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Behavioural responses in effect to chemical stress in fish: A review

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Abstract
Due to industrialization and urbanization many pollutants are being introduced directly and indirectly into aquatic ecosystem. Behavioural bioassay have been widely used in toxicity assessment. Bioassay based on behavior is faster, more sensitive and ecologically more relevant as assessing growth and reproduction need longer bioassay. Behavioural bioassay is more promising alternatives than lethality evaluating bioassay which are currently used for the risk assessment of toxicant. Behavioural changes provide early warning signals about the health of exposed population which other standard tests do not take in to consideration. These endpoints may be 10–100 times more sensitive than those derived from acute or chronic tests because chemicals can induce rapid behavioural responses in organisms even at very low concentrations. Behaviour is an organism-level effect defined as the action, reaction, or functioning of a system under a set of specific circumstances. We rationalize that a greater understanding of behavioural responses in effect to chemical stress may increase. Therefore in current scenario there is a need of developing newer and effective methods to study the behavioural responses. Behavioural changes in a fish form an efficient index to measure any alterations in the environmental conditions.

Keywords: Behaviour, toxicants, bioassay, stressors

Introduction
Nonspecific response in fish can be evoked by physical, chemical and perceived stressors and enable fish to cope with disturbances. It is important to link sub cellular responses with behavior, chemical stress, and higher levels of biological organization. Because behavior is a result of endogenous and exogenous processes, changes in such parameters can help to understand the health and viability of natural populations exposed to xenobiotics. Stressors evoke a nonspecific response in fish to adapt or cope with the disturbance. But if stress last for longer duration, it can threaten the wellbeing of fish [1]. Change in behavior can be noticed when the animal is exposed to a chemical concentration below than that can cause mortality [2]. [3] suggested that multidisciplinary research is required to increase the usefulness and significance of behavioral indicators.

Feeding behavior
Any change in fish behavior give information and knowledge regarding behavioural alterations which can be related to physiological biomarkers in aquatic species [4]. Behaviour links physiological function with ecological processes, and can be very sensitive to environmental stimuli and chemical exposure. Use of behavioural alterations in organisms in response to pollutant is increasingly studied in ecotoxicology for improving the determination of ecologically relevant risk end point. Behavioural changes related to feeding are having there ecological significance [5]. That results in effect on locating and reaching the food, which may affect population dynamics and ultimately the community structure. Swimming and avoidance behavior have direct effect on the fish appetite as both the activities are involved in determining the survival of fish such as getting food and avoiding adverse conditions [6]. After exposure to 19.44 ppm sublethal concentration of Cu *Catla catla* show reduced feed intake [7]. Reduced feeding behavior may reduce energy intake by the organism which effect its growth and reproduction.

Swimming Behaviour
Swimming behavior is considered as parameter to assess the physiological status of aquatic environment for presence of contaminants [8].

Swimming activity is one of the most common and easily measured behavioral responses during toxicological studies [9]. Because swimming is central to many aspects of fish biology, decreased performance could have important implications for interspecific and intraspecific interactions, ultimately decreasing fitness in affected individuals [10]. Behavioral changes shown by any organism in response to any chemical depend upon their mode of action [11]. Behavioral changes in response to certain chemicals like chlorpyriphos [12], chromium [13] polychlorinated biphenyls and tributyltin [14] have been investigated. Swimming activity has been previously used in number of studies to assess the toxicity of a compound. Swimming behavior effected by the metals by increasing the concentration of plasma ammonia which will decrease the level of Na^+, K^+, Ca^{2+} in plasma. These ions are involved in number of metabolic and physiological activities such as central and peripheral nervous activity, muscle contraction, transmission at neuromuscular junction etc.

