of naphthalene. Fish after acute exposure showed a tendency to increase specific oxygen consumption by virtue of naphthalene concentrations. After chronic exposures, however, a decrease was observed at the highest concentration evidencing a narcotic effect of naphthalene.

Patil *et al.* (2008) carried out a study on behavior and respiratory dysfunction as index on malathion toxicity in the freshwater fish, *Labeo rohita* (Ham). Carp fingerlings were exposed to different concentrations (6.0 to 10.1 µl /L) of malathion for 96 h. The acute toxicity value was found to be 9.0 µl /L and $1/10^{th}$ of LC_{50} (0.9 µl /L) was selected for sublethal studies. Oxygen consumption was studied in lethal (1, 2, 3 and 4 d) and the sublethal concentrations (1, 5, 10 and 15 d). An increase in oxygen consumption of 70.39 to 80.50%, 4.45 to 21.35 % were observed in lethal and sublethal concentrations of malathion respectively.

Montagna *et al.* (2008) studied oxygen consumption of freshwater crab, *Trichodactylus borellianus* exposed to chlorpyrifos and endosulfan insecticides. The effects of lethal and sublethal concentrations of chlorpyrifos and endosulfan on oxygen consumption rate of the crab *T. borellianus* were evaluated. A significant difference in oxygen consumption among times of exposure was registered in higher concentration (625 µg /L) of endosulfan. Lokaswamy *et al.* (2009) investigated on the impact of cypermethrin and Ekalux on respiratory activities of a freshwater fish *Tilapia mossambicus* under sublethal toxicity. It showed significant decrease in oxygen uptake.

Dube *et al.* (2010) studied on oxygen consumption in freshwater fish *Labeo rohita* (Ham) exposed to sodium cyanide. The toxicity tests were conducted by static renewal bioassay method on the juvenile fish. The LC_{50} value of sodium cyanide to *Labeo rohita* was

found to be 320 µg /L. One third (106 µg/L) and one fifth (64 µg/L) of the LC_{50} values were selected for sublethal studies. Oxygen consumption was observed in ($1/3^{rd}$ and $1/5^{th}$) sublethal concentrations for 15 days. A decrease in oxygen consumption was observed in $1/3^{rd}$ (11.62% and -4.52%) and $1/5^{th}$ (9.11% and -2.82 %) sublethal concentrations.

Patil and Yadav (2012) studied on the effect of sublethal concentration of Metasystox pesticide on fresh water crab, *Barytelphusa cunicularis*. The crabs were exposed to 0.125ppm ($1/10^{th}$ LC_{50} value of 48 hrs) and observation were made at 1, 7, 15 and 30 days. After sublethal exposure there was an initial increase in oxygen consumption for first seven days. However, a gradual decrease in rate of oxygen consumption was observed for the following days when compared to control crab.

Das and Gupta (2012) studied on the effect of endosulfan (EC 35) on oxygen consumption patterns and gill morphology of the Indian flying barb, *Esomus danricus*. It was exposed to three sublethal concentrations of endosulfan EC 35 (0.49, 0.049 and 0.0049 µg/L) to determine changes in the oxygen consumption patterns. The average rate of oxygen consumption in control fish after 28 days was found to be 39.06±0.146 ml/hr/100 g tissue). Rates of oxygen consumption after exposure to 0.49, 0.049 and 0.0049 µg/L of endosulfan after 28 days were 10.95, 14.85 and 19.67 ml/hr/100 g tissue, respectively. A significant negative dose-response of endosulfan was obtained. With increasing exposure duration, there was a corresponding decrease in the oxygen consumption up to day 28.

Rane *et al.* (2013) carried out study on the impact of acute and chronic toxicity on oxygen consumption of freshwater bivalve, *Lamellidens marginalis*. The bivalves were exposed to acute and chronic doses of organophosphate pesticide, Triazophos (40% EC). The rate of oxygen consumption for acute treatment was recorded after 24, 48, 72 and 96 hrs. While for chronic treatment, was recorded for 7, 14 and 21 days. The observations indicate that the rate of oxygen consumption was found to be decreased with increase in exposure period.

2.3.3. Food consumption rate

Growth of an organism is generally used as a sensitive and reliable endpoint in chronic toxicological investigations. Sublethal levels of a wide variety of toxicants have been found to slow down the growth of fish larvae or juveniles. This can be due to reduced food intake, but also due to increased metabolic expenditure for detoxification and maintenance of the normal body function. Feeding rates are one of the most important factors affecting fish performance and growth (Bert, 1979), and toxicants can affect growth of fish either directly or indirectly via effects on feeding or by increasing maintenance costs (Kooijman and Bedaux, 1996).

Pandian *et al.* (1983) studied on food utilization in the fish, *Channa striatus* exposed to sublethal concentration of DDT and Methyl Parathion. Sublethal concentrations of DDT and methyl parathion (MP) in the medium, significantly affected the rates of feeding, absorption and conversion in *Channa striatus*. Fish exposed to 250 ppb DDT and Methyl parathion consumed 23 and 50% less food than those exposed to pesticide-free water. James *et al.*

(1995) studied the toxic effects of copper and mercury on food intake and growth in *Heteropneustes fossilis*. Up on exposure to copper (750 ppb) and mercury (60 ppb) individually showed significant decrease in food consumption and growth.

De Boeck *et al.* (1997) studied on the effects of sublethal copper exposure on food consumption in common carp. Juvenile common carp were exposed for 28 days to three different sublethal copper concentrations (0.20 μM, 0.55 μM, and 0.80 μM). Food consumption was monitored on a daily basis during the exposure period. Exposure to copper at 0.80 μM affected feeding behavior in common carp. Even at the lowest copper concentration (0.20 μM), metabolic demand for the fish increased, challenging the carp with an increased demand for food.

Felista *et al.* (1998) studied on the toxic and sublethal effects of ammonium chloride on the freshwater fish, *Oreochromis mossambicus*. Rearing the fish by different increased sublethal concentrations of ammonium chloride, shown that the feeding rate decreased from 20.309 ± 0.506 mg/g/d (control) to 11.594 ± 0.479 mg/g/d at the highest sublethal concentration (100 mg/L).

Ali *et al.* (2003) studied on the effect of different sublethal concentrations of copper in water (0.15, 0.3 and 0.5 ppm) on the feed consumption of *Oreochromis niloticus*. Fish refused to accept the feed immediately after exposure and only began taking it up after about 4–5 h as compared with the control. Exposure of the fish to different copper concentrations in water significantly ($P < 0.05$) reduced their feed consumption as compared with the control.

Palanivelu *et al.* (2009) studied on the effect of urea on food utilization in the fresh water fish *Oreochromis mossambicus.* Rearing the fish in different increased sublethal concentrations of urea, shown a significant decrease in the feeding rate from 34.341 ± 7.067 mg/ g live fish /d (control) to 13.921 ± 2.315 mg /g live fish/d at the highest concentration of urea (22,000 mg /L).

Tim *et al.* (2004) studied on the cellular energy allocation and scope for growth in the estuarine mysid, *Neomysis integer* (Crustacea: Mysidacea) following chlorpyrifos exposure. In this study, they have estimated the egestion rate (indirect measurement of feeding rate) of mysids by exposing to different concentrations of chlorpyrifos pesticide. They noticed that egestion rates of *N.integer* were significantly affected by chlorpyrifos concentration. Egestion rates were significantly different from control mysids following each of the exposure periods at 0.100 µg chlorpyrifos/L and after 48 h at 0.072 µg chlorpyrifos/L.

Jezierska *et al.* (2006) studied on the effects of heavy metal exposures on feeding activity of common carp larvae were measured (as number of *Artemia* nauplii consumed within 10 minutes). The fish were exposed during embryonic or larval development to copper, cadmium, or mix of both metals (Cu – 0.2 mg /l, Cd – 0.2 mg /l, Mix – 0.1 mg/l of Cu + 0.1 mg/l of Cd). The results show that both embryonic and larvae exposed to heavy metals considerably shown impaired feeding activity. Niyogi *et al.* (2006) carried out a study on food selection, growth and physiology in relation to dietary sodium chloride content in rainbow trout (*Oncorhynchus mykiss*) under chronic waterborne Copper exposure. They have estimated the food consumption rate by exposing the rainbow trout to waterborne Cu for a period of 28

days. Food consumption rate was severely impaired in Cu-exposed fish relative to Cu-unexposed fish. The impairment was at maximum during the first 7 days of the exposure followed by a gradual recovery with time. However, the food consumption rate in Cu exposed fish still remained significantly reduced compared to Cu-unexposed fish until the end of the exposure.

Subathra *et al.* (2007) studied on the toxic effects of copper on bioenergetics and growth rates in fingerlings and adult of the fish, *Mystus vittatus* (Bloch, 1794). The fingerlings and adults were subjected to their respective higher (0.75 and 1.91 mg/L/g) and lower (0.47 and 1.20 mg /L/g) sublethal concentration of Cu for a period of 60 days. Food consumption was monitored on a daily basis during the exposure period. The rate of feeding of both the size groups under their respective higher and lower sublethal Cu concentration, led to decreasing trend with increasing periods of exposure. Floyd *et al.* (2008) determined the effect of pyrethroid insecticide esfenvalerate in larvae of the fat-head minnow (*Pimephales promelas*). Acute effect concentrations were determined, and in subsequent experiments, fish were exposed to the following measured sublethal concentrations: 0.072, 0.455, and 1.142 micro g/L of esfenvalerate. Food consumption was recorded daily. Fish exposed to 0.455 and 1.142 micro g/L of esfenvalerate exhibited impaired feeding ability as well as reduced growth compared to fish exposed to 0.072 micro g/L and control.

Manoharan *et al.* (2008) studied toxic and sublethal effects of endosulfan on *Barhus stigma* (Pisces: Cyprinidae). The LC_{50} was found to be 0·0043 ppm and the sublethal concentration was 0·003 ppm and below. The rate of feeding was reduced by 5·94% to 9·02%

in different sub-lethal concentrations. Parveen and Javed (2010) studied on the effect of water-borne copper on the growth performance of fish *Catla catla.* An experiment was conducted to determine the growth performance of *Catla catla* fingerlings during 90-day sublethal copper (Cu) exposure. During the exposed period the food consumption rate was measured and the treated fish exhibited significantly lower feed intake during study period.

Ferrari *et al.* (2011) studied on the energy balance of juvenile *Cyprinus carpio* after a short-term exposure to sublethal water-borne cadmium. Fish were exposed to a concentration of Cd (0.15 mg Cd/L) for 2 weeks. Food intake was determined. Significant decrease in the food intake in the fishes exposed to increased concentrations of cadmium. Saima *et al.* (2012) studied on the growth performance of metal stressed major carps. Growth responses of *Catla catla, Labeo rohita and Cirrhinus mrigala* were determined separately, under chronic exposure of mixture of metals (Fe+Zn+Pb+Ni+Mn) at sublethal concentrations ($1/3^{rd}$ of LC_{50}) for 12 weeks. Feed intake was determined. There was a significant impact on the feed consumption and growth of exposed fishes compared to control.

Ramesh *et al.* (2011) carried out a study on the effect of treated sago effluent on food utilization of the fish, *Clarias batrachus* . The fishes were exposed to sublethal concentrations of effluent(25%, 50% and 75%). The feeding rates recorded for control were higher than the various treated sago effluent concentrations. Javed (2013) studied on the chronic exposure impacts of waterborne and dietary nickel (Ni) and cobalt (Co) on the growth performance of juvenile major carps viz. *Catla catla, Labeo rohita* and *Cirrhinus mrigala* under static water bioassay. The growth of the fishes was monitored under chronic sublethal concentrations ($1/3^{rd}$ of LC_{50}) of waterborne and dietary Ni and Co, separately for 12 weeks. Feed intakes in all the three

fish species were significantly lesser due to waterborne Ni, whereas waterborne Co and dietary Ni exposure resulted in significantly higher feed intake by the fish. However, feed intakes were significantly better among control fish groups that caused significantly higher FCE (food conversion efficiency) than those exposed to waterborne or dietary metals.

2.3.4. Ammonia excretion rate

Ammonia is the main nitrogenous excretory product, accounts for about 80-98 % of all nitrogenous excretion in freshwater fish. It is the major end product of protein metabolism in fish. A study on the ammonia excretion rate was used to evaluate the changes in metabolism under environmental deterioration (Randall and Wright, 1987).

Chinni *et al.* (2000) studied on oxygen consumption and ammonia-nitrogen excretion in *Penaeus indicus* postlarvae exposed to lead. The PL were exposed to the sublethal concentration of lead for 30 days, parallel controls were also maintained. The medium was renewed for every 24 hr. Both control and exposed samples were taken at intervals of 24 hr, 48 hr, 96 hr, 10 days and 30 days for estimation of oxygen consumption, ammonia excretion. The decrease in oxygen consumption was significant ($P < 0.05$) from 24 hr up to 30 days exposure. In control PL, the rate of ammonia-N excretion increased with increasing time but the increase was not much in exposed PL.

