BEHAVIOURAL RESPONSES
INTRODUCTION

The physiology and behaviour of fish are closely associated, but this relationship presents a fragile link open to interference from outside disturbances. The aquatic ecosystem has been altered by human activities in many ways and it is relevant to address how human activities can and do, influence these interactions. As one of the most prevalent forms of anthropogenic disruption, chemical pollution will be the focus of the current interest. Chemical contaminants are sourced from many places including households, industry and agriculture ultimately find their way to the aquatic environments that fish inhabit and are thus a major concern for the health of aquatic ecosystems (Katherine and Rod, 2006).

Many chemical contaminants target specific physiological systems and exert their effects on behaviour via physiological pathways. In some cases, behavioural changes may be detectable before physiological changes are manifested and the use of fish behaviour as a sensitive and ecologically relevant tool for monitoring thresholds of effect has long been suggested (Little, et. al, 1985; Rand, 1985; Beitinger, 1990).

The fish serves as bio-indicator of water quality and this can be easily testified by morphological, physiological and behavioural changes in an altered environment. Since pesticides irrespective of their concentration are known to alter the aquatic environment, this certainly has profound effects on biochemical and physiological parameters, which in turn vary with the concentration of the pesticide and duration of exposure. Interestingly,
physiological and biochemical changes do influence behavioural patterns and this is more conspicuous in fish, particularly with reference to aquatic pollution. The interdependence of behaviour and physiology throughout the entire lifecycle of an individual fish presents many ways in which anthropogenic effects upon its environment can wield detrimental effects. So far literature on behavioural responses in fish under pesticide toxicity is rather limited. More recently, this line of work is also gaining impetus Prashanth and David (2010); Katherine and Rod, (2006).

The physiological basis for behavioural in fish is complex and thus understanding how these behaviours are affected by contaminants at the physiological level is not a simple matter. Disruption of memory, perception (i.e., sense organs) and locomotion could all play roles in the observed effects of toxicants on behaviour. A potential candidate for the inhibition of learning is the interference with cholinesterase activity (Hatfield and Johansen, 1972). Other malfunctions in the brain and the central nervous system (CNS) could also be involved.

In the laboratory, fish behaviour can be a sensitive marker of toxicant-induced stress (Prashanth and David, 2010; Atchison, et. al, 1987; Little, et. al, 1985). Toxic substance entering aquatic eco-system can have a wide range of adverse effects on animal communities, not all of which can be learned from standard toxicity tests (Henry and Atchison, 1986). The principle biological variables examined in the standard tests according to the same authors are changes in survival, growth and reproduction rate. Studies have documented
alteration in respiration, locomotion, social organization, reproduction tendency and predator avoidance (Katherine and Rod, 2006). Behaviour is an organismal level of all the above mentioned parameters including biochemical, physiological state of the animal under the influence of the environment. Further, behavioural study should have objectives that should (1) be easily observed in the laboratory or field (2) be sensitive to the chemicals of interest (3) be previously well-described (4) be ecologically relevant to species survival and (5) integrate several sensory and/or mechanical modalities. In addition, the method should be routinely available and simple to employ.

Behavioural tests typically have been precluded from the hazard assessment process because they are often labour intensive, subjective and difficult to quantify and field-verify. Several reviews, however, have demonstrated the sensitivity of fish behavioural toxicity tests and have suggested that these tests be added to the current hazardous evaluation process (Olla, et al, 1980; Westlake, 1984; Little, et al, 1985). Various behavioural changes that have been associated with organophosphorus and organochloride insecticides in a number of species of fish include loss of locomotor control (Raind, 1977), avoidance, failure to select optimal temperature, decreased feeding, increased aggression, elevated number of comfort movements and respiratory disruptions (Bull and Mc Inerney, 1974). Very little information is available on the behavioural toxicology of fish exposed to pyrethroid insecticides. In view of this, the effect of lethal and sub
lethal concentrations of cypermethrin on the general behavioural pattern of the fish, *Labeo rohita* are investigated in the present study.