Avoidance Behaviour

Avoidance behavior is shown by rainbow trout when exposed to oil-sand process effected water [15]. In an investigation done by [16] the control fish maintained in normal water were found to be active throughout the experiment, but the fish exposed to 4-nonylphenol showed abnormal behavior like erratic movement, increased operculum activity and reduced reflexes. High pigmentation as well as high mucus secretion was also observed. Avoidance behavior was shown by jerky moments and the fish often came to the surface for gulping air. Finally the fish settled at the bottom with loss of equilibrium and rolled its body prior to death. Different researchers have observed similar results in different fish species in response to various toxicants [17] found excessive mucus secretion, change in pigmentation, muscle fasciculation, refusal of feeding and respiratory distress in *Catla catla* in response to dimethoate. Similarly, gulping air, swimming at the water surface (surface phenomenon), loss of equilibrium, change in body colour, increase secretion of mucus and irregular swimming activity were observed by [18] in fish *Clarias batrachus* after exposure with copper sulphate. [19] reported faster opercular activity, jerky moments, erratic swimming and protrusion of eyes in gold fish after treatment with malathion and hinosan [20], observed neuotoxic effects like lateral side movement, loss of balance, movement in circular form with jerks and increased operculum activity in *Labeo rohita* exposed to chlorpyrifos [21]. Exposed fish *Cyprinus carpio* in a toxic media containing chlorpyrifos and visualized erratic, irregular and darting swimming movements, loss of equilibrium, hyper excitability and ultimately sinking of the fish to the bottom [22] had found a different swimming pattern like circular, spiral and upside down swimming in fish *O. niloticus* followed by domic acid injection and the symptoms were found to disappear after 3 hours of exposure.

Different studies in behavioural response after exposure to metals and pesticides

Affected fish with behavior alteration toward toxicant especially pesticides or heavy metal has been reported by [23, 24] visualized the signs of fenvalerate poisoning in fish, which led to loss of schooling behavior, surfacing, hyperactivity, erratic swimming, seizures, loss of buoyancy, high cough, secretion of mucus in gill, flaring of the gill arches, head shaking and restlessness before death [25] observed the behavioral responses in *C. carpio* after treatment with 0.5, 1.0 and 1.5 ppm concentrations of mercuric chloride ($HgCl_2$) for 8, 16, 24, 48, 72, 96 and 124 hrs. No change in behavioral activity was observed when fish were exposed to the lowest concentration (0.5 ppm) of $HgCl_2$, but on higher concentrations change in behavioral responses were seen. It was observed that $HgCl_2$ caused adverse effects on body colour, behavioral responses and haematological parameters in *C. carpio* [26] studied the variable behavioral pattern in fish, *O. niloticus* after exposure to different concentrations of Zn. Fish exposed to Zn showed sluggish movement, rapid operculum movement and loss of equilibrium. Congestion in gill, liver and kidney was observed after necropsy. The control group showed a normal pattern of swimming [27] suggested that after treatment with extracted microcystins, Carp (*Carassius auratus*) showed abnormal behavior. High doses induced death of the fish with a swollen belly. After 1-3 hours, uneasiness and frantic swimming were observed, while after 12-24 hours sluggish swimming with a quick burst of frenetic swimming were seen. On the other hand, control group exhibited normal swimming activity. Besides this decrease in RBCs count, haematocrit and Hb were also observed. A significant decrease in all the hematological indices except ESR was observed. No prominent change in MCH, MCHC and MCV were observed [28]. Observed abnormal behavior like fast swimming activity, excess secretion of mucus, hypersensitivity, jerks, increased pigmentation on the dorsal side and loss of equilibrium in *C. carpio* after exposure to chlorpyriphos [21].