Barbieri *et al.* (2009) studied on effect of 2,4-D (2,4-dichlorophenoxyacetic acid) herbicide on oxygen consumption and ammonium excretion of juveniles of cichlid *Geophagus*

brasiliensis. The average levels of ammonium excreted by *G. brasiliensis* exposed to 25 and 40 mg 2,4-D/L were 67.5% and 85.0% higher compared with the average value for the control animals. Amin *et al.* (2010) studied on the effects of copper on the physiological responses of the commercial crab, *Lithodes santolla* (Decapoda: Anomura) larvae. Larvae were exposed to sublethal concentrations (40, 80 and 160 µg /L) for 96 h. Oxygen consumption and ammonia excretion were measured. Oxygen consumption of treated groups (mean 46.92 ± 8.03 µg-atom O_2 /h /mg) did not differ significantly with control. Ammonia excretion decreased by 60% at higher Cu concentration (1.61 ± 0.65 µg-atom N-NH_3 /h /mg), leading to a 117% increase in the O:N ratio than that of controls.

Barbieri *et al.* (2010) studied on the effects of the organophosphate pesticide Folidol 600® on the freshwater fish, Nile Tilapia (*Oreochromis niloticus*). The ammonium excretion of fish exposed to 0.0, 0.1, 0.5, 1.0, 2.5, 5.0 and 10.0 mg Folidol 600®/L was 0.12, 0.18, 0.30, 0.33, 0.37, 0.36 and 0.33 µg/g/min, *i.e.*, an increase of 50%, 150%, 175%, 208%, 200% and 175% respectively, relative to the control. Santos *et al.* (2006) studied on the effects of naphthalene on metabolic rate and ammonia excretion of juvenile Florida Pompano, *Trachinotus carolinus*. Fish after acute exposures showed a tendency to increase specific oxygen consumption by virtue of naphthalene concentrations. After chronic (12 days) exposures, however, a decrease was observed at the highest concentration evidencing a narcotic effect of naphthalene. Ammonia excretion was reduced significantly, as compared to that of the controls, in all the exposed organisms.

Montagna and Collins (2008) studied on the ammonia excretion of the freshwater crab, *Trichodactylus borellianus* exposed to chlorpyrifos and endosulfan insecticides. The effects of lethal and sublethal concentrations of chlorpyrifos and endosulfan on ammonia excretion rate of the crab were evaluated. A significant increase in ammonia excretion was evidenced in fish exposed to 150 and 300 µg /L of chlorpyrifos. Vosloo *et al.* (2012) studied on the effect of sublethal copper levels on ammonia excretion rate of the marine bivalve, *Perna perna*. Animals were exposed to four environmentally relevant concentrations of 12.5, 25.0, 37.5 and 50.0 µg /L copper. Mussels excreted significantly higher levels of ammonia nitrogen when they were exposed to 37.5 µg /L and 50 µg/L copper compared to unexposed animals.

Naimo *et al.* (1992) studied on the sublethal effects of cadmium on physiological responses in the pocketbook mussel, *Lampsilis ventricosa.* Exposure of *Lampsilis ventricosa* (Barnes, 1823) to 0, 22, 111, and 305 µg/L of cadmium for 28 days in a proportional diluter resulted in a significant decrease in respiration rate, as cadmium concentration increased. Although variations in cadmium concentrations did not significantly affect food clearance rates or ammonia excretion rates, mussels exposed to 305 µg cadmium /L showed a decrease in ammonia excretion rate and a decrease in food clearance rate during the study period.

2.3.5. Oxygen : Nitrogen (O :N) ratio

A biomarker used to assess stress is the quantification of oxygen-nitrogen ratio (O : N), which indicates the physiological state of the organism exposed to xenobiotic. This

biomarker is the result of the quantification division of oxygen uptake and the quantification of ammonia excretion. This division of both biomarkers (oxygen uptake/ammonia-N excretion) generates an index which shows the metabolic changes in the organisms and the amount of energy available in them during periods of stress produced by pesticide contamination (Montagna and Collins, 2008). The respiration and excretion are major components of bioenergetics in aquatic organisms and such physiological energetics are studied by means of the oxygen: nitrogen (O : N) ratio. The O : N ratio measures balance between breakdown of protein and catabolism of carbohydrates and lipids. The ratio of O : N indicates the proportion of lipids and carbohydrate relative to protein which breaks down for energy metabolism (Stead and Thompson, 2003).

Oxygen consumption, ammonia-nitrogen excretion and metal accumulation in *Penaeus indicus* postlarvae exposed to lead were studied by Chinni *et al.* (2000). The PL were exposed to the sublethal concentration of lead for 30 days, parallel controls were also maintained. The medium was renewed for every 24 hr. Both control and exposed samples were taken at intervals of 24 hr, 48 hr, 96 hr, 10 days and 30 days for estimation of oxygen consumption, ammonia-N excretion. In addition, oxygen : nitrogen (O:N) ratios were also calculated as the ratio of atoms of oxygen consumed to atoms of nitrogen excreted at the above intervals. The decrease in oxygen consumption was significant ($P < 0.05$) from 24 hr up to 30 days exposure. In control PL, the rate of ammonia-N excretion increased with increasing time but the increase was not much in exposed PL. They have noticed a high O:N ratio in post larvae exposed to lead, when compared to control in all exposure days.

Barbieri *et al.* (2009) studied on the effect of 2,4-D (2,4-dichlorophenoxyacetic acid) herbicide on oxygen consumption and ammonium excretion of juveniles of cichlid, *Geophagus brasiliensis*. The acute toxicity of 2,4-D to *G. brasiliensis* in terms of the 24, 48, 72, and 96 h exposure to median lethal concentration (LC_{50}) were calculated to be 45.95, 32.49, 28.28, and 15.16 mg/L, respectively. Furthermore, it was found that exposure of fish to 40 mg/L 2,4-D caused reduction in oxygen consumption and ammonium excretion of 59% and 85%, respectively, in relation to the controls. The average levels of ammonium excreted by *G. brasiliensis* exposed to 25 and 40 mg 2,4-D/L were 67.5% and 85.0% higher compared with the average value for the control animals.

Kunwar *et al.* (2009) studied on influence of food ration, copper exposure and exercise on the energy metabolism of common carp (*Cyprinus carpio*). Fish acclimated to low (0.5% body weight) and high (5% body weight) food rations were exposed to 1 μM copper for a period of 28 days and then kept for further 14 days in copper free water to examine their recovery. Measurements of oxygen consumption, ammonia excretion and ammonia accumulation in plasma and muscle were done at various time intervals during the experimental period. Overall, oxygen consumption and ammonia excretion rates were significantly affected by food ration in both copper free and copper exposed fish.

Amin *et al.* (2010) studied on the effects of copper on the physiological responses of the commercial crab, *Lithodes santolla* (Decapoda: Anomura) larvae. Larvae were exposed to sublethal concentrations (40, 80 and 160 μg/L) for 96 h. Oxygen consumption, ammonia excretion, O:N atomic ratio were measured. Oxygen consumption of treated groups (mean 46.92 ± 8.03 μg-atom O_2 /h /mg) did not differ significantly with control. Ammonia excretion

decreased by 60% at higher Cu concentration (1.61 ± 0.65 µg-atom N-NH_3/h /mg), leading to a 117% increase in the O:N ratio than in the controls. The O:N ratio were significantly higher in treatments than in controls.

Barbieri *et al.*, (2010) studied on the effects of the organophosphate pesticide, Folidol 600® on the freshwater fish, Nile Tilapia (*Oreochromis niloticus*). The exposure of Tilapia to Folidol 600® caused an increase of 4%, 20% and 38.4% in oxygen consumption at 0.1, 0.5 and 1.0 mg L^{-1}, respectively. However, exposure to 2.5, 5.0 and 10 mg L^{-1} caused a decrease of 33.6%, 35.2% and 42.4% in oxygen consumption relative to the control. The ammonium excretion of fish exposed to 0.0, 0.1, 0.5, 1.0, 2.5, 5.0 and 10.0 mg Folidol 600®/L were 0.12, 0.18, 0.30, 0.33, 0.37, 0.36 and 0.33 µg/g/min, *i.e.*, 50%, 150%, 175%, 208%, 200% and 175% increase, respectively, relative to the control.

Santos *et al.* (2006) studied on the effects of naphthalene on metabolic rate and ammonia excretion of juvenile Florida Pompano, *Trachinotus carolinus* . Fish after acute exposures show a tendency to increase specific oxygen consumption by virtue of naphthalene concentrations. After chronic (12 days) exposures, however, a decrease was observed at the highest concentration evidencing a narcotic effect of naphthalene. Ammonia excretion was reduced significantly, as compared to that of the controls, in all the exposed organisms. The O: N ratio of fish exposed to different concentrations of Naphthalene was higher than that of the controls.

Montagna and Collins (2008) studied on the oxygen consumption and ammonia excretion of the freshwater crab, *Trichodactylus borellianus* exposed to chlorpyrifos and endosulfan insecticides. The effects of lethal and sublethal concentrations of chlorpyrifos and endosulfan on oxygen consumption and ammonia excretion rate of the crab were evaluated. A significant difference in oxygen consumption among times of exposure was registered in 625 µg/ L of endosulfan. A significant increase in ammonia excretion was evidenced in 150 and 300 µg /L of chlorpyrifos. The O:N ratio showed a decrease in chlorpyrifos and in 2500 µg /L of endosulfan. An increment in the O:N ratio was observed in the lower endosulfan concentrations.

Vosloo *et al.* (2012) studied on the effect of sub-lethal copper levels on oxygen consumption, ammonia excretion rates and O:N ratio of the marine bivalve *Perna perna.* Mussels were exposed to four environmentally relevant concentrations of 12.5, 25.0, 37.5 and 50.0 µg/ L copper. The average atomic equivalents of oxygen consumed and nitrogen excreted (O:N ratio) of mussels decreased with an increase in copper levels. Mussels excreted significantly higher levels of ammonia nitrogen when they were exposed to 37.5 µg /L and 50 µg/L copper compared to unexposed mussels. The specific oxygen consumption rate of mussels increased with increasing copper concentrations. The oxygen consumption rates of 37.5 µg /L and 50 µg /L copper were both significantly higher than the mean specific oxygen consumption rate of unexposed mussels.

The sublethal effects of cadmium on physiological responses of pocketbook mussel, *Lampsilis ventricosa* was studied by Naimo *et al.* (1992). Exposure of *Lampsilis ventricosa*

(Barnes, 1823) to 0, 22, 111, and 305 μg/L of cadmium for 28 days in a proportional diluter resulted in a significant decrease in respiration rate as cadmium concentration increased. Although, variations in cadmium concentrations did not significantly affect food clearance rates or ammonia excretion rates, mussels exposed to 305 μg cadmium /L showed a decrease in ammonia excretion rates and a decrease in food clearance rate during the study period. Oxygen-to-nitrogen ratios were significantly increased in mussels exposed to 111 and 305 μg cadmium per liter by day 28.

Materials and methods

III. MATERIALS AND METHODS

Toxicity tests (Bioassay tests) are performed to evaluate the impact of chemicals both in aquatic and terrestrial environments. Aquatic toxicology is the study of the effects of manufactured chemicals, anthropogenic and natural materials and their activities on aquatic organisms at various levels of organization, from sub cellular through individual organisms to communities and ecosystems (Rand, 1995). The objective of a toxicity test is to define the concentration at which a test chemical is capable of producing selected response, usually deleterious, in a population under controlled conditions of exposure. Death or mortality is generally used as criteria of a change in the 96-h test while, the extension of duration can be adopted for investigation of the other parameters like biochemical, physiological or behavioral changes. The most common of these toxicity terms are LD_{50} and LC_{50}. The LD_{50} for a specified animal is the amount of toxicant that must be in or on the body of that type of animal (usually injected or incorporated with feed) to kill half of the affected population within a given amount of time. LC_{50} is the amount of a material that comes in contact with the animal being tested that will kill one-half the population affected. Static renewal test and flow through test are the commonly used types of bioassay tests.

3.1. Test animals

Catla, (*Catla catla*) was selected for the present study because of its high economic value, notable contribution to freshwater aquaculture production and occurrence in the riverine stretches. The acute as well as chronic toxicity tests were conducted using the fingerlings of the fish (*Catla catla*) in the laboratory of the Department of Aquatic Environment Management, College of fisheries, Mangalore.

3.2. Maintenance of test animals

The fry of catla were collected and transported from Bhadhra fish farm, Shimoga to the college fish pond in well oxygenated polythene bags containing clean pond water under standard condition. Soon after arrival, they were released into the pond water after proper acclimation. The polythene bags carrying the catla fry were allowed to float on the pond water for one hour. Then, the fry were allowed to enter into the pond voluntarily by opening the bags. Catla fry were allowed to grow in the college fish pond till they attained fingerling size with artificial feeding. They were fed daily twice with oil cake and rice bran mixture to maintain healthy condition.

3.3. Laboratory conditioning of test animals

Fingerlings were collected and transferred to the laboratory and released into FRP (fiber reinforced plastic) tanks of 1000 L capacity. Aeration was provided in the tanks with natural photoperiod. The fishes were fed every 24 hrs with commercial floating feed called 'Economy'. The walls of the holding tank were thoroughly cleaned once in four days to avoid fungal growth. The excreta were siphoned off on a daily to prevent the buildup of ammonia in the medium. Each batch of the bulk collection of fishes was conditioned in holding tank water for 10 days before employing them for experiments. After the acclimation of 10 days, the fingerlings were starved for 24 hours, the active and healthy individuals were selected for the experiment. Fishes were transferred in dip nets and handling time was less than 15 seconds. While transferring and handling, care was taken so that the test animals were least stressed.