**RESULTS**

*Normal fish*

Control fishes maintained a fairly compact school, covering about one third of the bottom during the first five days of the 15 days experiment. By fifth day, the school became less compact covering up to two-third of the tank area. Fishes were observed to scrap the bottom surface. When startled, they instantly formed a tight school that was maintained briefly. They were sensitive to light and moved to the bottom of the tank when light was passed into the tank. Except a less response to form a dense school towards the end of the study, no other extraordinary behaviour was observed.

*Treated fish*

When the fish were exposed to the lethal concentration of cypermethrin, they migrated immediately to the bottom of the tank. The schooling behaviour was observed to be disrupted on the first day itself and the fish occupied twice the area than that of the control group. They spread out and appeared to be swimming independent of one another. Irregular, erratic and darting movements followed this with imbalanced swimming activity and non-stop movement of pectoral fins (fanning). The fish exhibited peculiar behaviour of trying to leap out from the pesticide medium, which can be viewed as an escaping phenomenon. The frequency of surfacing
phenomenon was greater on the second day of exposure wherein the fish frequently come to the water surface. 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. Partially extended fins and single wide opening of the mouth and opercular coverings accompanied by hyperextension of all fins were found; consequently the fish was in a state of excitement on the third day. The swimming behaviour was in a "corck screw" pattern rotating along horizontal axis and followed by 'S' jerk, partial jerk, sudden, rapid, non directed spurt of forward movement (Burst swimming); Nudge and Nip phenomenon, i.e., the movement of fish towards another fish (Nudge) and biting (Nip). The fish progressively showed signs of tiredness and lost positive rheotaxis characterized by weakness and apathy. On the 4 day they lost their equilibrium and response, to external stimuli such as touch and light followed by drowning to the bottom. Erection of dorsal and anal fins while motionless (Lateral display). They often barrel-rolled or spiraled at intervals and engulfed the air through mouth before respiration ceased. Prior to death, the pectoral and pelvic fins of affected fish were spread forward (anteriorly) and movements and respiration rate declined. The fish eventually died with their mouth and operculum wide opened. A change in colour of the gill lamellae from reddish to light brown with coagulation of mucus on gill lamellae was seen in dead fish.

In sub lethal treatment, the schooling behaviour of the fish was slowly disrupted during the first day. The ventilation rate was increased,
hyperactivity, excitement, hyperventilation etc, were not much influenced at 5 and 10 days. Further, the fish at 15 days of exposure exhibited balanced swimming and active feeding. The fish behaved in normal way.

DISCUSSION

The potential of contaminants to upset metabolic processes has implications not only for social behaviour but also for schooling behaviour. Migration is energetically demanding processes (Katherine and Rod, 2006). The migration of the fish to the bottom of the tank following the addition of cypermethrin clearly indicates the avoidance behaviour of the fish. Similar observations were reported earlier; Murthy (1987) observed avoidance behaviour in trout on exposure to pesticides. Virtually every species of fish contains a reflex response that makes a rapid, appropriate response possible. Eaton and Emberley (1991) in which a negative relation exists for the angle of initial orientation for the fish and the angle of the escape turn have demonstrated this. Nisar Ahmed, (1989) have also observed the avoidance nature by *Labeo rohita* on exposure to three pollutants viz, Endosulfan, Malathion and Sevin. Similar behaviour was reported by Belitginer and Freeman, 1983; Hartwell, *et. al*, (1989) in various species of fish.