Recorded the behavioral changes in Common carp after exposure to chlorpyrifos. After treatment the fish showed irregular, erratic and darting swimming movements, hyper excitability, loss of equilibrium and sinking at the bottom [23]. Visualized the behavioral changes in fish, Nile tilapia (*O. niloticus*) and catfish in response to copper sulphate (50, 60, 70, 80 100 and 120 mg/l). *O. niloticus* showed more severe effects, as compared to catfish. Avoidance behavior was observed by unsteady swimming pattern with jerky moments. Fish were observed to be suspended in vertical position with tail pointing downwards. Finally fishes sank in the bottom and became motionless [29]. tested biopesticide Neem for its behavioral response in fish *Labeo rohita* after acute exposure. The behavioral patterns observed during the experiment were slow opercular movement, erratic swimming, surfing behavior and fish was not able to maintain posture and equilibrium with time [30]. Studied the behavioral changes and histological alterations in *Trichogaster trichopterus* induced by paraquat. The fish became lethargic and displayed erratic swimming. The changes in liver were characterized by cytoplasmic vacuoles, cloudy swelling and hypertrophy, while gill exhibited hypertrophy and edema of secondary gill lamellae. Furthermore spleen showed accumulation in melano-macrophage centers and disorder in ellipsoid cells after exposure with 0.15 and 0.3 mg/l of paraquat. A link between different biomarkers (genotoxic, haematology, biochemical and behavior) had been presented by treating Amazon Fish (*Colossoma macropomum*) to Crude Oil [31]. After exposure 90% decrease in their response to alarm substance and a 60% decrease in swimming activity was observed as compared to control. No change was revealed in hematology. A significant increase in the DNA damage was found along with an increase of GST activity [32].

Used Zebrafish (*D. rerio*) for studying behavioural responses after exposure to sublethal concentrations of deltamethrin. Change in swimming pattern was observed in the first 5 hours. Hyperactivity and surfing was high in the groups treated with higher concentrations. Acute toxicity of cartap hydrochloride to fish *L. rohita* induced several behavioral changes after 6 days. The fish appeared to be in distress and exhibited erratic swimming movements. After 2 days operculum movement became fast and fish surface frequency for gasping air get increased. Along with this hyper mucous production, flaring of the gills and darting movement were noticed in the fish [33].

[19] Used 0, 1, 2, 4, 8 mgL^{-1} of hinosan and 0, 1, 2, 4, 16 mgL^{-1} of malathion on Goldfish (*C. auratus*) to study the effect on the behavioral response after 96 hours of exposure. Irregular, darting, spiral and erratic swimming movements were observed along with loss of equilibrium and bottom sinking [18]. Revealed that when fish *Clarias batrachus* was exposed to copper sulphate, marked behavioral changes were observed. Treatment of 33µg/l was given for 96 hours. Increased opercular activity, loss of equilibrium, surface behavior, rapid jerky movement and aggressiveness were seen. Increased ventilation rate and disruption of schooling behavior were also observed [17]. Revealed that 96 hours exposure of dimethoate (insecticide) led to behavioral changes in *Catla catla.* Change in body pigmentation, lower opercular movement, uncoordinated erratic swimming and excess mucus secretion, feeding refusal and respiratory distress were the main behavioral changes observed during the exposure [34]. Exposed fish rainbow trout with a mixture of herbicide and observed that fish became hypoactive and were at the bottom as compared to control. Swimming height decreased in exposed fish. Even short term exposure showed altered behavior as compared to the control group.

Table 1: Latest studies on behavioural changes in fish in response to different toxicants

Chemical/ Toxicant used	Fish	Behavioural changes observed	Reference
Biopesticide	Common Carp fry	Effect on movement Fish become sluggish after 72 hours of exposure and movement reduced with increase in concentration	Chandan and Chandra, 2018 [42]
Ammonia	Rainbow trout	Reduce or abolished the formation of dominanace hierarchies in juvenile Decreased growth	Grobler and Wood, 2018 [44]
Cd	Aquatic snail	Feeding activity reduced	Alonso and Valletines, 2018 [5]
Endosulphan	*Channa punctatus*	Decreased swimming activity, increased surfacing, higher opeculum activity, more mucus secreation	Harit and Srivastava, 2018 [45]
Triclosan	*Anabas testudineus*	Surfacing, air gulfing, reduced operculum movement, mucus deposition, bulged and hemorrhagic eyes	Priyatha and Chitra, 2018 [46]
Microplastic and murcury	European Seabass	Reduction in swimming velocity and resistance time of fish	Barboza *et al* 2018 [47]
Copper and Mercury	*Pomatoschistus microps*	Swimming resistance	Vieira *et al*, 2009 [48]
Bitter leaf Varonia amygdalina	*Clarius garipinus*	Erratic swimming led to loss of equilibrium, respiratory disturbance, lethargic	Olowolafe and Olufayo, 2018 [49]
Zinc and chromium	*Danio rerio*	Avoidance behavior, speed increase in lower concentration and increased in higher concentrations.	Lu *et al*., 2017 [50]