The freshwater source for all the experiments was bore well tap water. The water was collected and stored in 1000 L capacity plastic tub for ageing. The water parameters such as temperature and pH were measured by using standard mercury thermometer and pH meter respectively. The standard methods (APHA, 1992) were followed for the estimation of dissolved oxygen (Winkler's method) and hardness (EDTA method) as given in the standard methods. For the experiments, aquarium tanks of 20 L capacity were used and 18 liters of test solution was taken in them. Fingerlings of 3.91±0.2766 cm. in total length and weighing 0.44 ±0.04 g. were selected for the present study.

Table 2: Water quality parameters maintained during the experimental tenure

Temperature	28° C ± 1° C
Dissolved oxygen	7.0 - 7.5ppm
pH	6.9-7.3
Hardness	40 to 42 mg/l of CaCO3
Un-ionized ammonia	16 ± 2 µg/L

3.4. Toxicant

The pyrethroid pesticide deltamethrin was used to assess the toxicity and its impact on the physiological conditions of catla fingerlings (oxygen consumption rate, food consumption rate, ammonia excretion rate and oxygen: nitrogen ratio). Deltamethrin was obtained from the local market of Mangalore, Karnataka, India. It is sold under the trade name Butox containing 1.25% (w/v) of deltamethrin (active ingredient). It was supplied by Intervet India Pvt.Ltd, Pune. The expiry date of the test substance checked prior to initiation of the treatment and it was found to be suitable for the exposure. Stock solution was prepared by dissolving the

required amount of the chemical in deionised water. Stock solution was a light greenish liquid having strength of 12,500 ppb. Later calculated amount of stock solution was added to the test containers so as to get required pesticide concentrations.

3.5. Studies on lethal toxicity

Lethal toxicity study was carried out by following the standard guidelines of APHA (1992) to determine the lethal (LC_{50}) level of deltamethrin using static renewal system. In static renewal system, test organisms were exposed to a fresh solution of the selected concentration and at every 24 h by transferring the test organisms from one test chamber to another test chamber of the same concentration. Laboratory conditioned fishes of uniform sizes were selected to assess the lethal concentration of the toxicant. Total cessation of opercular activity, resting of organism on its sides and at the bottom of container were some of the indices of death which were considered as the main criteria for assessing the lethal effects. Mortality experiments were conducted for estimating LC_{50} values (the concentration which kills 50% of test animals).

All the tests were performed in triplicates and appropriate controls with 10 fish in each replicate for a period of 96 hours and the mortality was recorded at regular intervals. Before beginning the experiments, preliminary studies were conducted using various concentrations of test solution to select the concentrations which give 10% to 100% mortality in 96 h. The stock solution of deltamethrin was prepared for 12500 ppb using deionised water. From this stock solution, following concentrations (0.08, 0.09, 0.1, 0.2, 0.3, 0.4 and 0.5 ppb) were

prepared for testing the toxic effects on fishes. Dead fishes were removed immediately from the test medium. The animals were not fed during the experiment and the test solution was replenished totally every 24 h. Care was taken to leave the animals with minimum disturbance.

3.6. Sublethal toxicity studies

One fifth and one tenth of the acute toxicity value (LC_{50}) were selected as concentrations for sublethal studies according to Sprague (1971). Sublethal study is a comparative study in which organisms that are subjected to different treatments are observed for a long period or a substantial portion of their life span. Long- term effects that may be related to changes in appetite, growth, metabolism, reproduction and mutations are generally studied. Chronic tests usually include additional measures of effect such as growth or reproduction (ASTM, 2002). All the tests were performed in triplicate for a period of 16 days. Fish were exposed to both the sublethal concentrations for 4, 8, 12 and 16 days along with the control sets. Fishes were fed with commercial floating pellets during the test period. The test solution was replenished totally every 24 h. Behavioral responses, oxygen consumption rate, food consumption rate, ammonia excretion rate and oxygen : nitrogen ratios of fishes were studied in experimental tenures. The control and deltamethrin exposed fish were kept under continuous observation during experimental periods.

Plate 1: Photograph of deltamethrin pesticide and its stock solution

Plate 2: Experimental set up of 96 h LC_{50}

3.6.1. Studies on behavior of normal and exposed fish

Behavioral changes are physiological responses shown by the animal, which are often used as the sensitive measure of stress syndrome in the organism experiencing it, consequently the behavioral changes were observed in control and exposed fish. The experiments on the behavior of catla fingerlings were carried out in a glass aquarium of 20 L capacity. Both control and treated fish were used to evaluate the comparative behavioral changes under various concentrations. During this experiment the behavioral changes were critically observed.

3.6.2. Estimation of oxygen consumption rate

The rate of oxygen consumption can be used as a bio-detector in monitoring the physiological effects of pesticides and the oxygen consumption pattern will indicate the possible mapping of metabolic pathways influenced by the pesticide stress. During the present study oxygen consumption rate was studied as an indicator of sublethal toxicity effect. Oxygen consumption rate was recorded in sublethal concentrations ($1/10^{th}$ and $1/5^{th}$ of LC_{50} value) for 16 days (4^{th}, 8^{th}, 12^{th} and 16^{th} day). The animals were fed during the experiment and the test solution was replenished totally, for every 24 h. The experiments on the oxygen consumption rate of catla fingerlings were carried out in a glass aquarium of 20 liter capacity. The water surface of the control and test chambers were covered with a thin film of liquid paraffin, which prevents the diffusion of atmospheric air into the test medium. The amount of dissolved

oxygen in water for every 24 h. was estimated by Winkler's method (Golterman and Clymo, 1969). The difference in the dissolved oxygen content between initial and final water samples represents the amount of oxygen consumed by the fish. The oxygen consumption was based on the method as described by Thampi *et al.* (1994). The oxygen consumption rate (mg/l/gm/hr) was calculated as follows:

oxygen in water for every 24 h. was estimated by Winkler's method (Golterman and Clymo, 1969). The difference in the dissolved oxygen content between initial and final water samples represents the amount of oxygen consumed by the fish. The oxygen consumption was based on the method as described by Thampi *et al.* (1994). The oxygen consumption rate (mg/l/gm/hr) was calculated as follows:

$QO_2 = DO \times 1/BW \times 1/t$

QO_2 is the amount of oxygen (mg/l) consumed in the interval ''t'' (hr)

BW is the body weight of fish (g)

DO= Difference in DO

3.6.3. Estimation of food consumption rate

Food consumption rates or growth rates are often considered as reliable indicators of stress of organisms. The experiments on the food consumption rate of catla fingerlings were carried out in a glass aquarium of 20 liter capacity. Food consumption rate was studied in sublethal concentrations ($1/10^{th}$ and $1/5^{th}$ of LC_{50} value) for 16 days (4^{th}, 8^{th}, 12^{th} and 16^{th} day). For determining the food consumption rate, the fingerlings were fed once in 24 hours with dry feed pellet 'Economy'. After 15 minutes, the remaining food was removed. It was dried overnight at 60 °C in hot air oven and weighed to compare mean food consumption (De Boeck

et al., 1997). Intake was estimated as the difference between the given and the remaining food weight. Food consumption rate was expressed in feed consumed in g/g body wt of fish.

3.6.4. Estimation of ammonia excretion rate

Ammonia is the main nitrogenous excretory product, accounts for about 80-98 % of all nitrogenous excretion in freshwater fish. It is the major end product of protein metabolism in fish. A study on the ammonia excretion rate was used to evaluate the changes in metabolism under environmental deterioration (Randall and Wright, 1987). The experiments on the ammonia excretion rate of catla fingerlings were carried out in a glass aquarium of 20 L capacity. Ammonia excretion rate was studied in sublethal concentrations ($1/10^{th}$ and $1/5^{th}$ of LC_{50} value) for 16 days (4^{th}, 8^{th}, 12^{th} and 16^{th} day). The animals were fed during the experiment and the test solution was replenished totally every 24 h. The water surfaces of the control and test chambers were covered with a thin film of liquid paraffin, which prevents the diffusion of atmospheric air into the test medium. Ammonia excretion rate was estimated every 24 hrs by phenol hypochlorite method (Sallorzano, 1969). Absorbance is measured spectrophotometrically at 640 nm. Difference between the final and initial ammonia reading will give the ammonia excretion rate in 24 h. Ammonia excretion rate is expressed as mg/l ammonia-N/g body weight of fish/hr (Huang, 2004).

3.6.5. Estimation of Oxygen : Nitrogen ratio

Oxygen consumption and ammonia excretion are widely considered to be critical factors for evaluating the physiological responses of aquatic organisms (Claybrook, 1983; McMahon and Wilkens, 1983; McMahon, 2001). The changes in metabolic substrate usage can be measured by monitoring the oxygen: nitrogen (O:N) ratio of test organisms. The O:N ratio indicates the relationship between the amount of oxygen consumed by an organism and the amount of nitrogen excreted, and shows the relative role protein catabolism plays in the organism's energy budget. The oxygen: nitrogen ratios were calculated from the rates of oxygen consumption and rates of ammonia excretion in *Catla catla*. The oxygen: nitrogen ratio was calculated as the ratio of atoms of oxygen consumed to atoms of nitrogen excreted in the time interval (Widdows, 1985).

3.7. Statistical analysis

The LC_{50} value was estimated by Finney probit analysis method (1971). In the sublethal testing, Significance of differences was tested using one way ANOVA. Means were compared using Tukey's test (Zar, 1999). The differences were considered significant when $P < 0.05$.

Experimental results

IV. EXPERIMENTAL RESULTS

The experimental results obtained on the toxicity of pyrethroid pesticide deltamethrin on the fingerlings of *Catla catla* are presented. Experiments were conducted by static renewal bioassay technique based on the standard procedure for the measurement of pollutants toxicity on freshwater organisms (Sprague, 1969 and APHA, 1992).

4.1. Lethal toxicity (LC_{50})

Catla fingerlings weighing 0.44 ±0.04 g. were exposed to deltamethrin concentrations ranging from 0.08 to 0.40 ppb. The concentrations of deltamethrin for this study were selected based on a preliminary study in which, concentrations of deltamethrin which gives 10 % to 100% mortality of catla fingerlings in 96 h. Results of the study on lethal responses of catla fingerlings when exposed to different concentrations of pesticide are presented (Table 1). The 96 hr LC_{50} value of deltamethrin for catla was found to be 0.10 ppb (Table 1 and Fig 1). The percentage mortality of *Catla catla* exposed to deltamethrin at each 6 h interval is shown in Table 1. Results revealed that there was no death of test animals in control and fish mortality increased with the concentration of deltamethrin. Only a 13 % of fish death was observed at a lower concentration of 0.08 ppb after 48 h. The mortality rate of 97 % was observed after 96 h exposure at concentration of 0.40 ppb deltamethrin.

Table 3: Lethal concentration of deltamethrin (96 h LC_{50}) on catla fingerlings

Concentration (ppb)	No. of fishes used	Mean % Mortality	Conc. x 100	Log concentration	Probit value
0.08	10	13	8	0.90	3.87
0.09	10	27	9	0.95	4.39
0.10	10	40	10	1.00	4.75
0.20	10	63	20	1.30	5.33
0.30	10	80	30	1.47	5.84
0.40	10	97	40	1.60	6.88

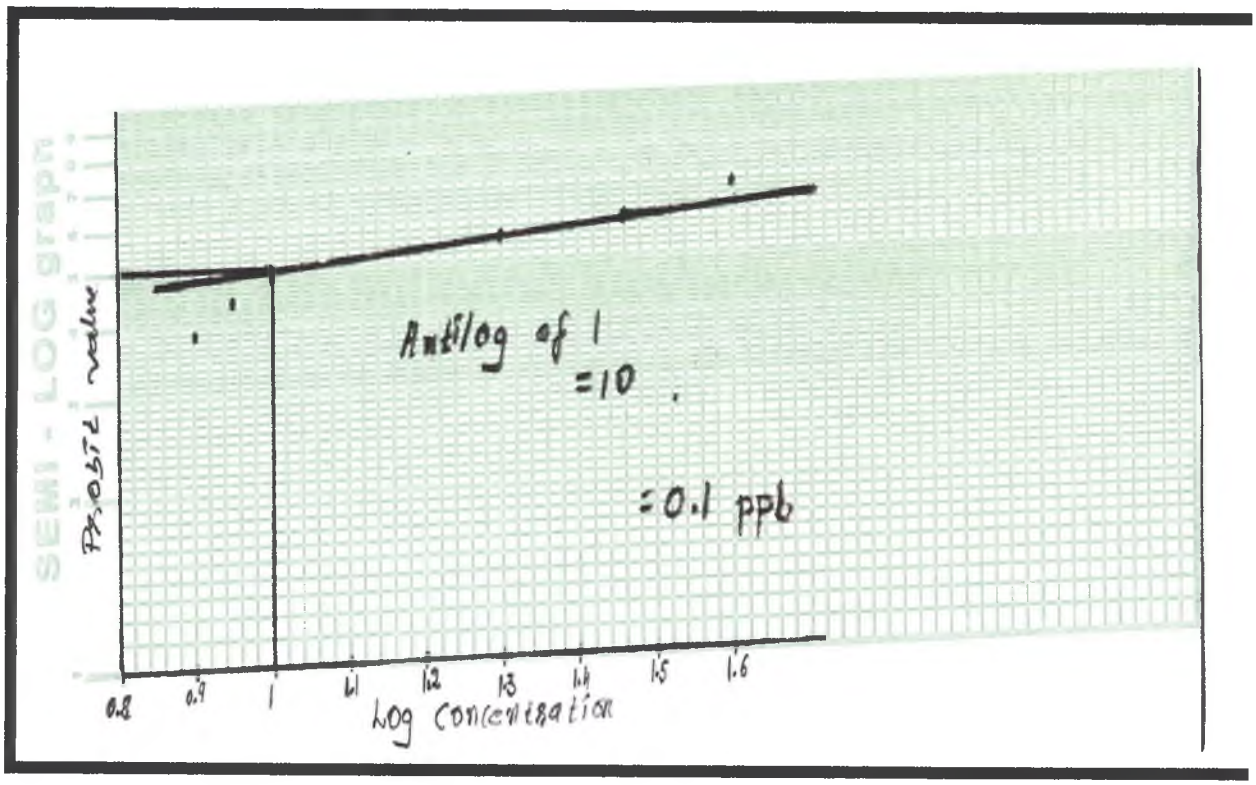


Fig 1.Graphical derivation of 96 h LC_{50} of deltamethrin in *Catla catla* fingerlings