The effects of anthropogenic contaminants on cognition have been investigated mainly through studying conditioned responses in the laboratory. For example, using a conditional avoidance technique, Weir and Hine (1970) looked at retention of a conditioned response in goldfish, *Carassius auratus*, after exposure to sodium arsenate (Na2HAsO4), lead nitrate (Pb(NO3)2), mercuric chloride (HgCl2), or selenium dioxide (SeO2).
Contaminants impaired performance at levels below the concentration at which 1% mortality of the population occurred following 48 hour exposure and 7 subsequent days in clean water. Of the four contaminants investigated, mercury (Hg) had the greatest effect, causing disruption of conditioning at 3 mg/l. In similar experiments, Hg and aromatic hydrocarbons have been found to impair even the conditioned learning (Salzinger, et. al, 1973; Purdy, 1989). Gold fish has been observed to avoid fenitrothion at low concentration of 10 mg/l (Scheter, 1975). It has been also reported by Folmar (1976) that Rainbow trout can detect and avoid copper sulphate, dalapon, 2,4-D (DMA), xylene and acrolein. The lethal concentration of herbicide glyphosate has been avoided by Rainbow trout (Hildebrand, et. al, 1982). Similar observation was reported by Swetharanyam, (1991) on the Oreochromis mossambicus exposed to endosulfan.

The schooling tendency of various fish species may help mediate rapid anticipatory responses to chemicals. Here, the three sensory systems that are likely to play key roles in rapid responses are vision, the lateral line system and olfaction (Brown and Magnavacca, 2003). Disruption of schooling behaviour of the fish, due to the lethal and sub lethal stress of the toxicant, results in increased swimming activity and entails increased expenditure of energy (Murthy, 1987). The disturbance of the schooling behaviour of the fish in treated media indicates that the group hydrodynamic effect of fish would help them to swim within the school has been lost. (Zuyer and Belyayen, 1970). A change in the normal physiological and bio-chemical aspects in the
treated fish in the present study could be attributed to the disruption of the schooling behaviour of the fish, which in turn leads to higher activities as suggested by (Murthy, 1987). Weis and Weis (1974) have reported that carbaryl has a marked effect on the schooling behaviour of the Atlantic silverside. Many workers have observed loss of such behaviour following pesticide exposures (Drummond, et. al, 1986).

The erratic swimming of the treated fish indicates loss of equilibrium. European eels, *Anguilla anguilla*, exposed to sublethal concentrations of thiobencarb for 4 days displayed inhibition of AChE activity in the brain, muscle and gill tissues (Fernandez-Vega, et. al., 2002). Behavioural effects were also observed, including reduced motility, loss of equilibrium, uncoordinated movements and an increase in the levels of respiratory frequency. Additionally, European eels exposed to sublethal fenitrothion for 4 days suffered a 44% reduction in brain AChE activity at 0.02 mg/l and 64% at 0.04 mg/l (Sancho, et. al., 1997). One week of recovery in clean water was not sufficient for AChE activity to return to control levels. The resultant behavioural changes in this study included erratic swimming and convulsions. Loss of equilibrium observed in the present study is likely that the region in the brain which is associated with the maintenance of equilibrium should have been affected as suggested by Drummond, et. al, (1986). Loss of equilibrium and erratic swimming are reported in blue gills exposed to dursban (Mehrle and Mayer, 1975). Santhakumar and Balaji, (2000) observed exciting and erratic movements in *Anabas testudineus* exposed to
monocrotophos. Similar observations were reported in *Hiteropeustes fossilis* exposed to rogor and endosulfan (Sabita and Yadav, 1995).

The surfacing phenomenon of fish observed under cypermethrin exposure might either be due to hypoxic condition of the fish as reported by Sambasiva Rao and Chandrasekara Rao, (1987) for *Channa punctaus*. The increased surfacing during the initial periods of exposure to cypermethrin concentrations suggests an elevated rate of metabolism. Changes in ventilation rate and surfacing frequencies are the general symptoms noticed in the fish after exposure to the pesticide and these activities help the fish to avoid contact with poison and fight against stress (Ray and Munshi, 1987). This fact is clearly evidenced in the present study. Chronic exposure of fin fish to aroclor was found to induce surfacing phenomenon of fish as pointed out by Hansen, *et. al*, 1972. Drummond, *et. al*, 1986, have recorded similar observation in fathead minnow treated with different chemical groups.