Behavior is both a sequence of actions, operating through the central and peripheral nervous systems and the cumulative manifestation of genetic, biochemical and physiological processes essential to life, such as feeding, reproduction and predator avoidance. It is the result of adaptation to changing environment and allows the organism to adjust internal and external stimuli to cope with the variable environment. It is a sensitive measure of an organism's response to stress including environmental contaminants. It serves as a link between physiological and ecological processes and may be ideal for studying environmental pollutant effects [3]. Fish are able to uptake and retain different xenobiotics dissolved in water via active or passive processes. Any change in the behavior of fish indicates the deterioration of water quality. The performance of the normal behavior of individual fish follows specific physiological sequences which are triggered by external stimuli acting via a neural network [3]. Disruption of these sequences before completion is likely to result in detrimental behavioral alterations. Inappropriate behavioral responses to environmental and physiological stimuli due to the toxic effects of aquatic contaminants can have severe implication for survival [3]. Different chemicals can differ in the types of behaviors that they affect depending on their mode of action.

The abnormal behaviors observed in the fish may be caused by the neurotoxic effects and also by the irritation to the perceptive system of the body. Toxicants may damage nerve cell bodies, axons, and myelin sheaths. At the biochemical level, they can alter the synthesis and release of neurotransmitters, which may be associated with behavioral changes. Organic chemicals as well as metals can affect neurotransmitters and behavior [35] have reported the deleterious effects of 4-NP on central nervous system as well as neuroendocrine homeostasis and cognitive functions. Inhibition of AChE activity due to the exposure of 4-NP activity in *Mytilus galloprovincialis* has been reported by [36]. So the abnormal behavior shown by fish may be due to abnormal level of neurotransmitters. These changes occur much earlier than mortality [2]. Jumping to and fro signify the avoidance reaction of the fishes to the toxicants. Fish avoid the area containing chemical so mostly fishes remain in the corners of the tank. The increase in surfacing and gulping of air from surface water after toxicant exposure could be an attempt of the animal to escape from the toxicant and to avoid breathing in the contaminated water. Secretion of excessive mucus is probably due to irritation of the skin due to direct contact with the toxicant. Mucus forms a layer between the body and toxicant to minimize irritating effect [37] and also inhibit the diffusion of oxygen during gaseous exchange [18]. Preference of upper layer may be due to elevated demand for oxygen due to respiratory stress in the exposed groups [38]. Lateral swimming and loss of equilibrium is probably due to the impairment of the nervous system [39]. Ultimately, fish sank into the tank bottom with a least operculum activity showing failure to fight with stress and ultimately the fish died. Inappropriate behavioral responses to environmental and physiological stimuli due to the toxic effect of aquatic contaminants can have severe implications for survival [40]. The most commonly observed links with behavioural disruption include cholinesterase (ChE) inhibition, altered brain neurotransmitter levels, sensory deprivation, and impaired gonadal or thyroid hormone levels. We conclude that future integrative, multidisciplinary research is clearly needed to increase the significance and usefulness of behavioural indicators for aquatic toxicology, and aim to highlight specific areas for consideration.

Behavioral characteristics are sensitive indicators of sublethal contamination and the end points frequently occur below concentrations that are chronically lethal and at lower concentrations than those that affect growth, so these should be routinely included in aquatic toxicity assessment programs.

In recent years a great advancement in studying the behavioural changes have been observed. Better quantification of behavior pattern has enabled due to development of video tracking technology. Automatic behavior tracking software View Point® automatic behaviour tracking system (Zebralab version 3.22, ViewPoint Life Science Inc., Montreal, CN) have been developed and is being used by number of reasearchers [41]. Moreover, scientific knowledge about the importance of behaviour for the health and fitness of organisms has increased [42].

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