4.2. Sublethal toxicity of deltamethrin

Catla fingerlings were exposed to deltamethrin in two sublethal concentrations, $1/10^{th}$ of LC_{50} *i.e.* 0.013 ppb and $1/5^{th}$ of LC_{50} *i.e.* 0.026 ppb. All the tests were performed in triplicate for a period of 16 days. Fish were exposed to both the sublethal concentrations for 4, 8, 12 and 16 days along with the control sets. Fishes were fed with commercial floating pellets during the test period. The test solution was replenished totally for every 24 h. Behavioral responses, oxygen consumption rate, food consumption rate, ammonia excretion rate and oxygen : nitrogen ratios of fishes were studied in experimental tenures.

4.2.1. Behavior of normal and exposed fish

Experiments were conducted to assess the behavioral changes of catla fingerlings without any toxicant and with two sublethal concentrations of deltamethrin as treatment. In the present study, the control fish behaved in a natural manner, *i.e.,* they were active for feeding and alert to slightest of the disturbance with their well synchronized movements. The behavior did not vary significantly between the control groups. Therefore, the results of these non exposure series were taken as standards for the whole test period. Exposures to lethal concentrations of deltamethrin caused significant visible behavioral changes in the fingerlings of *Catla catla.*

First changes in behavior were observed 15 min after exposure to the higher of the tested deltamethrin sublethal concentration (0.40 ppb). Behavioral response was dose dependent and decreased with decreased concentrations. When the fish were exposed to the lethal concentration of deltamethrin, they migrated immediately to the bottom of the tank. The

schooling behavior was observed to be disrupted in the first day itself and the fish occupied twice the area than that of the control group. They were spread out and appeared to be swimming independent of one another. Irregular, erratic and darting movements followed this with imbalanced swimming activity. Fishes exposed to less than 1 ppb showed normal behavior during exposure period, but afterwards abnormal behavior, such as less general activity and loss of equilibrium, when compared with the control group fishes. In all higher concentrations, they have exhibited loss of balance, swimming alteration, some clinical symptoms like loss of movement and coordination followed by hanging vertically in water, fish lying at the tank bottom and moving in one spot, subsequent short excitation periods with convulsions were also observed in the acute toxicity test of 96 h. Respiratory disruption was observed in the normal ventilating cycle (cough, yawn) with a more rapid, repeated opening and closing of the mouth and opercular coverings. The fish progressively showed signs of tiredness. On the 4^{th} day, they lost their equilibrium and response, to external stimuli such as touch and followed by drowning to the bottom. They often barrel-rolled or spiraled at intervals and engulfed the air through mouth before respiration ceases. The fish eventually died with their mouth and operculum opened. A change in colour of the gill lamellae from reddish to light brown with coagulation of mucus on gill lamellae was prominently seen in dead fishes. The behavioral changes were severe in fishes exposed to lethal concentrations than that of sublethal concentrations.

The fishes were exposed to sublethal concentrations ($1/10^{th}$ and $1/5^{th}$ of LC_{50}) for up to 16 days. In sublethal treatment, the schooling behavior of the fish was slowly disrupted during the first day. Less general activity, excitement, loss of equilibrium, swimming alterations were

Plate 3. Photograph showing the behavior of normal catla fingerlings under study

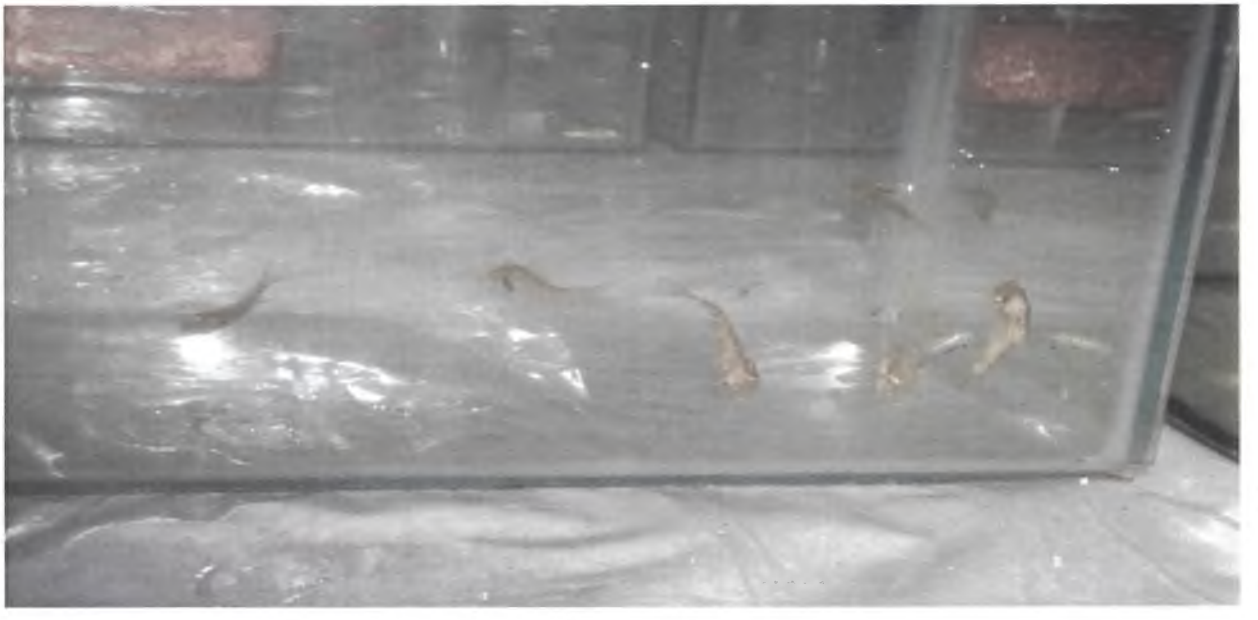


Plate 4. Photograph showing the behavior of catla fingerlings exposed to deltamethrin during the study

not much pronounced on exposure to the sublethal concentration of deltamethrin for the first few days. 7^{th} day onwardsthe inherent activity of the fish decreased with increasing concentration and duration of exposure. The loss of equilibrium and reduced feeding rate were more pronounced in fishes exposed to 0.02 ppb ($1/5^{th}$ of LC_{50}) than 0.01 ppb ($1/10^{th}$ of LC_{50}). In sublethal exposure, fish body became lean towards abdomen position compared to control fish and was found under stress, but that was not fatal. The treated fishes also showed fading of their body color.

4.2.2. Oxygen consumption rate

A variation in the oxygen consumption rate is an indicator of stress, which is frequently used to evaluate the changes in metabolism under environmental deterioration. It is clearly evident from the results that deltamethrin affected the oxygen consumption rate of catla fingerlings under sublethal concentrations. Fishes exposed to both sublethal concentrations (0.01 ppb and 0.02 ppb) showed a drastic decrease in the oxygen consumption rate compared to control (Fig. 2). Highest oxygen consumption rates were attained in control fishes on the 4^{th} day (0.0085±0.0005 mg/L/g/hr) and lowest were in 0.02 ppb concentration ($1/5^{th}$ of LC_{50}) on the 8^{th} day (0.0038±0.0001 mg/L/g/hr) (Table 2). Oxygen consumption rate was higher in control fishes and lower in fishes exposed to 0.02 ppb throughout the 16 days of experiment. Compared to fishes in 0.02 ppb concentration ($1/5^{th}$ of LC_{50}) oxygen consumption rate is higher in fishes of 0.01 ppb ($1/10^{th}$ of LC_{50}) but lesser than that of control fishes during the 16 days sublethal exposure period. One way ANOVA indicated that there is a significant difference ($P<0.05$) in the oxygen consumption rate between different concentrations (Table 3) and there is no significant difference between days (Table 5). Least square difference test (Tukey's test) has showed that there is a significant difference ($P<0.05$) between control and $1/5^{th}$ of LC_{50} and there is no significant difference between controls and $1/10^{th}$ of LC_{50}. Thus deltamethrin had a significant effect on the metabolic activity of catla fingerlings, since an increase in deltamethrin concentration induced a decrease in oxygen consumption rate at sublethal concentrations.

Table 4: Oxygen consumption rate (mg/l/g/hr) of catla fingerlings exposed at different concentrations of pesticide.

Days / Conc. (ppb)	4th	8th	12th	16th
Control	0.0085±0.0005	0.0081±0.0007	0.0075±0.0008	0.0076±0.0002
0.01 ppb	0.0071±0.0003	0.0067±0.0007	0.0064±0.0008	0.0067±0.0002
0.02 ppb	0.0049±0.0002	0.0038±0.0001	0.0049±0.0007	0.0043±0.0008

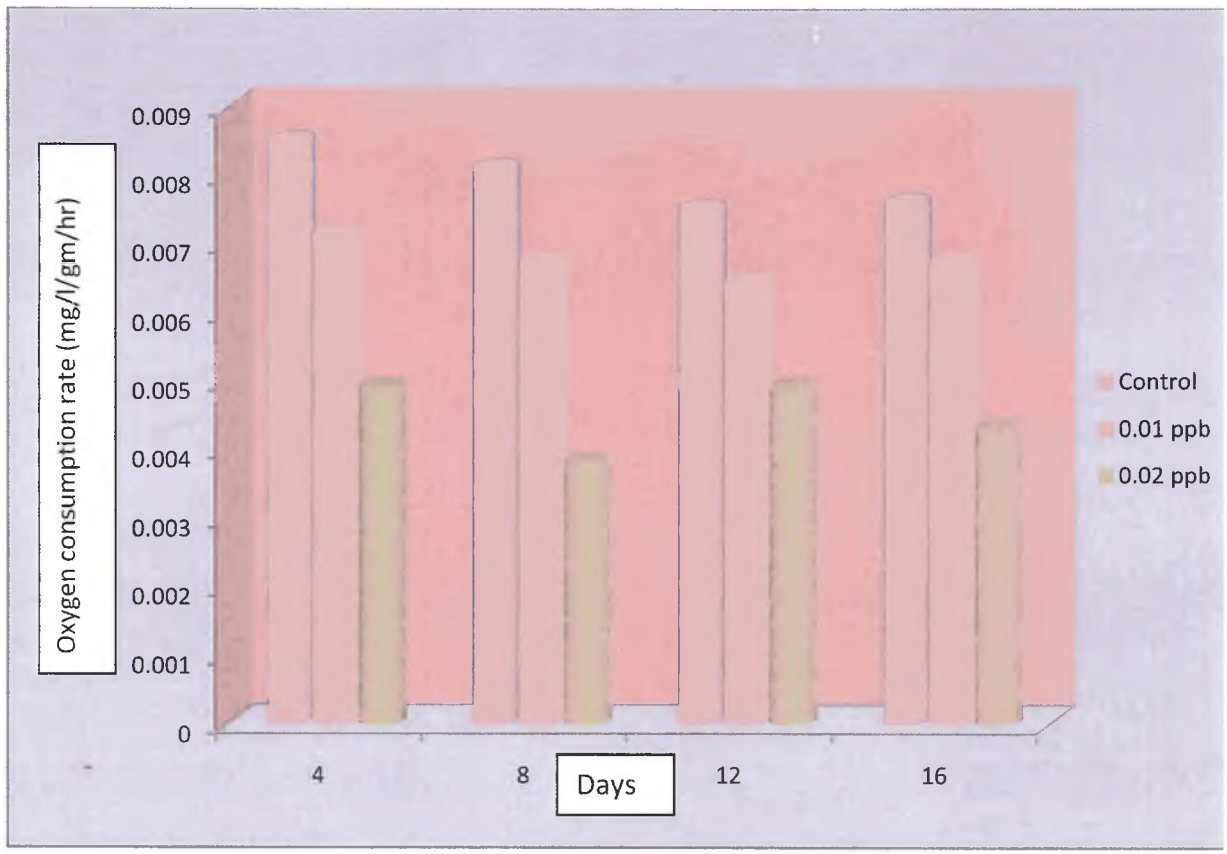


Fig 2: Variation in oxygen consumption rate (mg/l/g/hr) of catla fingerlings exposed at different concentrations of pesticide.