The increased ventilation rate by rapid, repeated opening and closing of the mouth and opercular coverings accompanied by partially extended fins (Caughing) was observed in the present study. This could be due to clearance of the accumulated mucus debris in the gill region, for proper breathing as suggested by Carlson and Drummond, (1978), caugh and yawns seem to be a more extreme effort to do the same (Cairns, *et. al*, 1982). *Per se* it can be explained by energetic constraints caused by the toxicants. Similar situation was observed by Carlson, *et. al*, (1982) in the bluegill *Lepomis mahrochirus*. Like wise, monocrotophas exposure has been reported to cause increased
opercular movements in *Anabas testudineus* (Santhakumar and Balaji, 2000). Schaumburg, *et. al*, (1967) have noticed a direct relationship between the frequency of coughing and the time of exposure in Rainbow trout. Caughing frequency in *Coho salmon* was observed increased, with increasing concentration of fenitrothion (Bull and Mc Inerney, 1974).

The hyperexcitability of the fish invariably in the lethal and sub lethal exposure of cypermethrin may probably be due to the hindrance in the functioning of the enzyme AChE in relation to nervous system as suggested by many authors (Moore, 1966; Narasimha, *et. al*, 1986; Shakul and Vadamalai, 1986; Agarwal and Balakrishnan, 1989). It leads to accumulation of acetylcholine which is likely to cause prolonged excitatory post synaptic potential. This may first lead to stimulation and later cause a block in the cholinergic system. David, (1995) has observed hyperactivity, in *Labeo rohita* exposed to fenvalerate affected central nervous system Mehrle and Mayer, (1975). Venugopalan and Sasibhushana Rao, (1979) reported the state of neuromotor system in the exposed fish. Pesticide exposures manifest into hyperactivity of muscles in Blunt-nose minnow (Mount, 1962); Gold fish (Grant and Mehrle, 1970); *Cyprinus carpio* (Toor and Kaur, 1974); *Barbus stigma* (Manoharan and Subbaiah, 1982. *Tilapia mossambica* (Deva Prakasa Raju, 2000) and *Anabas testudinus* (Madhab Prasad, *et. al.*, 2002).

According to Sambasiva Rao and Chandrasekara Rao (1987) behavioural patterns are also influenced by bio-chemical changes at the tissue level. The significant alterations observed in the bio-chemical constituents of gill, liver and muscle in the present investigation corroborate with the above
view that bio-chemical change at the tissue level of the treated fish contribute to the abnormal behaviour of the fish.

The accumulation and increased secretion to mucus in the fishes exposed to pesticide may be an adoptive responses perhaps providing additional protection against corrosive nature of the pesticides and they avoid the absorptions of the toxicant by the general body surface. This agrees to the earlier findings of Sadha (1993); Sabita and Yadav (1995); Santhakumar and Balaji (2000). Loss of positive rheotaxis of the fish is a good indication of toxic response. Similar observation has also been made in Tilapia renalli exposed to ‘bis’ by Chliamovitch and Khun, (1977). In the present study as evidenced by the results the abnormal changes in the fish exposed to lethal concentration of cypermethrin are time dependent. However, the normal behaviour of the fish at 10 and 15 days indicates adaptability of the fish to the sub lethal concentration. The physiological, biochemical and histological aspects in the sub lethal concentration of cypermethrin also support this.

Thus, as postulated by Murthy (1987), behavioural changes of the fish under insecticidal stress may have deleterious effects of making the fish fall an easy prey in their natural habitat and may affect the stability of the population. Behavioural characteristics are obviously sensitive indicators of toxicant effect. It is necessary, however, to select behavioural indices for monitoring that relates to the organisms behaviour in the field in order to derive a more accurate assessment of the hazards that a contaminant may pose in natural systems. If social hierarchies have developed evolutionarily as
a mechanism to efficiently ration limited resources (Food, Mates, Spawning sites etc) among individuals of a population (Krebs and Davis, 1978), than disruption of such a system by a toxicant could affect survival and reproduction within a population under natural conditions. Therefore, rank-related responses should be considered for species forming social organizations. If social interactions are not considered, only a certain portion of a population may be protected and the toxicity of contaminant may be underestimated. So this type of study can be useful to compare the sensitivity of the various species of aquatic animals and potency of chemicals to derive safe environmental concentration.