Table 5: ANOVA for the oxygen consumption rate (mg/l/g/hr) of catla fingerlings exposed at different concentrations of pesticide.

Source of Variation	Sum of squares	Degrees of freedom	Mean sum of squares	F -value	P-value	F critical
Treatment	2.5E-05	2	1.2E-05	63.3745	5E-06	4.25649
Error	1.7E-06	9	1.9E-07			
Total	2.6E-05	11				

P<0.05 Significant difference

Table 6: Summary of the ANOVA for oxygen consumption rate (mg/l/g/hr) of catla fingerlings exposed at different concentrations of pesticide.

Groups	Count	Sum	Average	Variance
Control	4	0.0317	0.007925	2.16E-07
0.01 ppb	4	0.0269	0.006725	8.25E-08
0.02 ppb	4	0.0179	0.004475	2.83E-07

Table 7: ANOVA for the oxygen consumption rate (mg/l/g/hr) of catla fingerlings exposed to pesticide between days.

Source of Variation	Sum of squares	Degrees of freedom	Mean sum of squares	F-value	P-value	F critical
Treatment	8.49E-07	3	2.83E-07	0.089035	0.964054	4.066181
Error	2.54E-05	8	3.18E-06			
Total	2.63E-05	11				

P<0.05 Significant difference

Table 8: Summary of the ANOVA for oxygen consumption rate (mg/l/g/hr) of catla fingerlings exposed to pesticide between days.

Groups	Count	Sum	Average	Variance
4^{th} day	3	0.0205	0.006833	3.29E-06
8^{th} day	3	0.0186	0.0062	4.81E-06
12^{th} day	3	0.0188	0.006267	1.7E-06
16^{th} day	3	0.0186	0.0062	2.91E-06

4.2.3. Food consumption rate

Food consumption rates or growth rates are often considered as reliable indicators of stress and welfare of an organism. It is clearly evident from the results that deltamethrin affected the food consumption rate of catla fingerlings under sublethal concentrations. Fishes exposed to both sublethal concentrations (0.01 ppb and 0.02 ppb) showed a drastic decrease in the food consumption rate compared to control (Fig. 3). Highest food consumption rates were attained in control fishes on the 4^{th} day (0.0114±0.015 g feed/g body wt.) and lowest were in 0.02 ppb concentration ($1/5^{th}$ of LC_{50}) on the 16^{th} day (0.0068±0.002 g feed/g body wt.) (Table 7). Food consumption rate was higher in control fishes and lower in 0.026 ppb throughout the 16 days of experiment. Compared to fishes in 0.02 ppb concentration ($1/5^{th}$ of LC_{50}) food consumption rate is higher in fishes of 0.01 ppb ($1/10^{th}$ of LC_{50}) but lesser than that of control fishes during the 16 days sublethal exposure period. One way ANOVA has indicated that, there is a significant difference ($P<0.05$) in the food consumption rate between different concentrations (Table 8) and there is no significant difference between days (Table 10). Least square difference test (Tukey's test) showed that there is a significant difference ($P<0.05$) between control and $1/5^{th}$ of LC_{50} and there is no significant difference between controls and $1/10^{th}$ of LC_{50}. Thus, deltamethrin had a significant effect on the functional activity of catla fingerlings since an increase in deltamethrin concentration induced a decrease in food consumption rate in sublethal concentrations.

Table 9: Food consumption rate (g feed/g body wt.) of catla fingerlings exposed at different concentrations of pesticide.

Days / Conc. (ppb)	4th	8th	12th	16th
Control	0.0114±0.015	0.0103±0.018	0.0096±0.001	0.0096±0.011
0.01 ppb	0.0108±0.015	0.0098±0.010	0.0089±0.010	0.009±0.004
0.02 ppb	0.009±0.003	0.008±0.001	0.0072±0.004	0.0068±0.002

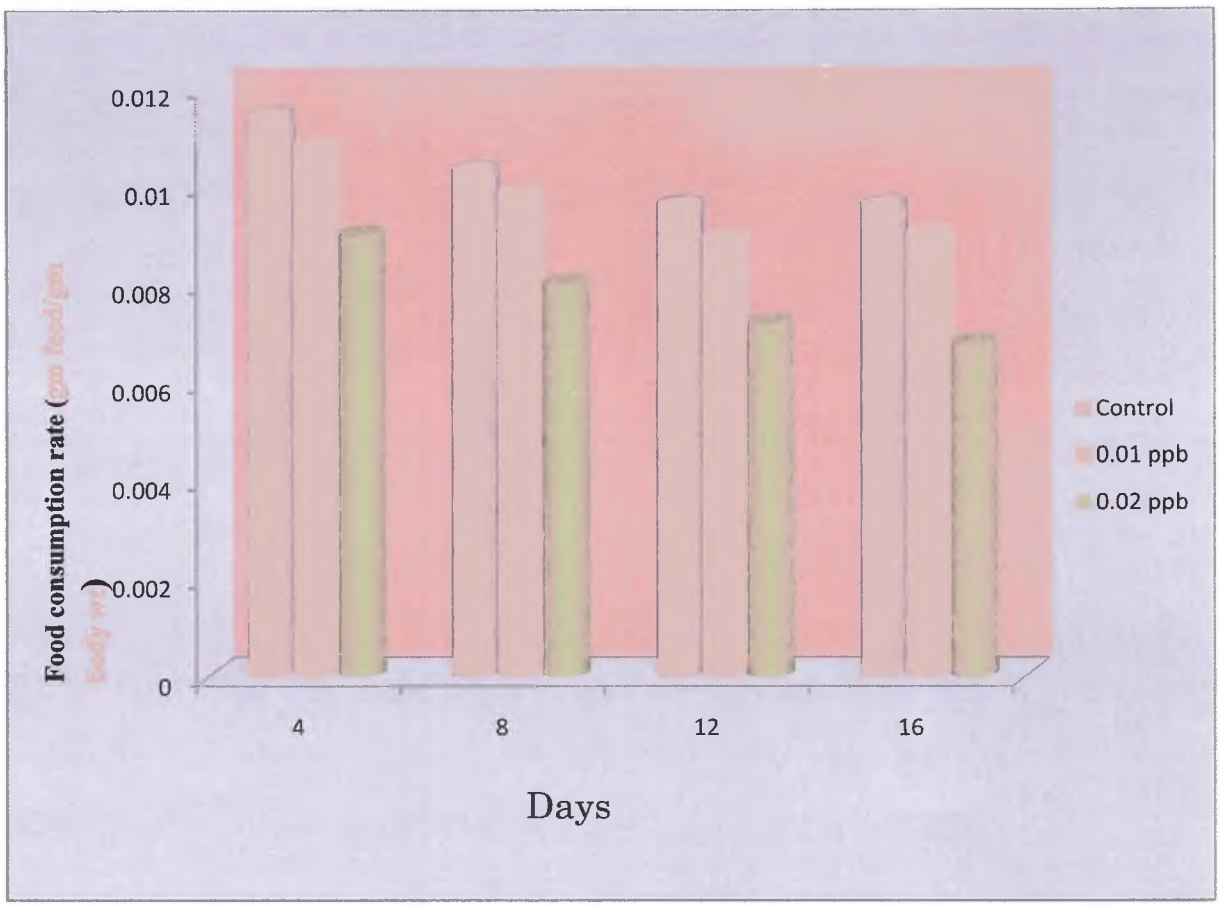


Fig 3: Variation in food consumption rate (gm feed/g body wt.) of catla fingerlings exposed at different concentrations of pesticide.

Table 10: ANOVA for the food consumption rate (g feed /g body wt.) of catla fingerlings exposed at different concentrations of pesticide.

Source of Variation	Sum of squares	Degrees of freedom	Mean sum of squares	F-value	P-value	F critical
Treatment	1.33E-05	2	6.67E-06	8.19215	0.009409	4.256495
Error	7.33E-06	9	8.14E-07			
Total	2.07E-05	11				

P<0.05 Significant difference

Table 11: Summary of the ANOVA for food consumption rate (g feed/g body wt.) of catla fingerlings exposed at different concentrations of pesticide.

Groups	*Count*	*Sum*	*Average*	*Variance*
Control	4	0.0409	0.010225	7.23E-07
0.01 ppb	4	0.0385	0.009625	7.76E-07
0.02 ppb	4	0.031	0.00775	9.43E-07

Table 12: ANOVA for the food consumption rate (g feed/g body wt.) of catla fingerlings exposed to pesticide between days.

Source of Variation	Sum of squares	Degrees of freedom	Mean sum of squares	F-value	P-value	F critical
Treatment	7.22E-06	3	2.41E-06	1.43254	0.303479	4.066181
Error	1.34E-05	8	1.68E-06			
Total	2.07E-05	11				

P<0.05 Significant difference

Table 13: Summary of the ANOVA for food consumption rate (g feed/g body wt) of catla fingerlings exposed to pesticide between days.

Groups	Count	Sum	Average	Variance
4th day	3	0.0312	0.0104	1.56E-06
8th day	3	0.0281	0.009367	1.46E-06
12th day	3	0.0257	0.008567	1.52E-06
16th day	3	0.0254	0.008467	2.17E-06

4.2.4. Ammonia excretion rate

A study on the ammonia excretion rate was used to evaluate the changes in metabolism under environmental deterioration. From the results it is clear that, deltamethrin affected the ammonia excretion rate of catla fingerlings under sublethal concentrations. Fishes exposed to both sublethal concentrations (0.01 ppb and 0.02 ppb) showed a drastic increase in the ammonia excretion rate compared to control (Fig. 4). The rate of ammonia excretion was higher in 0.02 ppb ($1/5^{th}$ of LC_{50}) on the 4^{th} day (0.0011±0.0012 mg/L/g/hr) and lower in control on the 16^{th} day (0.00025±0.0002 mg/L/g/hr) (Table 12). Ammonia excretion rate was higher in 0.02 ppb and lower in control throughout the 16 days of experiment. Compared to fishes in 0.02 ppb concentration ($1/5^{th}$ of LC_{50}) ammonia excretion rate is lower in fishes of 0.01 ppb ($1/10^{th}$ of LC_{50}) but higher than that of control fishes during the 16 days sublethal exposure period. One way ANOVA indicated that there is a significant difference ($P<0.05$) in the ammonia excretion rate between different concentrations (Table 13) and there is no significant difference between days (Table 15). Least square difference test (Tukey's test) has done and found out that there is a significant difference ($P<0.05$) between control and $1/5^{th}$ of LC_{50} and there is no significant difference between controls and $1/10^{th}$ of LC_{50}. Thus, deltamethrin had a significant effect on the metabolic activity of catla fingerlings since an increase in deltamethrin concentration induced an increase in ammonia excretion rate in sublethal concentrations.

Table 14: Ammonia excretion rate (mg/l/g/hr) of catla fingerlings exposed at different concentrations of pesticide.

Days / Conc. (ppb)	4th	8th	12th	16th
Control	0.00048±0.00012	0.00035±0.0001	0.00025±0.0001	0.00025±0.0002
0.01 ppb	0.00089±0.00041	0.00076±0.00052	0.00046±0.00033	0.00044±0.00033
0.02 ppb	0.0011±0.0012	0.0009±0.0007	0.00098±0.0006	0.00092±0.0002

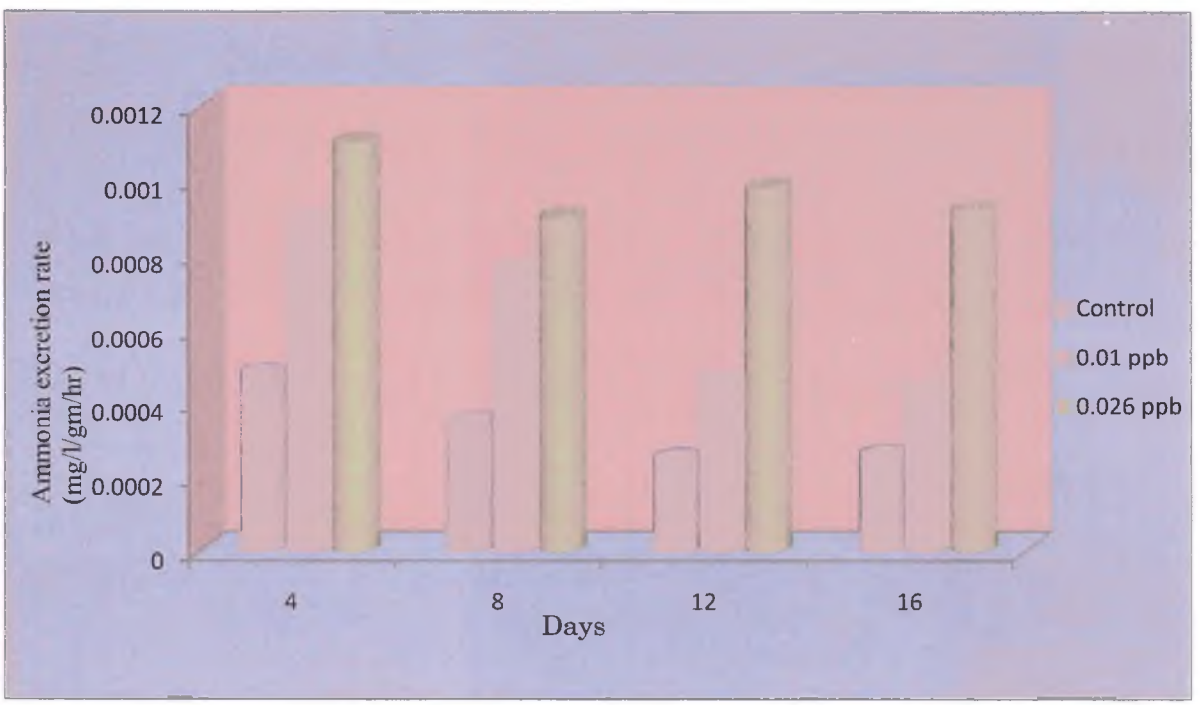


Fig 4: Variation on ammonia excretion rate (mg/l/g/hr) of catla fingerlings exposed at different concentrations of pesticide.

Table 15: ANOVA for the ammonia excretion rate (mg/l/g/hr) of catla fingerlings at different concentrations of pesticide.

Source of Variation	Sum of squares	Degrees of freedom	Mean sum of squares	F-value	P-value	F critical
Treatment	8.2E-07	2	4.1E-07	17.76851	0.00075	4.256495
Error	2.08E-07	9	2.31E-08			
Total	1.03E-06	11				

P<0.05 Significant difference

Table 16: Summary of the ANOVA for ammonia excretion rate (mg/l/g/hr) of catla fingerlings exposed at different concentrations of pesticide.

Groups	Count	Sum	Average	Variance
Control	4	0.00134	0.000335	1.14E-08
0.01 ppb	4	0.00255	0.000638	4.98E-08
0.02 ppb	4	0.0039	0.000975	8.1E-09

P<0.05 Significant difference

Table 18: Summary of the ANOVA for ammonia excretion rate (mg/l/g/hr) of catla fingerlings exposed to pesticide between days.

Groups	Count	Sum	Average	Variance
4th day	3	0.00247	0.000823	9.94E-08

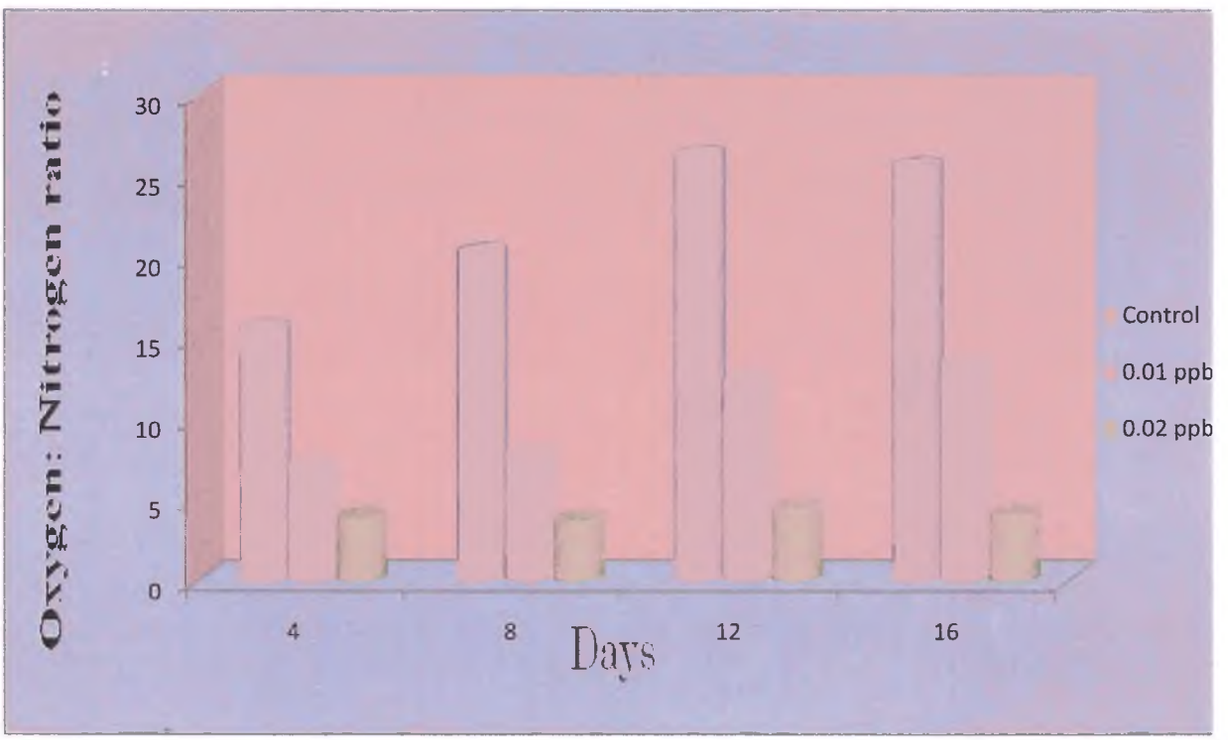


Fig 5: Variation on oxygen: nitrogen ratio of catla fingerlings exposed at different concentrations of pesticide.

Table 20: ANOVA for oxygen: nitrogen ratio of catla fingerlings exposed at different concentrations of pesticide.

Source of Variation	Sum of squares	Degrees of freedom	Mean sum of squares	F-value	P-value	F critical
Between Groups	662.0523	2	331.0262	27.96181	0.000137	4.256495
Within Groups	106.5466	9	11.83851			
Total	768.5989	11				

Table 21: Summary of ANOVA for oxygen: nitrogen ratio of catla fingerlings exposed at different concentrations of pesticide.

Groups	Count	Sum	Average	Variance
Control	4	87.56	21.89	25.40053
$1/10^{th}$ LC_{50}	4	40.18	10.045	10.02923
$1/5^{th}$ LC_{50}	4	16.03	4.0075	0.085758

Table 22: ANOVA for the oxygen: nitrogen ratio of catla fingerlings exposed to pesticide between days.

Source of Variation	Sum of squares	Degrees of freedom	Mean sum of squares	F-value	P-value	F critical
Treatment	68.84016	3	22.94672	0.262339	0.85068	4.066181
Error	699.7587	8	87.46984			
Total	768.5989	11				

Table 23: Summary of the ANOVA for oxygen: nitrogen ratio of catla fingerlings exposed to pesticide between days.

Groups	Count	Sum	Average	Variance
4^{th} day	3	26.36	8.786667	36.08803
8^{th} day	3	31.65	10.55	74.6076
12^{th} day	3	42.8	14.26667	122.8712
16^{th} day	3	42.96	14.32	116.3125

Discussion

V. DISCUSSION

Fish has been largely used to evaluate the quality of aquatic systems as bio indicators of environmental pollutants. The toxicity tests measure the integrated responses to the possible acute or chronic effects of contaminants. Acute toxicity is a significant reduction in survival of the exposed organisms within a relatively short time and can be expressed as the species specific median lethal concentration (LC_{50}). Chronic toxicity effects can occur at exposure levels far below the concentration that causes lethality. The concentrations of pesticides in surface waters generally range far below lethal concentrations for aquatic organisms. Deltamethrin has been widely used in intensive carp farming system for the treatment of external parasites such as Argulus, Lernae, Dactylogyrus etc. The major carps are prone to different parasitic diseases like Argulosis, Lernaeosis, Paradactylogyrosis and Dactylogyrosis in the intensive culture systems. Deltamethrin is widely used for the treatment of these parasitic diseases. Discussion on the toxicity of pyrethroid pesticide deltamethrin on the fingerlings of *Catla catla* is presented.

5.1. Lethal concentration (LC_{50})

The present study on lethal toxicity of deltamethrin indicated that the 96 hour LC_{50} value for fish of the experimental size group was 0.10 ppb. The result shows that, deltamethrin is indeed highly toxic to catla fingerlings. During the experimental period, fishes were found to be reacting strongly even to slightest disturbance in the test media. Such behavioral

abnormalities include loss of balance, swimming alteration, subsequent short excitation periods with convulsions were also observed in the acute toxicity test of 96 h. The values obtained from the current study are clear indications of the fact that, the LC_{50} is greatly influenced by the size of the test animal even though the influence of experimental situations like physico-chemical parameters cannot be ruled out.

The acute toxicity treatments showed strong negative effects on survival as pesticide concentration increased. This suggests dose-dependent survival and concentration graded lethality (Table 1). In laboratory trials, the USDA National Agricultural Pesticide Impact Assessment Program's EXTOXNET document reports deltamethrin's acute fish toxicity below 10 ppb. Present results are in good agreement with this report. Boateng *et al.* (2006) reported that young fish are more susceptible to deltamethrin toxicity and different species respond unlike to concentrations of chemicals. Similar ranges of deltamethrin LC_{50} values for fish have been reported by many researchers. Datta and Kaviraj (2003) found 24 and 96 h LC_{50} values for catfish, *Clarias gariepinus* as 0.015 ppb and 0.004 ppb, respectively. Svobodova *et al.* (2003) determined 96 h LC_{50} value for *C. carpio* as 0.058 ppb. Bradbury and Coats (1989) reviewed the toxicology of pyrethroids in fishes, invertebrates, amphibians, mammals, and birds and cited deltamethrin toxicity to *Salmo salar, Gambussia affinis*, and *O. mykiss* as 96 h LC_{50} values of between 0.50 and 1.97 ppb.

5.2. Sublethal toxicity of deltamethrin

The study on sublethal toxicity has shown that at concentration considerably below LC_{50} levels induce physiological disturbance, which would ultimately affect the life and activity of these fishes.

5.2.1. Behavior of normal and exposed fish

Experiments were conducted to assess the behavior of catla fingerlings without any toxicant and with two sublethal concentrations of deltamethrin. In the present study, the control fish behaved in a natural manner, *i.e.,* they were active for feeding and alert to slightest of the disturbance with their well synchronized movements (Plate 4). The behavior did not vary significantly between the control groups. Therefore, the results of these non exposure series were taken as standards for the whole test period. Exposures to lethal concentrations of deltamethrin caused significant visible behavioral changes in the fingerlings of *Catla catla* (Plate 5). The migration of the fish to the bottom of the tank following the addition of deltamethrin clearly indicates the avoidance behavior of the fish, which was also reported by Murthy (1987) in trout. The decrease in opercular movement and corresponding increase in frequency of surfacing of fish clearly indicated that fish adaptively shifted towards aerial respiration (by obtaining atmospheric oxygen) and the fish tried to avoid contact with the pesticide through gill chamber (Santhakumar *et al.*, 2000). The increased ventilation rate by rapid, repeated opening and closing of mouth and opercular coverings accompanied by partially extended fins was observed in the present study. This could be due to accumulation of mucus in the gill region for proper breathing (David *et al.*, 2000). Present work coincides

with the work done by David *et al.* (2000) on toxicity of fenvalerate to the freshwater fish, *Labeo rohita*. The hyper excitability of the fish invariably in the lethal and sub lethal exposure to deltamethrin may be attributed to the hindrance in the functioning of the enzyme acetylcholine esterase enzyme (AChE) in relation to nervous system as suggested by, Narasimha (1983) Agarwal and Balakrishnan (1989) and David (1995). It leads to accumulation of acetylcholine, which is likely to cause prolonged excitatory postsynaptic potential.

Koprucu *et al.* (2006) also reported that European catfish (*Silurus glanis*) exposed to acute concentrations of deltamethrin higher than 0.50 µg/L showed abnormal behavior such as loss of equilibrium, hanging vertically in the water, rapid gill movement, erratic swimming, swimming at the water surface, air gulping and fading in color of fingerlings. Similar observations were made in the present study. Changes in behavioral patterns exhibited by fish were possibly to counteract aquatic hypoxia condition possibly caused by the agrochemical (Kind *et al.*, 2002). In the present study catla fingerlings showed skin discoloration. Such types of changes were also observed in zebrafish after toxaphene intoxication (Ree *et al.*, 1997). Fishes were also found to secrete mucous along their opercular region, prior to death following deltamethrin poisoning. This phenomenon could be, a symptom of the inflammatory reaction of the gills to the pollutant.

Susan *et al.* (2010) carried out a study on acute toxicity, behavioral changes in the three major Carps, *Labeo rohita* (Ham), *Catla catla* (Ham) and *Cirrhinus mrigala* (Ham) exposed to fenvalerate. They observed that, toxicant exposed fish showed anomalous behavior like surfacing phenomenon, irregular, erratic and darting swimming movements, hyper excitability, loss of equilibrium and hitting to the walls of the test tank before finally sinking

to the bottom just before death. Similar results were also obtained by Josef *et al.* (2011)who carried out a study on behavioral responses of rainbow trout and common carp to pyrethroids (deltamethrin, cypermethrin and bifenthrin). They observed clinical symptoms like accelerated respiration, loss of movement and coordination, fish lying at the tank bottom and moving in one spot, subsequent short excitation periods with convulsions and movement in circles. The present study also revealed similar types of responses from fishes when exposed to deltamethrin.

5.2.2. Oxygen consumption rate

Depletion in oxygen content occurs in the medium when pesticides, chemicals, sewage and other effluents containing organic matter are discharged into the water bodies. In the aquatic environment, one of the most important manifestation of the toxic action of chemical is the over stimulation or depression of respiratory activity. Aquatic organisms like prawns, fish, bivalve, crab respire through gills. Such respiratory surfaces may lead to the alteration in the normal respiratory area which causes reduction in oxygen consumption and physiological imbalance in the organism (Mukke and Chinte, 2012).

Oxygen consumption of an animal is a very sensitive physiological process and changes in the respiratory activity have been used as indicator of stress in toxicant exposed animals. The measurements of oxygen consumption of animal therefore would provide an additional clue to the physiological mode of action of pesticide and pollutants. The gill lamellae of the fishes are ultimately associated with the toxicant in case of polluted

environment. The water current flowing around the gills; carries the toxicants directly, before all other internal organs (Jagadeeson and Vijayalekshmi 1999).

In the present study there was a decrease in opercular movements and the oxygen consumption with an increase in concentration of deltamethrin. Sublethal doses of synthetic pyrethroid have been reported to decrease oxygen consumption of fish (Kumaraguru *et al.*, 1981). This is coinciding with the result of Radhaiah and Jayantha Rao (1990) on the freshwater fish *Tilapia mossambicus* exposed to sublethal toxicity of pyrethroid insecticide, fenvalerate. The significant decrease in oxygen consumption is probably the result of alterations of energy metabolism (Olsen *et al.*, 2006).

Kalavathy *et al.*, (2001) studied on the toxic effects of the pesticide, dimethoate on the fish, *Sarotherodon mossambicus*. They found out that the fluctuated response in respiration may be attributed to reduction in gill permeability causing a drop in oxygen consumption for which the fish compensates by increasing the ventilation volume. In the current study also fluctuated response in respiration is reported in exposed fishes compared to control. Several authors suggested that the gills are the major respiratory organs and all metabolic pathways depend upon the efficiency of the gill for their energy supply and any damage to this vital organ causes a chain of destructive events, which ultimately lead to respiratory distress (Radhaiah, 1990 and Magare and Patil, 2000).

Natarajan *et al.* (1983) investigated the effect of sublethal concentration of Metasystox, an insecticide on the circadian rhythm of bimodal oxygen uptake in *Channa straitus*. They

explained that the decrease in oxygen consumption uptake from pesticide polluted water is mainly due to shrinkage of the respiratory epithelium, since there was swelling of the secondary lamellar tips. In the present study also oxygen consumption rate was lower in 0.02 ppb (higher sublethal concentration) throughout the 16 days of experiment when catla fingerlings were exposed to deltamethrin. Bradbury *et al.* (1986) studied on the toxicokinetics of fenvalerate in rainbow trout, *Salmo gairdneri*. A decrease in oxygen up take efficiency was noticed in rainbow trout exposed to fenvalerate. Similar situation was observed in *Cirrhinus mrigala* and *Labeo rohita*, exposed to fenvalerate (Mushiger *et al.*, 2002). The decrease in oxygen consumption appears to be a protective measure to ensure that there is low intake of the toxic substance.

Lokaswamy and Reddy (2009) who had studied on the impact of pyrethroid insecticide cypermethrin and Ekalux on respiratory activities of the fresh water fish, *Tilapia mossambicus*. Under sublethal toxicity, it showed significant decrease in oxygen uptake by the fishes. Similar observation was also reported in the present study. Kumaraguru and Beamish (1983) reported that the gill is the target organ for synthetic pyrethroid toxicity in fish. This toxicant will pass through the gills and interfering in the gill movement, which is directly proportional to the respiratory activity of the fish, primarily affecting the oxygen uptake.

Patil and Saivid (2008) carried out a study on respiratory dysfunction as index on malathion toxicity in the freshwater fish, *Labeo rohita* (Hamilton). Oxygen consumption was studied in the sublethal concentrations (1, 5, 10 and 15 d). Significant variation in respiration rate with increasing concentration and exposure time were observed here. The depletion in oxygen

5.2.3. Food consumption rate

Fishes in control continued to consume the feed throughout the experiment. In all experimental chambers no mortality was observed and the fishes remained in a good condition. The results revealed that the food consumption rate decreased significantly in the fishes exposed to deltamethrin when compared to the control. A decreasing trend of food consumption rate was observed with increasing concentration of deltamethrin. Similar results have been reported by Pandian and Bhaskaran (1983), when the fish was exposed to 250 ppb DDT and Methyl parathion, consumed 23 or 50 % less food than those exposed to pesticide free water. The decreased food intake may be due to damage caused to taste receptors. In this context, Heath (1995) is of the opinion that the fish subjected to long term exposure to pollutants exhibited reduction in the appetite. The mechanism for this has not been determined, but probably due to hormonal changes. These hormonal changes could cause a direct inhibition of eating.

A reduction in the food intake levels and/or disruption of the feeding behavior is a common feature of the behavioral responses to stress in fish. Under stressful conditions fish eat less and grow more slowly than unstressed fish. Stressful conditions are known also to induce reductions in feed conversion efficiency (FCE) that can lead to decreased growth rates even when food intake levels are maintained (Bernier et al., 2004; Bernier 2006).

Reduced food consumption frequently accompanies toxicant exposure, especially during the first days of the exposure period (Beyers and Sikoski 1994; Heath 1995). We

observed that 16 days of exposure to deltamethrin in sublethal concentration reduced the food consumption rate. The mechanism for suppression of feeding is unknown, but it may be related to physiological effects of the alarm phase of the general adaptation syndrome. Selye (1956, 1973) suggested that loss of appetite is an inherent characteristic of the body's nonspecific response to a stressor. Loss of appetite may be an example of a negative side effect of the stress response. Physiological changes that induce repair mechanisms may reduce ability or desire to process food, consequently, fish demonstrate a loss of appetite (Heath, 1995).

De Boeck *et al.* (1997) studied on the effects of sublethal copper exposure on food consumption in common carp. Juvenile common carp were exposed for 28 days to three different sublethal copper concentrations (0.20 µM, 0.55 µM, and 0.80 µM). Food consumption was monitored on a daily basis during the exposure period. Copper exposure to 0.80 µM affected feeding behavior in common carp. Presumably, the decrease in food intake may be due to increase in metabolic rate which may have been associated with tissue repair and development of defense and toxicant excreting mechanisms (De Boeck, 1997).

Felista *et al.* (1997) studied on the sublethal effects of ammonium chloride on the freshwater fish, *Oreochromis mossambicus*. They found that the feeding rate decreased from 20.309± 0.506 (mg/g/d) in control to 11.594 ± 0.479 (mg/g/d) at the highest sublethal concentration (100 mg/L). In present work similar observations were made. Highest food consumption rates were attained in control fishes on the 4^{th} day (0.0114±0.015 g feed/g body

wt.) and lowest were in 0.02 ppb concentration (1/5th of LC_{50}) on the 16^{th} day (0.0068±0.002 g feed/g body wt.) (Table 7). The decreased feeding rate at higher concentration of ammonium chloride may be due to loss of appetite, it is evident that the accumulation of nitrogenous metabolites in the medium affects appetite (Felista *et al.*, 1997).

Floyd *et al.* (2008) determined the effect of pyrethroid insecticide, esfenvalerate on larvae of the fat-head minnow (*Pimephales promelas*). Fish were exposed to the following measured sublethal concentrations: 0.072, 0.455, and 1.142 microg/L of esfenvalerate. Food consumption was recorded daily. Fish exposed to 0.455 and 1.142 microg/L of esfenvalerate exhibited impaired feeding ability as well as reduced growth compared to fish exposed to 0.072 micro g/L and controls. According to Kasthuri and Chandran (1997), food intake has been identified as a prime factor influencing growth by developing appetite and is found to be affected by toxicants.

Sheela *et al.* (1992) have observed the decreased rates of feeding, absorption, growth, metabolism and conversion efficiency of the fish *Channa striatus* exposed to fenvalerate and phosphamidon with increasing concentrations. Javed (2013) studied on the chronic exposure impacts of waterborne and dietary nickel (Ni) and cobalt (Co) on the growth performance of juvenile major carps viz. *Catla catla, Labeo rohita* and *Cirrhinus mrigala* were studied under static water bioassay. The growth of the fishes was monitored under chronic sublethal concentrations ($1/3^{rd}$ of LC_{50}) of waterborne and dietary Ni and Co, separately for 12 weeks. Feed intakes in all the three fish species were significantly lesser due to waterborne Ni, while waterborne Co and dietary Ni exposure resulted into significantly higher feed intake by the

fish. However, feed intakes were significantly better among control fish groups that caused significantly higher FCE (food conversion efficiency) than those exposed to waterborne or dietary metals. Metals have the ability to modify the feeding behavior of fish (James *et al.*, 2003) that would ultimately affect their growth (Hayat *et al.*, 2007).

Niyogi *et al.* (2006) carried out a study on food selection, growth and physiology in relation to dietary sodium chloride content in rainbow trout (*Oncorhynchus mykiss*) under chronic waterborne copper exposure. They have estimated the food consumption rate by exposing the rainbow trout to waterborne Cu for a period of 28 days. Food consumption rate was severely impaired in Cu-exposed fish relative to Cu-unexposed fish. Similarly in the present work, the significant impairment of food consumption was observed in fishes exposed to pesticide when compared to control.

Subathra and Karuppasamy (2007) studied on the toxic effects of copper on bioenergetics and growth rates in fingerlings and adult stages of the fish, *Mystus vittatus* (Bloch, 1794). The fingerlings and adults were subjected to their respective higher (0.75 and 1.91 mg/L) and lower (0.47 and 1.20 mg/L) sublethal concentration of Cu for a period of 60 days. Food consumption was monitored on a daily basis during the exposure period. The rate of feeding of both the size groups under their respective higher and lower sublethal Cu concentration, led to decreasing trend with increasing periods of exposure. Sarnowski (2004) revealed that the heavy metals might have disturbed the development of locomotive ability that might also adversely affect the feeding abilities.

5.2.4. Ammonia excretion rate

The excretion rate of ammonia allows evaluating the fish nitrogen balance and is a useful tool to determine the effects of environmental and nutritional factors on protein metabolism. Studies on ammonia excretion rates provide a wider view of fish metabolism (Fournier *et al.*, 2003).

Fishes exposed to both sublethal concentrations (0.01ppb and 0.02 ppb) showed a drastic increase in the ammonia excretion rate compared to control (Figure 4). In all experimental chambers no mortality was observed. The fishes remained in a good condition. The results revealed that the ammonia excretion rate increases significantly in the fishes exposed to deltamethrin when compared to the control (Table 13). An increasing trend of ammonia excretion rate was observed with increasing concentration of deltamethrin.

Similar results have been reported by Barbieri *et al.* (2010) when an organophosphate pesticide Folidol 600® exposed on the freshwater fish, Nile Tilapia (*Oreochromis niloticus*) in sublethal doses for 15 days. Ammonium is one of the final products following catabolism, principally of amino acids that might have an alimentary or muscular origin, depending on nutritional conditions. Nitrogen excreted by fish suggested a reduction of free amino acids catabolism and/or else a polypeptide synthesis increase. Another explanation could be the

toxicant effect on gill epithelium, causing a shift in the nitrogen excretion (Mayzaud and Conover 1988).

Huang *et al.* (2004) studied on the chronic toxicity of pesticide, lindane on juvenile

Parveen and Javed (2010) studied on the effect of water-borne copper on the growth performance of the fish, *Catla catla* for 90 days. They also found that water-borne Cu exposure enhanced oxygen consumption and ammonia excretion compared to control fishes. Environmental stress, affects metabolism and ammonia excretion in fishes, possibly as part of the adaptive response which allows survival under adverse conditions (Wright *et al.*, 1995).

Vosloo *et al.* (2012) studied on the effect of sublethal copper levels on ammonia excretion rate of the marine bivalve, *Perna perna*. Animals were exposed to four environmentally relevant concentrations of 12.5, 25.0, 37.5 and 50.0 µg/L copper. Mussels excreted significantly higher levels of ammonia nitrogen when they were exposed to 37.5 µg/L and 50 µg/L copper compared to unexposed animals. They reported that high levels of copper proved to cause sufficient stress in the animals that carbohydrate stores may be depleted and animals start to utilize protein as an energy source as seen from the elevated nitrogen excretion rates at high (37.5 and 50 ppb) copper levels. In the current study also catla fingerlings exposed to both sublethal concentrations (0.01 ppb and 0.02 ppb) of deltamethrin showed a drastic increase in the ammonia excretion rate compared to control.

Ferrari *et al.* (2011) reported a significant increase in ammonia excretion, when juveniles of *Cyprinus carpio* were exposed to sublethal water-borne cadmium (0.15 mg Cd /L) for 2 weeks. Proteins play a central role in the energy production during the stress caused by toxicants. Stress is known as one of the factors that increase ammonia excretion rate (Smith, 1971).

5.2.5. Oxygen :Nitrogen ratio (O : N ratio)

Oxygen consumption rate is a useful indicator of the overall metabolic rate of an animal (Schmidt-Nielsen, 1997), while ammonia excretion rates can indicate higher reliance on proteins in stressed animals (Bayne *et al.*, 1985). The ratios of oxygen consumption to ammonia-N excretion in atomic equivalents can provide information on changes in energy substrate utilization under various environmental regimes (Corner and Cowey, 1968).

It is clearly evident from the results that, deltamethrin affected the oxygen : nitrogen ratio of catla fingerlings under sublethal concentrations. Fishes exposed to both sublethal concentrations (0.01 ppb and 0.02 ppb) showed a drastic decrease in the O:N ratio compared to control (Figure 4). O:N ratio were higher in control fishes and lower in 0.02 ppb throughout the 16 days of the experiment. Thus deltamethrin had a significant effect on the protein catabolism of catla fingerlings, since an increase in deltamethrin concentration induced a decrease in O:N ratio in sublethal concentrations.

In the present work values of O:N ratio were in between 3 and 16 in both sublethal concentrations (0.01 ppb and 0.02 ppb). However the control organisms showed an increase in O:N ratio ranging from 15.49 to 26.25. According to Mayzaud and Conover (1988), the catabolism of pure protein would be related to theoretical values of O:N of between 3 and 16. It is inferred that individuals exposed to high concentrations of pesticides would use only protein as an essential source of amino acids. However, they could conclude that values fewer than 10 in the O:N ratio are deleterious to the organism. Catabolism of equal quantities of

proteins and lipids yields O:N values of between 50 and 60 whereas greater values of O:N corresponds to increases in lipid and carbohydrate catabolism. Studies have concluded that changes in the O:N ratio measured among test organisms can serve as a sensitive indicator, which provides for the relatively early detection of physiological impacts by contaminants (Schweer, 2002).

Montagna and Collins (2008) studied on the oxygen consumption and ammonia excretion of the freshwater crab, *Trichodactylus borellianus* exposed to chlorpyrifos and endosulfan insecticides. The O:N ratio showed a decrease in higher concentrations of chlorpyrifos. An increment in the O:N ratio was observed in the lower endosulfan concentrations. Their results coincide with the present study. Highest O:N ratio was attained in control fishes on the 12th day (26.25) and lowest was in 0.02 ppb concentration (1/5th of LC_{50}) on the 8th day (3.69) (Table 17). A high O:N ratio suggests primarily lipid or carbohydrate metabolism, and a low O:N ratio indicated a protein metabolism (Montagna and Collins, 2008).

Uliano *et al.* (2010) reported a significant decrease in the O:N ratio, when gambusia (*Gambusia affinis*) and zebrafish (*Danio rerio*) were exposed to salinity and temperature stress. The values of O:N ratio was found to be below 10 in all stressed conditions. O:N values recorded under these conditions for both species suggest a predominant protein catabolism. The use of protein as fuel is generally indicative of stressful conditions (Schmidt ., 1997).

Vosloo *et al.* (2012) studied on the effect of sublethal copper levels on oxygen consumption rate, ammonia excretion rate and O:N ratio of the marine bivalve *Perna perna*.

The average atomic equivalents of oxygen consumed and nitrogen excreted (O:N ratio) of mussels decreased with an increase in copper levels. Similar results were obtained in the present study. Proteins are seen as emergency fuel and used when other nutrients are not available (Bayne *et al.*, 1985). In the current study, high levels of deltamethrin proved to cause sufficient stress in the catla fingerlings that carbohydrate stores may be depleted and animals start to utilize protein as an energy source as seen from the reduced O:N ratio and elevated ammonia excretion rates at high (0.02 ppb) deltamethrin levels (Figure 4).

Fuhrer *et al.* (2012) studied on the O:N ratio of the bivalve, *Aulacomya ater* as a criteria for effects of organophosphate pesticide exposure. They found out that concentrations between 0.8 and 1.61 ppb stimulated ammonia excretion rate and decreased O:N ratio, with respect to the control group during 21 days of exposure. In their study, the higher tested concentration of 1.61 ppb of Lorsban 4E showed an average value of 14.8 ± 4.09 in the O:N ratio; thus, it was inferred that individuals exposed to high concentrations of pesticides would use only protein as an essential source of amino acids. However, they conclude that values under 10 in the O:N ratio are deleterious for the organism (Mayzaud and Conover, 1988) from which it follows that the chlorpyrifos concentration 1.61 ppb tested in the study (with values close to 10 in the O:N ratio) would have induced biochemical and physiological alterations on the organisms.

From the present study it is possible to arrive at the sublethal concentrations of deltamethrin affected the schooling behavior, the oxygen consumption rate, food consumption rate, ammonia excretion rate and oxygen : nitrogen ratio of catla fingerlings. The results are evident that, deltamethrin had significantly affected the metabolic activity, which induced in

the decrease of oxygen consumption rate and increased the ammonia excretion rate in the catla fingerlings. Also, deltamethrin impaired the functional activity and hence decreased food consumption rate and by altering the protein catabolism contributed to decrease in the oxygen : nitrogen ratio in catla fingerlings exposed to sublethal concentrations under experimental studies in the laboratory conditions.

Summary

VI. SUMMARY

During the present investigation an attempt has been made to find out the lethal concentration (LC_{50}) of deltamethrin on fingerlings of *Catla catla* and study the sublethal effects of deltamethrin on behavioral, oxygen consumption rate, food consumption rate, ammonia-N excretion rate and oxygen: nitrogen ratio of catla fingerlings. Water quality parameters were monitored regularly. The temperature of water was 28° C ± 1° C and the dissolved oxygen content was 7.0 - 7.5ppm.The pH of the water ranged from 6.9 to 7.3 and hardness varied between 40 and 42 mg/L of $CaCO_3$.

The findings of the study are summarized as below:

1. Lethal toxicity study was carried out by following the standard guidelines of APHA (1992) to determine the lethal (LC_{50}) level of deltamethrin using static renewal system. The 96 h LC_{50} for catla fingerlings was found to be 0.10 ppb.

2. Experiments were done to assess the behavior of catla fingerlings without any toxicant and with two sublethal concentrations of deltamethrin. The fishes were exposed to sublethal concentrations 0.02 ppb ($1/5^{th}$ of LC_{50}) and 0.01 ppb ($1/10^{th}$ of LC_{50}) for up to 16 days. In sublethal treatment, the schooling behavior of the fish was slowly disrupted on the first day. However, less general activity, excitement, loss of equilibrium, swimming alterations were not much pronounced on exposure to the sublethal concentration of deltamethrin for the first few days. From 7^{th} day onwards intensity of the behavioral activities of the fish decreased with increasing concentration and duration of exposure. The loss of equilibrium and reduced feeding rate were more pronounced in fishes exposed to 0.02 ppb ($1/5^{th}$ of

LC_{50}) than 0.010 ppb ($1/10^{th}$ of LC_{50}). In sublethal exposure, fish body became lean towards abdomen position compared to control fish and was found under stress, but that was not fatal. Moreover, the treated fishes showed fading of their body color during the experimental period.

3. The sublethal concentrations of deltamethrin affected the oxygen consumption rate of catla fingerlings. Fishes exposed at different sublethal concentrations (0.01 ppb and 0.02 ppb) have showed a drastic decrease in the oxygen consumption rate compared to control. Higher oxygen consumption rate was recorded in control fishes on the 4^{th} day (0.0085±0.0005 mg/l/g/h) and lower in 0.02 ppb concentration ($1/5^{th}$ of LC_{50}) on the 8^{th} day (0.0038±0.0001 mg/l/g/h). In general, oxygen consumption rate was higher in control fishes and lower in 0.02 ppb during the 16 days of experiment period. Compared to fishes in 0.02 ppb concentration ($1/5^{th}$ of LC_{50}) oxygen consumption rate was higher in fishes of 0.01 ppb ($1/10^{th}$ of LC_{50}) but lesser than that of control fishes during the 16 days sublethal exposure period. Thus, deltamethrin had a significant effect on the metabolic activity of catla fingerlings since an increase in deltamethrin concentration induced a decrease in oxygen consumption rate in sublethal concentrations.

4. Study revealed that deltamethrin affected the food consumption rate of catla fingerlings under sublethal concentrations. Fishes exposed to both sublethal concentrations (0.01 ppb and 0.02 ppb) showed a drastic decrease in the food consumption rate compared to control. Higher food consumption rate was recorded in control fishes on the 4^{th} day (0.0114±0.015 g feed/g body wt.) and lower in 0.02 ppb concentration ($1/5^{th}$ of LC_{50}) on the 15^{th} day

(0.0068±0.002 g feed/g body wt). Food consumption rate was higher in control fishes and lower in fishes exposed to 0.02 ppb throughout the 16 days of experiment. Compared to fishes in 0.026 ppb concentration ($1/5^{th}$ of LC_{50}) food consumption rate is higher in fishes of 0.013 ppb ($1/10^{th}$ of LC_{50}) but lesser than that of control fishes during the 16 days sublethal exposure period. Thus deltamethrin had a significant effect on the functional activity of catla fingerlings since an increase in deltamethrin concentration induced a decrease in food consumption rate in sublethal concentrations.

5. Sublethal concentrations of deltamethrin affected the ammonia excretion rate of catla fingerlings under sublethal concentrations. Fishes exposed to sublethal concentrations (0.01 ppb and 0.02 ppb) showed a drastic increase in the ammonia-N excretion rate compared to control. Highest ammonia-N excretion rates were observed in 0.02 ppb ($1/5^{th}$ of LC_{50}) on the 4^{th} day (0.0011±0.0012 mg/l/g/h) and lowest were in control on the 16th day (0.00025±0.0002 mg/l/g/h). Ammonia excretion rate was higher in 0.02 ppb and lower in control throughout the 16 days of experiment. Compared to fishes exposed in 0.02 ppb concentration ($1/5^{th}$ of LC_{50}) ammonia excretion rate was lower in fishes of 0.01 ppb ($1/10^{th}$ of LC_{50}) but higher than that of control fishes during the 16 days sublethal exposure period. Thus, deltamethrin had a significant effect on the metabolic activity of catla fingerlings since an increase in deltamethrin concentration has induced an increase in ammonia excretion rate in sublethal concentrations.

6. From the results it is evident that deltamethrin affected the Oxygen: Nitrogen ratio of catla fingerlings under sublethal concentrations. Fishes exposed to both sublethal concentrations

(0.01 ppb and 0.02 ppb) showed a drastic decrease in the O: N ratio compared to control. Higher ratio of oxygen : Nitrogen (O: N ratio) was recorded in control fishes on the 12^{th} day (26.25) and lower in 0.026 ppb concentration ($1/5^{th}$ of LC_{50}) on the 8^{th} day (3.69). O: N ratio was higher in control fishes and lower in 0.02 ppb throughout the 16 days of the experiment. Compared to fishes in 0.02 ppb concentration ($1/5^{th}$ of LC_{50}) O:N ratio is higher in fishes of 0.01 ppb ($1/10^{th}$ of LC_{50}) but lesser than that of control fishes during the 16 days sublethal exposure period. Thus, deltamethrin had a significant effect on the protein catabolism of catla fingerlings since an increase in deltamethrin concentration induced a decrease in O:N ratio in sublethal concentrations.

The acute toxicity of deltamethrin on catla fingerlings exposed for 96 h. was found to be 0.10 ppb. For sublethal toxicity study, the fishes were exposed to two sublethal concentrations ($1/10^{th}$ of LC_{50} *i.e.* 0.01 ppb and $1/5^{th}$ of LC_{50} *i.e.* 0.02 ppb) and compared with control fishes for a period of 16 days. The fingerlings of *Catla catla* exhibited erratic swimming, decreased rate of opercular movement, copious mucous secretion, and inability to maintain normal posture and balance with increasing exposure time. Oxygen consumption rate, food consumption rate, ammonia-N excretion rate, oxygen: nitrogen ratio and fish behavior were affected, when they were exposed to increasing concentration of deltamethrin during the study period.

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Abstract

Thesis title: "Lethal and Sublethal Toxicity of Deltamethrin on Fingerlings of *Catla catla* (Hamilton)"

THESIS ABSTRACT

A static renewal bioassay was conducted to determine the acute toxicity (LC_{50}) of synthetic pyrethroid pesticide, deltamethrin on fingerlings of *Catla catla*. The acute toxicity of deltamethrin on catla fingerlings exposed for 96 h was found to be 0.10 ppb. For sublethal toxicity study, the fishes were exposed to two sublethal concentrations ($1/10^{th}$ of LC_{50} *i.e.* 0.01 ppb and $1/5^{th}$ of LC_{50} *i.e.* 0.02 ppb) and compared with control fishes for a period of 16 days. The effect of this pesticide on the physiological conditions was remarkable. The test fish exhibited erratic swimming, decreased rate of opercular movement, copious mucous secretion, and inability to maintain normal posture and balance with increasing exposure time. Oxygen consumption rate, food consumption rate, ammonia excretion rate, oxygen : nitrogen ratio and fish behavior were affected, when it was exposed to increasing concentration of deltamethrin. There was a significant decrease in oxygen consumption rate and food consumption rate with an increase in concentration of deltamethrin when compared to control. Ammonia excretion rate showed a significant increase in the $1/5^{th}$ of LC_{50} *i.e.* 0.02 ppb when compared to control. Fishes exposed to both sublethal concentrations (0.01 ppb and 0.02 ppb) showed a drastic decrease in the O : N ratio compared to control. It is concluded that deltamethrin is highly toxic to fingerlings of *Catla catla* and severely affects their physiology and behavior.

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